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Magnesium Transport in *Salmonella typhimurium*: *mgtA* Encodes a P-Type ATPase and Is Regulated by Mg^{2+} in a Manner Similar to That of the *mgtB* P-Type ATPase

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***Salmonella typhimurium* has three distinct Mg^{2+} transport systems: CorA, MgtA, and MgtB, each encoded by its respective gene. *corA* and *mgtB* have been previously sequenced and characterized. This report details the sequence and properties of *mgtA*. Like *mgtB*, *mgtA* encodes a P-type ATPase. The *mgtA* gene encodes a slightly smaller protein than does *mgtB*, with a predicted molecular mass of about 95 kDa, running at 91 kDa on protein gels, which compares with values of 101 and 102 kDa, respectively, for the MgtB protein. The deduced amino acid sequence of MgtA is only 50% identical to that of MgtB, with a further 25% conservative amino acid substitutions, surprisingly low for such otherwise functionally similar proteins from the same organism. Codon usage for each gene is normal for *S. typhimurium*, however, indicating that neither gene is the result of a recent acquisition from another organism. A single open reading frame at *mgtA* encodes MgtA, in contrast to *mgtB*, which is shown to be an operon encoding (5′ to 3′) the 22.5-kDa MgtC and the MgtB proteins. Genetic constructs were used to show that deletion of MgtC does not alter the expression or transport properties of MgtB, making the role of the companion MgtC protein unclear. (The *S. typhimurium* homolog of *treR*, which encodes a putative repressor for trehalose uptake, is encoded by a gene adjacent to *mgtA*, and its sequence is also reported.) Finally, extremely strong Mg^{2+} regulation of the *mgtA* and *mgtB* promoters but not of the *corA* or *treR* promoters was demonstrated by cloning the appropriate DNA sequences with *luxAB* and measuring enhancement of light production as a function of extracellular Mg^{2+} concentration. Lowering the extracellular Mg^{2+} concentration from 10 mM to 1 or 10 μM elicited a transcriptional response of several thousandfold from both the *mgtA* and *mgtB* promoters.**

Magnesium is a vital biological divalent cation functioning both as cofactor and regulator of numerous proteins and as both a regulatory and stabilizing factor for membranes, ribosomes, and other cellular structures (3, 10, 14, 18, 19, 27, 28). Recent studies in mammalian and other systems have shown that Mg^{2+} transport and intracellular content, both free and total, are far more active metabolically than previously appreciated (6, 10, 13, 20, 28, 29, 32). Nonetheless, specific membrane transport proteins for Mg^{2+} cannot be studied effectively in mammalian systems because of a lack of appropriate techniques. In contrast, prokaryotic systems, with their highly developed genetic and molecular techniques, have allowed dissection of Mg^{2+} transporters.

In *Salmonella typhimurium*, three Mg^{2+} transport systems have been identified and designated CorA, MgtA, and MgtB, each encoded by its respective gene (16, 35). Transport via the CorA system is constitutive and of high capacity. *corA* encodes a single protein of about 40 kDa, capable of mediating Mg^{2+} influx. The CorA protein has no homology to any currently known protein (34). In contrast, *mgtB* encodes both MgtB, an Mg^{2+}-transporting P-type ATPase (33, 38), and MgtC, a 22.5-kDa protein of unknown function. Interestingly, MgtB has relatively poor similarity to other prokaryotic P-type ATPases but much greater similarity to eukaryotic P-type ATPases, especially the muscle sarcoplasmic reticulum Ca^{2+}-ATPases (25, 33, 38). The *mgtA* gene has not previously been characterized at the molecular level. Transcription of both *mgtA* and *mgtB* is tightly repressed under normal laboratory growth conditions. This repression, however, is relieved when the Mg^{2+} concentration in the medium is lowered (37) and upon phagocytosis into mammalian epithelial cells (26). Here we report that the nucleotide sequence of *mgtA* also encodes a P-type ATPase but that no gene encoding a protein similar to MgtC is present. Additional data suggest several similarities but also significant differences in these two genes and the two Mg^{2+}-transport ATPases. In addition, derepression of the promoters for both *mgtA* and *mgtB* by decreasing the extracellular Mg^{2+} concentration results in extremely large increases in both gene transcription and translation of functional transport protein.

MATERIALS AND METHODS

Plasmids and strains. The plasmids and strains used and their sources are listed in Table 1.

Reagents. Reagents were purchased from Sigma (St. Louis, Mo.), Aldrich (Milwaukee, Wis.), or Gibco-BRL (Life Technologies, Inc., Bethesda, Md.). Restriction endonucleases and T4 DNA ligase were purchased from Gibco-BRL and used as suggested by the manufacturer. Plasmid p*Trc*99A was obtained from Pharmacia LKB Biotechnology. The T7 Sequenase 2.0 kit was purchased from U.S. Biochemicals (Cleveland, Ohio), and the manufacturer's suggested double-stranded sequencing protocol and gel methods were followed. Synthetic oligonucleotide primers were custom ordered from Midland Reagent Co. (Midland, Tex.) or Oligos, Etc. (Wilsonville, Oreg.).

Buffers. LB broth was used for routine cultures, with antibiotics added as required, as previously described (16, 38). For luciferase assays as well as $^{63}Ni^{2+}$ transport assays, cells were grown in N minimal medium (16) supplemented with

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TABLE 1. Strains and plasmids used in this work

Strain	Relevant genotype/plasmid	Source or reference
TN2540	DB4926 *metE551 metA22 hisC47*(Amp^r) *trpB2 ilv-452 rpsL120 fla-66 xyl-404 galE496 hsdL6*($r^- m^+$) *hsdSA29*($r^- m^+$)	C. G. Miller
MM142	TN2540/pSPH25 (*corA*)	16
MM169	TN2540/pSPH37 (*mgtA* + *treR*)	16
MM171	TN2540/pSPH39 (*mgtC* + *mgtB*)	16
MM281	*DEL485*(*leuBCD*) *mgtB10*::MudJ *corA45*::MudJ *mgtA21*::MudJ *zjh-1628*::Tn*10*(Cam^r)	16, 35
MM387	*DEL485*(*leuBCD*) *corA185*::Tn*10*Δ 16Δ17(Tet^r)	34
MM1101	MM387/pTT5SE	This study
MM1102	MM387/pTT6SE	This study
MM1103	MM387/pTT-CALux	This study
MM1104	MM387/pTT-ALux	This study
MM1105	MM387/pTT-BLux	This study
MM1106	MM387/pTT-CLux	This study
MM1107	MM387/pTT-DLux	This study
MM1108	MM281/pTT39	This study
MM1109	MM281/pTT39d	This study

1 mM leucine, 0.1% casamino acids, 0.4% glucose, 1 μg of $FeSO_4$ per ml, and the indicated concentration of Mg^{2+}. Luciferase assay buffer contains 50 mM sodium phosphate buffer, pH 7.5, with 0.01% dodecyl aldehyde. Competent cells of both *Escherichia coli* and *S. typhimurium* were prepared with TSS buffer (30). For transformation by electroporation, *S. typhimurium* cells were prepared by the method of Miller (21).

Luciferase assay. The *luxAB* fragment was excised from pSB71 (24) as an *Eco*RI-*Sma*I fragment and ligated into pBluescript II KS(−) cut with *Eco*RI and *Hin*cII to generate pTT5. This plasmid had a high background of *luxAB* transcription, presumably due to the lack of a terminator. Consequently, the *luxAB* insert was reisolated from the pBluescript vector as either an *Eco*RI-*Apa*I or *Eco*RI-*Bam*HI fragment and ligated into p*Trc*99A cut with the same combination of restriction endonucleases to generate pTT5SE and pTT6SE, respectively. Promoter-*luxAB* plasmids were then constructed as described below. *S. typhimurium* strains carrying promoter-luciferase reporter constructs were grown overnight in LB broth (with no added Mg^{2+}) at 37°C. Subcultures were made by inoculating an aliquot of the original overnight cultures into N minimal medium without added Mg^{2+} to a final optical density at 600 nm (OD_{600}) of 0.025 or 0.05, as indicated. (Initial cell density over a range of OD_{600}s from 0.025 to 0.1 slightly altered the time course of transcriptional increases but did not significantly affect the shape, extent, or Mg^{2+} dependence of the transcriptional response.) Cultures were supplemented with Mg^{2+} as indicated for each experiment and grown at 37°C unless otherwise indicated. Fifty microliters of the subculture was taken at each time point and mixed with 500 μl of room temperature luciferase assay buffer in a 0.7-ml clear microcentrifuge tube. The capped tube was vortexed for 10 s and placed in a carrier scintillation vial, and luciferase activity was determined immediately by light counting in a Beckman LS7000 liquid scintillation counter for 0.5 min with a full channel setting. To correct for coincidence detection in the scintillation counter, the activity was calculated as the square root of the apparent counts per minute (cpm) and then normalized for cell density. For comparisons between experiments, data within a single dose-response curve were normalized to the activity at 100 mM Mg^{2+} (100%) at each assay time. Luciferase activity was linear over cell densities in the scintillation counter (as OD_{600}) from 0.001 to at least 1.0 as long as the total cpm was less than 10^6. Activities greater than this amount were measured by dilution, so that the total cpm used per cell aliquot was less than 10^6.

$^{63}Ni^{2+}$ transport. Plasmids carrying inserts to be tested were transformed by electroporation into MM281. Strain MM281 carries insertions at all three Mg^{2+} transport loci and thus requires 100 mM extracellular Mg^{2+} for growth and exhibits no detectable Mg^{2+} uptake (16, 35). The resulting strains were grown overnight in LB broth supplemented with appropriate antibiotics overnight. Cultures of 20 ml were started by 1:50 inoculation into N minimal medium containing 1 mM Mg^{2+}. After 8 h of growth, the cells were collected by centrifugation at 1,000 × *g* for 15 min and washed twice in the same volume of N minimal medium containing no added Mg^{2+}. New subcultures were then started from the washed cells by resuspension in N minimal medium containing 10 μM Mg^{2+} (or other concentrations as indicated) to a final OD_{600} of 0.1, followed by overnight incubation at 37°C. This concentration of added Mg^{2+} is limiting for growth. As the Mg^{2+} is depleted overnight, a large induction of *mgtA* and *mgtB* transcription occurs, providing a sufficient level of transport activity for ready measurement. This small amount of Mg^{2+} is added to allow some degree of initial cell growth to give sufficient cell mass for convenient measurement of transport. Control experiments with cells incubated overnight in medium without added Mg^{2+} gave similar results. For the assay, cells were collected and washed three times in N minimal medium without added Mg^{2+} before suspension in the same medium at a final OD_{600} of 1.0 for use in the transport assay. The transport assay was performed as previously described (15, 35).

Computer analysis. DNA sequences were assembled and analyzed by using GeneWorks or PCGENE (Intelligenetics, Mountain View, Calif.). The deduced amino acid sequences were compared with available sequences in the GenBank database via the National Center for Biotechnology Information BLAST (2) mail server (blast@ncbi.nlm.nih.gov). Final protein sequence alignment was done by hand.

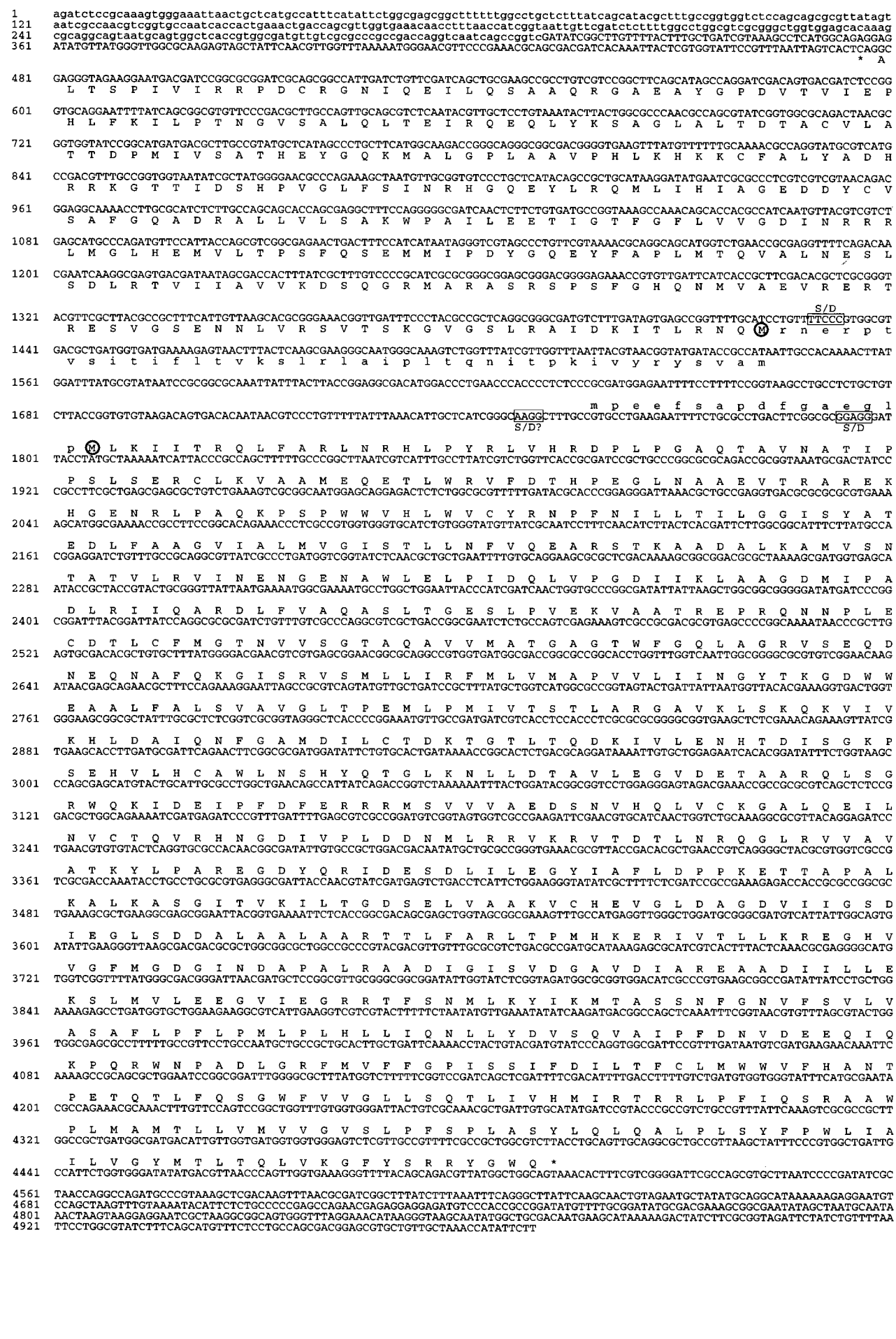
RESULTS

Sequence analysis of *mgtA*. The *mgtA* region was originally isolated as pSPH37 from an *S. typhimurium* chromosomal library by complementation of strain MM77 (16) by restoration of the ability to grow without supplementation of the medium with Mg^{2+}. This plasmid has previously been shown to encode two proteins with gel molecular masses of 37 and 91 kDa. The 37-kDa protein is found in the cytosol and is transcribed in the opposite direction from the 91-kDa protein, which is membrane bound. Only the 91-kDa protein is required for complementation of the Mg^{2+}-dependent phenotype of MM77 and similar strains and has been designated MgtA (16, 35, 36). Upon sequencing of the chromosomal insert in pSPH37, two open reading frames were found (Fig. 1). In agreement with the expression data, the encoded proteins are transcribed in opposite directions, with predicted molecular masses of 36 to 39 and 95 to 99 kDa, depending on the start sites chosen. The locations of the open reading frames were in agreement with the expression data from subclones described previously (36).

The deduced amino acid sequences were searched against the EMBL and GenBank databases. The sequence of MgtA was clearly similar to that of the superfamily of P-type ATPases, membrane ATPases responsible for cation flux (25). Not surprisingly, MgtA was most similar to its sibling Mg^{2+} transport P-type ATPase in *S. typhimurium*, MgtB (38); however, the overall similarity appears low for enzymes from the same organism mediating the same reaction, about 50% amino acid identity, with an additional 25% conservative amino acid substitutions. Codon usage at both *mgtA* and *mgtB* is normal for *S. typhimurium*, suggesting that both ATPases are encoded by typical *S. typhimurium* genes.

The alignment of the MgtA and MgtB proteins is shown in Fig. 2. The similarity is weakest in the N-terminal 100 to 150 amino acids, with a relatively constant similarity throughout the remainder of the proteins. In particular, MgtA and MgtB are quite similar in the 100 or so amino acids following the conserved phosphorylation site (Asp-377 in MgtA). While the phosphorylation site itself is highly conserved in all P-type ATPases, the sequence immediately following this site is quite variable in this family, even among otherwise closely related enzymes. During the preparation of the manuscript for this article, the *E. coli mgtA* sequence appeared in GenBank as part of the *E. coli* genome sequencing project. The *E. coli* and *S. typhimurium* MgtA amino acid sequences are 91% identical and 95% similar (see legend to Fig. 2), far higher than the similarity between the amino acid sequences of MgtA and MgtB from *S. typhimurium*.

Chromosomal structure at *mgtB*. The previously published sequence of the MgtB protein (38) revealed that it is likely encoded by the second gene of a small operon (see below) which also encodes the 22.5-kDa MgtC protein. MgtC shows no similarity to any known protein, including known β-subunits of P-type ATPases, and is likely membrane bound, as determined by hydropathy analysis. In contrast to MgtC, the 37-kDa



protein from plasmid pSPH37 is transcribed in the opposite direction from MgtA and shows no similarity to MgtC or to the known β-subunits of eukaryotic P-type ATPases. (The sequence 3′ to *mgtA*, as far as has been determined [Fig. 1], also shows no apparent additional open reading frame that might encode an MgtC-like protein.) Indeed, rather than encoding an apparent membrane protein, the open reading frame 5′ to *mgtA* encodes a 37-kDa protein with a high degree of similarity to many bacterial operon repressors, e.g., LacI, GalR, and MalR (5, 7, 17). Thus, the chromosomal structures at *mgtA* and *mgtB* are significantly different. We therefore investigated the locations of the promoters for these genes and the roles, if any, of the genes encoding the MgtC and 37-kDa proteins.

Promoter activity. From the sequence of the *mgtA* region, the promoters for the genes encoding MgtA and the 37-kDa protein should lie in the intervening DNA sequence. Computer analysis of the DNA sequence with PCGENE was consistent with this expectation. Similar analysis at *mgtB* indicated that the only likely promoter site was 5′ to the *mgtC* gene. The sequence between *mgtB* and *mgtC* was not predicted to have promoter activity. To confirm these predictions, potential promoter regions from the *corA* Mg^{2+} transporter (as a control), *mgtA*, and *mgtB* loci were subcloned from the initial chromosomal isolates in pSPH25, pSPH37, and pSPH39 (16, 35), respectively, into the luciferase reporter vector pTT5SE. In addition, the putative promoter sequence at *mgtA* was cloned in an orientation opposite to that of *mgtA* to determine potential promoter activity for the 37-kDa protein. The intervening sequence between the *mgtC* and *mgtB* genes was also cloned into the same vector to determine its ability to act as a promoter. The genetic maps of the three regions and the promoter-luciferase reporter constructs for all five potential promoter sites are given in Fig. 3. After confirmation of the insertion junctions by restriction mapping and sequence analysis, the plasmids were transformed into *S. typhimurium* JR501 to allow proper DNA modification. Plasmids prepared from JR501 transformants were transformed into *S. typhimurium* MM387 for assay of promoter activity. This strain carries a *corA*::Tn*10*(tet) insertion and is thus dependent on chromosomal copies of *mgtA* and *mgtB* for Mg^{2+} uptake (34). Promoter activity was assayed in the presence of 100 mM and 1 μM added Mg^{2+} in N minimal medium. Basal plasmid promoter activity with the vector alone (pTT5SE and pTT6SE) was not affected significantly by the Mg^{2+} concentration (Table 2). The positive control vector pTT6SE was highly induced by addition of IPTG (isopropylthiogalactopyranoside) to the medium, and this induction was not affected by the Mg^{2+} concentration (Table 2).

The Mg^{2+} sensitivity of the putative promoters of each Mg^{2+} transport system was then determined. We have previously shown that transcription of *corA*, measured by using a *corA*::*lacZ* fusion, is unaffected by the Mg^{2+} concentration. A similar result was found with pTT-CALux (Table 3), confirming that *corA* is not significantly regulated by the extracellular Mg^{2+} concentration. In contrast, protein fusions of *mgtA* and *mgtB* to *lacZ* had previously shown that a decrease in extracellular Mg^{2+} markedly stimulated their transcription (37, 38). However, the fusions were not sufficient to distinguish the location of the promoter for the putative *mgtCB* operon or to determine if transcription of the gene encoding the 37-kDa protein 5′ to *mgtA* was regulated by Mg^{2+}. Because of the homology of the 37-kDa protein to other operon repressors, it seemed possible that this particular repressor might be involved in the Mg^{2+} regulation of *mgtA* and *mgtB*. The results presented in Table 3 show that the promoter for the *mgtCB* region is 5′ to *mgtC*. The sequence between *mgtC* and *mgtB* (pTT-BLux) had no intrinsic promoter activity in the presence or absence of Mg^{2+}. In contrast, while the sequence 5′ to *mgtC* (pTT-CLux) has minimal intrinsic promoter activity at an extracellular Mg^{2+} concentration of 100 mM, a decrease to 10 μM Mg^{2+} (Table 3) or 1 μM Mg^{2+} (data not shown) elicited strong promoter activity. We conclude that *mgtCB* is indeed an operon and that the site of Mg^{2+} regulation of its transcription is encoded within the sequence in pTT-CLux, 5′ to the *mgtC* gene.

At *mgtA*, the insert sequence in the orientation towards *mgtA* (pTT-ALux) likewise had minimal intrinsic promoter activity at 100 mM extracellular Mg^{2+} but strong activity when extracellular Mg^{2+} was reduced to 10 μM (Table 3) or 1 μM (data not shown). In contrast, when this same sequence was inserted in the opposite orientation (pTT-DLux), as for transcription of the gene encoding the 37-kDa protein, the insert sequence showed good intrinsic promoter activity at 100 mM extracellular Mg^{2+} that was decreased somewhat when extracellular Mg^{2+} was lowered to 10 μM. Dose-response curves for from 100 mM to 1 μM extracellular Mg^{2+}, performed after 4, 8, or 24 h of incubation, confirmed the lack of significant Mg^{2+} regulation of the promoter activity of this sequence when oriented as in pTT-DLux (data not shown). We conclude that the promoter for *mgtA* is within the segment cloned. This promoter, when oriented towards *mgtA*, is highly sensitive to extracellular Mg^{2+}. This same sequence apparently contains an additional promoter oriented oppositely and regulating the gene encoding the 37-kDa protein. Transcription of this gene is not regulated significantly by Mg^{2+}. Since this gene encodes an operon repressor, an obvious possibility is that it mediates the Mg^{2+} regulation of *mgtA* and/or *mgtCB*. This is unlikely, however. During preparation of the manuscript for this article, the sequence of the corresponding gene from *E. coli* was submitted to GenBank. The 37-kDa protein has been identified as the product of *treR*, the repressor for trehalose utilization, while immediately 5′ to *treR* is the *E. coli* homolog of *mgtA* (5a).

Role of MgtC. The role of the protein encoded by *mgtC* is unclear. Its most likely role, based on studies of β-subunits of eukaryotic Na^+,K^+- and H^+,K^+-ATPases (11, 12, 22), would be to help insert the ATPase (or α) subunit into the membrane. If so, this function would only be relevant for the MgtB

FIG. 1. Nucleotide and derived amino acid sequences of the *mgtA* region of *S. typhimurium*. Nucleotides in potential coding regions are capitalized. Translation of the open reading frames for the 37-kDa protein (TreR) and for MgtA are given in the single-letter code under the nucleotide sequence. Note that the complementary strand is shown for the 37-kDa protein, and thus the protein sequence should be read in reverse, starting from position 1422. Potential Shine-Dalgarno sites are indicated by boxes. The most likely start codons are indicated by a circled methionine residue. For MgtA, the ATG codon at position 1806 is the most likely start site, based on the gel molecular mass of the MgtA protein and on the good Shine-Dalgarno site at positions 1793 to 1797. For the 37-kDa protein, the open reading frame contains in-frame start codons at positions 1542 and 1422. However, there is no potential Shine-Dalgarno site near the start codon at 1542. Moreover, the 40 amino acids potentially encoded by the region between nucleotides 1542 and 1422 show no homology to the GalR repressor family and do not have a counterpart in other members of the family. Amino acid homology in this gene family is highest at the N terminus. Finally, the gel molecular mass of the 37-kDa protein agrees with the start site at 1422 but would be almost 5 kDa too small for a protein beginning at nucleotide 1542. Thus, we conclude that the open reading frame for the 37-kDa protein begins at nucleotide 1422. This is consistent with the recently reported *E. coli* sequence of *treR* (accession number U07790) (5a). The GenBank accession number for the sequence of the *mgtA*/*treR* region as shown is U07843.

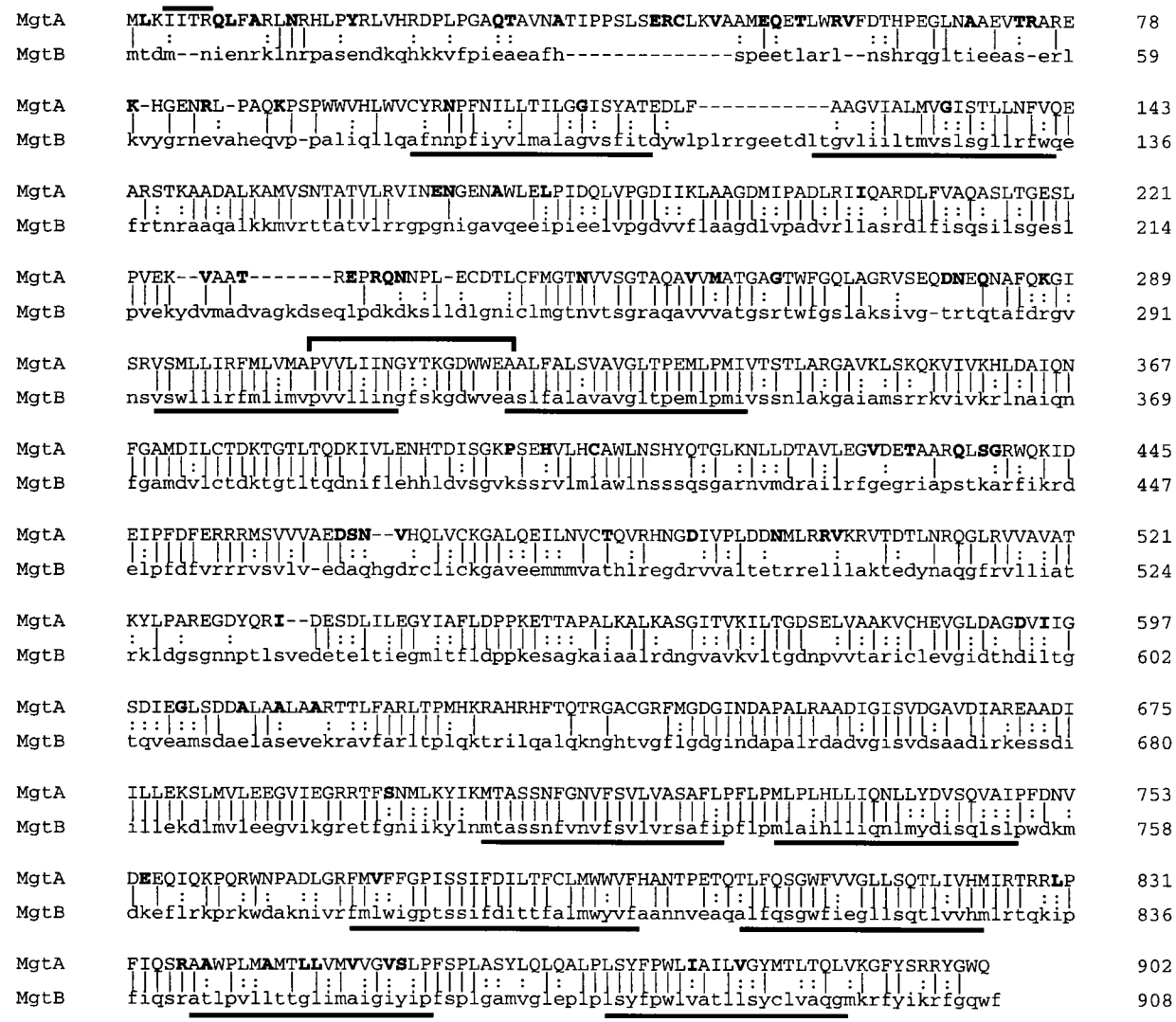


FIG. 2. Amino acid alignment of the MgtA and MgtB proteins. Alignment of the deduced amino acid sequences of MgtA (this report) and MgtB (37) (GenBank accession number J05728) was done by hand. Identities are shown by a vertical bar, and conserved residues are shown by a colon. The homology over the initial 130 amino acids of the two proteins is poor, and the alignment may not be optimal. Substitution of an amino acid between the two proteins was considered to be conservative according to the following groupings: G, A, S, and T; I, L, V, and M; H, R, and K; E, D, N, and Q; and W, F, and Y. No amino acid substitution for proline or cysteine was considered conservative. The membrane topology of MgtB was determined previously (33), is shown by the lines underneath the MgtB sequence, and is presumably identical in MgtA. During preparation of the manuscript for this article, Blattner and associates, as part of the *E. coli* genome project, submitted the sequence of the corresponding region of the *E. coli* chromosome to GenBank (accession number U14003). We had previously communicated the sequence of *mgtA* from *S. typhimurium* to confirm identification of the location of the *E. coli mgtA* sequence. Alignment of the *S. typhimurium* and *E. coli* sequences for *mgtA* shows at least 89% identity over the entire derived amino acid sequence, with an additional 5% conservative substitution and only one possible gap (horizontal line above MgtA). Positions at which the *S. typhimurium* and *E. coli* MgtA proteins differ are shown in boldface type in the MgtA sequence. In addition, there are possibly two single-base-pair frameshifts the *E. coli mgtA* nucleotide sequence, resulting in a complete lack of homology between amino acids 305 and 321, shown by a horizontal bracket. Otherwise, there are only two sections with more than two consecutive differences between the *S. typhimurium* and *E. coli* MgtA amino acid sequences. Assuming an error altering amino acid residues 305 and 321 in the *E. coli* sequence, correction of the shifts using the *S. typhimurium* sequence raises protein identity to 91% and similarity to 95%.

transport system. *mgtC* expression is not required for expression or function at the independent *mgtA* gene, since we have previously shown that a transposon insertion immediately 5′ to the start codon of *mgtC* abolishes expression of both *mgtA* and *mgtB*, with no effect on expression or transport by *mgtA* (35, 36). To investigate the role of *mgtC* in the MgtB transport system, we constructed two sibling vectors, one containing the promoter region and complete coding sequences of the *mgtCB* operon (pTT39) and the other (pTT39d) containing the promoter region and the complete coding sequence of *mgtB* but with most of the coding region for *mgtC* deleted (Fig. 4). Both plasmids were transformed into MM281, which carries mutations in all three *S. typhimurium* Mg^{2+} transporters and requires 100 mM Mg^{2+} for growth. The efficiency of transformation of MM281 to growth without supplemental Mg^{2+} was similar with the two plasmids (data not shown), suggesting that a functional Mg^{2+} transport system was produced by both. Thus, *mgtC* is not essential for function of the MgtB ATPase subunit. Uptake via MgtB in the presence or absence of MgtC was then investigated by using $^{63}Ni^{2+}$ as a surrogate for the unavailable $^{28}Mg^{2+}$. As shown in Fig. 5, the kinetic parameters for Ni^{2+} uptake were identical whether or not a functional MgtC protein was expressed. As a further test of transport, cation inhibition profiles for several divalent cations were de-

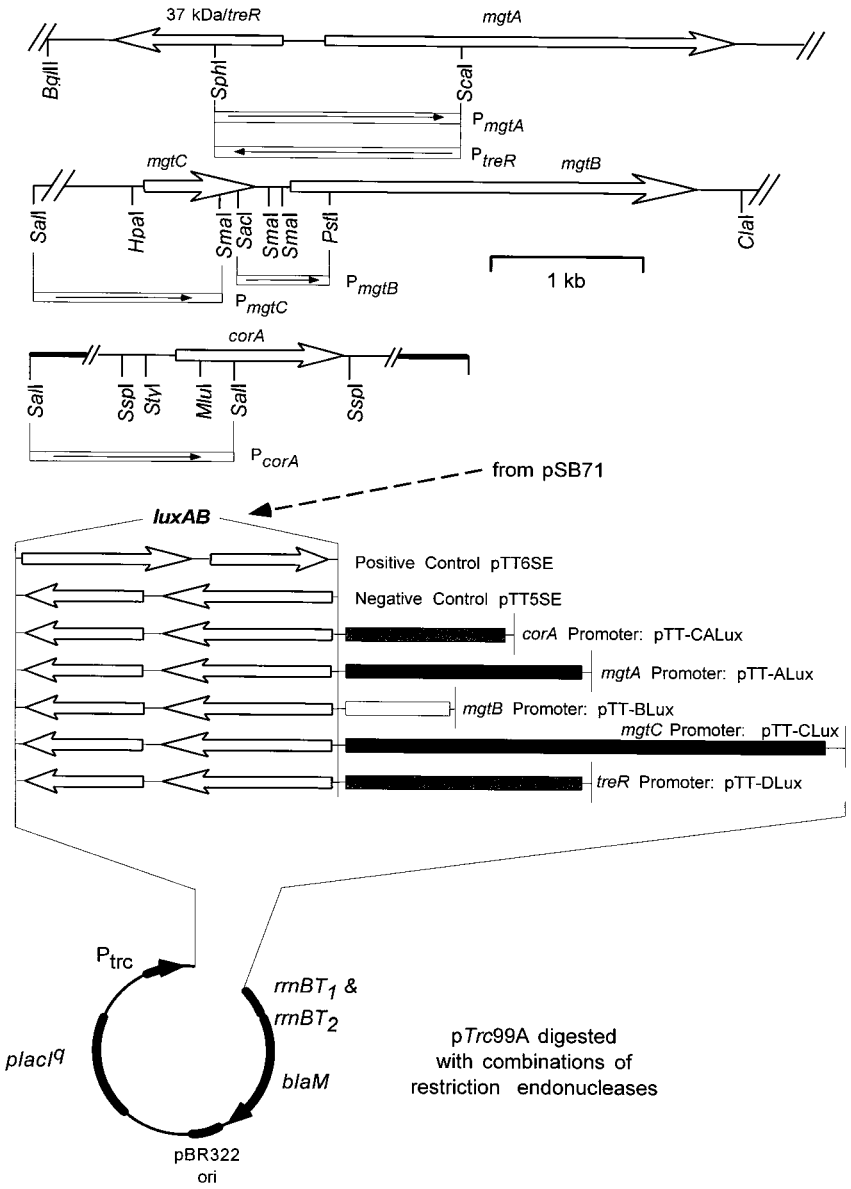


FIG. 3. Construction of the pTT-Lux vectors for determination of promoter (P) activity. The *corA*, *mgtA*, and *mgtB* DNAs were derived from pSPH25, pSPH37, and pSPH39, respectively (16, 35).

termined. Again, there was no difference in the presence or absence of MgtC (Fig. 6), indicating that the MgtC protein has no role in cation selectivity.

Since the apparent V_{max} for $^{63}Ni^{2+}$ uptake is identical with and without MgtC, this is presumptive evidence that MgtC is not required for insertion of the MgtB protein into the membrane. Additional evidence indicating that MgtC has no role in membrane insertion was obtained by using *mgtB*::*blaM* fusions. These fusions were generated previously for determination of the membrane topology of MgtB (33). Several of these fusions were electroporated into MM281 (which does not express the MgtC protein) and LT2 (wild type). All gave similar amounts of β-lactamase activity (33) whether functional MgtC was present or not (data not shown), indicating that the membrane topology of MgtB was not significantly altered. Thus, MgtC is

TABLE 2. Lack of Mg^{2+} effect on control promoter plasmids[a]

Plasmid	Addition (mM)	Fold change
pTT5SE (negative control)	Mg^{2+} (100)	
	Mg^{2+} (100) + IPTG	1.1
	Mg^{2+} (0.001)	2.5
	Mg^{2+} (0.001) + IPTG	2.5
pTT6SE (positive control)	Mg^{2+} (100)	
	Mg^{2+} (100) + IPTG	58
	Mg^{2+} (0.001)	1.6
	Mg^{2+} (0.001) + IPTG	70

[a] Control plasmids were grown and prepared as described in Materials and Methods and incubated for 8 h with the indicated additions before being tested for luciferase activity. Data were calculated as fold change, normalized separately for positive and negative controls to activity at 100 mM Mg^{2+}. Basal promoter activity was similar to that shown in Table 3.

TABLE 3. Effect of Mg^{2+} concentration on promoter activity of *S. typhimurium* Mg^{2+} transport genes[a]

Plasmid	5′ position of putative promoter sequence	Mg^{2+} concn in medium (mM)	Promoter activity (cpm)	Fold change
pTT-CALux	*corA*	100	58,600	
		0.01	100,800	1.8
pTT-ALux	*mgtA*	100	94	
		0.01	23,700	250
pTT-BLux	*mgtB*	100	14	
		0.01	24	1.7
pTT-CLux	*mgtC*	100	94	
		0.01	50,750	540
pTT-DLux	*treR*	100	1,300	
		0.01	541	0.4

[a] Cells carrying the indicated plasmid were grown as described in Materials and Methods and in Table 2, footnote *a*. Data shown are the square roots of the actual counts measured. The fold increase at 0.01 mM extracellular Mg^{2+} is normalized for each plasmid to the activity at 100 mM Mg^{2+}. The data shown are from a single experiment performed with triplicate aliquots. Several similar experiments with different incubation periods and with 0.001 or 0.01 mM extracellular Mg^{2+} gave comparable results. Variability between replicates within an experiment was ≅15% of the absolute value (and thus somewhat less when the square root is taken). The background activity was 10 to 15 cpm per vial and has not been subtracted prior to calculation since basal activity with some of the plasmids, especially pTT-BLux, was extremely low and often comparable to background activity.

not essential either for membrane insertion or for the transport function of MgtB.

DISCUSSION

***mgtA* versus *mgtB*.** With the sequence of *mgtA* now determined, all three of the known Mg^{2+} transport systems of *S. typhimurium* have now been characterized at the molecular level. The deduced protein sequence of the basal, constitutive Mg^{2+} transporter, CorA, is unlike that of any known protein and has a membrane topology unlike that of currently known transporters (34). In contrast, both of the regulated Mg^{2+} transporters of *S. typhimurium* belong to the same well-studied superfamily, the P-type ATPases (25, 33, 38; this report). Nonetheless, MgtA and MgtB are unusual members of this family. They are far more similar to mammalian P-type ATPases than to currently known prokaryotic P-type ATPases. Moreover, they transport the primary cation into the cell with its electrochemical gradient rather than against the gradient (33, 35, 37, 38). Despite this inward transport vector, their membrane topology, determined by fusion protein analysis to contain 10 membrane segments, is oriented identically to that of other P-type ATPases, with the majority of the protein being within the cytosol and little or no protein being present extracellularly (33).

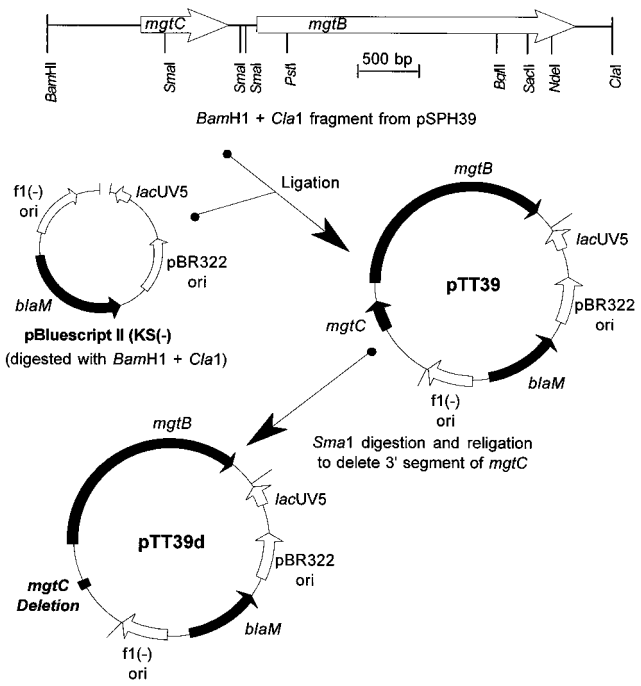


FIG. 4. Construction of pTT39 and pTT39d to test the effect of deletion of MgtC.

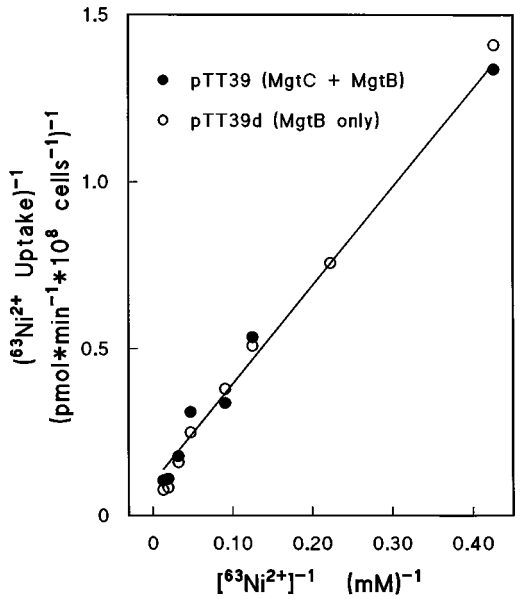


FIG. 5. Kinetics of $^{63}Ni^{2+}$ uptake by cells expressing MgtB and MgtC versus MgtB only. Influx, using $^{63}Ni^{2+}$ as a surrogate for the unavailable $^{28}Mg^{2+}$, was measured at multiple Ni^{2+} concentrations as previously described (15, 35) in strain MM281 carrying pTT39 (●) or pTT39d (○), making each pair of dose-response curves directly comparable. In the representative experiment shown, a single composite line is shown for clarity. The linear regression correlation coefficients were 0.987 and 0.985 for pTT39 and pTT39d, respectively. Two other experiments gave similar data.

Although MgtA and MgtB transport the same primary cation, they differ in a number of properties. Although each transports both Mg^{2+} and Ni^{2+}, their cation inhibition profiles are not similar. For example, Zn^{2+} and Ca^{2+} inhibit uptake via MgtA, but neither has any effect on MgtB. MgtB is markedly temperature sensitive, exhibiting no detectable transport in intact cells at 20°C, while MgtA is quite active at that temperature (35). The sequence and chromosomal structure of *mgtA* further differentiates these two Mg^{2+} transporters. The deduced protein sequence of MgtA is rather more different from MgtB than one would expect for two proteins from the same gene family performing the same basic function within the same species. Although they are each other's closest relative within the P-type ATPases, they exhibit only 50% identity. This contrasts sharply with the nearly complete identity of the *E. coli* and *S. typhimurium* MgtA amino acid sequences (see leg-

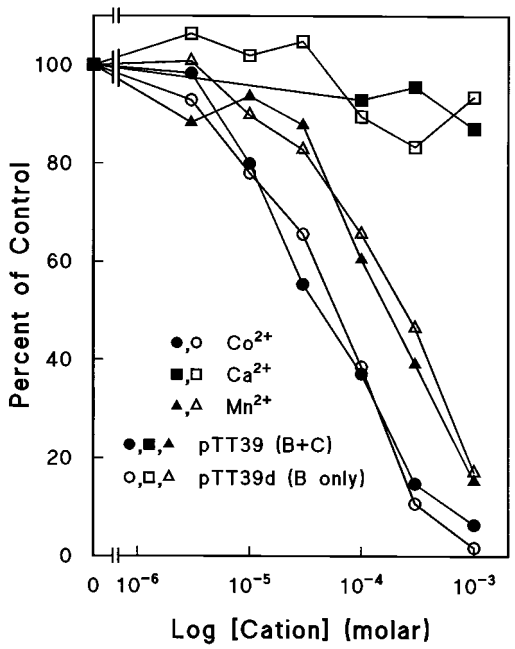


FIG. 6. Cation inhibition of $^{63}Ni^{2+}$ transport by cells expressing MgtB and MgtC versus MgtB only. Ni^{2+} uptake was determined in strain MM281 carrying either pTT39 (solid symbols) or pTT39d (open symbols) as described in the legend to Fig. 5. The final Ni^{2+} concentration was 20 μM. As shown in Fig. 5, the K_m for Ni^{2+} was similar for pTT39 and pTT39d. The ability of Co^{2+} (●,○), Ca^{2+} (■,□), and Mn^{2+} (▲,△) to inhibit uptake was determined. The data shown are from a single experiment repeated once with comparable results.

end to Fig. 2). Additionally, *mgtB* is part of a small operon with the companion *mgtC* gene, while there is no evidence for a similar gene at *mgtA*.

Function of MgtC. Currently, the function of MgtC is unclear. Putative roles for the small subunits of P-type ATPases include membrane insertion of the larger subunit and binding and presentation of extracellular cations to the ATPase subunit (11, 22, 31–33, 37). Neither role appears to be essential in the case of MgtB, since it is able to transport $^{63}Ni^{2+}$ (as a surrogate for $^{28}Mg^{2+}$) in the absence of MgtC, implying that MgtB can insert properly into the membrane without MgtC. The situation is further complicated by the lack of a similar putative subunit at *mgtA*, which raises the question of why one ATPase but not the other might utilize a β-subunit. Since functional transport via MgtB occurs and since neither the kinetic parameters nor cation inhibition profiles are altered in the absence versus the presence of MgtC, MgtB requires MgtC neither for its insertion into the membrane nor for its transport function. Further experiments will be necessary to suggest a specific role for MgtC and to determine why MgtA does not have such a companion.

***mgtA* and *mgtB* promoters.** The promoters for *mgtA* and *mgtB* have been localized, and each was shown to be regulated by extracellular Mg^{2+}. This suggests that the MgtA and MgtB transport systems could be acting as Mg^{2+} scavengers under conditions of Mg^{2+} deprivation. However, they are not true scavengers in the usual sense. For example, *E. coli* possesses several K^+ transport systems (1, 4, 8, 9). The normal constitutive TRK system has a K_m for K^+ uptake of about 1 mM. The transcriptionally regulated *kdp* P-type ATPase system, closely related to the MgtA and MgtB P-type ATPases, has a K_m for K^+ of 2 μM. In contrast, the K_ms of the *S. typhimurium* CorA, MgtA, and MgtB Mg^{2+} transporters for Mg^{2+} range only from 5 to 30 μM (14). Thus, if a scavenger system has a much higher affinity than other systems for the same substrate, MgtA and MgtB do not fit the criterion. Therefore, the regulation of *mgtA* and *mgtB* likely reflects, at least in part, some other function(s) for these transport systems. Preliminary evidence suggests that several chromosomal loci in *S. typhimurium* in addition to these two can be transcriptionally regulated by extracellular Mg^{2+} (23).

Additionally, some clues may emerge from a study of regulatory influences on these loci. As one example, we have previously shown that transcription of *mgtB* is greatly increased when a pathogenic strain of *S. typhimurium* is phagocytized into the vacuole of an epithelial cell line (26). Further, the temporal dependence of transcriptional regulation of these two loci by Mg^{2+} is quite complex, and several other divalent cations also influence transcription, whether or not they influence transport of Mg^{2+} via MgtA and/or MgtB (37).

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