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Mutants in Three Genes Affecting Transport of Magnesium in *Escherichia coli*: Genetics and Physiology

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Mutants in three genes affecting two Mg^{2+} transport systems are described. System I, for which Co^{2+}, Mn^{2+}, and Mg^{2+} are substrates, is inactive in *corA* mutants. *corB* mutants express system I after growth on high (10 mM) Mg^{2+} but not low (0.1 mM) Mg^{2+}. Both *corA* and *corB* mutants are resistant to Co^{2+} or Mn^{2+}. *corA* mutants are sensitive to Ca^{2+}. Transport system II is specific for Mg^{2+} and is repressed by growth on 10 mM Mg^{2+}. *mgt* mutations inactivate system II. Growth of *mgt* mutants is normal except on very low (1 μM) concentrations of Mg^{2+}. *corA mgt* strains exhibit no high-affinity, energy-dependent transport of Mg^{2+} and require 10 mM Mg^{2+} for optimal growth. The three genes are not linked. The *corA* locus is cotransducible with *ilv* at 75 min, *corB* is cotransducible with *pyrB* at 85 min, and *mgt* is cotransducible with *malB* and *mel* at 81 min on the genetic map.

Transport of Mg^{2+} by *Escherichia coli* is energy-dependent, saturable, and competitively inhibited by other divalent cations such as Co^{2+}, Mn^{2+}, and Zn^{2+} but not by Ca^{2+} (4, 6, 12). Although estimates of the K_m for Mg^{2+} have varied (4, 6, 12), simple Michaelis-Menten kinetics have always been observed.

Genetic analysis of Mg^{2+} transport has suggested that there are two transport systems. Mutants resistant to Co^{2+} (Cor) were found to have lost a constitutive transport system (system I) for Co^{2+} and Mg^{2+} (6, 7). The remaining Mg^{2+} transport in Cor mutants was not expressed after growth in high concentrations of Mg^{2+}, and therefore was attributed to a second, repressible, Mg^{2+}-specific transport system (system II).

Mutants resistant to Mn^{2+} (Mng) were found to have an increased K_i of Mg^{2+} for competitive inhibition of Mg^{2+} transport (13). They transported Co^{2+} and therefore probably retained system I. No evidence allowing interpretation of the *mng* mutations in terms of systems I and II has been reported.

The mutants reported in this study are most readily interpreted in terms of the two transport systems proposed by Nelson and Kennedy (7). Two unlinked genes, (*corA* and *corB*) affect system I, which appears to transport Mn^{2+} as well as Co^{2+} and Mg^{2+}. Mutations in a third gene (*mgt*) abolish system II.

MATERIALS AND METHODS

Bacteria. The bacterial strains used are listed in Table 1.

Media. Minimal medium was medium N (6), 0.1 M Tris-hydrochloride (pH 7.4), 1 mM KH_2PO_4, 5 mM KCl, and 1 g of $(NH_4)_2SO_4$, 1 mg of $FeSO_4$, 4 g of glucose, 2 mg of thiamine per liter with amino acids and $MgSO_4$ as indicated in the text. Buffer N omitted glucose, amino acids, thiamine, and $MgSO_4$. P1 broth contained 10 g of tryptone and 5 g of NaCl per liter, adjusted to pH 7.8, with sterile 50 mM $CaCl_2$ added after autoclaving.

Solid media were made with the addition of 10 g of BBL agar per liter or 20 g/liter for replica plating (Baltimore Biological Laboratory, Cockeysville, Md.), except when limiting concentrations of Mg^{2+} were desired. Plates solidified with 10 g of agarose (electrophoresis grade, Sigma Chemical Co., St. Louis, Mo.) per liter supported the growth of only small colonies unless Mg^{2+} was added. Agarose plates are necessary for demonstrating limitation of growth by low Mg^{2+} or inhibition by Co^{2+}, but BBL agar without added Mg^{2+} is adequate to prevent growth of *corA mgt* strains. Care must be taken not to add Mg^{2+} to agarose plates with the inoculum; the bacteria must be washed free of Mg^{2+} or diluted in Mg^{2+}-free buffer. The concentration of amino acids appeared to affect the activity of Co^{2+}, thereby affecting the concentrations at which the bacteria are inhibited. In addition, different unrelated strains were sensitive to different concentrations of Co^{2+} at the same total concentration of amino acids. For derivatives of strain S183-726, the total concentration of amino acids was 400 mg/liter, 100 mg/liter each of leucine, isoleucine, valine, and histidine, with 0.1 mM $CoCl_2$. When *corB* mutants were selected in other genetic backgrounds, amino acids were supplied to give a total of 100 to 300 mg/liter, so that 0.1 mM Co^{2+} inhibited the wild type.

P1 transduction. P1*kc* was obtained from April R. Robbins. Phage stocks were prepared from confluent plates (P1 broth) in a soft agar (6.5 g/liter) overlay, suspended in P1 broth and sterilized with $CHCl_3$. Recipient bacteria were grown in P1 broth and in-

TABLE 1. *E. coli strains*[a]

Strain	Relevant genetic markers	Source	Point of origin and direction of insertion
F⁻			
S183-726	*leu lac his strA rif*	A. R. Robbins	
MP2	*corA*	S183-726; spontaneous	
MP1	*corB*	S183-726; spontaneous	
BW1	*corA mgt*	MP2; methylmethanesulfonate	
BW 10	*mgt*	BW1; P1 tranduction	
A324	*str lacI pro*	S. E. Luria	
A324 *ilv*	*str lacI pro ilv*	A324; spontaneous	
DN53	*str lacI pro corA*	D. L. Nelson	
AN120	*str arg uncA*	E. P. Kennedy	
S148	*mel, malB*	B. Rotman	
AT2535	*pyrB*	B. Bachman	
Hfr			
KL209			81 min, ccw
BW113			8-9 min, ccw
W3212			9-10 min, cw
P3			14 min, ccw
KL99			21-24 min, cw
KL208			32-34 min, ccw
KL983			45 min, ccw
Hfr44			32-39 min, ccw
KL16			55-56 min, ccw
Ra-2			77-78 min, cw
P72			76-78 min, cw
KL25			72-75 min, cw
JC12			58-61 min, cw
CSH63			85 min, cw
CSH70			7 min, ccw
CSH61			14 min, ccw
PC0950	*purA*		8-9 min, ccw
F′			
AB1206			F′14
KLF33/JC1553			F′133

[a] Gene symbols are according to Taylor and Trotter (14), and points of origin and direction of transfer (cw, clockwise; ccw, counter clockwise) are according to K. B. Low (2). All F′ and Hfr strains were obtained from B. Bachman, except strains CSH63, CSH70, and CSH61, which were obtained from B. Rotman.

fected at a multiplicity of approximately one phage per cell for 30 min at 37 C. They were washed with 8.5 g of NaCl-2 g of sodium citrate per liter and plated directly on selective media. When phage were grown on the Ca^{2+}-sensitive *corA* strains, 5 mM $CaCl_2$ and 15 mM $MgSO_4$ were used instead of 50 mM $CaCl_2$.

Mutagenesis and penicillin selection. Methyl methanesulfonate and penicillin were used according to standard procedures (5), except that the bacteria were washed free of the mutagen or of high concentrations of Mg^{2+} instead of simply being diluted.

Transport assays. Transport of $^{28}Mg^{2+}$, $^{60}Co^{2+}$, or $^{54}Mn^{2+}$ was measured by millipore filtration as previously described (6), except that the bacteria were washed twice with buffer N before assay. $^{60}CoCl_2$ and $^{54}MnCl_2$ were purchased from New England Nuclear, Boston, Mass. and $^{28}MgCl_2$ from Brookhaven National Laboratory, Upton, Long Island, N.Y.

RESULTS

Isolation of *corA* mutants. Accumulation of Co^{2+} is lethal to *E. coli* and has been used to select Co^{2+}-resistant (Cor) mutants that fail to transport Co^{2+} (6, 7). We selected spontaneous Cor mutants by prolonged incubation at 37 C in growth medium without Mg^{2+} and with 1 mM Co^{2+}. After 4 h 0.01% of the cells could form colonies on minimal glucose. The survivors were grown in minimal medium and screened for the ability to transport Co^{2+}. Six of seven independent mutants (*corA*) were unable to transport Co^{2+} after growth on either 0.1 mM or 10 mM Mg^{2+} (Table 2). Transport of Mg^{2+} in *corA* mutants was repressed by growth in 10 mM Mg^{2+}, as it was in the Cor mutants of Nelson and Kennedy (7). Strain MP2 was cho-

TABLE 2. *Rates of entry of $^{28}Mg^{2+}$, $^{60}Co^{2+}$, and $^{54}Mn^{2+}$* [a]

Strain	[Mg^{2+}] during growth (mM)	Rate of entry (nmol/mg/min)			Transport system	
		Mg^{2+}	Co^{2+}	Mn^{2+}	I	II
S 183-726 (*cor*$^+$ *mgt*$^+$)	0.1	11.5	7.0	3.3	+	+
	10	12.5	19.4	4.1	+	−
MP2 (*corA*)	0.1	7.9	0.49	1.3	−	+
	10	1.4	0.16	0.22	−	−
MP1 (*corB*)	0.1	3.6	0.39	0.50	−	+
	10	7.7	14.0	1.9	+	−
BW10 (*mgt*)	0.1	11.5	10.0	5.4	+	−
	10	11.4	7.9	6.2	+	−
BW 1 (*corA mgt*)	10	0.41	0.13	0.02	−	−

[a] Uptake of $^{28}Mg^{2+}$, $^{60}Co^{2+}$, or $^{54}Mn^{2+}$ was measured as described in Materials and Methods, at 0.05 mM extracellular divalent cation. Data for each cation is from a separate experiment. Symbols: +, present; −, absent.

sen as a representative *corA* mutant. The seventh mutant, MP1 (*corB*), was unable to transport Co^{2+} after growth on 0.1 mM Mg^{2+} but exhibited normal transport activity after growth on 10 mM Mg^{2+} (Table 2). Transport of Mg^{2+} was not repressible in this strain. Transport of proline was normal in all the Cor mutants (not shown).

Isolation of *corB* mutants. *corB* mutants were isolated at high frequency from strain S183-726 or others by selecting cells able to form colonies on minimal medium containing 0.1 mM $CoCl_2$, without any added Mg^{2+}, and solidified with agarose to avoid adding Mg^{2+}. In strain S183-726, only *corB* mutants were isolated by this procedure. In other strains, however, both *corA* and *corB* mutants could be isolated on Co^{2+}-agarose plates. The slow growth of *corA* derivatives of strain S183-726 on Co^{2+}-agarose plates (Table 3) may account for a low frequency of selection.

Isolation of *corA mgt* mutants. If *corA* strains depend upon system II for transport of Mg^{2+}, a second mutation eliminating system II (*mgt*) should create a strain dependent on passive diffusion and therefore requiring high concentrations of Mg^{2+} for growth. A *corA* strain (MP2) was mutagenized with methyl methanesulfonate, and mutants able to grow on 200 mM Mg^{2+} but not on 0.05 mM Mg^{2+} were selected with penicillin. Strain BW1 was chosen as representative of 14 such Mg^{2+}-dependent strains. It was totally deficient in transport of Mg^{2+} (Table 2). Transport of proline or glutamine was normal in strain BW1 (not shown), implying that energy coupling was normal.

Construction of *corA*$^+$ *mgt* strains. The *corA* locus is cotransducible with *ilv* (Table 4). A *corA*$^+$ allele was introduced into strain BW1 (*corA mgt*) by P1 transduction from strain A324 *ilv*. Transductants (*corA*$^+$ or *mgt*$^+$) that had regained the ability to grow on 0.05 mM Mg^{2+} were screened for the requirement for isoleucine and valine; 6% were *ilv* and thus had to be *corA*$^+$ *mgt*. (The remaining 94% could have been *corA*$^+$ *ilv*$^+$ *mgt* or *corA mgt*$^+$ transductants or revertants.) The *ilv*$^+$ allele was then restored to the *corA*$^+$ *mgt* transductants by transduction from strain S183-726 (*corA*$^+$ *ilv*$^+$). Transport of Co^{2+} or Mn^{2+} in the *mgt* strain was constitutive (Table 2).

TABLE 3. *Properties of cor and mgt mutants of strain S183-726*[a]

Growth on	Wild type	*corA*	*corB*	*mgt*	*corA mgt*
10 mM $MgSO_4$	+	+	+	+	+
0.1 mM $MgSO_4$	+	+	+	+	−
0.001 mM $MgSO_4$	+	+	+	+	−
Limiting $MgSO_4$	+	+	+	±	−
0.1 mM $CoCl_2$	−	±[b]	+	−	ND
0.0025 mM $MnCl_2$	−	−	+	−	ND
0.0001 mM $MnCl_2$	−	±	+	−	ND
50 mM $CaCl_2$	+	−	+	+	ND

[a] Medium N, 10 g of BBL agar per liter, was used with 10 mM $MgSO_4$ and 0.1 mM $MgSO_4$. Medium N, 10 g of agarose per liter, was used with 0.001 mM $MgSO_4$ and with no added $MgSO_4$ for limiting $MgSO_4$, 0.1 mM $CoCl_2$, 0.0025 mM $MnCl_2$, and 0.0001 mM $MnCl_2$. P1 broth, 10 g of BBL agar per liter, was used with 50 mM $CaCl_2$. Symbols: +, Colony formation after 1 day at 37 C; ±, colony formation with slower growth after 3 to 5 days (*corA*, Co^{2+}) or 2 to 3 days (*mgt*, limiting Mg^{2+}); −, no growth after 3 to 5 days; ND, not done.

[b] In other genetic backgrounds, *corA* and *corB* mutants were equally resistant to Co^{2+}.

Phentoypes of the mutants. Growth in liquid medium was not noticeably affected by *corA*, *corB*, or *mgt* mutations, even at low concentrations of Mg^{2+} (Fig. 1). The double mutant BW1 (*corA mgt*), however, grew more slowly than the other strains on concentrations of Mg^{2+} less than 10 mM, in glucose minimal medium (Fig. 1) or in tryptone broth (not shown).

The mutants could be distinguished on solid media by several criteria (Table 3). Their re-

TABLE 4. *Mapping of corA by P1 transduction*

Donor	Recipient[a,b]	Selected marker	Frequency of unselected markers	
uncA ilv$^+$ *corA*$^+$ AN120	*uncA*$^+$ *ilv corA*	*ilv*$^+$	*uncA corA*	75/202
			uncA corA$^+$	9/202
			uncA$^+$ *corA*	68/202
			uncA$^+$ *corA*$^+$	50/202
uncA$^+$ *ilv corA*[a]	*uncA ilv*$^+$ *corA*$^+$ AN120	*uncA*$^+$	*ilv corA*	15/300
			ilv corA$^+$	81/300
			ilv$^+$ *corA*	0/300
			ilv$^+$ *corA*$^+$	204/300

[a] Spontaneous *ilv* derivatives of four independent *corA* mutants, including MP2, were used. The results describe the sum of 50 transductants from each of the four strains; no significant differences between strains were seen.

[b] When the recipient was *corA*, transduction was done in 5 mM Ca^{2+} and 15 mM Mg^{2+}; use of 50 mM Ca^{2+} artifactually doubled the recovery of *corA*$^+$ transductants. The concentration of Ca^{2+} did not affect the cotransduction frequencies when the donor was *corA*.

quirements for Mg^{2+} differed; *corA mgt* strains required 10 mM Mg^{2+} on plates as they did in liquid medium. *mgt* mutants were found to grow slowly on limiting Mg^{2+}, although they grew as well as the wild type on 0.01 mM Mg^{2+} in liquid culture. *corA* and *corB* strains grew as well as the wild type on limiting Mg^{2+} on solid or liquid medium.

corA and *corB* mutants were resistant to Co^{2+} or Mn^{2+} in limiting Mg^{2+} (Table 3). Differential sensitivity to Co^{2+} was reproducible only under carefully controlled conditions, which varied in detail for different genetic backgrounds (see Materials and Methods). Amino acids appeared to affect the Co^{2+} activity, probably by forming complexes. The total concentration of amino acids was chosen so that 0.1 mM Co^{2+} inhibited the wild type but not Cor mutants.

Ca^{2+} inhibited the growth of *corA* mutants, completely preventing growth in tryptone (P1 broth) and slowing growth in minimal medium. The cause of Ca^{2+} sensitivity is not known, but it appears to be a result of the *corA* mutation. Ca^{2+}-insensitivity and Co^{2+} transport were cotransduced by phage P1. One third of Ca^{2+}-resistant revertants had also recovered the ability to transport Co^{2+} and presumably were *corA*$^+$. The properties of the revertants and pseudorevertants have not been investigated further. The mechanism of suppression of the Ca^{2+}-sensitive phenotype of *corA* mutants may help elucidate the cause of Ca^{2+}-sensitivity.

Mapping of *corA*. Sensitivity to 50 mM Ca^{2+} was used to counterselect *corA* mutants in interrupted matings. *corA*$^+$ (resistance to Ca^{2+}) was transferred as an early marker by Hfr strains KL209 and KL25 but was not transferred by strains KL228 and P72. In P1 transductions a high frequency of Ca^{2+}-resistant cells in *corA* cultures prevented the use of *corA* as a selected marker. However, the *corA* locus was cotransducible with *ilv* and *uncA*, as shown by screening *ilv*$^+$ or *uncA*$^+$ transductants for Ca^{2+}-sensitivity (Table 4). The reciprocal three-factor cross showed that the order of the genes is *uncA ilv corA* (Fig. 2).

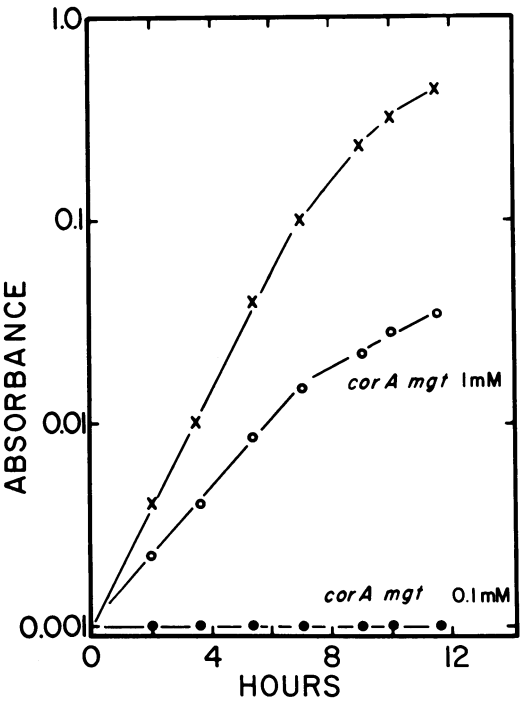

FIG. 1. *Growth of mutants as a function of Mg^{2+}. Cultures were grown in medium N with 0.05, 0.1, 1 or 10 mM $MgSO_4$ at 37 C. Symbols: ×, S183-726 (cor$^+$), MP2 (corA), MP1 (corB), or BW10 (mgt) at 0.05, 0.1, 1.0, or 10 mM $MgSO_4$; ●, BW1 (corA mgt) at 0.1 mM $MgSO_4$; ○, BW1 (corA mgt) at 1 mM $MgSO_4$.*

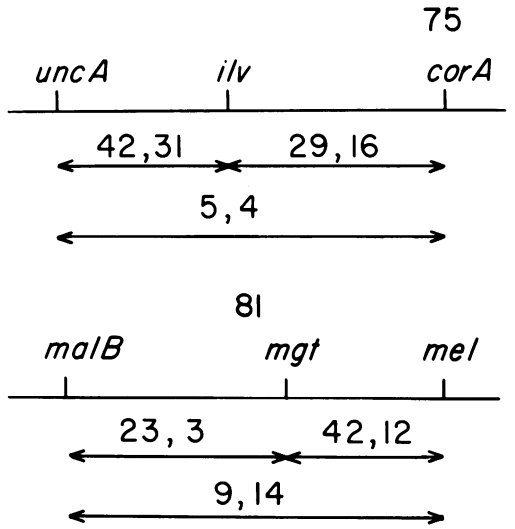

FIG. 2. *Genetic maps showing corA and mgt loci. The numbers represent percentage of cotransduction by phage P1.*

The *corA* allele was recessive to *corA*$^+$. Spontaneous *ilv* derivatives of several *corA* mutants were selected, and the episomes F'14 or F'133 were transferred from strains AB1206 or KLF33/JC1553, respectively. Each of five *ilv*$^+$ clones from each mating was resistant to Ca^{2+} and had normal Co^{2+} transport (not shown). The merodiploids could donate the *ilv*$^+$ marker but not *pro*$^+$ to strain A324 *ilv*. The episomal *corA*$^+$ allele therefore was dominant over the chromosomal *corA* gene in the merodiploids.

Mapping of *corB*. Hfr strains were mated with strain MP1, and *leu*$^+$ recombinants were screened for the Cor phenotype. *corB*$^+$ recombinants were found when strain Ra-2 or CSH 61 was the donor, showing that the *corB* locus was between 77 and 14 min. The *corB* mutation in strain MP1 was 99% cotransducible with *pyrB* (85 min) and 1% cotransducible with *purA*, as shown by screening 100 *pyrB*$^+$ transductants in strain AT2535 and 100 *purA*$^+$ transductants in strain PCO950.

Mapping of *mgt*. Transfer of either a *corA*$^+$ or a *mgt*$^+$ gene to a *corA mgt* double mutant conferred ability to grow on limiting Mg^{2+}. The Hfr strain Ra-2, which has *corA*$^+$ only as a late marker, gave recombinants capable of growth on limiting Mg^{2+}; the locus was shown by interrupted mating to be between *rif* and *leu* (min 79 to 1). P1 phage grown on strain S148 (*cor*$^+$ *mgt*$^+$ *malB mel*) were used to transduce strain BW1 to *corA*$^+$ *mgt* or *corA mgt*$^+$ by selecting transductants on limiting Mg^{2+}. Transductants were screened for sensitivity to Ca^{2+}, and the Ca^{2+}-resistant (*corA*$^+$ *mgt*) transductants, 40% of the total, were discarded. Of the 160 *corA mgt*$^+$ transductants from two transductions 23% were *mal* and 42% were *mel*. The *mgt* locus therefore lies between *malB* and *mel*, near 81 min.

The reciprocal cross, using phage P1 grown on strain BW1 to transduce strain S148 to *mal*$^+$ or *mel*$^+$, also showed the *mgt* locus to be cotransducible with both *malB* and *mel*. The frequency of cotranduction appeared lower, however, possibly because of the difficulty of screening for the *mgt* marker in the more rapidly growing strain S148. Of 100 *malB*$^+$ recombinants, three were scored as *mgt*; of 100 *mel*$^+$ recombinants 12 were *mgt*. Figure 2 summarizes the mapping of *mgt* and *corA*.

Specificity and expression of divalent metal ion transport. Mutations in either of the *cor* genes affected transport of Co^{2+} and Mn^{2+} concomitantly (Table 2). In *corA* strains, transport of both Co^{2+} and Mn^{2+} was defective after growth in 0.1 mM Mg^{2+} and was further reduced by growth in 10 mM Mg^{2+}. In *corB* strains, transport of both ions was increased by growth in 10 mM Mg^{2+}. In *mgt* strains and in the wild type transport of Co^{2+} and Mn^{2+} was constitutive. The fact that the rates of Co^{2+} and Mn^{2+} transport were not observed to vary precisely in parallel is unexplained, as is the extremely low rate of Mn^{2+} uptake by the *corA mgt* strain. It may be that the system II remaining in *corA* mutants transports Mn^{2+}; most of the Mn^{2+} transport, however, can be attributed to system I. The existence of a separate high-affinity transport system for Mn^{2+} (10, 11) (V_{max} = 0.01 to 0.04 nmol/mg per min) and the day-to-day variation in rates measured in different cultures may also contribute to the lack of proportionality of Co^{2+} and Mn^{2+} transport.

A single transport system, the constitutive system I of Nelson and Kennedy (7), thus seems to transport both Co^{2+} and Mn^{2+} as well as Mg^{2+}. In *corA* mutants this system is absent; in *corB* mutants it is expressed only after growth in high concentrations (10 mM) of Mg^{2+}. *mgt* mutations do not affect system I. The Mg^{2+}-specific, repressible system II comprises the total Mg^{2+} transport in *corA* and *corB* strains grown on 0.1 mM Mg^{2+} and is absent from *mgt* strains. *corA mgt* double mutants thus lack any active, saturable Mg^{2+} transport and are unable to grow without high concentrations of external Mg^{2+}. Table 2 includes an interpretation of the data in terms of the two transport systems.

Kinetics of Mg^{2+} transport. The two transport systems were not distinguishable by differences in affinity or maximal velocity. The slopes of the lines in Fig. 3 show that the wild

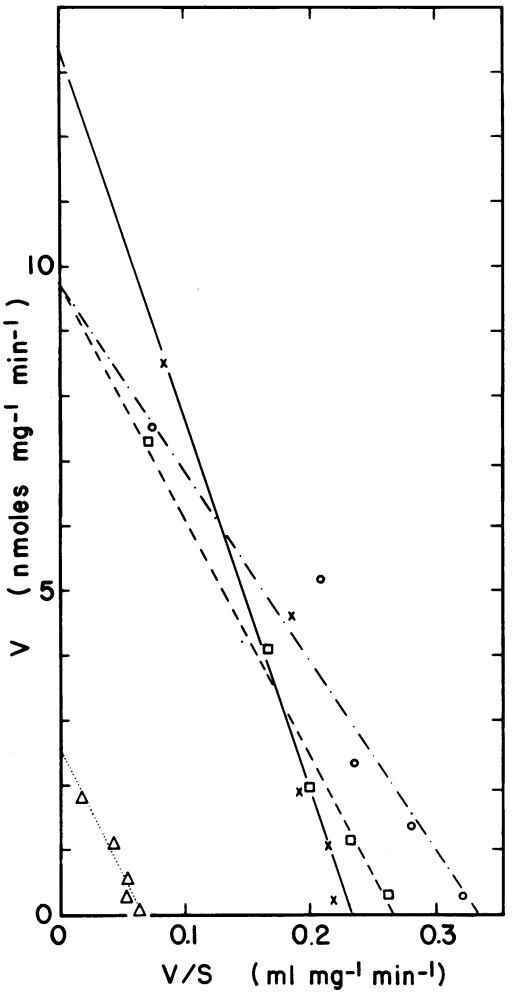

FIG. 3. *Kinetics of energy-dependent entry of $^{28}Mg^{2+}$. The bacteria were grown in medium N with 0.1 mM Mg^{2+}, harvested, and washed twice with buffer N without Mg^{2+}. The rate of entry of $^{28}Mg^{2+}$ at 1, 5, 10, 25, and 100 μM was measured during the first 6 min of incubation at 25 C. The rate in the presence of 10 μM carbonylcyanide m-chlorophenylhydrazone was subtracted. Symbols: ×, wild type; □, corA; △, corB; ○, mgt.*

type, *corA*, *corB*, and *mgt* strains all had nearly the same K_m for energy-dependent entry of Mg^{2+}. The values of K_m were 57 μM (wild type), 36 μM (*corA*), 37 μM (*corB*), and 28 μM (*mgt*). When measured in different cultures, K_m varied between 15 and 60 μM, but no consistant difference between strains was observed. The K_m was also not significantly different after growth of the four strains on 10 mM Mg^{2+}, although V_{max} varied in accordance with the rates given in Table 2. V_{max} in Fig. 3 was 13.4 (wild type), 9.6 (*corA*), 2.5 (*corB*), and 9.7 nmol/mg per min (*mgt*). In different experiments, V_{max} varied between 13 and 15 nmol/mg per min in the wild type, between 9 and 11 nmol/mg per min in *corA* or *mgt* strains, and between 2.5 and 5 nmol/mg per min in *corB*.

The rate of entry of $^{28}Mg^{2+}$ into the *corA mgt* strain was proportional to the concentration of $^{28}Mg^{2+}$ (Fig. 4). The uncoupler carbonylcyanide *m*-chlorophenylhydrazone (CCCP) did not inhibit the entry of $^{28}Mg^{2+}$ into *corA mgt* cells, although it did inhibit transport in the wild type and single mutants (Fig. 4). Entry of Mg^{2+} into *corA mgt* strains therefore appeared to occur by passive diffusion. Uncoupled transport was more rapid than entry of $^{28}Mg^{2+}$ into *corA mgt* cells, but was not saturable within the concentration range used. Lack of energy thus appears to leave some transport attributable to the *corA* and *mgt* gene products but functioning with lower affinity.

Retention of Mg^{2+} by *corA mgt* mutant. The apparent lack of transport of Mg^{2+} by the double mutant could be due to an inability to retain Mg^{2+} against a gradient. When grown in 10 mM $^{28}Mg^{2+}$, however, the *corA mgt* strain did not release $^{28}Mg^{2+}$ into Mg^{2+}-free medium (Fig. 5). Exchange of the internal $^{28}Mg^{2+}$ with low concentrations of external Mg^{2+} was defective in the double mutant.

DISCUSSION

The newly discovered *corB* and *mgt* mutants are most readily interpreted in terms of two transport systems catalyzing entry of Mg^{2+} into *E. coli*, a constitutive Mg^{2+}, Co^{2+}, and Mn^{2+}

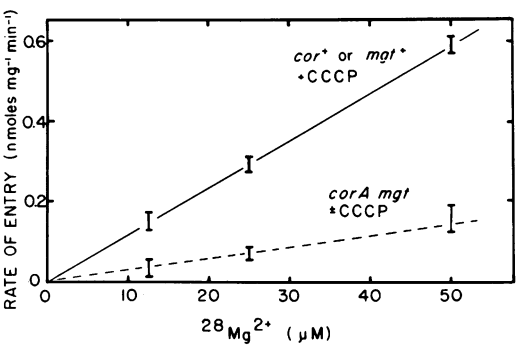

FIG. 4. *Kinetics of energy-independent entry of $^{28}Mg^{2+}$ and entry into corA mgt strain BW1. Strain BW1 was grown in medium N with 10 mM Mg^{2+}; other strains were grown with 10 mM or 0.1 mM Mg^{2+}. Transport was measured as in Fig. 2. The symbols indicate the range of values from several experiments. Symbols: (–) cor⁺ or mgt⁺ strains with 10 μM carbonylcyanide m-chlorophenylhydrazone (CCCP), a concentration inhibiting uptake in the wild type by at least 95%; (- - -) strain BW1 (corA mgt) with or without CCCP.*

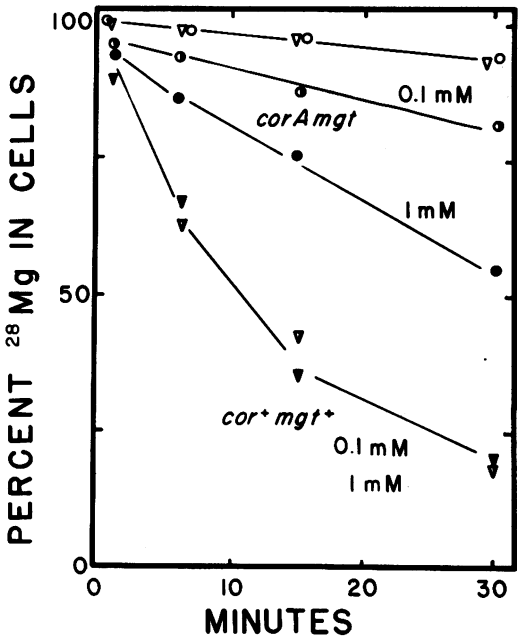

FIG. 5. *Retention and exchange of intracellular $^{28}Mg^{2+}$. The bacteria were grown in medium N with 10 mM $^{28}MgSO_4$. The cells were washed and resuspended in Mg^{2+}-free medium without amino acids. The indicated concentrations of $^{24}MgSO_4$ were added at zero time, and the ^{28}Mg remaining in the cells was determined by filtration. Symbols for strain S183-726: ▽, no $^{24}MgSO_4$; ▼, 0.1 mM $^{24}MgSO_4$; ▼, 1 mM $^{24}MgSO_4$. Symbols for strain BW1 (corA mgt): ○, no $^{24}MgSO_4$; ◑, 0.1 mM $^{24}MgSO_4$; ●, 1 mM $^{24}MgSO_4$.*

transport system I and a repressible, Mg^{2+}-specific system II (7). As originally suggested by Nelson and Kennedy (7), *corA* mutations abolish system I. *corB* may be a control gene; system I is constitutive or slightly inducible in the wild type but is not expressed at low extracellular concentrations of Mg^{2+} in *corB* mutants. System II, specific for Mg^{2+} and not expressed at high concentrations of Mg^{2+}, is missing from *mgt* mutants. Transport of Co^{2+} and Mn^{2+} (system I) is normal in *mgt* mutants, but repressible transport of Mg^{2+} is lacking.

corA mgt mutants lack both systems I and II and appear to allow only passive, energy-independent entry of Mg^{2+}. They grow slowly on 1 mM Mg^{2+}, a concentration that by extrapolation of the data of Fig. 4 should allow entry by diffusion at a rate of 2.8 nmol/mg per min at 25 C. That rate of diffusion is more than sufficient to explain the observed growth rate of strain BW1. To maintain a Mg^{2+} content of 155 nmol/mg (3) at the observed doubling time of 110 min for growth on 1 mM Mg^{2+}, the rate of net uptake must be 1 nmol/mg per min.

Kinetic evidence in support of two separate transport systems is less convincing than the genetic analysis. The K_m for Mg^{2+} is nearly the same in all strains grown under conditions that express either system alone or both together, and V_{max} is also very similar. Silver and Clark (12) and Nelson and Kennedy (6) have reported similar values of K_m in wild-type strains and also found no indication of kinetically distinguishable components. The K_m of 3 to 4 μM reported by Lusk and Kennedy (4) was not detected here. Cells grown on 0.01 mM Mg^{2+} may develop a lower K_m, or there may be differences between strains.

The values of K_m measured are those of exchange of $^{28}Mg^{2+}$ in the medium with intracellular Mg^{2+}, rather than net uptake. Net uptake must be more important for growth than an equimolar exchange and could exhibit different kinetic constants. Net uptake of K^+, for example, has been shown to have a much higher K_m than steady-state exchange (1, 9). The slow growth of *mgt* mutants on limiting Mg^{2+} indicates that system II is physiologically more important for growth on limiting Mg^{2+}, in spite of having the same K_m as system I for exchange of Mg^{2+}.

The two systems clearly differ in specificity; transport of Co^{2+} and Mn^{2+} is abolished in *corA* or *corB* mutants when transport of Mg^{2+} is only slightly altered. The rates of transport in the wild type were not equal to the sum of the rates attributed to systems I and II in mutants expressing a single system. However, the expression of each system may be controlled, so that a mutant synthesized different levels of its functional transport system than the wild type grown in the same medium.

The specificity of the transport systems can explain the resistance or sensitivity of the *cor* mutants to divalent metal ions. Since system I transports Co^{2+} and Mn^{2+}, *corA* and *corB* mutants are resistant to Co^{2+} and Mn^{2+} when system I is not expressed. System I is different from the high-affinity Mn^{2+} transport system discovered by Silver et al. (10, 11). *cor* and *mgt* mutants transported 10^{-7} M Mn^{2+} as rapidly as the wild type (not shown). The *cor* mutants were not resistant to Mn^{2+} under the conditions of Silver et al. (13), and thus were not the same as his Mn^{2+}-resistant (*mng*) mutants. Furthermore the *mng* locus has been mapped near 35 min, far from the *cor* and *mgt* loci.

Experiments are in progress to define the physiological role of the two transport systems. The slow growth of *mgt* suggests that system II may catalyze net uptake from low concentrations more efficiently than system I. The sensi-

tivity of *corA* mutants to Ca^{2+} suggests that system I may play a role in the exclusion of Ca^{2+} from the cell (8, 10, 15).

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ADDENDUM IN PROOF

The *corA* locus is near 83.8 min, *mgt* is near 91 min, and *corB* is near 94.7 min on the recently revised linkage map of *E. coli* K-12 (B. J. Bachman, K. B. Low, and A. L. Taylor. Bacteriol. Rev. 40: 116-167, 1976).

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