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Magnesium Transport in *Salmonella typhimurium*: Characterization of Magnesium Influx and Cloning of a Transport Gene

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The influx of Mg^{2+} in *Salmonella typhimurium* LT-2 was studied by both kinetic and genetic techniques. Wild-type cells grown in a high $MgSO_4$ concentration (10 mM) exhibited a K_m of 15 μM for Mg^{2+} influx, with a V_{max} of 0.25 nmol of Mg^{2+} per min per 10^8 cells. The apparent K_m decreased to 3 μM, and the V_{max} increased 60% after growth in a low $MgSO_4$ concentration (10 μM). Co^{2+} was a simple competitive inhibitor (K_i = 30 μM) of Mg^{2+} influx in cells grown in high Mg^{2+} concentrations but blocked only a portion of the Mg^{2+} influx in cells grown in low Mg^{2+} concentrations. Co^{2+} influx exhibited kinetics similar to those of Mg^{2+} influx (K_m = 30 μM; V_{max} = 0.5 nmol of Co^{2+} per min per 10^8 cells) but was not affected by growth conditions. Co^{2+} influx was competitively inhibited by both Mg^{2+} and Mn^{2+}. Mutations affecting Mg^{2+} uptake were isolated by selection for spontaneous resistance to toxic levels of Co^{2+}. One class of mutants designated *corA* mapped at 84 min near *metE* with the following gene order: *corA*, *metE*, *zie-3161*::Tn*10*, *pepQ*. A second class designated *corB* mapped at 98 min near *pyrB*. Mg^{2+} influx was decreased in a *corA* mutant strain (relative to that of the wild type) when grown in high Mg^{2+} concentrations but was restored when grown in low Mg^{2+} concentrations. Co^{2+} transport was completely abolished by the *corA* mutation under all growth conditions. Recombinant plasmids carrying the *corA* region from either *Escherichia coli* K-12 or *S. typhimurium* complemented the *corA* mutation in *S. typhimurium*, restoring uptake of both Co^{2+} and Mg^{2+} and conferring sensitivity to Co^{2+}. The *S. typhimurium corA* gene was localized to a restriction fragment of approximately 1.5 kilobases.

The Mg^{2+} ion is an essential cellular constituent with important roles in enzyme activation and the stabilization of cell structure (4, 6, 22). Active uptake of Mg^{2+}, however, has been studied in only a few bacterial species, i.e., *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. Studies in eucaryotic cells have also been limited (for reviews, see references 4, 6, and 17). In this report we describe the characterization and initial genetic analysis of the Mg^{2+} influx systems of *Salmonella typhimurium* LT-2.

Transport of Mg^{2+} in *S. typhimurium* would be expected to be similar to that observed in *E. coli* because the two genera exhibit considerable genetic homology. In *E. coli* K-12, Mg^{2+} influx occurs through two systems that are controlled by three distinct genetic loci (5, 10, 11, 13, 18, 20). The first system is expressed constitutively and transports Co^{2+} and probably Mn^{2+}, in addition to Mg^{2+}. Mutations affecting this system confer resistance to toxic levels of Co^{2+} and map at two unlinked loci: *corA* at 84 min and *corB* at 96 min (1, 13). The second system is reported to be specific for Mg^{2+} and is inducible by growth in medium with a limiting Mg^{2+} concentration (10 μM). Mutations abolishing this system map at a third locus, *mgt*, at 91 min. Strains deficient in both influx systems (*corA mgt*) require greater than 10 mM Mg^{2+} for normal growth (13).

Uptake of Mg^{2+} in *S. typhimurium* has been previously studied indirectly by Webb (24), who demonstrated that *S. typhimurium* and other gram-negative enteric bacteria accumulate Mg^{2+} from the growth medium in parallel with active growth. In this report we describe the kinetics of Mg^{2+} influx in *S. typhimurium* and the isolation and characterization of mutations that affect this influx. One locus involved in Mg^{2+} influx (*corA*) was isolated on a recombinant plasmid. This plasmid complemented the influx defect in the *corA* mutant strain.

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MATERIALS AND METHODS

Bacteria and phage. Bacterial strains and phage used in this study are listed in Table 1.

Media. The minimal medium used for routine testing of auxotrophic markers was E medium (21) supplemented with the required amino acids at 0.4 mM and glucose at 4 g/liter. The citrate in this medium forms complexes with divalent cations; therefore, the N medium described by Nelson and Kennedy (10) was used whenever the concentrations of Mg^{2+} or Co^{2+} were varied. When supplemented with 10 mM $MgSO_4$, this medium is designated NM medium. NM buffer is NM medium without amino acids or glucose. Rich media were LB and nutrient broth (15). Antibiotics were added to rich media at the following levels, in micrograms per milliliter: tetracycline, 25; ampicillin, 50; kanamycin, 50; chloramphenicol, 20. Rich and E media were solidified with agar (15 g/liter); top agar was used at 7 g/liter. Co^{2+} containing N medium was solidified with agarose (15 g/liter; Type I; Sigma Chemical Co., St. Louis, Mo.).

Isotope uptake assay. Radioactive isotope uptake assays with $^{28}Mg^{2+}$ or $^{60}Co^{2+}$ were performed by using a filtration assay (19). $^{28}Mg^{2+}$ was obtained as $MgCl_2$ in HCl solution from Brookhaven National Laboratories (Upton, N.Y.). $^{60}Co^{2+}$ was obtained as $CoCl_2$ in aqueous solution from Amersham Corp. (Arlington Heights, Ill.). Briefly, cells were grown to mid-log phase (optical density at 600 nm, 0.4 to 0.5) in N medium containing the desired Mg^{2+} concentration and harvested by centrifugation. The cells were washed twice with ice-cold N medium without Mg^{2+} or amino acids,

TABLE 1. Bacterial strains and phage used in this study

Strain	Genotype[a]	Reference or source
S. typhimurium		
LT-2		
TN1379	*leuBCD485*	8
TN2540	*metE551 metA22 hisC47*(Am) *trpB2 ilv-452 rpsL120 fla-66 xyl-404 galE496 hsdL6* ($r^- m^+$) *hsdSA29* ($r^- m^+$)	R. Maurer
AK103	(as TN2540) *zji-3103*::Tn*10*[b]	R. Maurer
AK161	(as TN2540) *zie-3161*::Tn*10*	R. Maurer
SP27	*corA27*	This study
SP52	*corA27 zie-3161*::Tn*10 metE551*	This study
SP54	*corA27 metE551 leuBCD485 zie-3161*::Tn*10*	This study
SP137	(as SP54), plasmid pBR328	This study
SP139	(as SP54), plasmid pSPH14	This study
SP145	(as SP54), plasmid pMS4	This study
E. coli		
KO21	*trpE9829*(Am) *tyr*(Am) *sup-126 thy his thr corA5738*	12
AB2575	*thi-1 ilvD188 relA1 spoT1* Hfr	3
MS4	*corA5738 thi-1 relA1 spoT1* Hfr	This study
TN2539	*hsdR514* ($HsdR^- HsdM^+$) *supE44 supF58 lacY1 galK2 galT22 metB1 trpR55*	9
Phage		
λ	*corA uvrD*	12
P22	*HT*105/1 *int*201	15
P1	*clr*1000	15

[a] Genotypes are presented according to standard notation, as described by Bachmann (1) and Sanderson and Roth (16).
[b] All Tn*10* elements are the transposition-defective mini-Tn*10*Δ16Δ17 described by Way et al. (23).

suspended in this medium to an optical density at 600 nm of 0.200 (10^8 cells per ml), and then kept on ice until use. For each experiment the cells were warmed to room temperature (22°C), and uptake was initiated by the addition of 1.0 ml of cells to a tube containing 1×10^5 to 5×10^5 cpm of isotope and sufficient $MgSO_4$ or $CoCl_2$ (10 to 20 μl of 100 × $MgSO_4$ or $CoCl_2$ stock solutions; total added volume, 25 μl) to yield the desired final concentration. To terminate uptake, 10 ml of ice-cold NM buffer was added, the suspension was filtered through 0.45-μm-pore-size nitrocellulose filters (BA85; Schleicher & Schuell, Inc., Keene, N.H.), the filters were washed with an additional 10 ml of buffer, and radioactivity was quantitated by gamma counting. For each experiment parallel assays were done at room temperature and on ice, with each point determined in triplicate; specific uptake was defined as the difference between cell-associated counts at the two temperatures. Linearity of initial uptake was verified for each strain and varied from at least 3 min in wild-type strains to less than 45 s in some plasmid-containing strains. Incubation times were adjusted for each strain tested so that the measured rates were true initial influx rates. Because all strains used for influx experiments were leucine auxotrophs, the absence of amino acids prevented cell division during the influx assay, while the presence of glucose allowed metabolic activity.

Isolation of Co^{2+}-resistant strains. Co^{2+} selective medium was N medium solidified with 15 g of agarose per liter containing 10 to 50 μM $CoCl_2$ and no added Mg^{2+} (13). After 10^7 cells were plated on selective plates and incubated at 37°C for 24 to 48 h, spontaneous Co^{2+}-resistant mutants (designated *cor*) were observed. Mutants isolated from single colonies were screened for their level of Co^{2+} resistance by streaking or replica plating strains to minimal medium containing Co^{2+}. In addition, a disk sensitivity test was used for routine confirmation of the *cor* phenotypes. A lawn of the strain to be tested was plated in LB top agar on fresh LB plates, and a 6-mm-diameter filter paper disk saturated with 40 μl of 100 mM $CoCl_2$ was placed in the center. The zone of growth inhibition around the disk was measured after overnight incubation.

Transduction. Transduction experiments were performed with P22 *HT int* and P1 *clr*100 by standard methods (15).

DNA manipulation. Chromosomal DNA was isolated by the method of Ebel-Tsipis et al. (2). Plasmid DNA was isolated by the rapid alkaline lysis technique (7). T4 DNA ligase was obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.); restriction endonucleases were obtained from Bethesda Research Laboratories (Gaithersburg, Md.), Boehringer Mannheim, and International Biotechnologies Inc., New Haven, Conn.); and bacterial alkaline phosphatase was obtained from Bethesda Research Laboratories. All enzymes were used according to the directions of the manufacturer. Transformations were performed by the calcium chloride procedure described by Maniatis et al. (7).

The ability of phage P22 to transduce small plasmids (16) allowed the screening of recombinant plasmids for complementation by transduction rather than transformation. A plasmid library of *S. typhimurium* DNA was constructed as follows. Chromosomal DNA from *S. typhimurium* strains was partially digested with *Sau*3AI and electrophoresed on a 0.5% agarose gel. DNA fragments of between 8 and 15 kilobases (kb) were electroeluted from the appropriate gel slice and ligated into pBR328, which was digested with *Bam*HI and dephosphorylated with bacterial alkaline phosphatase. The recombinant plasmids were used to transform *E. coli* TN2539, selecting for chloramphenicol resistance. The transformants were pooled, and the plasmid DNA was isolated and used to transform a *galE* strain of *S. typhimurium*, TN2540, again selecting for chloramphenicol resistance. These transformants were also pooled, and a P22 transducing phage lysate was prepared. The lysate was used to transduce appropriate mutant strains to antibiotic resistance, and the transductants were screened for complementation.

RESULTS

Influx of Mg^{2+} in wild-type strains. Wild-type cells were grown in NM medium, and Mg^{2+} uptake was determined in N medium containing 100 μM $MgSO_4$ and $^{28}Mg^{2+}$ as tracer (Fig. 1A). Cell-associated $^{28}Mg^{2+}$ increased with time in cells incubated at 22°C. There was no significant increase in cell-associated isotope, however, over at least a 20-min period at 0°C. Uptake of $^{28}Mg^{2+}$ was linear to 3 min in wild-type cells (Fig. 1A). The difference in cell-associated isotope between 22 and 0°C presumably reflects active transport, whereas cell-associated $^{28}Mg^{2+}$ at 0°C likely represents isotope binding to cellular structures or trapping on

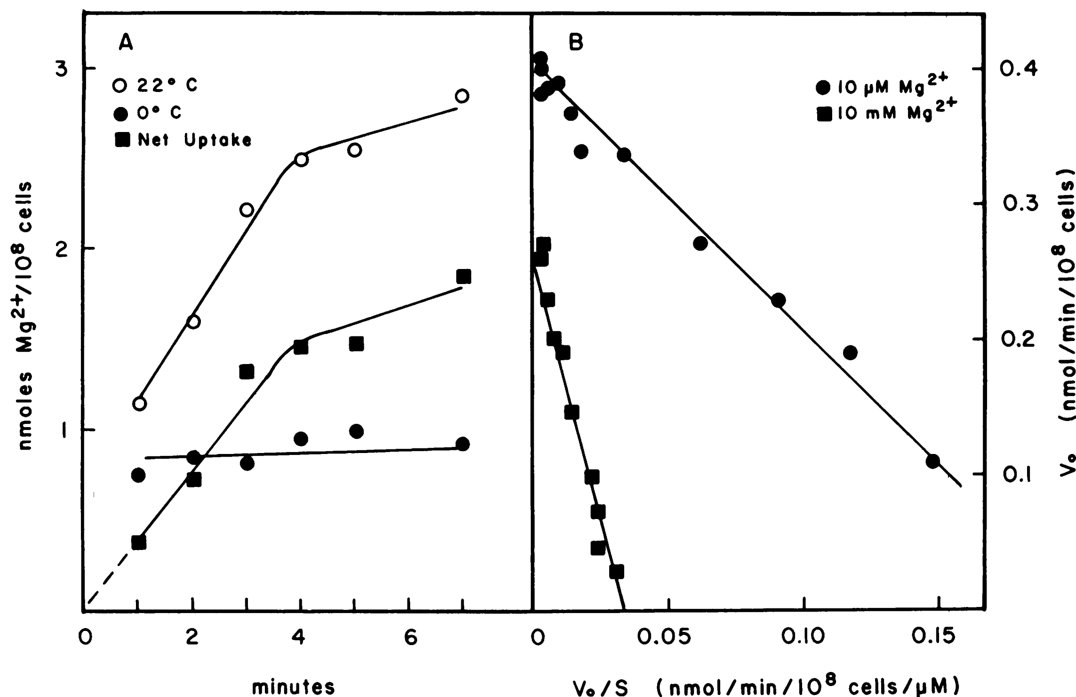

FIG. 1. Uptake of Mg^{2+} in wild-type *S. typhimurium* in the presence of 100 μM $MgSO_4$. (A) Cells were grown in the N medium with 10 mM $MgSO_4$, with uptake assays performed as described in the text. Cell-associated radioactivity is shown for the uptake of $^{28}Mg^{2+}$ at 22°C and 0°C and for the net difference. Cell-associated counts at 0°C remained essentially constant for at least 20 min (data not shown). (B) Concentration dependence of Mg^{2+} uptake in wild-type cells. Shown is an Eadie-Hofstee plot of initial influx rate versus Mg^{2+} concentration for cells grown in N medium containing either 10 μM $MgSO_4$ or 10 mM $MgSO_4$. The initial influx rate was determined with a 2-min incubation period. Abbreviations: V_0, initial velocity; S, ion concentration.

the filter. In support of this interpretation, at 0°C cell-associated $^{28}Mg^{2+}$ showed a nonsaturable dependence on the Mg^{2+} concentration, which is consistent with a large number of poor affinity-binding sites. Also, cell-associated $^{28}Mg^{2+}$ at 22°C was reduced to the 0°C level in the presence of metabolic poisons such as cyanide or carbonyl cyanide *m*-chlorophenylhydrazone.

The initial rate of Mg^{2+} influx in cells grown in 10 mM Mg^{2+} followed simple Michaelis-Menten kinetics, with a K_m of 15 μM Mg^{2+} and a V_{max} of 0.25 nmol of Mg^{2+} per min per 10^8 cells or 25 nmol of Mg^{2+} per min per mg of cell protein (Fig. 1B and Table 2). Cation selectivity was examined by evaluating other cations as inhibitors of Mg^{2+} influx in cells grown in NM medium and assayed in N medium containing 100 μM total Mg^{2+}. Calcium (1 mM) did not inhibit influx, while 1 mM Mn^{2+} or Ni^{2+} inhibited influx 30% and 70%, respectively. Co^{2+} abolished over 90% of influx at a concentration of 1 mM and acted as a simple competitive inhibitor of Mg^{2+} influx with a K_i of approximately 30 μM (data not shown).

Influx of Mg^{2+} in *E. coli* is mediated by two transport systems, one of which is inducible by growth in low Mg^{2+} concentrations (11). Therefore, we investigated this possibility in *S. typhimurium*. After growth of wild-type cells in N medium containing 10 μM $MgSO_4$, influx exhibited a significantly higher V_{max} (0.4 nmol of Mg^{2+} per min per 10^8 cells) and a lower K_m for Mg^{2+} (3 μM), suggesting that an inducible component is present (Fig. 1B and Table 2). Under these

TABLE 2. Influx kinetics of Mg^{2+} and Co^{2+} [a]

Strain	*corA* genotype	Growth medium (mM $MgSO_4$)	Plasmid	Mg^{2+} K_m (μM)	Mg^{2+} V_{max} (nmol/min per 10^8 cells)	Co^{2+} K_m (μM)	Co^{2+} V_{max} (nmol/min per 10^8 cells)
TN1379	*corA*+	0.01	None	3	0.40	30	0.40
		10	None	15	0.25	30	0.60
SP54	*corA27*	0.01	None	2	0.50	NM[a]	NM
		10	None	15	<0.05	NM	NM
SP137	*corA27*	10	pBR328	15	<0.05	NM	NM
SP139	*corA27*	10	pSPH14	15	0.95	15	5.0
SP145	*corA27*	10	pMS4	15	0.80	30	4.0

[a] NM, Not measurable. With wild-type cells grown in 10 mM Mg^{2+} and incubation of 10^8 cells per ml in 30 μM extracellular Co^{2+} and 10^5 cpm of $^{60}Co^{2+}$ per ml, specific uptake was about 2,500 cpm (e.g., see Fig. 4). In *corA* strains up to 5×10^5 cpm of $^{60}Co^{2+}$ was used under otherwise identical conditions, and less than 100 cpm of specific uptake was observed. Mg^{2+} influx exhibited a similar but somewhat lesser decrease, presumably because of the presence of the *mgt* Mg^{2+} influx system.

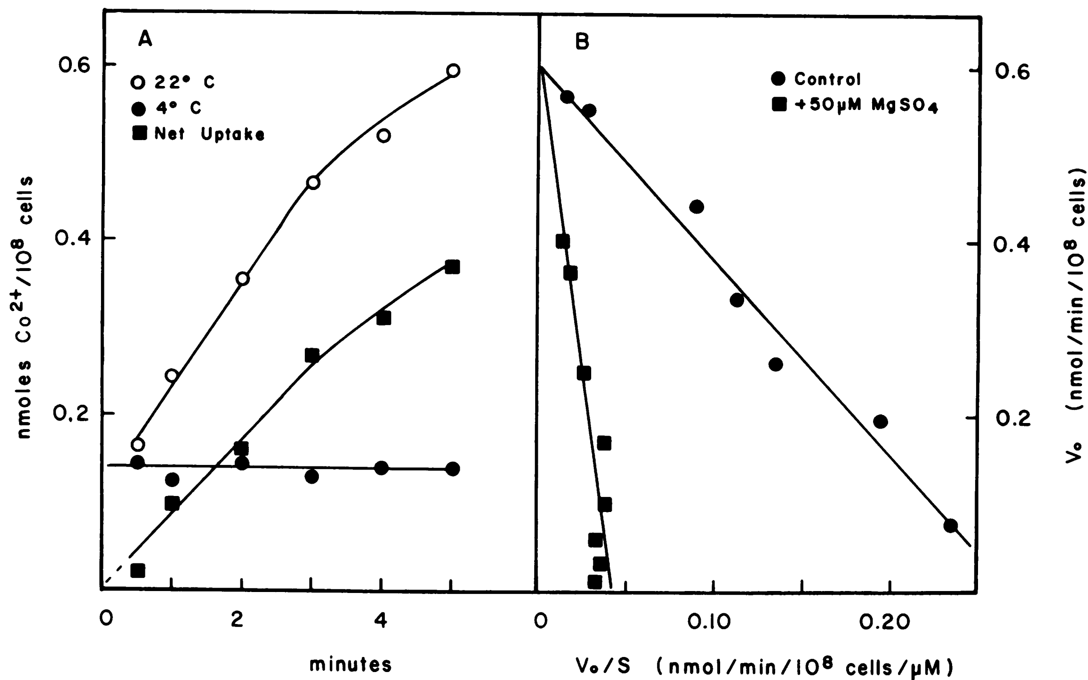

FIG. 2. Uptake of Co^{2+} in wild-type *S. typhimurium*. (A) Cells were grown in N medium with 10 mM $MgSO_4$. Uptake was measured in the presence of 30 μM $CoCl_2$. Co^{2+} uptake is shown for incubations at 22°C and 0°C and for the net difference. (B) Co^{2+} concentration dependence and Mg^{2+} inhibition of Co^{2+} influx in wild-type *S. typhimurium*. Cells were grown in N medium containing 10 mM $MgSO_4$, and the concentration dependence of Co^{2+} uptake was determined in either the absence or the presence of 50 μM Mg^{2+}. Specific influx was determined from 3-min incubation periods with isotope. Abbreviations: V_0, initial velocity; *S*, ion concentration.

conditions Co^{2+} inhibited only about two-thirds of the total Mg^{2+} influx (data not shown). Because Co^{2+} completely inhibited $^{28}Mg^{2+}$ uptake in cells grown in 10 mM Mg^{2+}, these data suggest that the inducible component of Mg^{2+} influx interacts with Co^{2+} only weakly.

Uptake of Co^{2+} in *S. typhimurium*. Uptake of $^{60}Co^{2+}$ was initially examined in wild-type cells grown in NM medium (10 mM Mg^{2+}). Uptake of $^{60}Co^{2+}$ was linear for 3 min and was abolished at 0°C (Fig. 2A). In cells grown in 10 mM Mg^{2+}, Co^{2+} influx exhibited a V_{max} of 0.6 nmol of Co^{2+} per min per 10^8 cells and a K_m of 30 μM (Fig. 2A and Table 2). Mg^{2+} was a competitive inhibitor with an approximate K_i of 10 μM (Fig. 2B). Co^{2+} influx was not increased when cells were grown in N medium with 10 μM $MgSO_4$ (data not shown). These results suggest that both Mg^{2+} and Co^{2+} are transported by the same system under growth conditions with high concentrations of Mg^{2+} and that the inducible component of Mg^{2+} influx is a distinct influx system.

Co^{2+} resistant mutants. Selection for Co^{2+} resistance in *E. coli* (13) resulted in the isolation of two classes of Mg^{2+} transport mutants. A similar selection process was applied to *S. typhimurium*, and spontaneous Co^{2+}-resistant colonies were observed after wild-type cells were plated on N medium containing 10 μM Co^{2+}. Forty such colonies were isolated and found to be resistant to Co^{2+} concentrations up to 25 μM. These mutations were designated *cor* for Co^{2+} resistance.

Because the two Co^{2+} resistance loci of *E. coli* K-12 are linked to *metE* and *argI* (13), possible linkages between these genes and the *S. typhimurium cor* alleles were exam-

TABLE 3. Transductional mapping of *corA*

Donor[a]	Recipient[b]	No. tested	Presence of recombinant		Class (no.)	Total mutants (%)	
			Met	Cor		*met*	*cor*
Cross 1							
AK161 (*metE zie-3161*::Tn*10*)	SP27 (*corA27*)	40	+	+	0		
			+	−	27		
			−	+	1		
			−	−	11	33	5
Cross 2							
SP52 (*metE corA zie-3161*::Tn*10*)	TN1379	253	+	+	147		
			+	−	0		
			−	+	93		
			−	−	14	43	6

[a] Only the relevant genotype is shown.
[b] Tetracycline resistance was the selected marker in both crosses.

ined. Tn*10* insertions near *metE* (*zie-3161*::Tn*10*, 35% linked) and *argI* (*zji-3103*::Tn*10*, 87% linked) provided tetracycline resistance as a selectable marker for determining P22 cotransduction frequencies. Two classes of *cor* mutations were identified based on linkage to these Tn*10* insertions. The first class, which was linked to *zji-3103*::Tn*10*, was tentatively designated *corB*. The second class, designated *corA*, was cotransducible with *zie-3161*::Tn*10* by 6% and with *metE* by 15%. This class was the focus of a subsequent study.

The location of *corA* relative to those of *metE* and *zie-3161*::Tn*10* was determined from three point crosses (Table 3). In both crosses one class of possible transductants was not observed. These rare classes probably resulted from double recombination events, and their absence is consistent with the following gene order: *corA*,*metE*,*zie-3161*::Tn*10*. This order is also consistent with higher cotransduction frequency of *metE* and Tn*10* (43%) relative to that of *corA* and Tn*10* (6%). This Tn*10* insertion is 4% cotransducible with *pepQ* (unpublished data), and thus the chromosomal order is *corA*, *metE*, Tn*10*, *pepQ*. One *corA metE* transductant was designated strain SP54 and was used for subsequent influx studies.

Tetracycline resistance could not be used as a selectable marker in minimal media with 10 μM $CoCl_2$. Under these conditions strains grew regardless of their *cor* genotype and independent of the presence of a Tn*10* transposon. These effects were attributed to the chelation of divalent cations by tetracycline, which reduced the effective concentrations of both drug and cation. Similar difficulties in scoring cobalt resistance were observed when high levels of amino acids were present in minimal Co^{2+} plates, presumably because of the formation of cation-amino acid complexes. The amino acid effect was eliminated by empirically determining a Co^{2+} level that completely inhibited the growth of *cor*$^+$ strains in the presence of amino acid supplements: 25 μM Co^{2+} and total amino acid concentrations under 1 mM gave reproducible results.

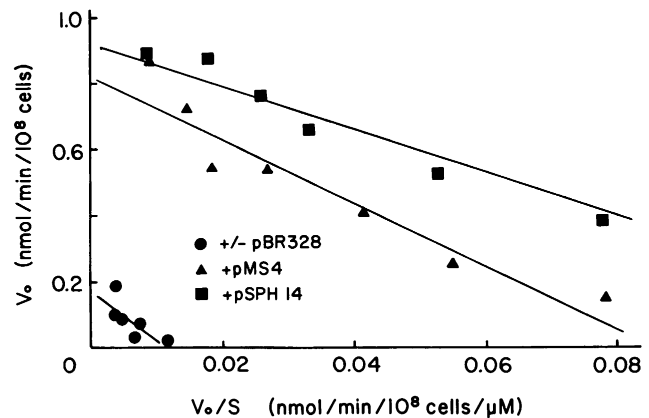

FIG. 4. Concentration dependence of Mg^{2+} influx in *S. typhimurium corA* strains containing various plasmids. Strains were grown in N medium with 10 mM $MgSO_4$ (to repress the *mgt* Mg^{2+} influx system) and with plasmids pBR328 (strain SP137), pSPH14 (strain SP139), and pMS4 (strain SP145). Specific influx was determined from 30-s incubation periods. Abbreviations: V_0, initial velocity; *S*, ion concentration.

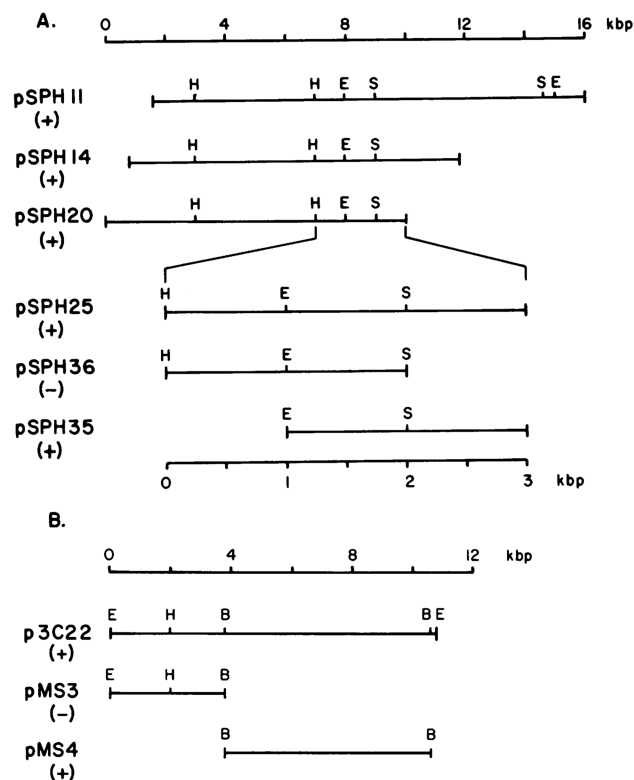

FIG. 3. Restriction endonuclease maps and *cor* phenotypes conferred by plasmids. (A) Restriction endonuclease maps of the plasmids derived from *S. typhimurium*. (B) Restriction endonuclease maps of plasmids derived from the lambda *corA uvrD* strain obtained from K. Oeda (11). Plasmids shown with a plus sign below the plasmid designation conferred Co^{2+} sensitivity on a *corA* strain of *S. typhimurium*, while plasmids shown with a minus sign below the plasmid designation failed to complement the *corA* mutation. Restriction nuclease sites are indicated by short vertical lines as follows: E, *Eco*RI; S, *Sal*I; H, *Hin*dIII; B, *Bam*HI. kbp, Kilobase pairs.

Influxes of Mg^{2+} and Co^{2+} in *corA* strains. When *corA* strain SP54 was grown in NM medium, the influx of Co^{2+} was not detectable (Table 2). In addition, the V_{max} for Mg^{2+} influx was reduced by over 80% in SP54 compared with that in wild-type cells. To determine if the *corA* mutation altered Mg^{2+} influx because of the inducible component of Mg^{2+} influx, influx was measured after strain SP54 was grown in N medium containing 10 μM $MgSO_4$. Under these conditions Mg^{2+} influx occurred at approximately the same rate as in wild-type cells, while Co^{2+} influx remained undetectable (Table 2). These results are consistent with the existence of two influx systems, a constitutive system that transports both Mg^{2+} and Co^{2+} and an inducible system that is highly selective for Mg^{2+}. Influx kinetics for cells grown on 10 mM Mg^{2+} should represent only the *corA* component. Furthermore, if a *corA* mutation abolishes the activity of this influx system, the kinetics measured in *corA* strains should represent the behavior of the Mg^{2+}-selective system. Thus, Mg^{2+} influx via the *corA* system exhibited a K_m of 15 μM, while Mg^{2+} influx through the inducible Mg^{2+}-selective system had a K_m of about 3 μM (Table 2).

To isolate mutations in the putative second influx system, a *corA* strain was mutagenized and enriched with penicillin for mutants that required high Mg^{2+} concentrations for growth. Strains isolated by this procedure require at least 10 mM Mg^{2+} for normal growth (S. P. Hmiel et al., manuscript

in preparation) and have been tentatively assigned the designation *mgt* (Mg^{2+} transport). When these strains were transduced to *corA*$^+$, they exhibited wild-type Mg^{2+} requirements, growing normally in medium with Mg^{2+} concentrations of less than 1 mM. This characteristic allowed us to isolate the wild-type *corA* gene directly from a chromosomal library.

Cloning of the *corA* gene. The *S. typhimurium* chromosomal library was screened for recombinant plasmids carrying *corA* by complementation of the growth requirements of the *corA mgt* mutant host strain. Plasmids could be grouped into three classes with different-sized inserts and overlapping restriction nuclease maps (Fig. 3). When transduced with a *corA* strain, these plasmids restored Co^{2+} sensitivity. Co^{2+} sensitivity in such strains was actually increased over that of the wild-type cells, as measured by the disk sensitivity test (see above). Table 2 shows Mg^{2+} and Co^{2+} influx data in *corA* strains grown in NM medium (10 mM Mg^{2+}) with or without plasmids. The parent *corA* strain exhibited no significant influx of Co^{2+} and very little influx of Mg^{2+} (due to repression of the inducible *mgt* system). Uptake of both Co^{2+} and Mg^{2+} (Table 2 and Fig. 4) was restored to strain SP139, a *corA* strain containing plasmid pSPH14. Influx of Mg^{2+} in strain SP139 exhibited a K_m value similar to that seen in wild-type cells, but V_{max} was increased almost twofold. Similarly, the V_{max} for Co^{2+} influx was increased severalfold in strain SP139 compared with that in wild-type cells.

Plasmid pSPH25 was constructed from pSPH20 by deletion of a 8-kb *Hin*dIII fragment. This plasmid also complemented the *corA* phenotype and was further subcloned by deletion of the *Eco*RI fragment to generate pSPH35 or by deletion of the *Sal*I fragment to generate pSPH36 (Fig. 3). pSPH36 did not complement the *corA* mutation, but pSPH35 retained this ability. Thus, the *corA* gene was localized to a fragment of approximately 1.5 kb.

A recombinant lambda phage containing the *E. coli* K-12 *corA-uvrD* region was obtained from K. Oeda (12). The phage insert was subcloned into pBR328. The resulting recombinant plasmid complemented the *corA* mutation in *E. coli* K-12 strain KO21. This strain proved difficult to transform and propagate, however, so the *corA* mutation was transduced into strain AB2575 by using phage P1. The resulting strain (MS4) exhibited Co^{2+} resistance identical to that of strain KO21 and was used as a host in subsequent experiments. Subcloning of the original insert showed that a 6.5-kb *Bam*HI fragment cloned in pBR328, designated pMS4 (Fig. 3), retained the ability to complement the *E. coli corA* strain.

To investigate the similarity of the Mg^{2+} influx systems of *E. coli* and *S. typhimurium*, the *E. coli corA* gene was introduced into a *S. typhimurium corA* mutant. Plasmid pMS4 was used to transform strain TN2540, and a P22 transducing lysate from one of the transformants was used to transfer the plasmid to strain SP54. Disk sensitivity tests indicated that Co^{2+} sensitivity was restored to the *S. typhimurium corA* strain by this procedure. As with the *S. typhimurium*-derived pSPH35, pMS4 induced increased Co^{2+} sensitivity in the *corA* mutant compared with that in wild-type *S. typhimurium*. Influxes of Co^{2+} and Mg^{2+} in the *corA* strain were restored by pMS4 to levels much greater than those observed in wild-type cells and were similar to those observed with the *S. typhimurium* plasmids (Table 2 and Fig. 4). The influx of Mg^{2+} in the *S. typhimurium corA* strain exhibited a similar K_m and an increased V_{max} in the presence of pMS4 compared with that of wild-type cells.

DISCUSSION

Results of this study provide substantial support for the presence of two distinct Mg^{2+} uptake systems in *S. typhimurium* and are in substantial agreement with previous findings in the related organism *E. coli* (13). Both organisms exhibit a Co^{2+}-sensitive component which can be eliminated by mutations at a locus linked to *metE*. This component transports a Co^{2+} as well as Mg^{2+}, with similar uptake kinetics for both ions in the two species. However, the kinetic evidence for the second uptake system is more compelling in *S. typhimurium*. When this species is grown in 10 μM Mg^{2+} medium, there is a consistent increase in the V_{max} as well as a change in the affinity for Mg^{2+} uptake (Fig. 1B). This uptake component is present in the *corA* mutant strain grown under similar conditions. In comparison, both uptake components in *E. coli* have similar K_m and V_{max} values, with the inducible component evident only in the *corA* mutant strains. These results might suggest that there are considerable similarities between the *corA* uptake systems of the two species, with the possibility of substantial differences in the inducible uptake components. Comparisons of the *S. typhimurium* and *E. coli corA* plasmids further demonstrate the similarities in this uptake system in the two species. The *E. coli corA* plasmid complemented the *S. typhimurium corA* mutation but restored Mg^{2+} uptake with kinetics more like those reported for the *E. coli corA* system. A further comparison of these systems awaits the establishment of the nucleotide and amino acid sequences of these two loci.

An alternative interpretation of these results could classify the *corA* uptake as a Co^{2+} influx system that also happens to transport Mg^{2+}, while the actual Mg^{2+} uptake system is the second, inducible system. Although enteric bacteria may require Co^{2+} as a micronutrient, the Co^{2+} influx observed is probably physiologically unimportant for two reasons. First, the observed affinity of the system for Co^{2+} (K_m = 30 μM) was approximately equal to the toxic concentration for this ion in minimal media, and the influx system would therefore be maximally active at Co^{2+} levels that would kill the cell. Second, and more importantly, the levels of free Co^{2+} in the usual environments of enteric bacteria are orders of magnitude below the K_m of this transport system, which would necessitate other methods for Co^{2+} accumulation. The Co^{2+} requirement of *E. coli*, and presumably of *S. typhimurium*, is most likely fulfilled via uptake of cobalamin through a distinct high-affinity binding protein and influx system (14). Thus, the Co^{2+} influx reported here can be considered a specific probe for the constitutive Mg^{2+} influx system in both *S. typhimurium* and *E. coli*.

Complementation of the *corA* mutations by recombinant plasmids, as assessed by restoration of Mg^{2+} and Co^{2+} influx, indicates that we isolated the *corA* gene. There are at least three possibilities as to the nature of this gene. It could be a structural gene for a component of the influx system, a gene with a product that can interact with the actual Mg^{2+} transport protein(s) to restore the transport defect, or a gene with a product that regulates the expression of the structural gene. The first explanation is most likely based on the uptake results. Both the *S. typhimurium* and the *E. coli corA* plasmids produced severalfold increases in the V_{max} for both Co^{2+} and Mg^{2+} transport, with no change in K_m. In addition, they conferred an increased sensitivity to Co^{2+} toxicity compared with that of the wild-type strain. These effects suggest a gene dosage effect that is due to the presence of the *corA* locus on a multicopy plasmid. While these results

strongly suggest that the *corA* gene is a structural protein for the transport system, the other possibilities cannot be excluded at this time.

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