Journal of Bioenergetics and Biomembranes, Vol. 24, No. 3, 1992

MgtA and MgtB: Prokaryotic P-Type ATPases That Mediate Mg^{2+} Influx

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Received February 23, 1992; accepted February 25, 1992

The gram-negative bacterium *Salmonella typhimurium* possesses three distinct Mg^{2+} transport systems, encoded by the *corA*, *mgtA*, and *mgtB* loci. The CorA transport system is the constitutive Mg^{2+} influx system. It can also mediate Mg^{2+} efflux at very high extracellular Mg^{2+} concentrations. In contrast, the MgtA and MgtB Mg^{2+} transport systems are normally expressed only at low extracellular Mg^{2+} concentrations. A strain of *S. typhimurium* was constructed by mutagenesis which lacks Mg^{2+} transport and requires 100 mM Mg^{2+} for growth. Using this strain, both the MgtA and MgtB transport systems were cloned by complementation of the strains inability to grow without Mg^{2+} supplementation. After sequencing and further genetic analysis, the MgtB system appears to be an operon composed of the *mgtC* and *mgtB* genes (5′ to 3′). The downstream *mgtB* gene encodes the 102 kDa MgtB protein which by sequence analysis is clearly a P-type ATPase. Interestingly, while MgtB has relatively poor homology to other known prokaryotic P-type ATPases, it is highly homologous to mammalian reticular Ca^{2+}-ATPases. MgtC is a 22.5 kDa hydrophobic membrane protein that lacks homology to any known protein. Transposon insertions in this gene abolish uptake by the MgtB transport system. We hypothesize that MgtC is a subunit of the MgtB ATPase involved either in proper insertion of MgtB into the membrane or possibly in binding of extracellular Mg^{2+} for delivery to the ATPase subunit. The sequence of the MgtA gene has recently been completed, and it too is a P-type ATPase more similar to eukaryotic than prokaryotic P-type ATPases. Expression of both MgtA and MgtB are highly regulated by the concentration of extracellular Mg^{2+}. Transcription of *mgtB* can be increased about 1000 fold by lowering Mg^{2+} from 1 mM to 1 μM. Likewise, when *mgtB* is expressed from a multicopy plasmid, a similar decrease in extracellular Mg^{2+} greatly increases transport. Under growth conditions of limiting Mg^{2+}, MgtB becomes the dominant Mg^{2+} influx system in *S. typhimurium.* Even so, since MgtB (and MgtA) mediate only influx of Mg^{2+}, it is unclear why the cell requires energy from ATP to mediate Mg^{2+} entry into the cell down a large electrochemical gradient. Further studies of the structure–function and energetics of these novel Mg^{2+} influx P-type ATPases should yield insights into the function of P-type ATPases in general as well as information about the regulation of cellular Mg^{2+} fluxes.

KEY WORDS: Magnesium; transport; ATPase, P-type ATPase; *Salmonella typhimurium*; genetics; influx; sequence regulation; homology; prokaryote.

INTRODUCTION

Mg^{2+} is the second most abundant cation within cells and by far the most abundant divalent cation. It plays important roles in the structural properties of cells, interacts with ATP as the required or preferred substrate for over 300 enzymes, and binds to specific metal sites on a multitude of proteins to alter their properties.[1-6] Despite its importance, little is known

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about how intracellular Mg^{2+} is controlled, including how Mg^{2+} flux across membranes is mediated. A major reason for this limited knowledge is that the techniques available for the study of Mg^{2+} lag far behind those for other ions. Microelectrodes and dyes sufficiently sensitive and selective for Mg^{2+} have not yet been optimized. $^{28}Mg^{2+}$, the only isotope useful for transport studies, is rarely available, has a 21-hour half-life, and is extremely expensive.

The determination of the structure, mechanism, and control of Mg^{2+} transport systems would be of great value, not only because of the importance of Mg^{2+} itself, but also for comparison to other transport systems. Because of the paucity of sensitive techniques with which Mg^{2+} transport can be measured, classical biochemical means cannot be used to purify a Mg^{2+} transport protein. Moreover, despite the great advances in mammalian molecular biology, techniques are not yet available for isolation of a Mg^{2+} transporter from a mammalian cell. Consequently, we have used the powerful genetic techniques available in prokaryotes to identify mutations and subsequently chromosomal loci involved in Mg^{2+} transport. The model organism chosen has been the gram-negative bacterium *Salmonella typhimurium*. Our data indicate that *S. typhimurium*, and likely most gram-negative bacteria, possesses three distinct Mg^{2+} transport systems, designated the CorA, MgtA, and MgtB, transporters.[7–12]

The CorA system is composed of four distinct chromosomal loci, *corA*, *corB*, *corC*, and *corD*. The *corA* locus is composed of a single gene expressing the 42-kDa CorA protein[13] which by itself can mediate Mg^{2+} influx. Concomitant expression from the *corB*, *corC*, and *corD* loci will allow Mg^{2+} efflux via CorA[12] in addition to influx. The CorA transport complex is the major, constitutive Mg^{2+} transporter in *S. typhimurium* (and likely all gram-negative bacteria) and is apparently the cell's only Mg^{2+} efflux system. Under normal growth conditions in the laboratory, the major role of the CorA system is to mediate Mg^{2+} influx, at least at extracellular Mg^{2+} concentrations below 0.3–0.5 mM. At extracellular concentrations of 1 mM or higher, the CorA system function as a gated Mg^{2+}–Mg^{2+} exchanger.[8,12] The CorA protein, capable of mediating influx by itself, represents a novel transport system, with no sequence homology to any known protein and with no apparent structural similarity to any known transport system.

In contrast, the MgtA and MgtB systems are clearly members of a well-studied family of transport proteins, but their function within the organism is not immediately clear. By sequence analysis, both MgtA and MgtB are P-type ATPases (Ref. 11 and unpublished observations). However, they appear to mediate the influx of Mg^{2+} down its electrochemical gradient even in aerobically grown cells whose $\Delta\psi$ plus $\Delta\mu^{+}$ may total -200 mV negative inside. Moreover, the K_a for Mg^{2+} influx via these two systems is not significantly different from that of the CorA system, thus rendering unlikely the possibility that they function (solely) as scavenger systems. This article is a brief review of what we have currently discovered about the function and expression of those two novel ATPases.

TRANSPORT PROPERTIES OF THE MgtA AND MgtB SYSTEMS

Influx

The MgtA and MgtB transport systems each mediate the uptake of both Mg^{2+} and Ni^{2+} (Table I). Because of the extreme cost and relative unavailability of $^{28}Mg^{2+}$, $^{63}Ni^{2+}$ has been used extensively to characterize uptake by each system.[10] The rank order of potency of inhibition by other divalent cations is identical for both Ni^{2+} and Mg^{2+} uptake, Mg^{2+} and Ni^{2+} are competitive inhibitors of the other's uptake, and deletion of the Mg^{2+} transport genes abolishes Mg^{2+} and Ni^{2+} uptake by each system. Mn^{2+} and Co^{2+} are also competitive inhibitors of uptake for both systems but cannot be transported by either. Ca^{2+} is a competitive inhibitor of uptake via MgtA with a K_i approximately 10-fold greater than the K_m for Mg^{2+}; in contrast, Ca^{2+} fails to inhibit uptake via MgtB even when added at concentrations more than 1000-fold greater than the K_m's for Mg^{2+} or Ni^{2+} uptake. In absolute terms, both MgtA and MgtB have a significantly higher V_{max} for Mg^{2+} uptake than for Ni^{2+}. In contrast, the K_m values for uptake of Mg^{2+} and Ni^{2+} are approximately equal for MgtB. With MgtA, the K_m for Ni^{2+} uptake is about 5-fold lower than the K_m for Mg^{2+} uptake.[8,10]

It might be argued from the transport data that the MgtA and/or MgtB systems physiologically mediate the uptake of Ni^{2+} rather than Mg^{2+}. However, at the Ni^{2+} concentrations required for uptake, Ni^{2+} is quite toxic to *S. typhimurium* and most other gram-negative organisms, rapidly causing cell death. Ni^{2+} uptake via Mg^{2+} transport systems is quite common in chemoorganotropic bacteria.[14,15]. The physiological requirement for Ni^{2+} in these bacteria is

Table I. Parameters of $^{28}Mg^{2+}$ and $^{63}Ni^{2+}$ Uptake by the MgtA and MgtB Mg^{2+} Transport Systems[a]

Property	MgtA	MgtB
A. $^{28}Mg^{2+}$ uptake		
V_{max} (pmol min^{-1} 10^8 $cells^{-1}$)	115	75
K_m (μM)	29	6
Apparent K_i (μM)		
Ca^{2+}	300	> 30,000
Co^{2+}	40	8
Mn^{2+}	PI	40
Ni^{2+}	30	13
Zn^{2+}	7	NI
B. $^{63}Ni^{2+}$ uptake		
V_{max} (pmol min^{-1} 10^8 $cells^{-1}$)	14	30
K_m (μM)	5	2
Apparent K_i (μM)		
Ca^{2+}	1000	> 10,000
Co^{2+}	4	4
Mg^{2+}	5	5
Mn^{2+}	10	33

[a]Kinetic parameters are for strains of *S. typhimurium* carrying only the Mg^{2+} transport system indicated; e.g., for MgtA, both the *corA* and *mgtB* genes were inactivated by transposon insertions.[8,10] Experiments were performed on cells grown at 37°C without added Mg^{2+}, conditions which cause near maximal derepression of both *mgtA* and *mgtB* transcription. For comparison, if the cells are grown at 10 mM extracellular Mg^{2+}, the V_{max} decreases to < 1 pmol min^{-1} 10^8 $cells^{-1}$ for both MgtA and MgtB uptake. PI = partial inhibition; Mn^{2+} inhibits $^{28}Mg^{2+}$ uptake via MgtA by only 35%. NI = no inhibition at the highest tested Zn^{2+} concentration (0.1 mM). For Ca^{2+} inhibition of MgtB uptake, an accurate K_i cannot be determined since at concentrations of extracellular Ca^{2+} greater than 10 mM, the cells aggregate extensively.

quite low and could easily be met merely by leakage through a Mg^{2+} transport system. This is in contrast to the substantial Ni^{2+} requirements of chemolithotropic bacteria which have very high affinity inducible Ni^{2+} uptake systems which are not selectively inhibited by Mg^{2+}.[16-18] Finally, as described in the next section, the exquisite physiological regulation of the *mgtA* and *mgtB* chromosomal loci by Mg^{2+} further shows that these two transporters are physiologically Mg^{2+} uptake systems.

Efflux

Neither the MgtA nor the MgtB transport system can mediate Mg^{2+} efflux. In strains carrying functional MgtA and/or MgtB transport systems, i.e., lacking the CorA transport system, no $^{28}Mg^{2+}$ efflux can be detected even at very high extracellular Mg^{2+} concentrations. Thus the CorA transport system, represented by the four distinct chromosomal loci *corA*, *corB*, *corC*, and *CorD*, is the sole transport system in *S. typhimurium* capable of Mg^{2+} efflux.[8,12] This result is important because it allows the conclusion that MgtA and MgtB mediate only the influx of Mg^{2+}, down its electrochemical gradient.

REGULATION OF MgtA AND MgtA EXPRESSION

It was noted early in our studies of Mg^{2+} transport in *S. typhimurium* that the apparent capacity for Mg^{2+} uptake was a function of the Mg^{2+} concentration in the medium that the cells had been grown in, with very low Mg^{2+} concentrations causing an increase in uptake capacity. Once all three Mg^{2+} transport systems were cloned and appropriate strains constructed carrying only one of the three transporters,[8-10] it became clear that the CorA system was not regulated by extracellular Mg^{2+}, but the *mgtA* and especially the *mgtB* genes were exquisitely sensitive to the concentration of Mg^{2+} that the cells were exposed to. The regulation of *mgtA* and *mgtB* expression is apparently at the transcriptional level as demonstrated by gene fusions which express β-galactosidase from the *mgtA* or *mgtB* promoters (Fig. 1). At extracellular Mg^{2+} concentrations greater than about 1 mM, little transcription of *mgtA* or *mgtB* is seen. However, as the extracellular Mg^{2+} concentration in the growth medium is progressively lowered to 1 μM, transcription of *mgtA* and *mgtB* increase substantially. In growth medium containing 10 μM added Mg^{2+}, transcription of *mgtA* and *mgtB* is approximately 25–30-fold greater than at 10 mM extracellular Mg^{2+}. The transcription of *mgtA* increases only slightly as the Mg^{2+} concentration is lowered further. In great contrast, as the extracellular Mg^{2+} concentration is decreased from 10 to 1 μM, transcription of *mgtB* increases dramatically, 30–40-fold over the level observed at 10 μM extracellular Mg^{2+} and approximately 1000-fold compared to the transcription level at 10 mM extracellular Mg^{2+}.

It is of interest that although extracellular Ca^{2+} is not a particularly potent inhibitor of uptake via MgtA and does not inhibit uptake via MgtB at all, it represses transcriptions at both loci[10] at concentrations between 0.1 and 1 mM (Table II). However, the very large increase in transcription of *mgtB* at extracellular

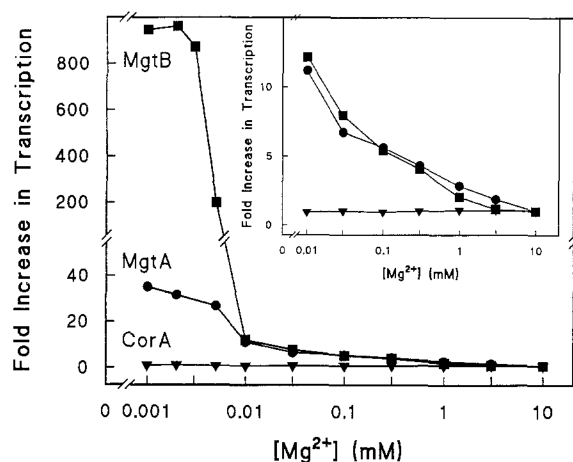

Fig. 1. The effect of Mg^{2+} concentration in the growth medium on β-galactosidase production from *lacZ* fusions to the promoters of the *S. typhimurium* Mg^{2+} transport systems. Cells were grown in N-minimal medium containing the indicated Mg^{2+} concentration to a density of about 10^8 cells/ml and aliquots immediately frozen for assay of β-galactosidase. The inset shows data from 10 μM to 10 mM extracellular Mg^{2+} on a larger scale for clarity. The basal activity of β-galactosidase for the three systems measured at 10 mM extracellular Mg^{2+} ranged from 0.5 to 2.2 units of β-galactosidase.

Mg^{2+} concentrations between 1 and 10 μM is not significantly inhibited by Ca^{2+}, suggesting that this transcriptional regulation involves a different mechanism than the transcriptional regulation above 10 μM extracellular Mg^{2+}. It is unclear physiologically why Ca^{2+} should inhibit transcription of these Mg^{2+} transport systems. Mn^{2+} can also repress transcription of each system. In contrast, Co^{2+} and Ni^{2+} do not repress transcription even though Co^{2+} is a competitive inhibitor of uptake and Ni^{2+} is a substrate for

Table II. Ability of Extracellular Ca^{2+} to Inhibit Transcription of *mgtA* and *mgtB*[a]

Mg^{2+} concentration (μM) during cell growth	MgtA	MgtB
	(Percent inhibition of transcription by Ca^{2+})	
1	77	18
5	76	92
10	80	90
20	72	90

[a]Cells carrying protein fusions expressing β-galactosidase from either the *mgtA* or *mgtB* promoters were grown in the extracellular Mg^{2+} concentration indicated and in the presence or absence of 1 mM Ca^{2+} to a cell density of approximately 10^8 cells/ml and the β-galactosidase activity measured. The data are expressed as the percent inhibition of β-galactosidase activity (and thus transcription) by Ca^{2+} at each indicated Mg^{2+} concentration.

the transport systems. Thus, the ability of a divalent cation to interact physically with either of these transport systems does not correlate with its ability to regulate the system's transcription.

THE SEQUENCE AND PUTATIVE STRUCTURE OF MgtB

The *mgtB* locus of *S. typhimurium* maps to approximately 80.5 min on the chromosome[7] and appears to consist of the *mgtC* and *mgtB* genes (5′ to 3′), arranged most likely as an operon.[11] Both genes are transcribed in the same direction and have been shown to express proteins of 22.5 kDa and 101 kDa for *mgtC* and *mgtB*, respectively. Both proteins are predicted to be membrane bound from their hydropathy plots. This prediction has been confirmed by biochemical analysis for *mgtB*,[8] while preliminary protein fusion data indicate that *mgtC* is also an integral membrane protein (unpublished observations).

Comparison of the amino acid sequence of *mgtB* predicted from its nucleotide sequence indicates that it belongs to the P-type ATPase gene family.[19] P-type ATPases are ubiquitous cation-transport systems whose members, with the addition of MgtB, are known to transport all of the common biological cations, i.e., Mg^{2+}, Ca^{2+}, Na^+, K^+, and H^+. The open reading frame of 908 amino acids predicted for *mgtB* would encode a protein of 102 kDa, which is in good agreement with the apparent molecular weight of 101 kDa of *mgtB* obtained via expression in *E. coli* maxicells.[13] Introduction of a plasmid carrying the *mgtB* gene into a strain of *S. typhimurium* deficient in all three Mg^{2+} transport systems is sufficient to restore growth on low extracellular Mg^{2+} concentrations and to restore Mg^{2+} uptake with kinetic parameters and a cation inhibition pattern identical to that seen with an intact chromosomal copy of *mgtB*.[8,10]

Although prokaryotic P-type ATPases have been described,[20–24] it is striking that MgtB is not exceptionally homologous to other currently known prokaryotic P-type ATPases (Fig. 2). For example, MgtB exhibits only about 18% identity to the KdpB subunit of the *kdp* K^+ influx P-type ATPase from *E. coli.* Using conservative substitutions, another 18% of the sequence shows some similarity. However, KdpB is only 682 amino acids in length, considerably shorter than MgtB. Rather than a total similarity of 36% when comparing only the KdpB sequence, if the full length of the MgtB sequence is used for comparison,

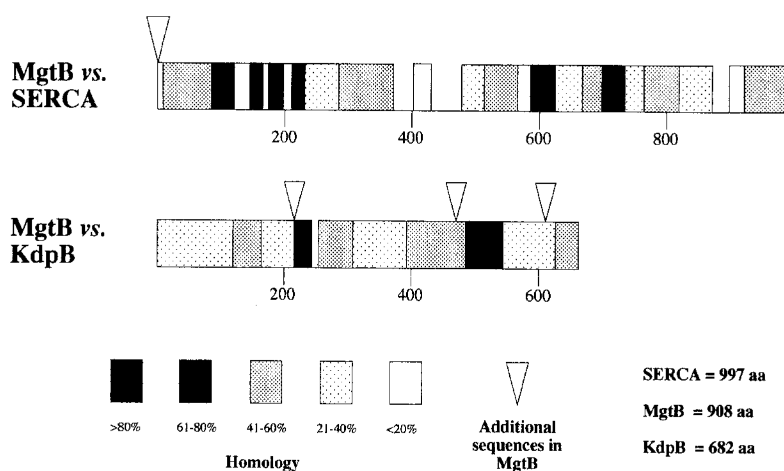

Fig. 2. Comparison of the *S. typhimurium* MgtB amino acid sequence with other P-type ATPases. The amino acid sequences of MgtB,[11] the KdpB subunit of the *kdp* K^+-ATPase of *E. coli*, and the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) of rabbit skeletal muscle[26,47] were aligned as previously described.[11] The percent amino acid identity plus the amino acid similarity was calculated for contiguous blocks of 20 or more amino acids. The wedges above each sequence represent sequences in MgtB that appear to be insertions or additional sequence not found in the other protein. The single insertion shown for the MgtB versus SERCA comparison represents an N-terminal extension in MgtB of about 10 amino acids. By comparison, the three insertions shown for the MgtB versus KdpB comparison represent well over 100 amino acids. In addition, even within the regions illustrated as having similarity, several gaps had to be inserted in the KdpB sequence to obtain alignment. The overall similarity of KdpB to MgtB is approximately 30% while that of MgtB and SERCA is approximately 50%.

the similarity drops to about 30%. Only two short regions of about 20 amino acids in KdpB and MgtB show as much as 65% homology. Comparison with other described prokaryotic P-type ATPases shows far less homology than with KdpB. In great contrast, MgtB shows much greater similarity to the sarco(endo)plasmic reticular Ca^{2+}-ATPases of yeast and mammalian skeletal muscle.[25–29] Sequence length is also closer with the reticular Ca^{2+}-ATPase (SERCA) being 950–1000 amino acids in length compared to 908 amino acids for MgtB. Sequence identity is approximately 25%, and conservative substitutions raise the overall similarity to 50%. Moreover, there are significant stretches of amino acids with more than 80% identity. MgtB is also significantly more similar to other eukaryotic P-type ATPases such as the various Na^+, K^+-ATPases of the plasma membrane and the stomach H^+, K^+-ATPase.[30–32] Interestingly, although MgtB is obviously closely related to the reticular Ca^{2+}-ATPases, the plasma membrane Ca^{2+}-ATPases[33,34] are rather more distant and are only slightly more related than the prokaryotic P-type ATPases. Nonetheless, MgtB is more similar to any of the known eukaryotic P-type ATPases than it is to any reported prokaryotic P-type ATPase. Indeed, generation of an evolutionary tree for the P-type ATPases (Fig. 3) using the algorithm of Feng and Doolittle[35] gives the sophistic answer that *S. typhimurium* and mammals are closely related. Presumably the similarity of MgtB to eukaryotic and particularly Ca^{2+} transporting P-type ATPases of mammalian muscle indicates that the P-type ATPase gene family has multiple branches and that current sequence data are not sufficient to separate the branches in any meaningful manner. Regardless, it is clear that MgtB and MgtA (which is 75% identical to MgtB) form a new class of prokaryotic P-type ATPases with great similarity to mammalian Ca^{2+}-ATPases.

Sequence similarities are echoed in the predicted membrane structures of these proteins, based primarily on hydropathy plots. The first four putative membrane loops occur in similar positions within the N-terminal 300 amino acids in all known members of the P-type ATPase gene family. Likewise, putative loops 5 and 6 occur in similar positions at a fairly constant distance C-terminal to the aspartyl residue that accepts the phosphate from ATP during transport. However, whereas the prokaryotic KdpB protein has

DNA "distance" Tree of Several P-type ATPases
(arbitrarily rooted)

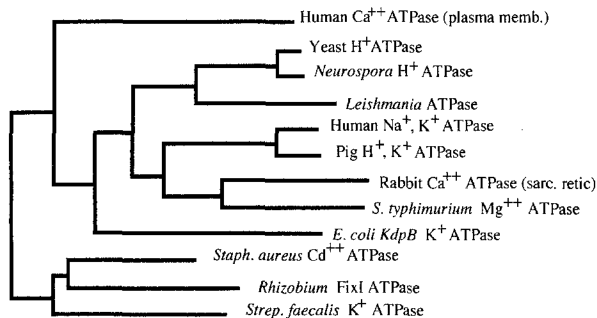

Fig. 3. Evolutionary tree diagram comparing MgtB with other P-type ATPases. MgtB was compared to a variety of other P-type ATPases by the algorithm of Feng and Doolittle.[35] I am grateful to Dr. Simon Silver (University of Illinois College of Medicine) for making this comparison. The results give the obviously fallacious conclusion that *Salmonella typhimurium* ATPases and mammalian reticular Ca^{2+}-ATPases evolved at approximately the same time. The tree serves the purpose, however, of illustrating how closely the Mg^{2+}-ATPases of the prokaryotic *S. typhimurium* are to mammalian P-type ATPases and, conversely, how unrelated the Mg^{2+} ATPases are to other prokaryotic ATPases. The results suggest that there are several subfamilies of transporters within the P-type ATPase gene family and that additional sequences, perhaps from yeast, will be necessary to construct a more reasonable tree.

at most one additional membrane loop, MgtB and the eukaryotic P-type ATPases apparently have a minimum of two and likely four additional membrane loops in the C-terminal portion of the sequence. Regardless of whether one favors the hypothesis of Serrano and colleagues[36] favoring eight membrane loops or that of MacLennan and coworkers suggesting 10 membrane loops,[27] it is clear that MgtB, although a prokaryotic protein, is much more similar in its structure to eukaryotic and especially the mammalian muscle Ca^{2+} P-type ATPases than it is to known prokaryotic P-type ATPases.

MacLennan and colleagues have described mutagenesis experiments[37] based on their membrane model[27] for the muscle sarcoplasmic reticulum Ca^{2+}-ATPase that appear to identify six amino acid residues within the membrane responsible for Ca^{2+} binding during the transport process. A comparison of these amino acids among the various P-type ATPases provides a basis for the design of some interesting mutagenesis experiments (Table III). It should be noted that a similar comparison among these residues cannot be made with prokaryotic P-type ATPases other than MgtB because the poorer homology precludes accurate alignment.

The *first* of the six residues always occurs in the N-terminal putative transmembrane segment 4 as predicted by hydropathy plots and is always preceeded by a proline. This residue is a glutamate in MgtB and in eukaryotic P-type ATPases which transport cations other than protons. The H^+-ATPases, such as the protozoan and fungal enzymes, have a hydrophobic amino acid in this position. The *second* putative important intramembrane residue always occurs at a peak of hydrophobicity within transmembrane segment 5. This residue is either an alanine or a glutamic acid in the eukaryotic P-type ATPases whereas it appears to be an asparagine in MgtB.

The *third through fifth* conserved residues occur as a cluster near the C-terminus of transmembrane loop 6. The *third* is perhaps the most interesting in this cluster. P-type ATPases which transport a *divalent* cation have an asparagine at this position. However, those P-type ATPases which transport a monovalent ion (including proton) have a variety of residues in place of the asparagine. Thus, the presence of an asparagine at the third position among these putative intramembranous residues appears diagnostic of a divalent cation P-type ATPase.

The *fourth* residue is an alanine in the fungal and yeast H^+-ATPases, and an asparagine in the *Leishmania* H^+-ATPase. However, in those P-type ATPases which transport a metal ion, residue 4 is a threonine or a methionine. Thus, for the ATPases transporting cations other than protons, residue 4 has a side chain possessing a polar atom containing free electrons, i.e., the oxygen of the threonine hydroxyl or the sulfur of the methionine. MgtA and MgtB both have tyrosines in this position, the tyrosine hydroxyl preserving the motif of a side chain with free electrons. An unhydrated Mg^{2+} ion has a far smaller ionic radius (0.65 Å) than other common biological cations (Na^+, 0.95 Å; Ca^{2+}, 0.99 Å; and K^+, 1.33 Å). Tyrosine is a much larger amino acid than threonine or methionine. If one assumes that the "pore" or binding "pocket" of a P-type ATPase is similar among the various members of the family, the larger tyrosine residue in a Mg^{2+}-transporting ATPase would be required for proper binding and orientation of the smaller cation.

The *fifth* residue is an aspartate in all P-type ATPases even though this overall region shows no exceptional conservation of sequence. Presumably this residue could stabilize and partially compensate the positive charge on the cation via an electrostatic bond with the aspartyl carboxyl group.

Table III. Comparison of Amino Acids Putatively Involved in Cation Binding within Transmembrane Regions of P-Type ATPases[a]

P-type ATPase	Putative metal binding residues						Reference
	1	2	3	4	5	6[b]	
Mg^{2+}-ATPase, *S. typhimurium, mgtB*	**E337**	**N718**	**N743**	**Y746**	**D747**	E818	11
Mg^{2+}-ATPase, *S. typhimurium, mgtA*	**E315**	**N695**	**N715**	**Y718**	**D719**	V790	Unpublished data
Ca^{2+}-ATPase, rabbit muscle (FT)	**E309**	E771	**N796**	T799	**D800**	E908	26, 37, 47
Ca^{2+}-ATPase, rabbit muscle (ST)	**E309**	E770	**N796**	T799	**D800**	E907	26, 37, 47
Ca^{2+}-ATPase, rat kidney	**E309**	E771	**N796**	T799	**D800**	E908	25
Ca^{2+}-ATPase, human teratoma	**E440**	A871	**N891**	M894	**D895**	?	33
Ca^{2+}-ATPase, rat brain, isoform 1	**E433**	A870	**N888**	M891	**D892**	?	34
Ca^{2+}-ATPase, rat brain, isoform 2	**E412**	A848	**N869**	M872	**D873**	?	34
Ca^{2+}-ATPase, rat brain, isoform 3	**E417**	A853	**N874**	M877	**D878**	?	48
Ca^{2+}-ATPase, *Drosophila*	**E309**	E771	**N796**	T799	**D800**	E908	49
Ca^{2+}-ATPase, *Artemia*	**E312**	E775	**N800**	T803	**D804**	E912	50
Ca^{2+}-ATPase, *S. cerevisiae*, PMR1	**E329**	A749	**N774**	M777	**D778**	D856	51
Na^+/K^+-ATPases, 10 isoforms[c]	**E332**	E784	D808	T811	**D812**	V922	30, 31
H^+/K^+-ATPase, rat gastric	**E343**	E795	E820	T823	**D824**	E936	32
H^+-ATPase, *S. cerevisiae*, PMA1 or PMA2	V336	E703	A726	A729	**D730**	E803	28, 29
H^+-ATPase, *Candida albicans*, PMA1	V313	E680	A703	A706	**D707**	E780	52
H^+-ATPase, *Schizosaccharomyces pombe*	V334	E701	A724	A727	**D728**	E803	53
H^+-ATPase, *Neurospora crassa*	V335	E703	A726	A729	**D730**	E805	54, 55
H^+-ATPase, *Leishmania donovani*, two isoforms	I315	A686	T716	N719	**D720**	D806	56, 57

[a]The original version of this table, comparing five P-type ATPases, was published by Clarke *et al.*[37] from site-directed mutagenesis data of charged or polar residues predicted to be within transmembrane segments. Mutagenesis of any of the six residues shown for the rabbit sarcoplasmic reticulum Ca^{2+}-ATPase abolishes both Ca^{2+} and ATP-dependent phosphorylation of the ATPase as well as Ca^{2+} transport. Alteration of other predicted intramembrane charged residues did neither.[37] Examples of most known eukaryotic P-type ATPases are shown in comparison to MgtA and MgtB. Alignments were determined as previously described.[11] Prokaryotic P-type ATPases other than MgtA and MgtB are not shown since their lesser homology precludes accurate alignment. FT and ST refer to fast twitch and slow twitch skeletal muscle, respectively. PMA1, PMA2, and PMR1 are gene designations. The underlined amino acids of MgtA and MgtB denote residues that differ from the sarcoplasmic reticulum Ca^{2+}-ATPases. Bold-faced residues denote groups of residues discussed in the text.

[b]Residue 6 in plasma membrane Ca^{2+}-ATPases is not assigned because of a C-terminal extension in these proteins that is not sufficiently similar to other members of this family to allow unambiguous alignment.

[c]Residues in the alpha subunit of Na^+, K^+-ATPases from sheep (shown), pig, rat (three isoforms), chicken, human, *Torpedo californica, Drosophila,* and *Artemia* are all identical except in position 6 where rat isoform 2 has an alanine and *Drosophila* and *Artemia* have isoleucines.

The *sixth* and last of these intramembrane conserved residues occurs within transmembrane segment 8. It is almost always a glutamate or an aspartate and thus might be hypothesized to be required to form an electrostatic bond as suggested for residue 5. However, two interesting exceptions occur. The Na^+, K^+-ATPases have a hydrophobic amino acid in this position, usually a valine. Moreover, in sharp contrast to MgtB and most other P-type ATPases, our preliminary sequence data suggests that residue 6 is a valine in MgtA. Thus, even if residue 6 forms an electrostatic bond in most P-type ATPases, this cannot be an absolute requirement for transport. Moreover, it suggests that a large part of the cation selectivity differences seen between MgtA and MgtB (Table I) might be explained by this one amino acid difference. Delineation of the role of these putative intramembranous residues via mutagenesis experiments coupled with careful transport studies should elucidate important information about the ion specificity of this family of transport systems.

THE POSSIBLE ROLE OF MgtC

The role of the protein encoded by the *mgtC* gene is unclear. Our preliminary data indicate that it is a 22.5-kDa integral membrane protein. Among currently well-studied P-type ATPases, those enzymes which mediate transport of an ion from the cytosol (either out of the cell or into an intracellular compartment) do not appear to have or require a second or β subunit. However, those P-type ATPases which mediate flux of ion into the cytosol all appear to have (and require) a β-subunit. Since MgtB mediates Mg^{2+}

influx, it seems likely that MgtC forms a required subunit of MgtB.

The role of MgtC or indeed of any β-subunit for the P-type ATPases is unclear. Experiments with the β-subunit of the Na^+, K^+-ATPase have suggested that it is required for proper insertion and/or stabilization of the ATPase subunit in the membrane[38–41] although there may be exceptions.[42] However, another or additional possibility is that a β-subunit is required essentially as an ion-binding protein that delivers ion to the ATPase subunit. There is a significant body of evidence that little if any of the amino acid chain of the ATPase subunit extends out of the membrane into the extracellular space.[43–46] Thus, it is possible that there is little protein with which to form an ion-binding pocket. The β-subunit would then perform the function of a binding protein or ion receptor. This obviously speculative hypothesis can readily be tested with MgtB due to the ease of genetic manipulation in prokaryotes.

IMPLICATIONS FROM STUDIES OF MgtA AND MgtB

The identification of MgtB and MgtA raise several interesting questions. First, it is unclear why a cell would need to use ATP to energize the transport of positive cation like Mg^{2+} down its electrochemical gradient. Since all physiological data currently indicate that MgtB and MgtA are Mg^{2+} transport systems, it seems unlikely that the energy requirement is due to the need for countertransport of another ion. Indeed, even if that were the case, Mg^{2+} itself could supply that energy. Elucidation of the reason(s) for using an ATPase will obviously require further study.

Lastly, identification of MgtB and MgtA as the first described Mg^{2+} transport systems opens the possibility for identifying and cloning presumably similar Mg^{2+} transporters from eukaryotes. The ubiquitous nature of the P-type ATPases as a gene family strongly implies that similar transport systems for Mg^{2+} exist in eukaryotic cells. We have tentatively identified Mg^{2+} transporters from the yeast *Saccharomyces cerevisiae* and are determining whether any of them are members of the P-type ATPase family.

ACKNOWLEDGMENTS

Research in this laboratory was supported by grants GM39447 and HL18708 from the National Institutes of Health. I am grateful to Dr. Tao Tao for assistance with the figures and to Dr. Simon Silver for generating the tree comparison of ATPase sequences.

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