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Magnesium Transport in *Salmonella typhimurium*: $^{28}Mg^{2+}$ Transport by the CorA, MgtA, and MgtB Systems

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Three loci in *Salmonella typhimurium* (*corA*, *mgtA*, and *mgtB*) code for components of distinct Mg^{2+} transport systems (S. P. Hmiel, M. D. Snavely, J. B. Florer, M. E. Maguire, and C. G. Miller, J. Bacteriol. 171:4742–4751, 1989). Strains carrying one wild-type and two mutant alleles of the three loci were constructed to study the kinetics and specificity of ion transport of each system in isolation. The transport systems had different K_m and V_{max} values for Mg^{2+} uptake, and each was inhibited by other divalent cations in a distinct rank order of potency: for CorA, $Mg^{2+} > Mn^{2+} > Co^{2+} > Ni^{2+} > Ca^{2+}$; for MgtA, $Zn^{2+} \geq Mg^{2+} > Ni^{2+} \approx Co^{2+} > Ca^{2+}$; and for MgtB, $Mg^{2+} \approx Co^{2+} \approx Ni^{2+} > Mn^{2+} \gg Ca^{2+}$. Other differences among the three systems were apparent. The CorA transport system functioned as a Mg^{2+}-Mg^{2+} exchange system, mediating both efflux and influx of Mg^{2+}. Neither the MgtA nor the MgtB system could mediate Mg^{2+} efflux. Transport via the MgtB system was very temperature sensitive; Mg^{2+} was transported at 37°C but not at 20°C. The MgtA and the MgtB transport systems were found to be regulated by the extracellular concentration of Mg^{2+}.

Transductional mapping data indicate that at least three genetic loci in *Salmonella typhimurium* affect Mg^{2+} transport: *corA*, *mgtA*, and *mgtB* (2, 3). Genetic (2) and expression (14) data have suggested that each of these three loci represents a distinct transport system capable of supporting Mg^{2+} movement through the membrane. A strain harboring mutations at all three loci does not accumulate $^{28}Mg^{2+}$ and requires a concentration of extracellular Mg^{2+} in excess of 10 mM in order to grow (2). Introduction of each locus independently in such triply mutant strains restores growth on low-Mg^{2+} medium, restores $^{28}Mg^{2+}$ uptake, and imparts a distinct sensitivity to growth inhibition by other divalent cations. Furthermore, isolated clones of each locus express discrete membrane-localized products (14). This report presents $^{28}Mg^{2+}$ transport data confirming the presence of three distinct transport systems for Mg^{2+}. A strain carrying only one of the three genes expresses a Mg^{2+} transport system with characteristic kinetics, distinctive cation inhibition properties, and differential regulation by concentration of extracellular Mg^{2+}. Comparison of these results with previous data for *Escherichia coli* (4, 8–13) and *Rhodobacter capsulatus* (4, 5) indicate essential similarity between the constitutive CorA systems of these gram-negative organisms but suggest significant differences in the repressible Mgt systems.

MATERIALS AND METHODS

Strain construction. The bacterial strains used are listed in Table 1. Construction of strains MM196, MM224, and MM227 is described elsewhere (2, 3). The *corA45*::Mu dJ insertion from pSPH45 was moved into the chromosome of strain TN1379 by homologous recombination as described elsewhere (2) to form MM199. The *mgtA21*::Mu dJ insertion from pJF3 (2) was similarly introduced into TN1379 to produce MM271. MM252 was constructed from MM196 (2) by first introducing a *metE* mutation by cotransduction with the linked insertion *zie-3162*::Tn*10*Δtet (6) and then transducing the resulting Met^- strain (MM249) to Met^+ with a P22 (*HT*12/4 *int*-3) transducing lysate on MM199 ($metE^+$ *corA*). The Met^+ transductants were screened for Co^{2+} resistance and tetracycline sensitivity.

Assay of Mg^{2+} transport. Uptake using $^{28}Mg^{2+}$ has been described previously (3). Mg^{2+} uptake was measured in strains of *S. typhimurium* that carry one wild-type and two mutant alleles of the three genes *corA*, *mgtA*, and *mgtB* (2). This allowed dissection of the properties of each individual transport system without interference from the others. For clarity, the Mg^{2+} transport system present is referred to as CorA, MgtA, or MgtB rather than by the longer genotypic designation of defective systems, e.g., as the CorA system rather than as $corA^+$ *mgtA mgtB*. The phenotype of each of the strains carrying only a single Mg^{2+} transport system was always verified the day before each transport assay by using a disk sensitivity test to determine the pattern of growth inhibition by divalent cations, a pattern that is characteristic for each strain (2). The apparent K_i for inhibition by other divalent cations was determined from the cation concentration giving 50% inhibition (IC_{50}) (Fig. 1) and the apparent K_m for Mg^{2+} uptake (Table 2), using the formula $K_i = IC_{50} \cdot \{K_m/(K_m + [Mg^{2+}])\}$, where $[Mg^{2+}]$ was 0.08 or 0.10 mM, depending on the specific experiment.

For measurement of $^{28}Mg^{2+}$ efflux, appropriate strains were grown in N medium containing 20 μM Mg^{2+} to an optical density at 600 nm of 0.1 to 0.15 and diluted with 0.5 volume of fresh N medium. $^{28}Mg^{2+}$ was added (6 μCi/50 ml), and the cells were incubated for an additional 80 to 120 min at 37°C (final optical density at 600 nm of about 0.20). At t = 0 and times thereafter, duplicate 1-ml samples were filtered and washed as described for measurement of influx. To initiate efflux, 1.0 M Mg^{2+} was added to a final concentration of 10 mM at t = 0. For determination of the effect of volume dilution on Mg^{2+} efflux, cells were grown as described above. Samples (2 ml) of cells incubated for 80 to 100 min in $^{28}Mg^{2+}$ were diluted to 200 ml in fresh N medium containing

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TABLE 1. Bacterial strains used

Strain	Genotype	Source or reference
TN1379	Δ*leuBCD485*	7
TN2373	*polA2 ara-9*	1
AK3162	*metE551 metA22 hisC47*(Am) *trpB2 ilv-452 rpsL120 fla-66 xyl-404 galE496 hsdL6*(r^- m^+) *hsdSA* (r^- m^+) *zie-3162*::Tn*10*	6
MM196	Δ*leuBCD485 mgtB10*::Mu dJ	2
MM199	Δ*leuBCD485 corA45*::Mu dJ	This study
MM224	Δ*leuBCD485 mgtB10*::Mu dJ *mgtA5 zjh-1628*::Tn*10*Δcam	2
MM227	Δ*leuBCD485 corA27 mgtA5 zia-3123*::Tn*10*Δtet	2
MM249	Δ*leuBCD485 metE551 mgtB10*::Mu dJ *zie-3162*::Tn*10*Δtet	This study
MM252	Δ*leuBCD485 mgtB10*::Mu dJ *corA45*::Mu dJ	This study
MM271	Δ*leuBCD485 mgtA21*::Mu dJ	This study

20 μM or 10 mM Mg^{2+}, and 20-ml portions were filtered and washed at the indicated times.

RESULTS

Kinetic parameters of $^{28}Mg^{2+}$ uptake. At 20°C, the K_m of the CorA system for Mg^{2+} was about 15 μM, with a V_{max} of 250 pmol min^{-1} 10^8 $cells^{-1}$ (Table 2). The K_m of the MgtA system was similar to that of the CorA system, but the V_{max} at 20 °C was an order of magnitude less (Table 2). Uptake of $^{28}Mg^{2+}$ via the MgtB system could not be detected when assayed at 20°C (Table 2). Further experiments using fivefold-higher $^{28}Mg^{2+}$ specific activity at either 0.02 or 0.1 mM extracellular Mg^{2+} again failed to show uptake at 20°C (data not shown). At 37°C, the extremely rapid rate of uptake by the CorA system precluded accurate determination of kinetic parameters; uptake was linear with time for less than 30 s. The K_m of the MgtA system was approximately 30 μM Mg^{2+} at 37°C, about double that at 20°C, whereas the V_{max} increased almost fivefold, to about 115 pmol min^{-1} 10^8 $cells^{-1}$. In contrast to the absence of detectable uptake at 20°C, uptake via MgtB at 37°C was readily detectable, with a K_m of 6 μM and a V_{max} of approximately 75 pmol min^{-1} 10^8 $cells^{-1}$. This latter value was quite variable, however, probably because of different degrees of repression of *mgtB* (see below). As the affinities of the three systems were comparable, the major point of comparison is their different capacities. The high V_{max} of the CorA system, even though measured at 20°C, suggests that the CorA system is the main transport system responsible for movement of Mg^{2+} into the cell. The maximal velocities of the MgtA and MgtB systems were comparable to one another at 37°C but significantly less than the V_{max} of the CorA system, probably by an order of magnitude.

Cation inhibition of $^{28}Mg^{2+}$ uptake. Certain divalent cations inhibit growth of strains carrying only a single wild-type locus for Mg^{2+} transport (2). To examine the relationship between this growth inhibition and the ability to affect Mg^{2+} transport, the effect of these cations on $^{28}Mg^{2+}$ uptake was determined (Fig. 1). Ca^{2+} was a poor inhibitor of uptake by the CorA and MgtB systems but inhibited uptake by the MgtA system with a K_i of 0.3 mM. The mode of inhibition by Ca^{2+} was not determined. Co^{2+} was a competitive inhibitor

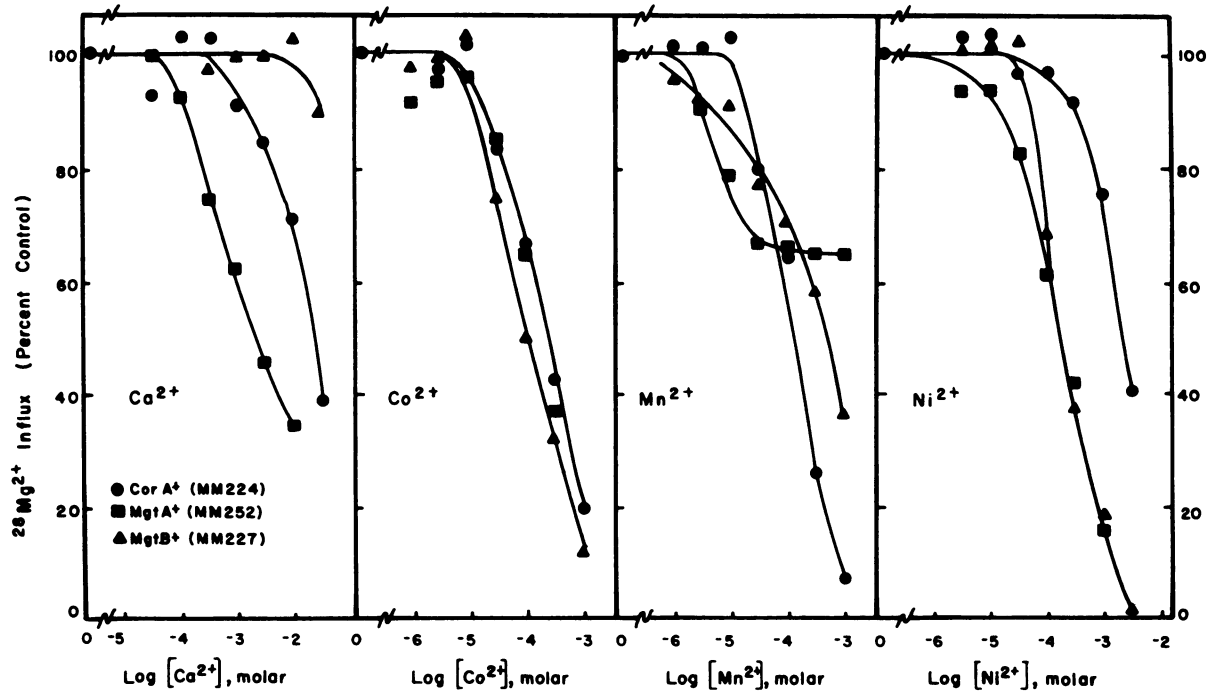

FIG. 1. Divalent cation inhibition of Mg^{2+} uptake. Uptake was measured (3) by using triplicate samples of cells added to buffer containing 0.08 or 0.10 mM Mg^{2+} and the indicated concentration of divalent cation. Incubation times were 3 min at 20°C for CorA (strain MM224) and 20 min at either 20 or 37°C for both MgtA (MM252) and MgtB (MM227) cells. The amount of $^{28}Mg^{2+}$ was 125,000 cpm/ml for MM224 and 350,000 cpm/ml for MM252 and MM227. A background determined by measuring uptake either at zero time (MM224) or after incubation for the specified time at 4°C (MM252 and MM227) was subtracted. Total uptake was at least three times background in all cases.

TABLE 2. *S. typhimurium* Mg^{2+} transport

Transport system	Temp (°C)	Kinetic parameter[a]		Apparent K_i for cation inhibition (μM)				
		K_m (μM)	V_{max} (pmol min^{-1} 10^8 $cells^{-1}$)	Ca^{2+}	Co^{2+}	Mn^{2+}	Ni^{2+}	Zn^{2+}
CorA	20	15	250	5,000	50	30	500	NI[b]
	37	ND[c]	ND					
MgtA	20	12	24	300	40	PI[d]	30	7
	37	29	115					
MgtB	20		<2					
	37	6	75	NI	8	40	13	NI
Triple mutant	20 and 37		<2					

[a] Averages of data from at least three experiments for each transport system. Kinetic parameters were determined from Eadie-Hofstee plots, using at least six and usually eight concentrations of Mg^{2+}. For the MgtA system, the values and standard deviations from six experiments at 20°C were $K_m = 12 \pm 7$ μM and $V_{max} = 24 \pm 6$ pmol min^{-1} 10^8 $cells^{-1}$. Replication for experiments on the CorA system was similar. In various experiments, the K_m of the MgtB system for Mg^{2+} was consistently below 10 μM, but the V_{max} ranged from 15 to 75 pmol min^{-1} 10^8 $cells^{-1}$ (see text for discussion).

[b] NI, No significant inhibition at the highest cation concentration that did not cause acute cell toxicity. Concentrations of Zn^{2+} greater than 0.1 mM caused rapid cell lysis. In the case of Ca^{2+} inhibition of the MgtB system, cell clumping occurs at Ca^{2+} concentrations greater than 10 mM, rendering interpretation of transport inhibition data difficult.

[c] ND, Not determined. Uptake via the *S. typhimurium* CorA transport system at 37°C is linear for only 15 to 30 s and is too fast for accurate determination of kinetic parameters. See text for further discussion.

[d] PI, Partial inhibition (see Fig. 1).

of Mg^{2+} influx through the CorA (3) and MgtA systems (data not shown); however, Co^{2+} was most potent as an inhibitor of transport by the MgtB system. Ni^{2+} inhibited $^{28}Mg^{2+}$ uptake via the CorA system but was a significantly more potent inhibitor of uptake via both the MgtA and MgtB systems. For all three systems, the mode of Ni^{2+} inhibition was competitive (data not shown). Mn^{2+} completely inhibited CorA- and MgtB-mediated $^{28}Mg^{2+}$ uptake but inhibited only 35% of uptake via the MgtA system even at very high Mn^{2+} concentrations. Zn^{2+} (data not shown) potently inhibited MgtA-mediated uptake at concentrations up to 0.1 mM but failed to inhibit uptake via the CorA and MgtB systems. At concentrations greater than 0.1 mM, Zn^{2+} was acutely toxic to all *S. typhimurium* strains regardless of which Mg^{2+} transport system(s) was present. This result is consistent with the previous demonstration that growth inhibition by Zn^{2+} is equivalent regardless of which Mg^{2+} transport locus is present (2). The modes of inhibition by Mn^{2+} of uptake via the CorA and MgtB systems and by Zn^{2+} of uptake via the MgtA system were not determined.

The data of Fig. 1 allow determination of IC_{50} values. To evaluate the rank order of potency of the cations for each system, the IC_{50} values must be corrected for the different affinity for Mg^{2+} of each transport system (see Materials and Methods). The calculated apparent K_is (Table 2) showed three distinct affinity patterns for divalent cations. The rank orders of potency were as follows: for CorA, $Mg^{2+} > Mn^{2+} > Co^{2+} > Ni^{2+} > Ca^{2+}$; for MgtA, $Zn^{2+} \geq Mg^{2+} > Ni^{2+} \approx Co^{2+} > Ca^{2+}$; and for MgtB, $Mg^{2+} \approx Co^{2+} \approx Ni^{2+} > Mn^{2+} \gg Ca^{2+}$.

Mg^{2+} repression of Mg^{2+} uptake. Previous studies in *E. coli* (4, 9, 10) and *S. typhimurium* (2, 3) have indicated that a component of Mg^{2+} uptake is repressible by growth in media containing high concentrations of extracellular Mg^{2+}. In *S. typhimurium*, Mg^{2+} repression of Mg^{2+} uptake was exhibited by both the MgtA and MgtB systems. Cells were grown at extracellular Mg^{2+} concentrations of 0.02 to 10 mM, washed, and then assayed at 0.1 mM Mg^{2+}. The initial rate of Mg^{2+} uptake via the MgtB system decreased at extracellular Mg^{2+} concentrations above about 300 μM (Fig. 2 and data not shown). Similarly, uptake by the MgtA system was much greater in cells grown in 20 μM Mg^{2+} than in cells grown in 10 mM Mg^{2+} (Fig. 3). With the MgtA system, these changes were apparent whether assayed at 20 or 37°C. Although net uptake of $^{28}Mg^{2+}$ via MgtB could be demonstrated at 37°C in cells grown in 10 mM Mg^{2+}, the initial rate of uptake was relatively small and was variable in different experiments; overall, the data suggest about a 10-fold increase in uptake via MgtB upon cell growth in 20 μM Mg^{2+} compared with cell growth in 10 mM Mg^{2+}. The fold increase was similar for MgtA. In contrast, identical experiments with strains carrying only the CorA system showed no significant effect of Mg^{2+} concentration in the growth medium on the initial rate of Mg^{2+} uptake (data not shown).

Mg^{2+} efflux studies. Strains carrying two wild-type alleles and one mutant allele of the three Mg^{2+} transport systems were used to study Mg^{2+} efflux. The CorA system was necessary for Mg^{2+} efflux (Fig. 4). In its absence, essentially no $^{28}Mg^{2+}$ was released from the cells. Moreover, the presence or absence of a mutant allele of *mgtA* or *mgtB* made no difference in the rate of efflux; the half-time ($t_{1/2}$) of efflux in a CorA strain was about 9 min regardless of whether mutant alleles of *mgtA* or *mgtB* were present (Fig. 4 and data not shown). Furthermore, neither the MgtA nor MgtB system appeared capable of functioning as a Mg^{2+} efflux sys-

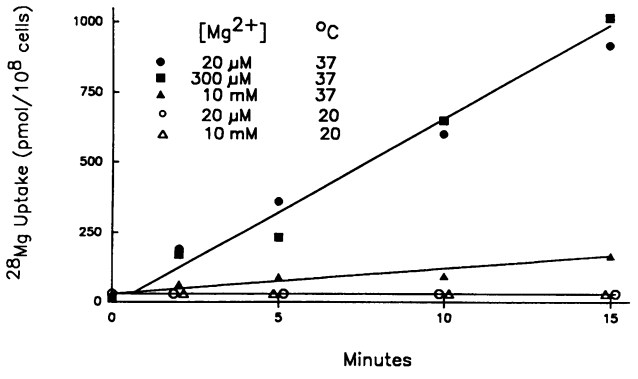

FIG. 2. Mg^{2+} uptake in the MgtB strain MM227 after growth in medium containing the indicated concentrations of Mg^{2+}. Cells were assayed for $^{28}Mg^{2+}$ uptake at 100 μM Mg^{2+} at either 20 or 37°C.

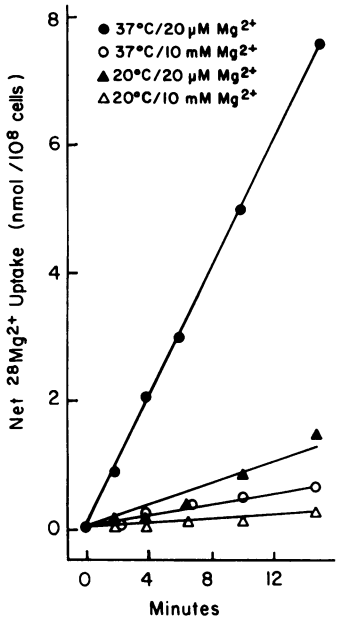

FIG. 3. Mg^{2+} uptake in the MgtA strain MM252, measured after growth in medium containing either 20 μM or 10 mM Mg^{2+}. Cells were assayed for $^{28}Mg^{2+}$ uptake at 100 μM Mg^{2+} at either 20 or 37°C.

tem, since a *corA mgtA*$^+$ *mgtB*$^+$ strain (MM199) exhibited essentially no Mg^{2+} efflux (Fig. 4).

In a Mg^{2+}-Mg^{2+} exchange system, the extracellular concentration of Mg^{2+} should alter the rate of efflux. This was tested by terminating uptake of $^{28}Mg^{2+}$ by two different methods: (i) cells were diluted into a large volume of medium containing 20 μM Mg^{2+} and (ii) nonradioactive Mg^{2+} was added to cells to a final concentration of 10 mM with or without dilution. Under such conditions, uptake of $^{28}Mg^{2+}$ (but not Mg^{2+}) is reduced to essentially zero, and loss of $^{28}Mg^{2+}$ from the cells is a measure of efflux. No significant

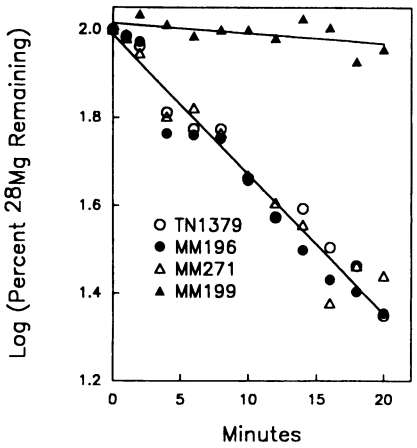

FIG. 4. Mg^{2+} efflux in strains lacking one Mg^{2+} transport system. Efflux was measured in strains TN1379 (wild type), MM196 (*corA*$^+$ *mgtA*$^+$ *mgtB*), MM271 (*corA*$^+$ *mgtA mgtB*$^+$), and MM199 (*corA mgtA*$^+$ *mgtB*$^+$). The line through TN1379, MM196, and MM271 is a compound regression line through all three sets of points. It has a correlation coefficient of 0.985 and a $t_{1/2}$ of 9 min. The line for MM199 has a correlation coefficient of 0.99 and a $t_{1/2}$ of at least 3 h.

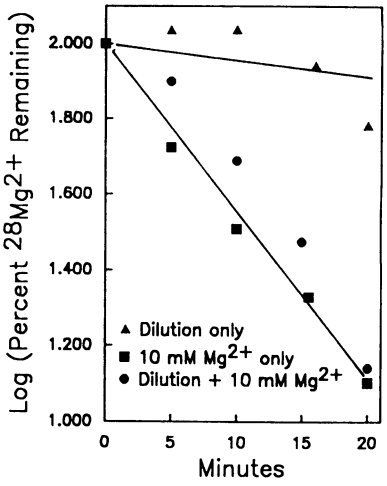

FIG. 5. Mg^{2+} efflux in wild-type strain TN1379 after dilution and/or addition of Mg^{2+}. Cells were diluted 100-fold in incubation buffer containing 20 μM Mg^{2+}, diluted 100-fold in incubation buffer plus 10 mM Mg^{2+}, or made 10 mM in Mg^{2+} without dilution, as indicated. The filtered sample size was 20 ml for diluted cells and 0.2 ml for cells treated with 10 mM Mg^{2+} only.

efflux of $^{28}Mg^{2+}$ was observed after dilution of cells while maintaining extracellular Mg^{2+} at 20 μM ($t_{1/2} > 3$ h). However, addition of Mg^{2+} to 10 mM or cell dilution combined with addition of 10 mM Mg^{2+} resulted in $^{28}Mg^{2+}$ efflux with a $t_{1/2}$ of 9 min (Fig. 5). These data suggest that the CorA transport system functions as a Mg^{2+}-Mg^{2+} exchange system and are in accord with previous studies on Mg^{2+} efflux in *E. coli* (8, 12).

DISCUSSION

The transport data presented above extend the evidence for our previous conclusion that the three *S. typhimurium* loci *corA*, *mgtA*, and *mgtB* represent distinct Mg^{2+} transport systems (2, 3, 14). Strains constructed to contain one functional and two inactivated loci demonstrated distinct cation transport characteristics for each locus. Differences are apparent in the kinetic parameters for Mg^{2+} transport, in the temperature dependence of uptake, in the pattern of inhibition of Mg^{2+} transport by other divalent cations, in the ability to mediate Mg^{2+}-Mg^{2+} exchange, and in regulation of transport by extracellular Mg^{2+}. This interpretation of the transport data complements previous genetic studies (2, 3). First, each locus maps to a distinct position on the *S. typhimurium* chromosome. Second, introduction of any one locus into strains that are dependent on high extracellular concentrations of Mg^{2+} for growth and lack detectable Mg^{2+} transport both relieves the Mg^{2+} dependence and restores Mg^{2+} transport. Third, divalent cation inhibition of growth discriminates among strains containing only one wild-type locus. Finally, isolated clones of each locus express distinct gene products in maxicells, all of which are tightly associated with the membrane (14). Together, the transport and genetic data establish that *S. typhimurium* possesses three discrete Mg^{2+} transport systems.

The strains containing a single wild-type Mg^{2+} transport system are operationally most easily distinguished by determining growth inhibition by divalent cations in a disk sensitivity test (2). For example, growth of strains containing only the MgtB system is markedly inhibited by Ca^{2+} and Mn^{2+}, whereas growth of strains containing either the CorA or the

TABLE 3. *E. coli* and *R. capsulatus* Mg^{2+} transport

Transport system	Temp (°C)	Kinetic parameter[a]		Apparent K_i for cation inhibition (μM)					References
		K_m (μM)	V_{max} (pmol min^{-1} 10^8 $cells^{-1}$)	Ca^{2+}	Co^{2+}	Mn^{2+}	Ni^{2+}	Zn^{2+}	
E. coli									
CorA	25	18	400	NI[b]	400	500	NR[c]	NR	10
CorA	25	28	970	NR	37	29	NR	NR	9–11
	37	38	1,700						
Mgt	25	36	960	NR	46	45	NR	NR	9–11
	37	39	1,650						
corA mgt	25	ND[d]	≈15						9–11
R. capsulatus	25	55	90	NR	200	300	NR	NR	5

[a] The V_{max}s were given in the original references in nanomoles per minute per milligram (dry weight) (4, 5; Jasper, Ph.D. thesis) or nanomoles per minute per milligram of protein (10). An approximate conversion factor of 0.2 mg (dry weight) or 0.1 mg of protein = 10^8 cells has been used. Data are estimated from Fig. 4 of Park et al. (10) and Fig. 7 of Wong (Ph.D. thesis); these data are not sufficient to estimate accurately a K_m, although uptake appears to have a similar affinity to the other systems listed. See text for further discussion.

[b] NI, No inhibition.

[c] NR, Not reported.

[d] ND, Not determined.

MgtA transport system is unaffected by these cations. A priori, the simplest explanations for inhibition of growth by these cations are (i) inhibition of specific Mg^{2+} transport, with or without concomitant transport of the inhibiting cation through the Mg^{2+} transport system; (ii) inhibition of transport at other, non-Mg^{2+} transport systems, with toxicity resulting from the limited availability of Mg^{2+}; and (iii) repression of a Mg^{2+} transport system similar to the Mg^{2+}-mediated repression of *mgtA* and *mgtB*. However, a comparison of the ability of various cations to inhibit growth versus their ability to inhibit Mg^{2+} uptake suggests that such simple explanations may not be sufficient or that, at the least, combinations of these mechanisms are required. For cells containing only the CorA system, Co^{2+} and Ni^{2+} are good inhibitors of growth as well as competitive inhibitors of Mg^{2+} transport. However, although Mn^{2+} is a more potent inhibitor of Mg^{2+} uptake than is Co^{2+} or Ni^{2+}, it fails to inhibit growth of a CorA-only strain even when present at a high concentration (2). In contrast, Mn^{2+} is very inhibitory to growth of a MgtB-only strain but only a modest inhibitor of Mg^{2+} uptake in the MgtB system. Finally, Ni^{2+} and Co^{2+} are as potent as Mg^{2+} with regard to Mg^{2+} transport in a strain carrying only the MgtB system, yet neither cation inhibits growth of such a strain. Thus, the mechanism(s) of growth inhibition by various divalent cations of strains deficient in Mg^{2+} transport is more complicated than the explanations suggested above.

Mg^{2+} efflux in *S. typhimurium* appears to be mediated primarily by the CorA transport system. Little $^{28}Mg^{2+}$ is released from cells for at least 20 min in the absence of a wild-type *corA* allele (Fig. 4). However, after 60 min, approximately 35% of the intracellular $^{28}Mg^{2+}$ is lost. These results are consistent with those of Nelson and Kennedy for *E. coli* (8). The basis of this CorA-independent efflux remains to be determined. The *mgtA* and *mgtB* loci play no role in Mg^{2+} efflux, as evidenced by the lack of effect of mutations at these loci on $^{28}Mg^{2+}$ efflux.

The existence of such an exchange system predicts that increasing the extracellular Mg^{2+} concentration should increase the rate of efflux, and the data of Fig. 5 confirm this. However, the effect of Mg^{2+} on efflux is not simply via an exchange reaction but apparently involves some degree of regulation of the Mg^{2+} efflux process. If the sole effect of extracellular Mg^{2+} is to influence the turnover rate of the exchanger, then a change in the extracellular Mg^{2+} concentration should alter the rate of efflux to essentially the same degree that it alters influx. At 20 μM extracellular Mg^{2+}, a concentration near the K_m of the CorA system for influx, the rate of Mg^{2+} uptake should be approximately 50% the rate at a supramaximal extracellular Mg^{2+} concentration (10 mM). Therefore, if efflux is governed solely by the turnover of the transporter, then the relative rates of efflux at 10 mM versus 20 μM extracellular Mg^{2+} should be about 2:1. The data (Fig. 5) indicate a ratio much greater than this. The actual mechanism of regulation of efflux by extracellular cation remains to be determined. It may be, as Nelson and Kennedy suggest (8), that extracellular divalent cation displaces bound intracellular $^{28}Mg^{2+}$ and makes it available for release. It is also possible that the CorA transport system itself responds to extracellular divalent cation in some way. Elucidation of the mechanism must await more detailed studies, probably using a reconstituted transport system.

There are both differences and similarities among the Mg^{2+} transport systems of *S. typhimurium* and those previously reported for *E. coli* and *Rhodobacter capsulatus* (4, 5, 8–13; Table 3). The CorA systems of *S. typhimurium* and *E. coli* are comparable in that they transport both Mg^{2+} and Co^{2+}, mediate Mg^{2+} efflux, are inhibited by Mn^{2+}, have similar affinities for Mg^{2+}, and map to analogous chromosomal positions. This conclusion is supported by the finding that the cloned *E. coli corA* gene will complement a *corA* mutation in *S. typhimurium* (3). Data for Co^{2+} and Mn^{2+} inhibition of Mg^{2+} uptake in *S. typhimurium* are also similar to those reported for *E. coli* (10); higher values had been reported earlier (11, 12), but these have subsequently been questioned by the original authors (4). The V_{max} of uptake in *E. coli* appears to be slightly higher than in *S. typhimurium*, although differences in assay conditions may contribute to this apparent difference. Mg^{2+} uptake by *R. capsulatus* (5) is competitively inhibited by Mn^{2+} and Co^{2+}, suggesting the possibility of an uptake system(s) in this species similar to those seen in *S. typhimurium* and *E. coli*. The high V_{max} suggests that the reported uptake is most likely via a system analogous to the CorA systems (4, 5; P. L. P. Jasper, Ph.D. thesis, Washington University, St. Louis, Mo., 1975).

Comparisons between the *S. typhimurium* MgtA and MgtB transport systems and Mgt-mediated transport in *E. coli* reveal similarities but also some significant differences. Only one Mgt system was genetically characterized in *E. coli* (10). Since comparable cation inhibition data were not reported, it is not possible to determine which, if either, of the *S. typhimurium* Mgt systems corresponds to the *E. coli mgt*

system. It is possible that neither *S. typhimurium mgt* system is analogous to the *E. coli mgt* system, since neither of the *S. typhimurium* loci maps to a chromosomal position analogous to the *E. coli mgt* locus. Nonetheless, a reexamination of the earlier *E. coli* data suggests strongly that *E. coli* possesses a third system capable of transporting Mg^{2+}. Uptake of $^{28}Mg^{2+}$ in an *E. coli corA mgt* strain was reported to be about 0.15 nmol min^{-1} mg of $protein^{-1}$ (or about 15 pmol min^{-1} 10^8 $cells^{-1}$) and was not inhibited by prior addition of the uncoupler carbonylcyanide *m*-chlorophenylhydrazone (10). These results were interpreted as indicating energy-independent uptake of Mg^{2+} rather than active transport. However, other data from these authors indicate that this relatively low level of uptake is increased more than 10-fold when the *E. coli corA mgt* strain is shifted from growth in 10 mM to growth in 1 mM extracellular Mg^{2+} (M. H. Park, Ph.D. thesis, Brown University, Providence, R.I., 1977; B. B. Wong, Ph.D. thesis, Brown University, 1979). The originally very low level of $^{60}Co^{2+}$ uptake in this strain was not increased under these growth conditions. The increase in the rate of Mg^{2+} uptake in the *E. coli* double mutant is similar to that seen with the *S. typhimurium* MgtA or MgtB transport system upon a decrease in extracellular Mg^{2+} concentration. Thus, although no additional (third) Mg^{2+} transport system has been identified genetically in *E. coli*, transport data suggest that such a system exists. If so, then *E. coli* and *S. typhimurium* each possess a single high-capacity Mg^{2+}-Mg^{2+} exchange system (CorA) and two additional Mg^{2+} transport systems repressible by high concentrations of extracellular Mg^{2+}.

It is unclear why three distinct Mg^{2+} transport systems exist in *S. typhimurium*. The CorA system is not regulated by extracellular Mg^{2+}, has moderate affinity for Mg^{2+}, mediates both influx and efflux, and has by far the highest capacity of the three Mg^{2+} transport systems. Thus, the CorA system is presumably the primary system through which the bacterium obtains Mg^{2+}. While the ability of CorA to mediate Mg^{2+} efflux might argue against this conclusion, the influx and efflux processes differ in dependence on the extracellular concentration of Mg^{2+} (Fig. 4 and 5), effectively uncoupling influx from efflux and allowing net accumulation of Mg^{2+} via CorA. The MgtA and MgtB systems would both be expressed primarily under conditions of low extracellular concentrations of $^{28}Mg^{2+}$ but cannot be considered scavenger systems for Mg^{2+}, since the K_m of each is not remarkably different from that of the CorA system. The significant differences in these latter systems are the greater capacity of the MgtA system compared with a fivefold-higher affinity for the MgtB system. Nonetheless, the capacity of the MgtA system is sufficiently greater than that of the MgtB transport system that at Mg^{2+} concentrations of about 1 to 10 μM, the MgtA system transports at least as much Mg^{2+} as does the MgtB system despite the lower affinity of the MgtA system. Since both systems are repressible by Mg^{2+}, this suggests the possibility that MgtA and MgtB are differentially regulated under some other, as yet unidentified environmental condition. Regardless of their respective roles, the characterization of three distinct Mg^{2+} transport systems in *S. typhimurium* and construction of isogenic strains containing only a single such Mg^{2+} transport system should greatly facilitate investigations of the mechanism of Mg^{2+} flux and the intracellular role of Mg^{2+}.

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