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# Magnesium Transport in *Salmonella typhimurium*: Genetic Characterization and Cloning of Three Magnesium Transport Loci

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***Salmonella typhimurium*** **strains lacking the CorA $Mg^{2+}$ transport system retain $Mg^{2+}$ transport and the ability to grow in medium containing a low concentration of $Mg^{2+}$. Mutagenesis of a *corA* strain followed by ampicillin selection allowed isolation of a strain that required $Mg^{2+}$-supplemented media for growth. This strain contained mutations in at least two loci in addition to *corA*, designated *mgtA* and *mgtB* (for magnesium transport). Strains with mutations at all three loci (*corA*, *mgtA*, and *mgtB*) exhibited no detectable $Mg^{2+}$ uptake and required 10 mM $Mg^{2+}$ in the medium for growth at the wild-type rate. A wild-type allele at any one of the three loci was sufficient to restore both $Mg^{2+}$ transport and growth on 50 μM $Mg^{2+}$. P22 transduction was used to map the *mgt* loci. The *mgtA* mutation was located to approximately 98 map units (cotransducible with *pyrB*), and *mgtB* mapped at about 80.5 map units (near *gltC*). A chromosomal library from *S. typhimurium* was screened for clones that complemented the $Mg^{2+}$ requirement of a *corA mgtA mgtB* mutant. The three classes of plasmids obtained could each independently restore $Mg^{2+}$ transport to this strain and corresponded to the *corA*, *mgtA*, and *mgtB* loci. Whereas the *corA* locus of *S. typhimurium* is analogous to the *corA* locus previously described for *Escherichia coli*, neither of the *mgt* loci described in this report appears analogous to the single *mgt* locus described in *E. coli*. Our data in this and the accompanying papers (M. D. Snavely, J. B. Florer, C. G. Miller, and M. E. Maguire, J. Bacteriol. 171:4752–4760, 4761–4766, 1989) indicate that the *corA*, *mgtA*, and *mgtB* loci of *S. typhimurium* represent three distinct systems that transport $Mg^{2+}$.**

$Mg^{2+}$ is required for membrane integrity and ribosome function and is essential for cell growth (6, 10, 25, 26, 28). Free $Mg^{2+}$ ion is a cofactor of many important enzymes and may have important regulatory functions (6, 25, 26). In *Escherichia coli* and *Salmonella typhimurium*, $Mg^{2+}$ transport is mediated by at least two genetically distinct transport systems (8, 10, 16–18, 21). Both species exhibit a constitutive component of $Mg^{2+}$ transport inhibited by $Co^{2+}$. Mutations at the *corA* locus eliminate this transport activity and confer resistance to $Co^{2+}$. In *E. coli*, an additional repressible $Mg^{2+}$ transport system is encoded by the *mgt* locus (16–18). *E. coli* strains with mutations at both the *corA* and *mgt* loci exhibit markedly decreased $Mg^{2+}$ transport and require 10 mM $Mg^{2+}$ in the growth medium to achieve maximal growth rates. The *E. coli* CorA and Mgt systems show similar kinetic parameters for $Mg^{2+}$ transport (18), although the $Mg^{2+}$ selectivity of Mgt is greater than that of CorA.

At least two systems capable of $Mg^{2+}$ transport were also identified in *S. typhimurium* (8). $Mg^{2+}$ transport by the CorA systems of the two species exhibited similar kinetics, was inhibited by $Co^{2+}$, and was eliminated by mutations that mapped near the *metE* locus. In addition, a plasmid carrying the *E. coli corA* gene complemented *corA* mutations in *S. typhimurium* (8). *S. typhimurium* strains with *corA* mutations exhibited decreased $Mg^{2+}$ transport when grown in medium containing high $Mg^{2+}$ concentrations (8). Strains lacking both the CorA system and this second, repressible system would be expected to lack specific $Mg^{2+}$ transport, and their growth rates should be dependent on the environmental $Mg^{2+}$ concentration. This report describes the isolation from a *corA S. typhimurium* strain of a mutant that requires $Mg^{2+}$ for growth. This mutant carries lesions at two loci, *mgtA* and *mgtB*, which affect $Mg^{2+}$ transport. Our data in this and the accompanying reports (23, 24) suggest that the three loci related to $Mg^{2+}$ transport represent three distinct $Mg^{2+}$ transport systems.

## MATERIALS AND METHODS

**Bacteria and plasmids.** Bacterial strains used are listed in Table 1. Plasmid construction is described below. Bacteriophage P22 (*HT*12/4*int*-3) was used for transduction. A strain designation of MM has been obtained from the *Salmonella* Genetic Stock Center for the *S. typhimurium* strains described herein. Our previous work (8) used the tentative strain designation SP. All strains from that paper with an SP prefix retain the same strain number but now have an MM prefix.

**Media and disk sensitivity testing.** Media used have been described by Hmiel et al. (8). MacConkey agar base (Difco Laboratories, Detroit, Mich.) was supplemented with 1% lactose (15). Except for the ampicillin selections (see below), which used 100 or 200 mM $Mg^{2+}$, growth at high $Mg^{2+}$ refers to medium containing 10 mM $Mg^{2+}$, and growth at low $Mg^{2+}$ refers to medium containing 50 μM added $Mg^{2+}$ (except LB medium; see below). Although 50 μM $Mg^{2+}$ has been used as the standard low-$Mg^{2+}$ medium, all strains that grow in this medium also grow in medium containing 10 μM $Mg^{2+}$. In this paper, $Mg^{2+}$-dependent refers to strains that grow on high- but not low-$Mg^{2+}$ medium, and $Mg^{2+}$-independent refers to strains able to grow on low-$Mg^{2+}$ medium. Unless stated otherwise, LB broth and plates contained no added $Mg^{2+}$. The level of $Mg^{2+}$ in LB medium is insufficient to support growth of $Mg^{2+}$-dependent strains but optimally supports growth of other strains; therefore, LB medium

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TABLE 1. *S. typhimurium* strains used

| Strain | Genotype | Reference or source |
|---|---|---|
| TN1010 | *argI547 pyrB64 leuD798 pepN96 zjh-831*::Tn*5 pepA1 (fol-101)* | C. G. Miller |
| TN1379 | Δ*leuBCD485* | 13 |
| TN2258 | *hisD9953*::Mu dJ(Lac Kan) *hisA9944*::Mu dI(Lac Amp) (TT10288) | 9 |
| TN2373 | *polA2 ara-9* | 7 |
| TN2540 | *metE551 metA22 hisC47*(Am) *trpB2 ilv-452 rpsL120 fla-66 xyl-404 galE496 hsdL6* ($r^-$ $m^+$) *hsdSA29*($r^-$ $m^+$) (DB2546) | R. Maurer |
| TN3017 | $Hut^+$ *gltC* (TR6093/BB42) | J. R. Roth |
| TN3018 | *ilvB101 ilvG236 pan-187 ara-9* (TR6421) | J. R. Roth |
| TN3020 | *zhj-1075*::Tn*10 gltC* (TT8963) | J. R. Roth |
| TN3243 | *pyrE26* | C. G. Miller |
| TR2962 | *leuD798 fol-101 pyrB64 argI547* | J. R. Roth |
| AK3048 | As TN2540, *zia-3048*::Tn*10*Δtet | 12 |
| AK3104 | As TN2540, *zia-3104*::Tn*10*Δtet | 12 |
| AK3123 | As TN2540, *zia-3123*::Tn*10*Δtet | 12 |
| AK3125 | As TN2540, *zia-3125*::Tn*10*Δtet | 12 |
| AK3205 | As TN2540, *zia-3205*::Tn*10*Δtet | 12 |
| AK3295 | As TN2540, *zia-3295*::Tn*10*Δtet | 12 |
| AK3306 | As TN2540, *zia-3306*::Tn*10*Δtet | 12 |
| MM27 | *corA27* | 8 |
| MM54 | Δ*leuBCD485 metE551 corA27 zie-3161*::Tn*10* | 8 |
| MM77 | *corA27 mgtA5 mgtB16* | This study |
| MM82 | *corA27 mgtB16 zjh-1625*::Tn*10* | This study |
| MM116 | *corA27 mgtB16 zjh-1628*::Tn*10*Δcam | This study |
| MM130 | *corA27 leuD789 argI547 pyrB64 metE551 pepA1 pepN96 zjh-831*::Tn*5 zie-3161*::Tn*10*Δtet | This study |
| MM136 | *corA27 mgtA5 zjh-1628*::Tn*10*Δcam *mgtB16* | This study |
| MM159 | Δ*leuBCD485 corA27* | This study |
| MM196 | Δ*leuBCD485 mgtB10*::Mu dJ | This study |
| MM197 | Δ*leuBCD485 mgtB11*::Mu dJ | This study |
| MM200 | Δ*leuBCD485 corA27 mgtB11*::Mu dJ | This study |
| MM201 | Δ*leuBCD485 corA27 mgtB10*::Mu dJ | This study |
| MM206 | Δ*leuBCD485 corA27 mgtA5 mgtB10*::Mu dJ *zjh-1628*::Tn*10*Δcam | This study |
| MM208 | Δ*leuBCD485 corA27 mgtA5 mgtB11*::Mu dJ *zjh-1628*::Tn*10*Δcam | This study |
| MM223 | Δ*leuBCD485 corA27 mgtB1630*::Tn*10*Δcam | This study |
| MM224 | Δ*leuBCD485 mgtA5 mgtB10*::Mu dJ *zjh-1628*::Tn*10*Δcam | This study |
| MM227 | Δ*leuBCD485 corA27 mgtA5 zjh-1628*::Tn*10*Δcam *zia-3123*::Tn*10*Δtet | This study |
| MM257 | Δ*leuBCD485 mgtB1630*::Tn*10*Δcam *mgtB10*::Mu dJ | This study |
| MM258 | $gltC^+$ *zia-3048*::Tn*10*Δtet | This study |
| MM277 | *leuD798 fol-101 pyrB64 zjh-1625*::Tn*10* | This study |
| MM278 | Δ*leuBCD485 corA45*::Mu dJ *mgtA21*::Mu dJ | This study |
| MM299 | Δ*leuBCD485 mgtA27*::Mu dJ | This study |
| MM323 | Δ*leuBCD485 mgtB1630*::Tn*10*Δcam | This study |

without or with added $Mg^{2+}$ (to 10 mM) was a convenient medium for selection. $Mg^{2+}$ was added as $MgSO_4$.

A complication in the selection procedures was the concurrent use of $Mg^{2+}$ and tetracycline. The levels of each agent must be balanced, since tetracycline-$Mg^{2+}$ chelation will reduce the available concentration of tetracycline to a level too low for significant growth inhibition and $Mg^{2+}$ to a level too low to support adequate growth. In practice, LB plates without additional $Mg^{2+}$ containing 25 μg of tetracycline per ml could be used to select simultaneously for $Tet^r$ and the ability to grow on low-$Mg^{2+}$ medium. However, this medium gave highly variable numbers of transductants in crosses involving $Mg^{2+}$-dependent strains and was not used in mapping crosses in which certain classes of recombinants might be unexpectedly counterselected.

**Disk sensitivity assay.** A 0.1-ml sample of an overnight culture was added to 3 ml of soft agar and poured on an LB plate with or without 10 mM $Mg^{2+}$. Cation solutions were added to disks in a volume of 40 μl and placed on a plate immediately while still wet to avoid potential oxidation of susceptible cations. Sensitivity of strains to a given cation was determined by measuring the diameter (in millimeters) of the clear ring devoid of growth around a disk after overnight incubation. The total diameter of the ring of growth inhibition minus the 6-mm diameter of the filter disk is reported as the average of three independent experiments for $Co^{2+}$, $Mn^{2+}$, and $Ni^{2+}$ and two experiments for $Ca^{2+}$ and $Zn^{2+}$. For diameters less than 10.5 mm, the range or standard deviation was less than 1 mm; for diameters over 10.5 mm, the range or standard deviation was 1 to 2 mm in all cases.

**Genetic techniques.** P22 transduction was used to move plasmids between strains (20). Random populations of transposons Tn*10*, Tn*5*, and Tn*10*Δcam (27), which confer resistance to tetracycline, kanamycin, and chloramphenicol, respectively, were prepared as described previously (5), with each random population derived from at least 1,000 independent insertions. Tn*10*-directed Hfr formation and conjugation crosses were performed as described by Chumley et al. (4). The collection of Tn*10*Δtet insertions (27) and its use for mapping has been described by Kukral et al. (12).

**Isolation of $Mg^{2+}$-dependent strains.** Strain MM27 (*corA* [8]) was mutagenized with diethylsulfate (19). Mutagenized cultures were grown overnight in N minimal medium supplemented with 100 mM $MgSO_4$, washed in medium without added $Mg^{2+}$, diluted 1:100 into N minimal medium (8)

containing 500 μg of ampicillin per ml and 50 μM $MgSO_4$, grown for 4 h, collected on a membrane filter (GSWP; millipore Corp., Bedford, Mass.), washed with an equal volume of 0.9% saline, suspended in 5 ml of N minimal medium containing 100 mM $Mg^{2+}$, and grown overnight. The cultures were plated on N minimal medium containing 200 mM $Mg^{2+}$ to yield about 100 to 200 colonies per plate, grown overnight, and replica plated to N minimal medium plates containing either 50 μM $MgSO_4$ or 200 mM $Mg^{2+}$. Colonies that grew only on the 200 mM $Mg^{2+}$ plates were characterized further.

**DNA manipulations and plasmid isolation.** DNA manipulations were performed as described previously (8). To isolate plasmids complementing the $Mg^{2+}$-dependent phenotype, a P22 lysate prepared on pooled transformants from a pBR328 library of *S. typhimurium* chromosomal DNA was used (8). With this lysate as donor, strain MM77 was transduced simultaneously to $Cam^r$ and growth on low-$Mg^{2+}$ medium on nutrient agar-chloramphenicol plates. Selection for $Cam^r$ alone gave about $10^4$ more transductants than did simultaneous selection for $Cam^r$ and $Mg^{2+}$ independence.

**Integration of plasmids in *polA* strains.** Plasmids with the ColE1 origin of replication cannot replicate autonomously in *polA* strains (11) and can be maintained only through integration into the chromosome (7). Plasmids that contain cloned chromosomal sequences can integrate by homologous recombination and, once integrated, can be mapped as ordinary chromosomal loci. This property was used to confirm the identity of a cloned gene by forcing its integration in a *polA* strain and determining the map position of the integration using P22 transduction. Plasmids with cloned *mgt* loci were transduced into TN2373, a strain carrying the *polA2* mutation (29), with selection for the plasmid antibiotic resistance (usually chloramphenicol).

**Isolation of Mu dJ insertions into cloned DNA.** The Mu dI1734 element (3), referred to as Mu dJ, is a transposition defective version of the Mu dI phage of Casadaban and Cohen (2). It lacks the Mu transposase and normally will not transpose from its site of integration. However, transpositions can occur when the Mu transposase is provided by temperature induction of a *c*ts Mu dI prophage present in the same strain. A strain containing both Mu dI and Mu dJ (TN2258) has been constructed by Hughes and Roth (9). When a plasmid is present during such induction, it also is a target for Mu d transposition. Superinfection with P22 during the Mu induction results in formation of a P22 lysate wherein some transducing particles contain plasmids with a Mu dJ insertion. Plasmids that have acquired a Mu dJ element confer resistance to kanamycin and can be recovered in a subsequent transduction with simultaneous selection for $Kan^r$ and $Cam^r$. This procedure has been used successfully to introduce Mu dJ into plasmids in *S. typhimurium* (C. G. Miller, unpublished results). To exchange a plasmid::Mu dJ fusion into the chromosome, the plasmid was transduced into the *polA* strain TN2373 with selection for $Kan^r$. P22 lysates were prepared from isolated transductants, and an appropriate recipient strain (such as TN1379) was transduced to $Kan^r$. The transductants were screened for loss of the plasmid markers ($Cam^r$, $Amp^r$, or both), and any that had lost the vector markers were kept for further characterization.

**$Mg^{2+}$ uptake assay.** $^{28}Mg^{2+}$ was obtained from Brookhaven National Laboratories (Upton, N.Y.) as $MgCl_2$ in NaCl solution, with an initial specific activity of approximately 100 mCi/mg of Mg. $Mg^{2+}$ uptake was assayed as described previously (8).

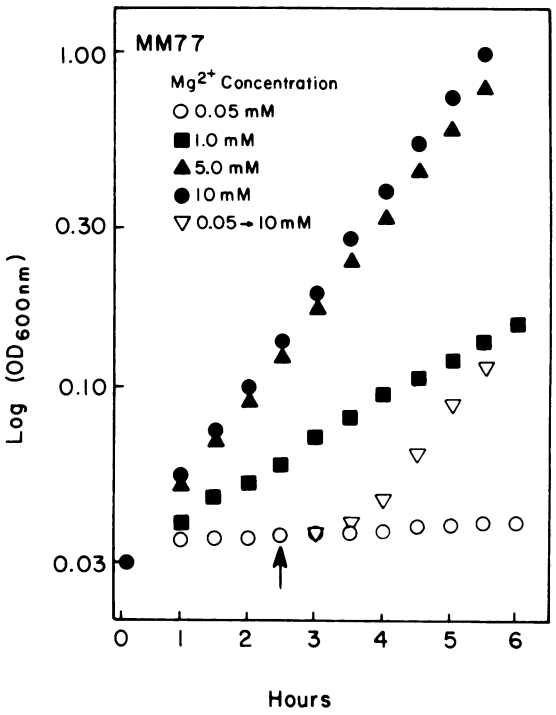


FIG. 1. Growth curve of the $Mg^{2+}$-dependent strain MM77. Cultures were grown overnight in N medium containing 10 mM $Mg^{2+}$, washed three times in N medium without added $Mg^{2+}$, and resuspended in N medium without added $Mg^{2+}$ at 100 times the initial density. A sample was then diluted 1:100 into N medium containing the indicated amount of $MgSO_4$ and incubated at 37°C, and the optical density at 600 nm was determined at the indicated times. After 2.5 h, the culture containing 50 μM $Mg^{2+}$ was split, $MgSO_4$ was added to 10 mM to one portion, and incubation was continued.

## RESULTS

**Isolation and properties of $Mg^{2+}$-dependent strains.** Mutations at the *corA* locus eliminated the component of $Mg^{2+}$ transport inhibited by $Co^{2+}$ but did not affect a component that was repressible during growth in high-$Mg^{2+}$ medium (8). Strains with mutations eliminating all $Mg^{2+}$ transport components should lack specific $Mg^{2+}$ transport and should require $Mg^{2+}$ supplementation for growth. Mutants requiring such supplementation were isolated after diethylsulfate mutagenesis and subsequent ampicillin enrichment (see Materials and Methods). Seven $Mg^{2+}$-dependent isolates (not independent) were obtained by screening 2,500 survivors of the enrichment. All failed to grow on plates containing N minimal medium supplemented with 50 μM $Mg^{2+}$, grew slowly with 1 mM $Mg^{2+}$, and grew at approximately the wild-type rate in 10 mM $Mg^{2+}$. One of the isolates (MM77) was chosen for further study, and the lesion responsible for its $Mg^{2+}$ requirement was tentatively designated *mgt*. The growth characteristics of this strain are shown in Fig. 1. MM77 did not grow in low-$Mg^{2+}$ (50 μM) medium, but growth resumed after a short lag when $Mg^{2+}$ was added. $MgSO_4$ and $MgCl_2$ were equally effective in supporting growth. In contrast, the growth rate of both wild-type and *corA* strains was constant over a wide range of $Mg^{2+}$ concentrations (10 μM to 50 mM; data not shown).

Uptake of $^{28}Mg^{2+}$ in strain MM77 after growth in N minimal medium containing 10 mM $Mg^{2+}$ was no greater than 0.003 nmol $min^{-1}$ $10^8$ $cells^{-1}$ and was less than 3% of

the transport observed in the wild-type strain (0.11 nmol $min^{-1}$ $10^8$ $cells^{-1}$) grown under similar conditions and assayed in parallel. Furthermore, when a culture of MM77 was grown in medium containing 10 mM $Mg^{2+}$, washed in medium without added $Mg^{2+}$, and suspended in medium without added $Mg^{2+}$ for 60 min, no additional $Mg^{2+}$ uptake was observed. This implies the absence of any repressible component of $Mg^{2+}$ uptake in MM77 and is in contrast to results obtained by using the wild-type or *corA* strains (8; data not shown).

**Identification of two distinct *mgt* mutations.** Transposon insertions linked to *mgt* were sought by using a transducing lysate grown on a pool of random transposon insertions (Tn*10*Δtet or Tn*10*Δcam) to transduce MM77 to $Tet^r$ or $Cam^r$ and screening the transductants for $Mg^{2+}$-independent growth. Two transposon insertions, *zjh-1625*::Tn*10* and *zjh-1628*::Tn*10*Δcam, unlinked to the *corA* locus were cotransducible with a locus that could confer on MM77 the ability to grow on medium containing 50 μM $Mg^{2+}$. The *zjh-1628*::Tn*10*Δcam element was 24% linked to the locus (selecting for $Cam^r$ and scoring $Mg^{2+}$ dependence). The linkage of *zjh-1625*::Tn*10* to this locus was at least 13%. The uncertainty in linkage is due to the difficulty in selecting $Tet^r$ on plates containing the high $Mg^{2+}$ concentrations required by MM77 (see Materials and Methods). Since transposons *zjh-1625*::Tn*10* and *zjh-1628*::Tn*10*Δcam were determined to be 8% cotransducible, they are likely linked to the same locus.

To reconstruct a strain with the $Mg^{2+}$-dependent phenotype, P22 transductions were performed, using as donor a strain containing the mutant *mgt* allele and the cotransducible element *zjh-1628*::Tn*10*Δcam. Strains MM27 (*corA27*) and MM54 (*corA27 metE*) were the recipients, with selection for $Cam^r$ on high-$Mg^{2+}$ plates. No $Mg^{2+}$-dependent transductants were identified out of 1,817 $Cam^r$ transductants in 15 independent transductions. Given a cotransduction frequency of 24%, about 450 $Mg^{2+}$-dependent transductants would be expected. The simplest explanation for this result is that the $Mg^{2+}$-dependent growth phenotype of strain MM77 was due to the presence of at least two mutations in addition to *corA*. The locus defined by *zjh-1625*::Tn*10* and *zjh-1628*::Tn*10*Δcam was therefore designated *mgtA*, and experiments aimed at uncovering an additional mutation were undertaken.

The collection of Tn*10*Δtet insertions developed by Kukral et al. (12) was screened to identify insertions linked to additional loci able to restore to MM77 the ability to grow on low-$Mg^{2+}$ medium. Seven of the lysates in strains carrying these insertions were found to transduce MM77 simultaneously to $Tet^r$ and growth on low-$Mg^{2+}$ medium. Transductants from crosses using three of these seven transposon insertions regained $Co^{2+}$ sensitivity. These insertions (*zie-3161*, *zie-3162*, and *zie-3235*) had previously been identified as cotransducible with both the *metE* and *corA* loci (8, 12). $Mg^{2+}$-independent transductants carrying the remaining four Tn*10*Δtet transposons (*zia-3048*, *zia-3123*, *zia-3125*, and *zia-3295*) remained $Co^{2+}$ resistant. These insertions were not cotransducible with *zjh-1628*::Tn*10*Δcam (an *mgtA*-linked insertion) and were therefore assumed to define a new locus, *mgtB*. These results indicated that at least one other mutation was present in strain MM77. A parallel series of investigations to isolate plasmids that restored $Mg^{2+}$-independent growth to MM77 provided evidence that this assumption was correct.

**Isolation of plasmids carrying *mgt* loci.** An *S. typhimurium* chromosomal library (8) was screened for plasmids that could restore the ability of MM77 to grow in low-$Mg^{2+}$ medium. Eleven $Mg^{2+}$-independent colonies were isolated; two were $Co^{2+}$ sensitive, indicating that they harbored plasmids carrying the *corA* locus. Plasmid DNA was isolated from the remaining nine transductants. Restriction endonuclease mapping yielded two distinct maps. Plasmids pSPH37 and pSPH39, representing each of the classes, did not share any restriction fragments, implying that they must carry different regions of the chromosome (Fig. 2). To determine the chromosomal location of the cloned DNA in these plasmids, each plasmid was integrated into the chromosome of the *polA* strain TN2373, and the integrated plasmids were tested for linkage to transposons near *mgtA*. The integrated plasmid pSPH37 was 22% cotransducible with *zjh-1625*::Tn*10* and therefore carries DNA homologous to the *mgtA* region. The integrated plasmid pSPH39 was not cotransducible with this transposon, suggesting that it carries the *mgtB* locus. This interpretation was confirmed by using insertion mutations into the cloned sequence of pSPH39 (see below).

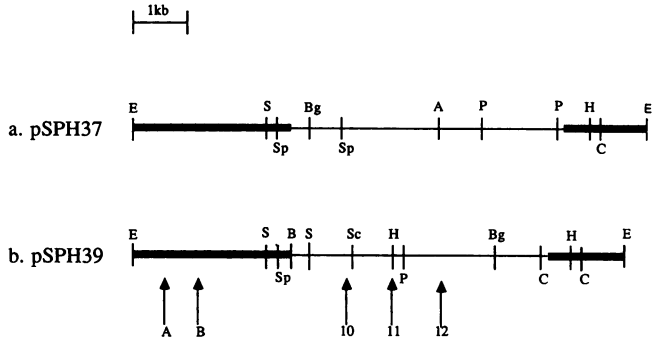


FIG. 2. (a) Restriction map of the *mgtA* plasmid pSPH37; (b) restriction map of the *mgtB* plasmid pSPH39. The plasmids contain the indicated DNA fragment cloned into the *Bam*HI site of pBR328. Positions of the Mu dJ insertions in pSPH39 are given by the numbers 10 through 12, representing plasmids pMM10, pMM11, and pMM12, respectively, and cause loss of complementation. Two additional insertions represented by A and B are in pBR328 DNA and do not cause loss of complementation. Symbols and abbreviations: ——, insert DNA; ■, pBR328 DNA; E, *Eco*RI; S, *Sal*I; Sp, *Sph*I; B, *Bam*HI; Bg, *Bgl*II; Sc, *Sac*I; H, *Hin*dIII; P, *Pst*I; A, *Ava*I; C, *Cla*I. The junctions of the insert and pBR328 DNA represent *Sau*3A sites.

**Mapping of the *mgtA* locus.** It seemed logical that one or both of the *S. typhimurium mgt* loci might map to a locus analogous to the single *E. coli mgt* locus at 92 map units (17). However, transposons linked to *mgtA* or *mgtB* were not cotransducible with either the *malE* locus at 91 map units or the *melAB* locus at 93 map units in *S. typhimurium*. Therefore, the approximate map position of the *mgtA* locus was determined by using the insertion *zjh-1625*::Tn*10* as a region of homology to target formation of Hfr strains with an origin of transfer near *mgtA* (4). The results of conjugational crosses using these Hfr strains indicated that the transposon insertion was between approximately 98 and 0 map units. P22 cotransduction experiments were performed to define the location further (Table 2). The results showed that *mgtA* was cotransducible with *pyrB* and *argI*. A three-point cross using MM299 as donor and TR2962 as recipient (Table 3) was performed, selecting for $Kan^r$, $Pyr^+$, or $Arg^+$ separately and scoring each class of transductant for the two unselected markers. The results unambiguously demonstrated a gene order of *mgtA pyrB argI*. Overall, our data are consistent with the order *zjh-1628*::Tn*10*Δcam *mgtA pyrB zjh-1625*::Tn*10 argI pepA* (Tables 2 and 3; Fig. 3A; 14).

TABLE 2. Transductional crosses for mapping *mgtA*

| Donor (genotype[a]) | Recipient (genotype[a]) | Marker | | No. tested | No. with unselected marker | % Linkage |
|---|---|---|---|---|---|---|
| | | Selected | Unselected[b] | | | |
| MM116 (*corA27 mgtB16 mgtA*$^+$ *zjh-1628*::Tn*10*Δcam) | MM77 (*corA27 mgtA5 mgtB16*) | Cam$^r$ | $Mg^{2+}$-Ind | 50 | 12 | 24 |
| MM116 (*pyrB*$^+$ *argI*$^+$ *zjh-1628*::Tn*10*Δcam) | TR2962 (*pyrB64 argI547*) | Cam$^r$ | Pyr$^+$ | 49 | 7 | 14 |
| | | | Arg$^+$ | 49 | 4 | 8 |
| MM116 (*zjh-1628*::Tn*10*Δcam) | MM82 (*corA27 mgtB16 zjh-1625*::Tn*10*) | Cam$^r$ | Tet$^s$ | 50 | 4 | 8 |
| MM82 (*pyrB*$^+$ *argI*$^+$ *zjh-1625*::Tn*10*) | TR2962 (*pyrB64 argI547*) | Tet$^r$ | Pyr$^+$ | 200 | 61 | 31 |
| | | | Arg$^+$ | 200 | 188 | 94 |
| MM82 (*pyrB*$^+$ *argI*$^+$ *pepA*$^+$ *zjh-1625*::Tn*10*) | TN1010 (*argI547 pyrB64 zjh-831*::Tn*5 pepA1*) | Tet$^r$ | Pyr$^+$ | 76 | 32 | 42 |
| | | | Arg$^+$ | 76 | 68 | 89 |
| | | | PepA$^+$ | 76 | 34 | 45 |
| MM277 (*pyrB64 zjh-1625*::Tn*10*) | MM299 (*mgtA27*::Mu dJ *pyrB*$^+$) | Tet$^r$ | Kan$^s$ | 88 | 20 | 23 |
| | | | Pyr | 88 | 23 | 26 |

[a] Only the relevant genotype is shown.
[b] $Mg^{2+}$-Ind, $Mg^{2+}$ independence. See Materials and Methods for further definition and discussion.

**Isolation and chromosomal integration of *mgtB*::Mu dJ insertion mutations.** The transposons linked to *mgtB* all carried tetracycline resistance and were difficult to use for mapping because of $Mg^{2+}$-tetracycline chelation. Therefore, Mu dJ (Kan$^r$) insertions into *mgtB* were sought in order to obtain a more usable marker for selection. Five insertions of Mu dJ into pSPH39 were isolated as described in Materials and Methods and designated pMM10 through pMM14. They were characterized by restriction endonuclease analysis and tested for the ability to restore growth on low-$Mg^{2+}$ medium to MM77. Three of the five plasmids (pMM10 to pMM12) were unable to restore $Mg^{2+}$ independence to MM77, suggesting that the Mu dJ insertions in the plasmids had disrupted the locus responsible for complementing $Mg^{2+}$ dependence. Two plasmids, pMM13 and pMM14, carry an insertion in a region of the plasmid not required for complementation. Each of the five plasmids represented a distinct insertion of Mu dJ (Fig. 2B). The three *mgtB*::Mu dJ insertions that eliminated complementation were exchanged into the chromosome by homologous recombination (7; Materials and Methods), and their linkage to the four Tn*10*Δtet insertions previously postulated to be linked to *mgtB* was determined. The results of these crosses confirmed that *mgtB11*::Mu dJ mapped at the chromosomal site previously designated *mgtB* (see below). Three additional Tn*10*Δtet insertions from the collection of Kukral et al. (12) were also identified as linked to *mgtB* (Table 4). A random Tn*10*Δcam population was screened for insertions linked to *mgtB11*::Mu dJ. An insertion, originally designated *zia-1630*::Tn*10*Δcam, was identified in this screen and was shown to be 85% cotransducible with *mgtB11*::Mu dJ (Table 5). The results of additional crosses indicated that this insertion inactivated *mgtB*, and it has therefore been designated *mgtB1630*::Tn*10*Δcam.

**Mapping of *mgtB*.** To map *mgtB*, transposons *zia-3123*::Tn*10*Δtet and *zia-3125*::Tn*10*Δtet were used for Tn*10*-directed Hfr formation (4). Both insertions were localized to the region between the *pyrE* and *metE* loci at 79.7 and 85 map units, respectively. Transductional crosses with markers in this region (*pyrE*, *gltC*, *ilvB*, *apeR*, *dnaA*, and *ilvG*) failed to show any linkage with the *mgtB*::Mu dJ insertion. Crosses between these markers and *mgtB1630*::Tn*10*Δcam and *mgtB*-linked insertion mutations were therefore carried out. The results of these crosses (Table 5) showed that *mgtB1630*::Tn*10*Δcam and *zia-3048*::Tn*10*Δtet were cotransducible with *gltC*. The two-point distances from these

TABLE 3. Three-point cross for *mgtA* mapping

| Cross | Donor (genotype[a]) | Recipient (genotype[a]) | Selected marker | Total no. selected | Recombinant class | | No. of recombinants |
|---|---|---|---|---|---|---|---|
| 1 | MM299 (*mgtA27*::Mu dJ) | TR2962 (*pyrB64 argI547*) | Kan$^r$ | 69 | Pyr | Arg | |
| | | | | | + | + | 36 |
| | | | | | + | − | 20 |
| | | | | | − | + | 0 |
| | | | | | − | − | 13 |
| 2 | MM299 (*mgtA27*::Mu dJ) | TR2962 (*pyrB64 argI547*) | Pyr$^+$ | 88 | Arg | Kan | |
| | | | | | + | r | 40 |
| | | | | | + | s | 27 |
| | | | | | − | r | 10 |
| | | | | | − | s | 11 |
| 3 | MM299 (*mgtA27*::Mu dJ) | TR2962 (*pyrB64 argI547*) | Arg$^+$ | 88 | Pyr | Kan | |
| | | | | | + | r | 16 |
| | | | | | + | s | 10 |
| | | | | | − | r | 0 |
| | | | | | − | s | 62 |

[a] Only the relevant genotype is shown.

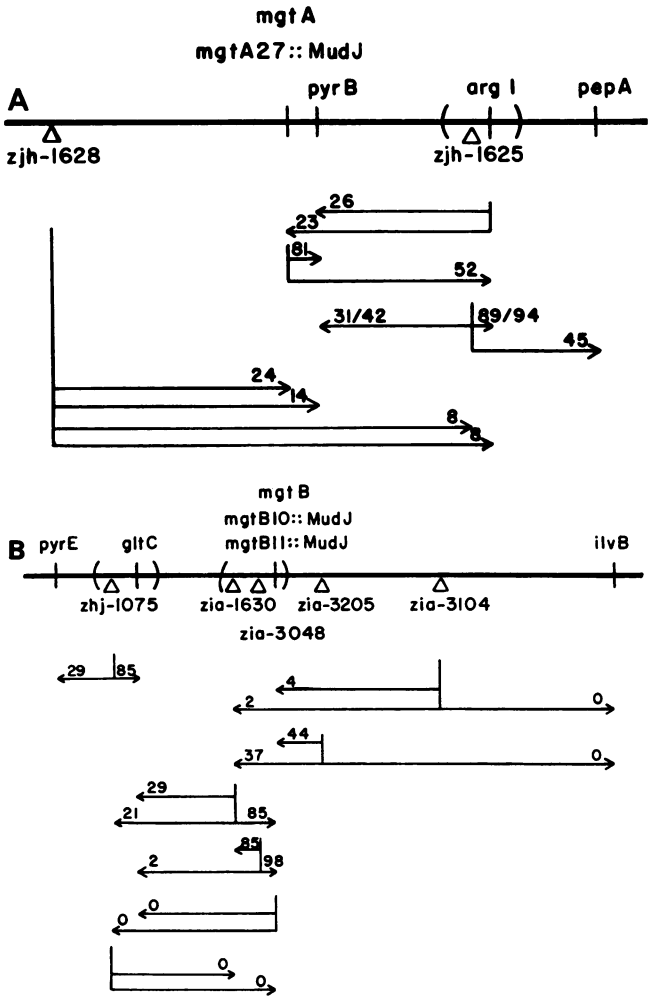


FIG. 3. (A) Gene order near *mgtA*. For reference, the current map assignment of *pyrB* is 98 map units (20). (B) Gene order near *mgtB*. Crosses shown involving the *mgtB* locus used the *mgtB*::Mu dJ construct. The current map assignment of *gltC* is 80 map units (20). There are also four additional Tn*10*Δtet insertions that are cotransducible with *mgtB* (*zia-3123*::Tn*10*Δtet, *zia-3125*::Tn*10*Δtet, *zia-3295*::Tn*10*Δtet, and *zia-3306*::Tn*10*Δtet; Table 4), but their orientation with regard to *mgtB* and the other markers shown has not been determined. See text and Tables 2 to 5 for derivation of the maps. All transductions were done by using P22 as described in Materials and Methods. Arrows point from the selected marker to the unselected marker(s). The number on each line is the percentage of transductants with the unselected marker(s). Markers are spaced according to their approximate map distance after conversion of the percent cotransduction according to the formula of Wu (30). Our data are insufficient to establish the gene order for markers shown in parentheses.

crosses suggest the order *pyrE* (*zjh-1075*::Tn*10* *gltC*) (*mgtB1630*:Tn*10*Δcam *zia*-3048::Tn*10*Δtet *mgtB*) *zia-3205*::Tn*10*Δtet *zia-3104*::Tn*10*Δtet *ilvB*, where the markers in parentheses cannot be clearly ordered with respect to each other (Fig. 3B). These data also suggest that the distance between *gltC* and *ilvB*, currently believed to be about 0.7 map units (20), may be substantially greater, perhaps as much as 1.7 map units.

**Construction and phenotypes of strains with *corA*, *mgtA*, and *mgtB* mutations.** Using insertions linked to each of the three $Mg^{2+}$ transport loci (*corA*, *mgtA*, and *mgtB*), attempts were again made to construct a strain requiring high $Mg^{2+}$ levels for growth. Starting with TN1379 as parent, a *corA* mutation (*corA27*) and two different insertions in *mgtB* (*mgtB10*::Mu dJ and *mgtB11*::Mu dJ) were each independently introduced, giving strains MM159, MM196, and MM197, respectively. The *mgtB*::Mu dJ from MM196 or MM197 was then transduced into MM159 by selection for $Kan^r$, resulting in strains MM201 and MM200 (each *corA mgtB mgtA*$^+$). Finally, the *mgtA5* mutation from MM136 was moved into MM200 or MM201 by selecting for $Cam^r$ carried by the linked transposon *zia-1628*::Tn*10*Δcam. Fewer than 2% of several hundred $Cam^r$ transductants exhibited a $Mg^{2+}$-dependent phenotype similar to that observed in the original $Mg^{2+}$-dependent strain, MM77. A $Mg^{2+}$-dependent transductant from each cross was purified and saved as MM206 or MM208.

TABLE 4. Cotransduction of *mgtB11*::Mu dJ and Tn*10*Δtet insertions

| Transposon insertion | Mu dJ marker[a] | No. tested | No. losing Mu dJ | % Linkage[b] |
|---|---|---|---|---|
| *zia-3048* | $Lac^+$ | 344 | 337 | 98 |
| *zia-3104* | $Lac^+$ | 80 | 9 | 11 |
| *zia-3123* | $Lac^+$ | 263 | 253 | 96 |
| *zia-3125* | $Lac^+$ | 521 | 507 | 97 |
| *zia-3205* | $Lac^+$ | 80 | 30 | 36 |
| *zia-3295* | $Lac^+$ | 80 | 3 | 4 |
| *zia-3306* | $Kan^r$ | 88 | 9 | 10 |

[a] MM200 was the recipient in all crosses, with selection for $Tet^r$ on MacConkey tetracycline medium. The donor strains are described elsewhere (12). Transductants were tested for loss of Mu dJ by scoring for $Kan^r$ (*zia-3306*), by scoring the Lac phenotype directly on MacConkey indicator medium, or both. Additional crosses (data not shown) measured linkage of these transposons and the *mgtB10*::Mu dJ insertion in MM201, with results identical to those shown. Data shown are for a single set of transductions.

[b] Values shown differ slightly for some insertions (e.g., *zia-3205*) from those shown in Fig. 3 and in the text, since the latter data were obtained from additional experiments.

The frequency with which $Mg^{2+}$-dependent strains were obtained was considerably less than expected, given the 24% cotransduction frequency of *mgtA* and *zia-1628*::Tn*10*Δcam. This result raised the possibility that *corA mgtA mgtB* mutant strains may have to acquire additional mutations in order to grow normally. Another approach to the construction of a triply mutant strain became available when the *mgtB1630*::Tn*10*Δcam was identified. With this insertion as donor in a cross with a recipient carrying Mu dJ insertion alleles of both *corA* and *mgtA*, all $Cam^r$ transductants should be the desired triple mutants. When this cross (MM323 [*mgtB1630*::Tn*10*Δcam] × MM278 [*corA45*::Mu dJ *mgtA21*::Mu dJ]) was carried out, varying the concentration of $Mg^{2+}$ in the transduction plates (N medium with 1, 10, 50, or 100 mM $Mg^{2+}$), $Cam^r$ transductants were obtained only on the 50 and 100 mM $Mg^{2+}$ plates. When these transductants were streaked on the standard $Mg^{2+}$-supplemented medium (10 mM $Mg^{2+}$), only a few large colonies grew up above a faint background growth. No colonies appeared on plates not supplemented with $Mg^{2+}$. Normally growing streaks were obtained from all such $Cam^r$ transductants on plates containing 100 mM $Mg^{2+}$. Apparently, such triple mutants require extremely high levels of $Mg^{2+}$ but revert at high frequency to growth at a somewhat lower $Mg^{2+}$ level (10 mM).

Strains containing only a single wild-type allele of one of the three loci related to $Mg^{2+}$ transport were also constructed: MM224, *corA*$^+$; MM201, *mgtA*$^+$; and MM227, *mgtB*$^+$ (Table 6). The sensitivity of each of these strains to

TABLE 5. Transductional crosses used in mapping *mgtB*

| Donor (genotype[a]) | Recipient (genotype[a]) | Marker | | No. tested | No. with unselected marker | % Linkage |
|---|---|---|---|---|---|---|
| | | Selected | Unselected | | | |
| TN3020 (*zhj-1075*::Tn*10 gltC*$^+$) | TN3243 (*pyrE26 gltC*) | Tet$^r$ | Pyr$^+$ | 176 | 51 | 29 |
| | | | GltC$^+$ | 176 | 150 | 85 |
| MM223 (*gltC mgtB1630*::Tn*10*Δcam) | TN3020 (*gltC*$^+$*zhj-1075*::Tn*10*) | Cam$^r$ | GltC$^-$ | 264 | 78 | 29 |
| | | | Tet$^s$ | 264 | 55 | 21 |
| | TN3243 (*pyrE26*) | Cam$^r$ | Pyr$^+$ | 88 | 0 | 0 |
| | MM196 (*mgtB10*::Mu dJ) | Cam$^r$ | Kan$^s$ | 155 | 133 | 85 |
| AK3048 (*gltC zia-3048*::Tn*10*Δtet) | MM223 (*mgtB1630*::Tn*10*Δcam) | Tet$^r$ | Cam$^s$ | 206 | 175 | 85 |
| | MM196 (*mgtB10*::Mu dJ) | Tet$^r$ | Kan$^s$ | 88 | 86 | 98 |
| | TN3017 (*gltC*$^+$) | Tet$^r$ | GltC$^-$ | 176 | 5 | 2 |
| MM196 (*mgtB10*::Mu dJ *gltC*) | MM258 (*gltC*$^+$ *zia-3048*::Tn*10*Δtet) | Kan$^r$ | Tet$^s$ | 33 | 32 | 97 |
| | TN3017 (*gltC*$^+$) | Kan$^r$ | GltC$^-$ | 176 | 1 | 0.5 |
| | TN3020 (*gltC*$^+$ *zhj-1075*::Tn*10*) | Kan$^r$ | GltC$^-$ | 34 | 0 | 0 |
| | | | Tet$^s$ | 34 | 0 | 0 |
| MM200 (*mgtB11*::Mu dJ *gltC*) | TN3017 (*gltC*$^+$) | Kan$^r$ | GltC$^-$ | 176 | 0 | 0 |
| TN3020 (*zhj-1075*::Tn*10*) | MM257 (*mgtB10*::Mu dJ *mgtB1630*::Tn*10*Δcam) | Tet$^r$ | Kan$^s$ | 88 | 0 | 0 |
| | | | Cam$^s$ | 88 | 0 | 0 |
| | MM223 (*mgtB1630*::Tn*10*Δcam) | Tet$^r$ | Cam$^s$ | 122 | 0 | 0 |
| | MM200 (*mgtB11*::Mu dJ) | Tet$^r$ | Kan$^s$ | 122 | 0 | 0 |
| AK3104 (*ilvB*$^+$ *zia-3104*::Tn*10*Δtet) | MM257 (*mgtB10*::Mu dJ *mgtB1630*::Tn*10*Δcam) | Tet$^r$ | Kan$^s$ | 103 | 4 | 4 |
| | | | Cam$^s$ | 103 | 2 | 2 |
| | TN3018 (*ilvB101 ilvG236*) | Tet$^r$ | IlvB$^+$ | 100 | 9 | 9 |
| AK3205 (*ilvB*$^+$ *zia-3205*::Tn*10*Δtet) | MM257 (*mgtB10*::Mu dJ *mgtB1630*::Tn*10*Δcam) | Tet$^r$ | Kan$^s$ | 108 | 47 | 44 |
| | | | Cam$^s$ | 108 | 40 | 37 |
| | TN3018 (*ilvB101 ilvG236*) | Tet$^r$ | IlvB$^+$ | 100 | 0 | 0 |

[a] Only the relevant genotype is shown. The mutation *gltC*$^+$ confers the ability to grow on glutamate as a sole carbon source (GltC$^+$). The wild type (*gltC*) cannot use glutamate as a sole carbon source (1).

various cations was determined by using a disk sensitivity test (Fig. 4 and Table 6). The results indicated that the presence of a *corA*$^+$ allele was necessary for inhibition of growth by $Co^{2+}$ regardless of the presence of wild-type or mutant *mgtA* and *mgtB* alleles. $Ni^{2+}$ (1 μmol per disk) inhibited growth of the wild-type and *corA*$^+$ strains but had no significant effect on the *mgtA*$^+$ or *mgtB*$^+$ strain. These latter two loci could be distinguished by increasing $Ni^{2+}$ to 2 μmol per disk, whereupon growth of the *mgtA*$^+$ strain was inhibited. Higher $Ni^{2+}$ concentrations inhibited growth of all strains. The results are consistent with the amount of $^{63}Ni^{2+}$ taken up by the different strains (M. D. Snavely, C. G. Miller, and M. E. Maguire, unpublished observations). Whereas growth of a *mgtB*$^+$ strain was relatively resistant to $Co^{2+}$ and $Ni^{2+}$, $Ca^{2+}$ and $Mn^{2+}$ were markedly inhibitory at all concentrations tested. These latter two cations were completely without effect on growth of the wild-type, *corA*$^+$, and *mgtA*$^+$ strains even at 20 μmol per disk. $Zn^{2+}$ inhibited the growth of all strains equally and was not tested further. Growth inhibition by $Ca^{2+}$, $Co^{2+}$, $Mn^{2+}$, or $Ni^{2+}$ could be reversed by adding 10 mM $Mg^{2+}$ to the growth medium (data not shown). Each of the strains containing only a single wild-type allele exhibited a unique phenotype with regard to divalent cation sensitivity. This allowed formulation of a

TABLE 6. Cation sensitivity and $Mg^{2+}$ uptake

| Strain | Genotype[a] | Cation sensitivity[b] | | | | | Growth[c] | $Mg^{2+}$ uptake[d] (nmol $min^{-1}$ $10^8$ $cells^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | | $Ca^{2+}$ | $Co^{2+}$ | $Mn^{2+}$ | $Ni^{2+}$ | $Zn^{2+}$ | | |
| Wild type, TN1379 | *corA*$^+$ *mgtA*$^+$ *mgtB*$^+$ | R | S | R | S | S | + | 0.31 |
| Double mutants | | | | | | | | |
| MM224 | *corA*$^+$ *mgtA5 mgtB10*::Mu dJ | R | S | R | S | S | + | 0.23 |
| MM201 | *corA27 mgtA*$^+$ *mgtB10*::Mu dJ | R | R | R | S | S | + | 0.034 |
| MM227 | *corA27 mgtA5 mgtB*$^+$ | S | R | S | R | S | ± | 0.014 |
| Triple mutant, MM206 | *corA27 mgtA5 mgtB10*::Mu dJ | ND | ND | ND | ND | ND | − | <0.002 |

[a] All strains carry the Δ*leuBCD485* mutation in addition to the markers shown.

[b] Relative to that of the wild-type strain, as assayed by a disk sensitivity assay (see Materials and Methods). The data presented in Fig. 4 show the complete dose-response relationship for the cations tested. The data shown here indicate resistance (R) or sensitivity (S) to specific amounts of each cation on a disk. In all cases, this was achieved by adding 40 μl of a solution of the concentration indicated in parentheses: 20 μmol of $Ca^{2+}$ (0.5 M), 2 μmol of $Co^{2+}$ (0.05 M), 2 μmol of $Mn^{2+}$ (0.05 M), 2 μmol of $Ni^{2+}$ (0.05 M), and 2 μmol of $Zn^{2+}$ (0.025 M). These concentrations were selected from the results shown in Fig. 4 so as to allow a simple phenotypic assay for the presence or absence of each of the wild-type alleles. ND, Not determined. (Since MM206 requires $Mg^{2+}$ for growth and since 10 mM $Mg^{2+}$ reverses the effect of the various cations, cation sensitivity cannot be determined for this strain.)

[c] Ability to grow on LB plates without a $Mg^{2+}$ supplement. +, Normal growth on LB plates in the absence of added $Mg^{2+}$; −, growth only in the presence of 10 mM added $Mg^{2+}$; ±, significantly slower growth without added $Mg^{2+}$ than with 10 mM $Mg^{2+}$.

[d] In all strains except MM206, $Mg^{2+}$ uptake was assayed for 2 min at 100 μM $Mg^{2+}$. In MM206, $Mg^{2+}$ uptake was assayed for 5 min at 25 μM $Mg^{2+}$ (see Results for further discussion). Background values obtained by incubating parallel samples at 4°C for the appropriate period were subtracted. The experiment shown for MM206 reflects a net uptake of less than 100 cpm over a background of 600 cpm (see text). For all other strains, gross uptake was at least double that of the background samples.

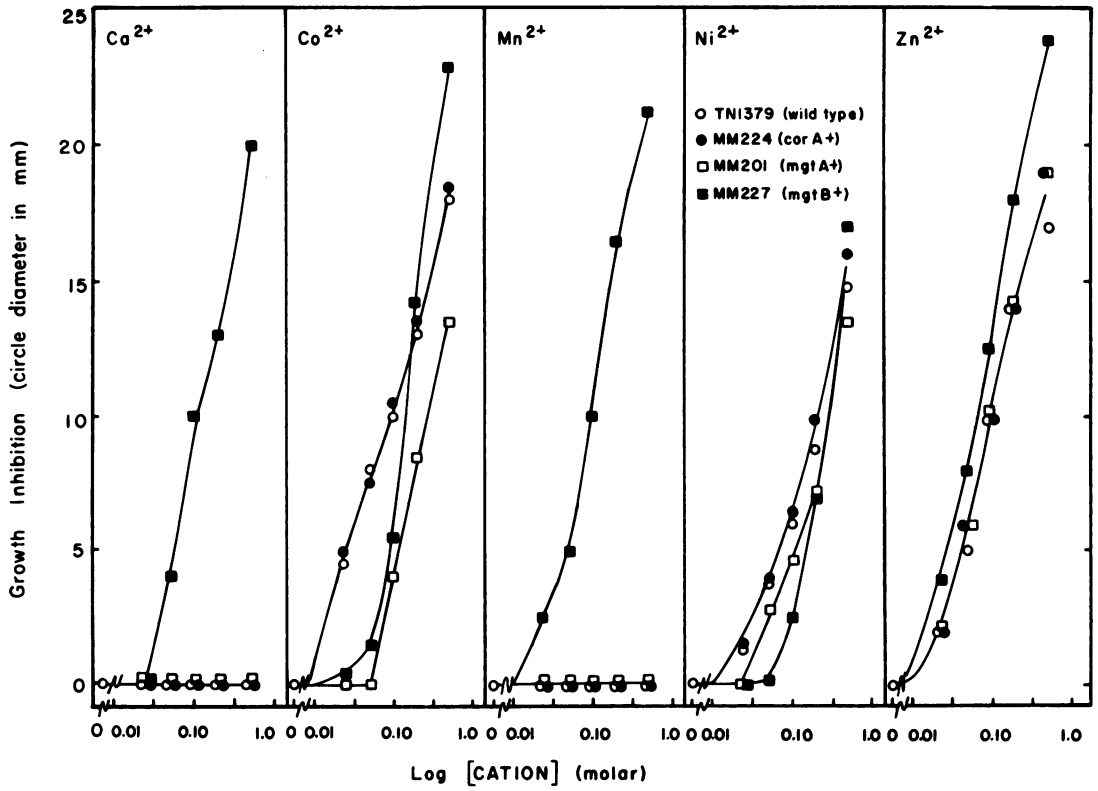


FIG. 4. Disk sensitivity test of strains containing only one $Mg^{2+}$ transport system. Disk sensitivity tests were performed as described in Materials and Methods, using 40 μl of solutions of each cation at concentrations of 25, 50, 100, 200, and 500 mM, which represents 1, 2, 4, 8, and 20 μmol of added cation.

simple means to distinguish each of the strains containing a single $Mg^{2+}$ transport locus from each other and from the triply mutant strain, using the disk sensitivity assay (Table 6).

$^{28}Mg^{2+}$ uptake in strain MM206 (*corA mgtA mgtB*) was essentially absent under the conditions tested (Table 6) and was comparable to that of the originally isolated $Mg^{2+}$-dependent strain, MM77. Further experiments attempted to measure specific $Mg^{2+}$ uptake in MM206 or MM77 at various $Mg^{2+}$ concentrations between 0.01 and 1 mM, at multiple specific activities of $^{28}Mg^{2+}$, and at 37 or 20°C; in no case was uptake at 20 or 37°C significantly different from that at 4°C. Finally, when MM206 was grown at a permissive $Mg^{2+}$ concentration and then shifted to a lower $Mg^{2+}$ concentration for a period before assay to allow derepression of any remaining $Mg^{2+}$ transport component that might be present, no $Mg^{2+}$ uptake could be detected. Thus, within the sensitivity of the transport assay, these data indicate that all specific $Mg^{2+}$ transport systems had been eliminated by the mutations carried by MM77 and MM206. Thus, mutations in three distinct genetic loci are necessary and sufficient to eliminate specific $Mg^{2+}$ transport in *S. typhimurium*.

The presence or reintroduction of the wild-type alleles of any one of the three identified loci, *corA* (MM224), *mgtA* (MM201), or *mgtB* (MM227), restored the ability of MM206 to grow on low-$Mg^{2+}$ medium and correspondingly restored a significant and readily detectable level of $Mg^{2+}$ uptake (Table 6).

## DISCUSSION

This and our previous report (8) demonstrate that there are three distinct loci involved in $Mg^{2+}$ transport in *S. typhimurium*: *corA*, *mgtA*, and *mgtB*. Each of these three loci probably represents a distinct $Mg^{2+}$ transport system. First, as discussed above, no $Mg^{2+}$ transport is detected in the absence of the *corA*, *mgtA*, and *mgtB* loci. Second, reintroduction of any one of the three loci restores growth on medium containing low $Mg^{2+}$ concentrations and restores some degree of $Mg^{2+}$ uptake. Third, strains containing only one of the three wild-type loci (Table 6) each exhibit a unique phenotype with regard to growth inhibition by other divalent cations, suggesting that the cation selectivity of each system is different. Additional data in support of this hypothesis are presented in the accompanying reports (23, 24).

The distinct cation sensitivity provides information both as to the characteristics of each of the three putative transport systems and as to the involvement of the transport process in the toxic effect of the various cations. The growth inhibition observed (Fig. 4 and Table 6) could be due to inhibition of $Mg^{2+}$ transport, with or without transport of the inhibitory cation in place of $Mg^{2+}$. Either effect might lead to a decrease in intracellular $Mg^{2+}$ and subsequent $Mg^{2+}$ starvation. If, however, the inhibitory cation were transported by a $Mg^{2+}$ transport system, then once the cation is inside the cell, its growth inhibition might also be due to direct toxicity by any of a number of mechanisms.

$Co^{2+}$ is transported by the *corA* gene product (8), and its toxicity is clearly dependent on the presence of a wild-type *corA* allele; thus, its deleterious effect is presumably due to interference with some aspect(s) of cell metabolism (10, 16, 18) rather than to interference with $Mg^{2+}$ transport per se. Although $Mn^{2+}$ is transported by the *E. coli* CorA system (17, 18, 21), no $Mn^{2+}$ toxicity is observed in the *S. typhimurium* wild type or the *corA*$^+$ *mgtA* mgtB mutant strain

(MM224) under the conditions used in these studies. $Mn^{2+}$ inhibition of the *corA mgtA mgtB*$^+$ mutant strain (MM227) is therefore likely due to inhibition by $Mn^{2+}$ of $Mg^{2+}$ transport via the MgtB system. Although an argument could be made for $Ca^{2+}$, since its pattern of growth inhibition is like that of $Mn^{2+}$, $Ca^{2+}$ is a very poor inhibitor of $^{28}Mg^{2+}$ uptake by all three transport systems. Thus, the mechanism of $Ca^{2+}$ growth inhibition is currently unclear.

$Ni^{2+}$ inhibits growth of the wild-type strain and of strains carrying a single $Mg^{2+}$ transport gene in the order wild type $\geq$ *corA* > *mgtA* > *mgtB*. These transport systems exhibit the same order with respect to capacity to transport $^{63}Ni^{2+}$ (M. D. Snavely, C. G. Miller, and M. E. Maguire, unpublished observations). Therefore, growth inhibition is likely related to the amount of $Ni^{2+}$ taken up into the cell and thus to direct $Ni^{2+}$ toxicity rather than to $Ni^{2+}$ inhibition of $Mg^{2+}$ transport and indirect toxicity through $Mg^{2+}$ deprivation. $Zn^{2+}$ toxicity is presumably independent of $Mg^{2+}$ transport, since its patterns of growth inhibition are similar regardless of the presence or absence of any particular $Mg^{2+}$ transport system.

The results of the crosses designed to reconstruct a triply mutant strain (*corA mgtA mgtB*) by using insertions at each locus indicate that such a strain requires very high levels of $Mg^{2+}$, on the order of 50 to 100 mM. It is likely, therefore, that other triply mutant strains (MM77 and MM206) contain an additional mutation that confers the ability to grow at lower (10 mM) $Mg^{2+}$ concentrations. Each of these strains, however, is absolutely dependent on $Mg^{2+}$ for growth and, under the conditions tested, the level of $Mg^{2+}$ uptake in each is below detectability. Return of a wild-type allele of *corA*, *mgtA*, or *mgtB* to these strains restores both $Mg^{2+}$-independent growth and specific $Mg^{2+}$ transport. In addition, strains containing only one wild-type allele show unique patterns of cation sensitivity. These strains therefore provide useful and reliable information about the three $Mg^{2+}$ transport loci described, but further analysis will be required to characterize the phenotype of the strains requiring very high levels of $Mg^{2+}$ and the presumed mutations that lower this level.

The ability of MM77 and MM206 to grow at the wild-type rate in medium containing 10 mM $Mg^{2+}$ implies that sufficient $Mg^{2+}$ can be obtained by some means. This may reflect the movement of $Mg^{2+}$ through transport systems that are poorly expressed or are poor carriers of $Mg^{2+}$. This latter possibility could include transport systems that physiologically transport cations other than $Mg^{2+}$ or that transport $Mg^{2+}$ only when faced with a greater than normal $Mg^{2+}$ gradient. The ability of the triply mutant strains to survive several hours of exposure to low $Mg^{2+}$ concentrations (Fig. 1) and then resume growth upon addition of $Mg^{2+}$ suggests that $Mg^{2+}$ deprivation is not immediately lethal and that *S. typhimurium* can retain accumulated $Mg^{2+}$ for a considerable period of time (24).

The presence of three separate $Mg^{2+}$ transport loci, *corA*, *mgtA*, and *mgtB*, in *S. typhimurium* differs from the situation reported for *E. coli*, in which only two loci (*corA* and *mgt*) were described (18, 21). This is somewhat surprising, since the CorA systems of the two species have similar kinetic properties and map to analogous locations on the chromosome. Neither the *mgtA* nor the *mgtB* locus of *S. typhimurium* maps to a chromosomal region analogous to the location of the *E. coli mgt* locus. Therefore, the relationship, if any, of the *S. typhimurium* and *E. coli mgt* loci is unclear. It is likely that loci in addition to *corA*, *mgtA*, and *mgtB* are involved in $Mg^{2+}$ uptake. The *corB* locus characterized in *E. coli* confers resistance to $Co^{2+}$, although to a lesser degree than that conferred by mutations at the *corA* locus (18). In *S. typhimurium*, mutations to $Co^{2+}$ resistance at two additional loci distinct from either *corA* or *corB* have been identified (8; M. M. Gibson, C. G. Miller, and M. E. Maguire, unpublished observations). Silver and colleagues (22) have also described a manganese resistance locus (*mng*) in *E. coli*. This mutation was selected by isolating colonies resistant to 10 mM $Mn^{2+}$ and was localized to about 39 map units on the chromosome. These strains have an altered $K_i$ for $Mn^{2+}$ inhibition of $Mg^{2+}$ uptake. Such a mutation in *S. typhimurium* has not been identified. Concentrations of $Mn^{2+}$ up to 0.5 M do not inhibit growth of the wild-type strain (Fig. 4), as determined by a disk test; however, growth inhibition of *S. typhimurium* by $Mn^{2+}$ has been observed in liquid medium, so the possibility of an *mng* locus in *S. typhimurium* remains. Of most interest, in both *E. coli* (18, 21) and *S. typhimurium* (8) is a repressible component of $Mg^{2+}$ uptake, represented in *E. coli* by the *mgt* locus. In *S. typhimurium*, both *mgtA* and *mgtB* are repressible by $Mg^{2+}$ in the growth medium (24). This finding implies the existence of one or more other genes that mediate this regulation. Preliminary experiments (M. D. Snavely, C. G. Miller, and M. E. Maguire, unpublished observations) confirm the existence of such loci. The cloning of the *corA*, *mgtA*, and *mgtB* loci and the construction of strains carrying only one wild-type locus provide the foundation with which to investigate these problems and the necessary tools for studies of the regulation and mechanism(s) of $Mg^{2+}$ transport.

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