Molecular Microbiology (1991) 5(11), 2753–2762

Magnesium transport in *Salmonella typhimurium*: the influence of new mutations conferring Co^{2+} resistance on the CorA Mg^{2+} transport system

M. M. Gibson,[1†] D. A. Bagga,[1] C. G. Miller[2‡] and M. E. Maguire[1]*

[1]Department of Pharmacology, and [2]Department of Molecular Biology and Microbiology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106, USA.

Summary

The CorA Mg^{2+} transport system of *Salmonella typhimurium* mediates both influx and efflux of Mg^{2+}. Mutations at the *corA* locus (83.5 min) confer resistance to Co^{2+}. Using transposon mutagenesis, three additional Co^{2+} resistance loci (*corB*, *corC*, and *corD*) were found and mapped to 55, 15, and 3 min, respectively, on the *S. typhimurium* chromosome. No mutations corresponding to the reported *corB* locus at 95 min in *Escherichia coli* were obtained. The *corB*, *corC*, and *corD* mutations confer levels of Co^{2+} resistance intermediate between those of the wild-type and *corA* mutations.

Isogenic strains were constructed containing combinations of transposon insertion mutations in each of the three Co^{2+}-resistance loci to assess their influence on the CorA Mg^{2+} transport system. The V_{max} and K_m values for $^{28}Mg^{2+}$ or for $^{57}Co^{2+}$ and $^{63}Ni^{2+}$ influx, analogues of Mg^{2+} transported by the CorA system, were changed less than twofold compared with the wild-type values, regardless of the mutation(s) present. However, while efflux of $^{28}Mg^{2+}$ through the CorA system was decreased threefold in strains carrying one or two mutant alleles among *corB*, *corC*, or *corD*, efflux was completely abolished in either a *corA* or a *corBCD* strain. Thus, although the *corA* gene product is necessary and sufficent to mediate Mg^{2+} influx, Mg^{2+} efflux requires the presence of a wild-type allele of at least one of the *corB*, *corC* or *corD* loci.

Received 18 June, 1991; revised 29 July, 1991. Present addresses: †Microbial Genetics Laboratory, Institute of Molecular Medicine, ICRF Laboratories, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK; ‡Department of Microbiology, University of Illinois, Urbana, Illinois 61801, USA. *For correspondence. Tel. (216) 368 6186; Fax (216) 368 3395.

Introduction

Three genetically distinct and independent transport systems, CorA, MgtA and MgtB, serve to mediate the transport of Mg^{2+} across the cell membrane of *Salmonella typhimurium* (Hmiel *et al.*, 1986; 1989). The CorA system is constitutively expressed and is the predominant system for accumulation of Mg^{2+} under normal growth conditions in this organism and, probably, most Gram-negative bacteria (Hmiel *et al.*, 1986; Snavely *et al.*, 1989a,b). The MgtA and MgtB transport systems are expressed under conditions of relative Mg^{2+} starvation (Snavely *et al.*, 1989b; 1991).

Wild-type *S. typhimurium* is sensitive to the cytotoxic effects of Co^{2+} ions. Hmiel *et al.*, (1986; 1989) previously isolated both spontaneous and MudJ insertion mutations within a gene whose product was required for the transport of both Mg^{2+} and Co^{2+}. Mutations in this gene, designated *corA*, impart resistance to high levels of Mg^{2+} in the growth medium, lack detectable Co^{2+} uptake, and show diminished levels of Mg^{2+} uptake. Similar mutations had previously been identified in *Escherichia coli* (Silver, 1969; Lusk and Kennedy, 1969), and the *corA* locus maps to about 83 min in both species. Further, cloned *corA* genes from *E. coli* or *S. typhimurium* encode functionally similar gene products, as shown by their ability to complement *corA* mutations in either species. Finally, at least in *S. typhimurium*, the CorA transport system mediates uptake of Ni^{2+} in addition to Mg^{2+} and Co^{2+}, and strains carrying a mutant *corA* allele show a complete absence of Mg^{2+} efflux (Snavely *et al.*, 1989b; 1991).

Park *et al.* (1976) identified an additional locus in *E. coli*, designated *corB*, mutations in which give rise to resistance to lower concentrations of Co^{2+} relative to a *corA* strain. This locus was shown to map to 95 min on the *E. coli* chromosome (cotransducible with the *pyrB* gene). In addition, a regulatory role for *corB* was implicated as it appeared to be required for the expression of *corA* at low (100 μM) levels of extracellular Mg^{2+}. We therefore sought to identify additional Co^{2+} resistance mutations in *S. typhimurium* to determine whether any such mutations were related to the CorA Mg^{2+} transport system. This paper reports the characterization of three additional loci in *S. typhimurium*, mutations in which impart Co^{2+} resistance. All three mutations have minimal influence on cation

uptake via the CorA system but appear to alter Mg^{2+} efflux via CorA.

Results

Isolation of insertion mutations in corB

Transposon mutagenesis was carried out on *S. typhimurium* LT2 using the mini-Tn*10* elements, Tn*10*Δcam and Tn*10*Δkan (Way*et al.*, 1984). Co^{2+}-resistant mutants from a pool of Tn*10*Δcam insertions were selected on minimal-glucose agar plates containing 100 μM $CoCl_2$. Some of these mutants were purified and shown to contain a single copy of the mini-Tn*10* element using P22 to transduce the antibiotic resistance into a clean background. In all cases, the strains also acquired a Co^{2+}-resistant phenotype, indicating that the Co^{2+}-resistant phenotype resulted from the insertion. Radial streaking showed that all of these mutants exhibited levels of resistance to Co^{2+} intermediate between those of the wild-type and a *corA* strain (MM199). The new Co^{2+}-resistant mutants do not contain *corA* mutations. The chloramphenicol-resistant insertion mutants (MM400–402) were tranduced to kanamycin rsistance with P22 grown on MM199 (*corA45*::MudJ). All Kan^R transductants screened retained resistance to chloramphenicol, indicating that the new mutations are unlinked to *corA*. This locus was tentatively designated *corB*.

Additional Co^{2+}-resistant insertion mutants isolated from a Tn*10*Δkan pool were also shown to carry *corB* mutations, as determined by the linkage between the Tn*10*Δcam and Tn*10*Δkan transposons in the two sets of strains. P22 lysates of strains MM400, MM401, and MM402 were used to transduce a *corB18*::Tn*10*Δkan insertion mutant (MM405) to chloramphenicol resistance (Chl^R) and transductants were screened for Kan^R. All Chl^R transductants tested were sensitive to kanamycin, indicating that the Tn*10*Δkan element had been lost by homologous recombination and was therefore in the *corB* locus (Table 1).

Chromosomal map location of corB

The approximate map position of the *S. typhimurium corB* locus was initially determined by Hfr conjugational crosses with a number of F^- auxotrophic recipient strains. The *corB4*::Tn*10*Δcam mutation was transduced into Hfr strains TN2015 and TN2016. Following conjugation, prototrophic recombinants were screened for inheritance of the Chl^R marker. A location near *tyrA* was indicated since 85% of the $tyrA^+$ recombinants acquired resistance to chloramphenicol. The *corB* locus was subsequently more precisely mapped by P22 contransduction analysis. A P22 lysate of strain MM402 (*corB25*::Tn*10*Δcam) was used to transduce the *tyrA* strain MM737 to Chl^R. The *corB25*::Tn*10*Δcam was shown to be 29% linked to the *tyrA* gene, demonstrating that the *corB* locus has a chromosomal map location of about 55.5 min. Other markers within this region were subsequently analysed for possible linkage to *corB*. The *corB* locus was also linked to *pheA* and to the Tn*10*Δtet elements (Kukral *et al.*, 1989) *zfe-3028*, *-3181*, and *-3222* (Table 1) thus confirming a map location of 55.5 min.

Isolation of insertion mutations in corC

Analysis of additional *cor* insertion mutants isolated from the Tn*10*Δcam transposon pool identified an additional

Table 1. Transductional crosses for mapping *corB*, *corC*, and *corD*

Donor genotype[a]	Recipient genotype	Marker selected	Marker unselected	No. screened	Percent linkage
corB crosses					
MM402 (*corB25*::Tn*10*Δcam)	MM739 (*tyrA44*)	Cam^R	Tyr^+	150	29
MM402 (*corB25*::Tn*10*Δcam)	MM737 (*pheA35*)	Cam^R	Phe^+	125	17
MM405 (*corB18*:Tn*10*Δkan)	MM739 (*tyrA44*)	Kan^R	Tyr^+	100	32
AK3181 (*zfe3181*::Tn*10*Δtet)	MM402 (*corB25*::Tn*10*Δcam)	Tet^R	Cam^s	150	61
AK3028 (*zfe3028*::Tn*10*Δtet)	MM402(*corB25*::Tn*10*Δcam)	Tet^R	Cam^s	88	22
AK3222(*zfe3222*::Tn*10*Δtet)	MM402 (*corB25*::Tn*10*Δcam)	Tet^R	Cam^s	115	3
MM402 (*corB25*::Tn*10*Δcam)	MM405 (*corB18*::Tn*10*Δkan)	Cam^R	Kan^s	50	100
corC crosses					
MM404 (*corC8*::Tn*10*Δcam)	TR3681 (*nag-1*)	Cam^R	Nag^+	200	31
MM404 (*corC8*::Tn*10*Δcam)	TT2342 (*zbf-99*::Tn*10*)	Cam^R	Tet^s	150	71
MM404 (*corC8*::Tn*10*Δcam)	JF2043 (*zbf-5123*::Tn*10*)	Cam^R	Tet^R	480	60
corD crosses					
MM406 (*corD13*::Tn*10*Δtet)	TN1379 (*ΔleuBCD485*)	Tet^R	Leu^+	150	14
MM406 (*corD13*::Tn*10*Δtet)	TN1040 (*leuD798 fol-101*)	Tet^R	Leu^+	88	13
MM406 (*corD13*::Tn*10*Δtet)	TN1040 (*leuD798 fol-101*)	Tet^R	Tmp^s	176	76
MM406 (*corD13*::Tn*10*Δtet)	TT12897 (*pyrA2414*::MudJ)	Tet^R	Kan^s	700	2

a. Only the relevant genotype is shown. Tmp^S, trimethoprim-sensitive.

cor locus, distinct from *corA* and *corB*. The Chl^R marker in these particular mutants was not linked to the *tyrA* gene, unlike *corB*, or to a Mu*dJ* insertion within *corA*. Phenotypic analysis of this class of insertion mutants, by radial streaking around a filter-paper disc containing $CoCl_2$, revealed their pattern of growth to be identical to that of the *corB* insertiion strains, exhibiting an intermediate level of resistance to Co^{2+}. This locus was tentatively designated *corC*.

Chromosomal map location of the corC *locus*

Hfr donor strains TN2015 and TN2016 were transduced to Chl^R with P22 grown on the *corC8*::Tn*10*Δcam insertion strain, MM404. Approximately 70% of $purE^+$ recombinants were shown to be Chl^R, indicating a map location for *corC* near 12 min. The *corC* locus was then more precisely mapped by P22 cotransduction. The *corC* locus was found to be 25% linked to the *nag* gene, thus placing *corC* at about 15 min on the chromosomal map. This location was confirmed using strain TT2342, which harbours a Tn*10* 75% linked to the suppressor gene, *supE*, located at 15.5 min. The *corC8*::Tn*10*Δcam insertion was 72% cotransducible with this *supE*-linked Tn*10*. In addition, *corC8*::Tn*10*Δcam was 60% linked to *zbf-5123*::Tn*10*. This latter insertion is 80% cotransducible with a putative *fur-1* locus that also maps to 15 min (J. W. Foster, personal communication) (Table 1).

Isolation of an additional Co^{2+}*-resistant mutation*

To determine if additional mutations conferring resistance to low concentrations of Co^{2+} could be isolated, 25 spontaneous Co^{2+}-resistant mutants were selected by plating 2×10^8 wild-type cells onto minimal-glucose plates containing of 125 μM $CoCl_2$. Resistant colonies were randomly picked and purified on Luria-Bertani (LB) agar plates. The resistance phenotype was assessed and confirmed by radial streaking around a disc of $CoCl_2$. P22 transductional crosses were then performed to identify *corA*, *corB*, and *corC* mutations in this group of spontaneous mutations. To identify *corA* mutations, the transduction donor carried *zie-3162*::Tn*10*Δtet (Kukral *et al.*, 1989), 25% contransducible with $corA^+$ (Hmiel *et al.*, 1986); tetracycline-resistant (Tet^R) transductants were selected using each of the 25 Co^{2+}-resistant strains as recipient. Inheritance of Co^{2+} sensitivity at a frequency similar to that for contransduction of the insertion and *corA* was taken to mean that the Co^{2+}-resistance mutation was in *corA*. Mutations in *corB* and *corC* were identified similarly using *zfe-3181*::Tn*10*Δtet (61% linked to $corB^+$) and *zbf-99*::Tn*10* (71% linked to $corC^+$). Spontaneous Co^{2+}-resistance mutations, classified by the crosses outlined above, were seen to encompass four distinct linkage groups, comprising mutations in *corA*, *corB*, *corC*, and an additional locus unlinked to any previously identified one.

Isolation of a putative insertion mutation in the corD *locus and chromosomal map location*

As with the *corB* and *corC* loci, the Co^{2+}-resistance level conferred by spontaneous *corD* mutations was intermediate between that of the wild-type and a *corA* mutant. Since no *corD* insertion mutations had been found in the pools that had produced *corB* and *corC* mutations, another pool of insertions was constructed using Tn*10*Δtet. Mutants resistant to 100 μM Co^{2+} were isolated from this pool and tested for linkage to each of the previously isolated classes, *corA*, *corB*, and *corC*. One of this new group of insertions was not linked to any of these previously identified loci and was designated *corD13*::Tn*10*Δtet.

In order to ascertain the chromosomal map location of the *corD13*::Tn*10*Δtet insertion mutation from strain MM406, it was transferred into the Hfr donor strains SA534 (the tetracycline-sensitive (Tet^S) parental strain of TN2015) and SA966 (the Tet^S parental strain of TN2016) by transduction to Tet^R. Appropriate crosses using these Hfr strains suggested that the map position of *corD13*::Tn*10*-Δtet was probably near the *leu* biosynthetic operon at 3 min. P22 cotransduction analysis indicated that *corD13*::Tn*10*Δtet was 14% linked to the *leu* operon, 76% linked to *fol-101*, and 2% linked to *pyrA* (Table 1), confirming a location at about 3 min (Table 1), and that it is a locus distinct from the *corA*, *corB* or *corC* loci. We are in the process of determining if the spontaneous Co^{2+}-resistant mutations tentatively designated *corD* are the same as the *corD13*::Tn*10*Δtet insertion mutation, although our data suggest that this is the case.

Construction of an isogenic series of cor *strains*

An isogenic series of Co^{2+}-resistant mutants was constructed to determine the effect of the *corB*, *corC* or *corD* mutations, and combinations thereof, on transport via the CorA transport system. A *corBCD* triple mutant strain was constructed by initially transducing the *corC8*::Tn*10*Δcam strain MM404 to Kan^R with P22 grown on MM405 (*corB18*::Tn*10*Δkan). Transductants exhibiting both Kan^R and Chl^R were isolated (e.g. MM407) and subsequently transduced to Tet^R with a P22 lysate of MM406 (*corD13*::Tn*10*Δtet) to give a strain (MM410) harbouring insertion mutations in *corB*, *corC*, and *corD*. The construction of strains deficient in any two of these three *cor* loci (e.g. MM408 and MM409) was carried out utilizing the same set of mutations.

Quantification of Co^{2+} *resistance and growth dependence in* cor *strains*

A qualitative measure of the ability of the various *cor*

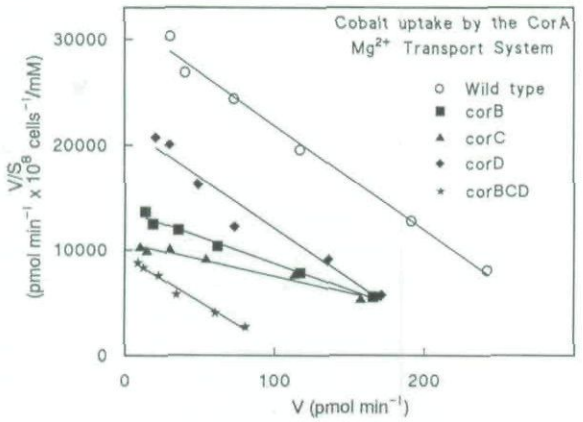

Fig. 1. $^{57}Co^{2+}$ uptake kinetics of a wild-type strain and strains carrying *corB*, *corC*, *corD*, and *corBCD* insertion mutations. Cation uptake was measured for 5 min at room temperature as previously described (Grubbs *et al.*, 1989). Strains were LT2 (wild type), MM405 (*corB*, MM404 (*corC*)), MM406 (*corD*) and MM410 (*corBCD*). The data are plotted as V/S versus V so that the *X*-axis intercept is V_{max} and the slope of the line is the $-1/K_m$. A single experiment is shown. These and the results of two additional experiments are summarized in Table 2.

strains to grow in the presence of Co^{2+} in the growth medium was determined (Table 2). As expected, *corA* strains were significantly more resistant to extracellular Co^{2+} than were strains harbouring *corB*, *corC* or *corD* mutations. The results were in complete agreement with resistance properties determined from the radial streak tests (data not shown) and confirmed the intermediate resistance phenotype of the *corB*, *corC*, and *corD* strains. In addition, these results indicate that resistance to Co^{2+} is not cumulative, the level of resistance afforded by any combination of the individual *corB*, *corC* or *corD* insertion mutations being equal to that of the most resistant of the individual mutations. Thus, a *corBCD* strain is no more Co^{2+} resistant that a strain carrying only a *corC* insertion mutation. Growth of *corB*, *corC*, *corD*, or *corBCD* strains of LB agar plates was indistinguishable from that of a wild-type strain and required no Mg^{2+} supplementation. Growth of *corC* or *corBCD* strains in N-minimal liquid media containing 50 μM Mg^{2+} usually showed a 30–60 min lag period before entry into logarithmic growth compared with wild-type or other *cor* strains (data not shown), but the lag was variable and not evident in N-minimal liquid media containing 10 mM Mg^{2+}.

Table 2. $^{57}Co^{2+}$ uptake kinetics and growth inhibition by Co^{2+} in *corB*, *corC*, and *corD* mutants.

Strain	K_m (percent of wild type)	V_{max} (percent of wild type)	*n*	Minimal inhibitory Co^{2+} concentration[a] (μM)
LT2	100[b]	100[b]	3	60
corA	ND	ND	–	325
corB	191 ±13	114 ±34	3	100
corC	279 ±31	103 ±30	3	150
corD	102 ±3	77 ±24	3	125
corBCD	97 ±17	43 ±7	3	150

a. Shown above is the minimal concentration of $CoCl_2$ that will completely inhibit growth of a liquid culture of the indicated strain as described in the *Experimental procedures*. Strains harbouring *corBC* or *corCD* mutations were completely inhibited by 150 μM Co^{2+}, while a strain harbouring *corBD* mutations was completely inhibited by 125 μM Co^{2+}.
b. The wild-type strain LT2 had a K_m and a V_{max} of 320 pmol min^{-1} 10^{-8} cells. The minimal uptake of $^{57}Co^{2+}$ that could be detected is less than 1 pmol min^{-1} 10^8 cells^{-1}.

Effect of corB, corC *and* corD *mutations on cation influx*

We have previously shown that the CorA transport system can mediate uptake of Co^{2+} (Hmiel *et al.*, 1986) and Ni^{2+} (Snavely *et al.*, 1991) in addition to uptake of Mg^{2+}. The ability to use Co^{2+} or Ni^{2+} is extremely useful because $^{28}Mg^{2+}$, the only usable radioisotope of Mg^{2+}, is not routinely available and currently costs over $30 000 per mCi. Therefore the K_m and V_{max} for $^{57}Co^{2+}$ or $^{63}Ni^{2+}$ influx in *S. typhimurium* strains carrying mutations in the *corB*, *corC*, and/or *corD* loci were determined for comparison with uptake in wild-type cells. A representative $^{57}Co^{2+}$ experiment comparing wild-type and mutant strains is shown in Fig. 1, while kinetic data for $^{57}Co^{2+}$ uptake are summarized in Table 2. The V_{max} for Co^{2+} influx was essentially unaffected by the presence of single mutations in *corB*, *corC* or *corD*. Even in a *corBCD* strain, the V_{max} for Co^{2+} influx was decreased only by half. Likewise, the presence of mutations in *corB*, *corC* or *corD* had little effect on the V_{max} of $^{63}Ni^{2+}$ uptake, with the presence of any one or all three mutations decreasing the V_{max} by about 50% (Fig. 2 and Table 3). A single $^{28}Mg^{2+}$ uptake experiment (data not shown) indicated that the

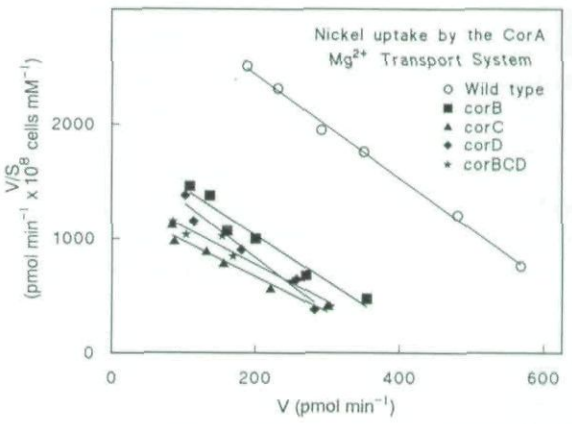

Fig. 2. $^{63}Ni^{2+}$ uptake kinetics of a wild-type strain and a strain carrying *corBCD* insertion mutations. Cation uptake was measured for 5 min at 37°C as previously described (Grubbs *et al.*, 1989). Strains used were LT2 (wild type), MM405 (*corB*), MM404 (*corC*), MM406 (*corD*) and MM410 (*corBCD*). A single experiment is shown. The data are plotted as V/S versus V so that the *X*-axis intercept is V_{max} and the slope of the line is the $-1/K_m$. These and the results of additional experiments are summarized in Table 3.

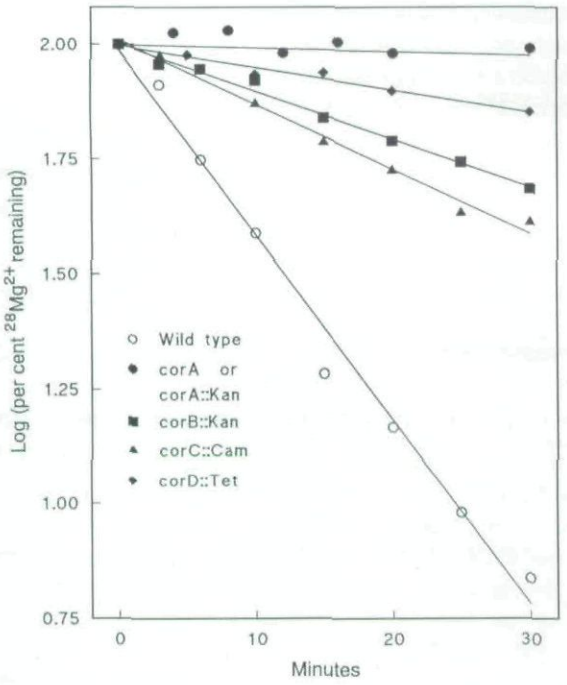

Fig. 3. Effect of insertions at single *cor* loci on $^{28}Mg^{2+}$ efflux. Efflux was measured at 37°C in strains LT2 (wild type), MM199 (*corA*), MM405 (*corB*), MM404 (*corC*), and MM406 (*corD*) as described (Grubbs *et al.*, 1989). The data shown are the averages of at least three experiments for each strain, except *corD*, for which a single experiment is shown. Efflux was measured simultaneously in four distinct strains grown and incubated in $^{28}Mg^{2+}$ in parallel. Variability was less than ±7% at each point. Excluding the data for MM199 because of the lack of detectable efflux, the correlation coeficients derived from linear regression analysis were >0.96 for each individual experiment and >0.97 for the composite line shown. An exception is the datum for MM406, where the $T_{1/2}$ in the presence of *corD*::Tn*10*Δtet was rather variable. In five experiments, the $T_{1/2}$ ranged from 20–90 min. While within each individual experiment the correlation coefficient for the linear regression line was >0.95, only a single representative experiment is shown because of the overall variability. Some of the data for strain MM199 were obtained in separate experiments, one of which has been published (Snavely *et al.*, 1989b).

V_{max} for Mg^{2+} influx was not significantly altered in a *corBCD* strain.

The cor mutations also had minimal effects on K_m. For $^{63}Ni^{2+}$ influx, the presence of any one or all mutations did not alter K_m significantly (Table 3). In slight contrast, the K_m for $^{57}Co^{2+}$ uptake was increased twofold in a *corB* strain, threefold in a *corC* strain, and was unaffected in a *corD* strain. Furthermore, despite the small but reproducible change in K_m in *corB* or *corC* strains, the K_m for $^{57}Co^{2+}$ uptake in *corBCD* strains was unaltered from that observed in the wild type (Table 2). Additional experiments indicated that Mg^{2+} inhibited $^{63}Ni^{2+}$ influx in *corB*, *corC*, *corD* or *corBCD* strains with a K_i identical to that observed for a wild-type strain (data not shown): this is an indication that the K_m for Mg^{2+} uptake was unaltered by the presence of these mutations.

These results show that the presence of insertion mutations at the *corB*, *corC* and/or *corD* loci have minimal effects on cation influx via the CorA transport system. Further, influx of Mg^{2+} requires only the presence of a wild-type allele of *corA*. This is presumptive evidence that wild-type alleles of *corB*, *corC* and/or *corD* are not required for expression or functioning of the *corA* gene product.

Effect of corB, corC *and* corD *mutations on cation efflux*

Since the effect of these newly identified *cor* mutations on cation uptake was relatively minor, the role of the gene products of these loci in the functioning of the CorA transport system remained unclear. Neither $^{57}Co^{2+}$ nor $^{63}Ni^{2+}$ are useful for measurement of efflux through the CorA transport system, probably because these transition metal cations bind extremely tightly to proteins and nucleic acids within the cell. Consequently, $^{28}Mg^{2+}$ was used to characterize efflux through the CorA system. Strains harbouring insertion mutations in *corB*, *corC* or *corD* showed a $T_{1/2}$ for Mg^{2+} efflux of 30–60 min compared with a $T_{1/2}$ of 9–10 min for wild–type cells (Fig. 3). As previously reported, a mutation in *corA* alone abolished efflux (Fig. 3 and Snavely *et al.*, 1989b). Interestingly, a strain harbouring mutations at both the *corB* and *corC* loci had no additional effect on the rate of $^{28}Mg^{2+}$ efflux (Fig. 4.). Similarly, simultaneous mutations in both *corC* and *corD* also had no greater effect than a mutation in *corC* alone (Fig. 4). In contrast to these relatively modest effects on efflux of single or double mutatins, a strain harbouring mutations at all three loci (*corB*, *corC*, and *corD*) showed complete abolition of $^{28}Mg^{2+}$ efflux (Fig. 4). Thus, Mg^{2+} efflux requires a wild-type allele of *corA* and at least one wild-type allele amongst *corB*, *corC* or *corD*.

Mg^{2+} efflux via the CorA transport system requires relatively high extracellular Mg^{2+} concentrations. At low extracellular Mg^{2+} concentrations, the efflux rate is greatly diminished in *E. coli* (Silver and Clark, 1971) and is undetectable in *S. typhimurium* (Snavely *et al.*, 1989b). In addition, previous work using *E. coli* had demonstrated that Mn^{2+} could stimulate Mg^{2+} efflux (Silver and Clark, 1971). We therefore tested the ability of other divalent

Table 3. $^{63}Ni^{2+}$ uptake kinetics in *corB*, *corC*, and *corD* mutants.

Strain	K_m	V_{max} (percent of wild type)	*n*
LT2	100[a]	100[a]	3
corA	ND	ND	–
corB	90	62	2
corC	116	49	2
corD	92	46	2
corBCD	126 ±23	58 ±10	3

a. *The wild-type strain* LT2 *had a* K_m *of* 200 μM *and a* V_{max} *of* 700 *pmol* min^{-1} 10^8 $cells^{-1}$. The minimal uptake of $^{63}Ni^{2+}$ that could be detected is about 1 pmol min^{-1} 10^8 $cells^{-1}$.

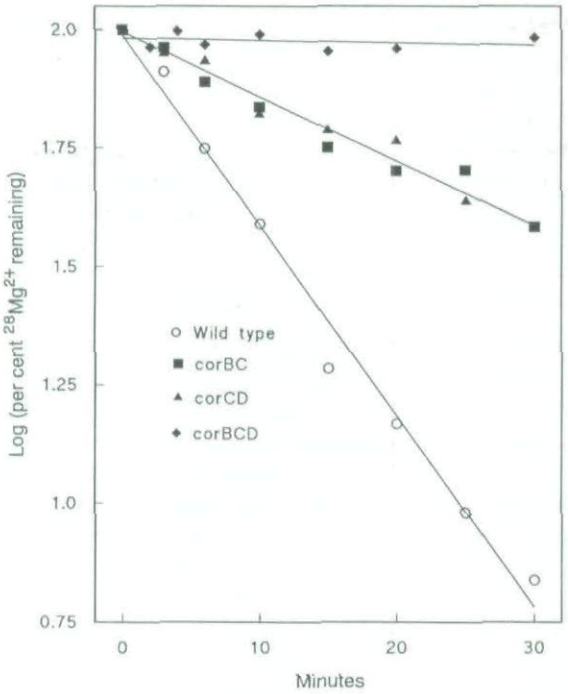

Fig. 4. Effect of multiple *cor* mutations on $^{28}Mg^{2+}$ efflux. Efflux was measured in strains LT2 (wild type), MM407 (*corBC*), MM408 (*corCD*) and MM410 (*corBCD*) as described previously (Grubbs *et al.*, 1989) and in the legend to Fig. 3. The data for the wild-type strain are identical to those in Fig. 3. The data for MM407 and MM408 are the averages of two independent experiments, while those for MM410 are for three experiments. Variability was less than ±5% at each point. The correlation coefficients derived from linear regression analysis was >0.96 for each individual experiment and >0.97 for the average line shown, except for the data with MM410 because of the lack of detectable efflux. Efflux was not measured in a *corBD* strain because MM409 had not been constructed when we were able to obtain a shipment of $^{28}Mg^{2+}$.

cations to stimulate $^{28}Mg^{2+}$ efflux. The data in Fig. 5 indicate that high extracellular concentrations of Mg^{2+} (and to a lesser degree, Mn^{2+}) can stimulate $^{28}Mg^{2+}$ efflux. In contrast, neither Co^{2+} nor Ni^{2+} was able to stimulate $^{28}Mg^{2+}$ efflux, even though, at the concentrations used, Co^{2+} and Ni^{2+} influx via the CorA system was essentially maximal. This result serves to dissociate the ability of Mg^{2+} to stimulate efflux from its actual transport through the CorA transport system.

The ability of Mg^{2+} to stimulate efflux via CorA is also a function of extracellular Mg^{2+} concentration. In the absence of added Mg^{2+}, no $^{28}Mg^{2+}$ efflux is evident in a wild-type strain (Fig. 6), whereas with the addition of increasing concentrations of extracellular Mg^{2+}, efflux occurs linearly with time at a rate that increases as the extracellular Mg^{2+} concentration increases (Fig. 6). Since the rate of efflux was linear at different Mg^{2+} concentrations, the experiment shown in Fig. 7 was feasible wherein the amount of efflux at a single time point was measured as a function of extracellular Mg^{2+} concentration. Our interpretation of these data is that the phenotypic effect of single *corB*, *corC*, and *corD* mutations is not actually to abolish efflux but to greatly increase the extracellular Mg^{2+} concentration required to initiate efflux via CorA. In the *corBCD* strain, efflux cannot be stimulated except at extremely high extracellular Mg^{2+} concentrations. However, this effect in the *corBCD* strain may not be completely specific since 100 mM extracellular Mg^{2+} also stimulates slight $^{28}Mg^{2+}$ efflux from a *corA* strain.

Discussion

Composition of the CorA Mg^{2+} transport system

Data presented above indicate that the CorA Mg^{2+} transport of *S. typhimurium* is influenced by four genetic loci: *corA*, *corB*, *corC*, and *corD*. The requirement for *corA* is well established (Hmiel *et al.*, 1986; 1989). The evidence in this and previous reports strongly indicates that the *corA* gene product is a protein that mediates the movement of Mg^{2+} across the membrane. First, V_{max} for transport is increased, without any effect on K_m, when the CorA protein is expressed from a multicopy plasmid (Hmiel *et al.*, 1986). Second, Mg^{2+} influx still occurs in a *corBCD* strain. Thus, the single gene product of the *corA* locus appears to mediate membrane flux of Mg^{2+}.

The basis for the Co^{2+}-resistance phenotype of *corB*, *corC* and *corD* mutations is not completely clear. It seems likely, *a priori*, that Co^{2+} resistance would be due to

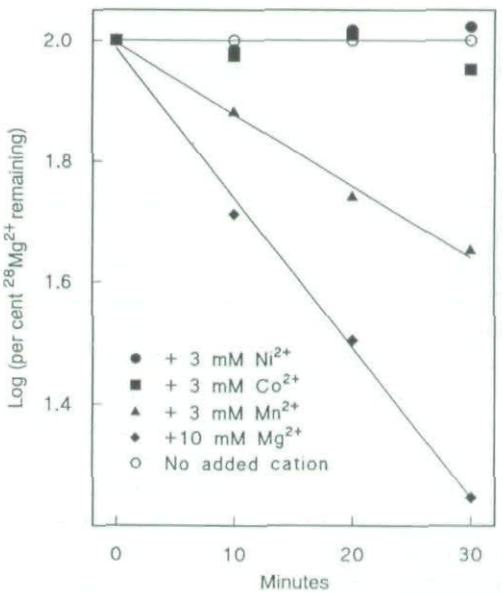

Fig. 5. Effects of Mg^{2+}, Mn^{2+}, Co^{2+}, and Ni^{2+} on $^{28}Mg^{2+}$ efflux from a wild-type strain. Efflux was measured in strain LT2 as described (Grubbs *et al.*, 1989), except that efflux was initiated by resuspension of the cells in 10 mM Mg^{2+} or 3 mM of either Mn^{2+}, Co^{2+}, or Ni^{2+}.

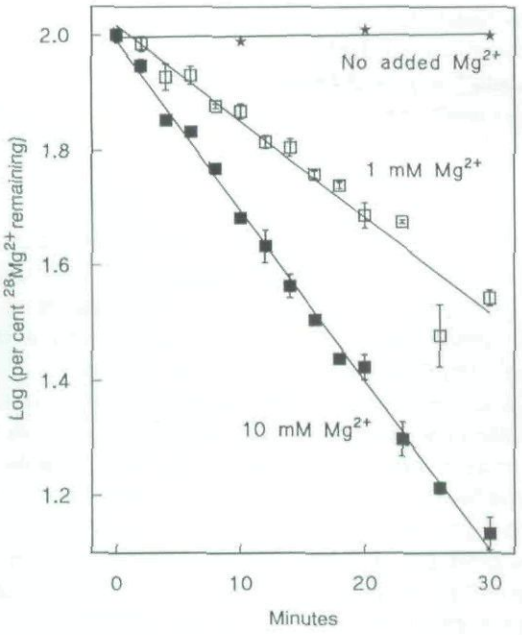

Fig. 6. The effect of extracellular Mg^{2+} concentration on the rate of $^{28}Mg^{2+}$ efflux. Efflux was measured in strain LT2 as described (Grubbs *et al.*, 1989) except that efflux was initiated by resuspension of the cells in 0, 1 or 10 mM Mg^{2+}.

altered transport of the cation, as is clearly the case for *corA* mutations (Hmiel *et al.*, 1986; Snavely *et al.*, 1989b). Growth inhibition by Co^{2+} probably results from interactions with a variety of target sites. Co^{2+} binds tightly to most proteins and to nucleotides, including ATP. It is thus difficult to imagine a single mutation that would diminish the affinity of all of these targets for Co^{2+}. The level of resistance conferred by these mutations is intermediate between that of the wild type and that of a *corA* strain. The findings that *corB, corC* and *corD* have small effects on cation influx, larger effects on cation efflux, and that a *corBCD* strain is completely deficient in Mg^{2+} efflux suggest that these mutations affect the intracellular concentration of Mg^{2+} and that they do so by affecting the CorA transport system. A Co^{2+}-resistant phenotype might then occur because an increased intracellular Mg^{2+} concentration would better compete with Co^{2+} for relevant sites. Alternatively, and more plausibly, diminished capacity for Co^{2+} influx might be sufficient for a Co^{2+}-resistant phenotype by virtue of the smaller amount of Co^{2+} entering the cell. This interpretation is in accord with the influx data reported here. The decrease in Co^{2+} influx for strains carrying *corB, corC, and corD* mutations is similar; likewise, all three mutations confer a similar degree of Co^{2+} resistance. Furthermore, while a *corBCD* strain shows no additional deficit in Co^{2+} influx relative to a strain carrying only one such *cor* mutation, a *corBCD* strain does not have a greater degree of Co^{2+} resistance.

It is not clear how the mutations described in this paper relate to the previously reported *corB* locus in *E. coli* (Park *et al.*, 1976) which was cotransducible with the *pyrB* gene (mapping at about 98 min on the *S. typhimurium* chromosome). In *S. typhimurium*, the *mgtA* Mg^{2+} transport system maps to a chromosomal location analogous to the *E. coli corB* locus (Hmiel *et al.*, 1989). However, no mutations at *mgtA* confer a Co^{2+}-resistance phenotype. Moreover, mutations at the *E. coli corB* locus apparently alter expression of the *corA* gene product and confer Ca^{2+} sensitivity, neither of which has been observed with *mgtA, corB, corC*, or *corD* mutations in *S. typhimurium*. It is possible that the *E. coli corB* locus is not in an analogous position on the *S. typhimurium* chromosome. This implies that one of the *S. typhimurium cor* loci described in this paper may be similar to the *E. coli corB* locus, or that we have not yet identified the appropriate *S. typhimurium cor* locus; additional work will be required to clarify this point.

Possible components of the CorA system and their functions

The transport data indicate that the *corA* gene product (CorA) is both necessary and sufficient for mediation of Mg^{2+} influx. Since CorA is capable of mediating both influx

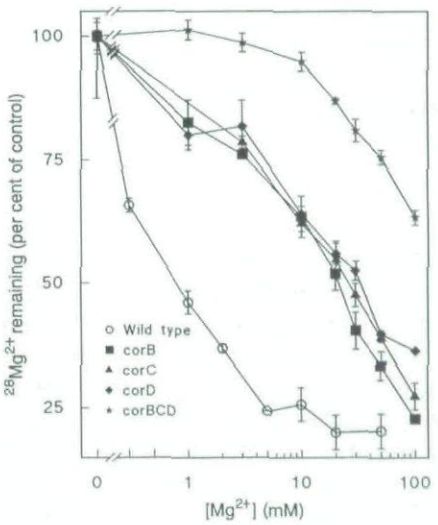

Fig. 7. The effect of extracellular Mg^{2+} concentration on $^{28}Mg^{2+}$ efflux in strains carrying insertions in *corB, corC*, and/or *corD*. Efflux was measured as described (Grubbs *et al.*, 1989) in strains LT2, MM405 (*corB*), MM404 (*corC*), MM406 (*corD*) or MM410 (*corBCD*), except that cells were resuspended in buffer containing no added Mg^{2+}. At t=0, efflux was initiated by the addition of 3.6 ml of cells to 0.4 ml of buffer containing 10× the final indicated Mg^{2+} concentration. At t=20 min, 1.0 ml triplicate samples were filtered to determine the $^{28}Mg^{2+}$ remaining in the cells. The rate of $^{28}Mg^{2+}$ efflux at 1 or 10 mM extracellular Mg^{2+} is linear with time for at least 40 min under these conditions. The amount of cell-associated $^{28}Mg^{2+}$ at t=0 was not altered by the concentration of extracellular Mg^{2+}. The data are plotted as percentages of the cell-associated $^{28}Mg^{2+}$ present at t=0.

and efflux, the role(s) of the gene products of the *corB*, *corC*, and *corD* loci in this transport system is(are) unclear. Their absence has no significant effect on K_m or V_{max} for the influx of $^{57}Co^{2+}$, $^{63}Ni^{2+}$, or $^{28}Mg^{2+}$ via CorA. Further, in a strain harbouring mutations at all three new *cor* loci, the residual level of Mg^{2+} uptake by CorA at extracellular Mg^{2+} concentrations approximating the K_m for Mg^{2+} is still more than an order of magnitude greater than the level of uptake mediated by either the MgtA or the MgtB Mg^{2+} transport system (Snavely *et al.*, 1991). Therefore, a strain carrying mutations in *corB*, *corC*, and *corD* is in no way limited in its ability to accumulate Mg^{2+} under any normal (laboratory) growth conditions. Thus none of these three loci seem to be intimately involved in or required by the influx process. Conversely, however, these three new Co^{2+}-resistance loci seem integral to the ability of *corA* to mediate and/or regulate Mg^{2+} efflux. While individual mutations at the *corB*, *corC* or *corD* loci have no effect or only moderate effects on the rate of Mg^{2+} efflux, their combination results in complete abolition of Mg^{2+} efflux. Nonetheless, since a wild-type allele of *corA* is still present, Mg^{2+} influx can continue. Thus, CorA is necessary but not sufficient for mediation of Mg^{2+} efflux; the presence of a wild-type allele of at least one of the *corB*, *corC* or corD loci is additionally required for Mg^{2+} efflux.

Since *corA* alone is required for Mg^{2+} influx, the energy transduction functions of this transport system which are required for influx must also reside within the CorA protein. However, little can be concluded currently about the specific roles of the individual *corB*, *corC* or *corD* gene products. At least two functions could reside within the products of these three loci: (i) a coupling function between efflux of cation and an energy source, and (ii) a cation sensor function.

An energy coupling function amongst *corB*, *corC*, and *corD* is implied because efflux of Mg^{2+} requires transport against the electrochemical gradient. Thus, efflux must be coupled either to ATP hydrolysis or to transport of another ion with its electrochemical gradient. Studies are currently underway to delineate these possibilities further using *unc* mutations to uncouple ATP from the H^+ gradient. A cation sensor function is implied by the gating of efflux by extracellular cation concentration. At an extracellular Mg^{2+} concentration equal to the K_m for influx, no efflux is detectable. Thus, at an extracellular Mg^{2+} concentration equal to 50% of the maximal influx rate, *corB*, *corC* and/or *corD* may interact with *corA* in such a manner as to prevent Mg^{2+} efflux. Only at relatively high extracellular Mg^{2+} concentrations does Mg^{2+} efflux become 'activated'. A sensor or activation function is also implied by the ability of Mg^{2+} and Mn^{2+} but not Co^{2+} or Ni^{2+}, to stimulate or 'activate' $^{28}Mg^{2+}$ efflux. Since at an extracellular concentration of 3 mM, both Co^{2+} and Ni^{2+} are being transported at a maximal rate by the *corA* gene product, it is not the act of transport through the CorA protein that regulates activation of efflux. Rather, the inability of Co^{2+} and Ni^{2+} to stimulate $^{28}Mg^{2+}$ efflux suggests that they either do not interact with the putative Mg^{2+} sensor or act as inhibitors.

Comparison with other cation transport systems

The most obvious system with which CorA can be compared is the Trk K^+ transport system of *E. coli* (Bakker *et al.*, 1987; Walderhaug *et al.*, 1987; Bossemeyer *et al.*, 1991; Dosch *et al.*, 1991) which is encoded and/or regulated by the gene products of four unlinked loci: *trkA*, *trkE*, *trkG*, and *trkH*. The Trk transport system, however, mediates only influx of K^+. The effect of mutations at the *trkA*, *trkE*, or *trkH* loci reduces the V_{max} of K^+ uptake by about a factor of 10 without having any significant effect on the K_m. In each case, a second mutation at another *trk* locus produces a further reduction in the V_{max} of K^+ uptake. TrkA is a peripheral membrane protein that is loosely bound to the cytoplasmic side of the *E. coli* inner membrane and requires functional *trkG*, *trkH*, and/or *trkE* gene products for its anchorage to the cytoplasmic membrane (Bossemeyer *et al.*, 1989). Thus, while the CorA and Trk systems are alike in being influenced by gene products of multiple unlinked loci, the transport characteristics (exchange versus influx) and the effect of mutations at individual or multiple loci differ.

K^+ efflux in *E. coli* is mediated by two distinct efflux systems encoded by the *kefB* and *kefC* loci (Bakker *et al.*, 1987; Walderhaug *et al.*, 1987; Meury and Robin, 1990). These loci apparently encode two different glutathione-gated K^+ channels, neither of which appear to have any relationship to the Trk K^+ influx system. The data presented above indicate that the gene products of the *corB*, *corC* or *corD* loci require a wild-type allele of *corA* to mediate efflux; therefore *corB*, *corC*, and/or *corD* do not represent an independent Mg^{2+} efflux pathway comparable to the K^+ efflux loci, *kefB* and *kefC*. Together, these data indicate that the CorA Mg^{2+} transport system represents a novel and, to date, unique example of a cation membrane transport system.

Experimental procedures

Bacterial strains and growth media

All strains used in this study are derivatives of *S. typhimurium* LT2 unless otherwise stated; their genotype and sources are listed in Table 4. Bacteria were grown at 37°C with aeration in LB medium or on LB agar plates (Miller, 1972), except where stated otherwise. Antibiotics were used at the following concentrations: ampicillin (Amp), 100 μg ml^{-1}; kanamycin sulphate (Kan) , 50 μg ml^{-1}; tetracycline (Tet), 25 μg ml^{-1}; chloramphenicol (Chl), 20 μg ml^{-1}. In minimal agar plates or broth, added antibiotic concentrations were one-half of the above levels. Minimal medium was based on

Table 4. Bacterial strains used.

Strain	Genotype	Source/ /Reference
AK3028	As TN2540, *zfe-3028*::Tn*10*Δtet	Kukral *et al* (1989)
AK3181	As TN2540, *zfe-3181*::Tn*10*Δtet	Kukral *et al* (1989)
AK3222	As TN2540, *zfe-3222*::Tn*10*Δtet	Kukral *et al* (1989)
MM199	Δ*leuBCD485, corA45*::MudJ	Snavely *et al* (1989b)
MM400	*corB4*::Tn*10*Δcam	This study
MM401	*corB24*::Tn*10*Δcam	This study
MM402	*corB25*::Tn*10*Δcam	This study
MM403	*corC1*::Tn*10*Δcam	This study
MM404	*corC8*::Tn*10*Δcam	This study
MM405	*corB18*::Tn*10*Δkan	This study
MM406	*corD13*::Tn*10*Δtet	This study
MM407	*corB18*::Tn*10*Δkan, *corC8*::Tn*10*Δcam	This study
MM408	*corC8*::Tn*10*Δcam, *corD13*::Tn*10*Δtet	This study
MM409	*corB18*::Tn*10*Δkan, *corD13*::Tn*10*Δtet	This study
MM410	*corB18*::Tn*10*Δkan, *corC8*::Tn*10*Δcam, *corD13*::Tn*10*Δtet	This study
MM737	*tyrA44*	*C. G. Miller*
MM739	*pheA35*	C. G. Miller
JF2043	*bciA1*::MudJ, *fur-1, zbf-5123*::Tn*10*	J. F. Foster
SA534	HfrK4 *serA13, rfa*-3058	K. E. Sanderson
SA966	HfrK19 *leuBCD39, ara-7*	K. E. Sanderson
TN1040	*leuD798, fol-101,zji-842*::*Tn5*	*C. G. Miller*
TN1379	Δ*leuBCD485*	C. G. Miller
TN2015	HfrK4 *serA13, rfa*-3058, *zxx-888*::Tn*10*	C. G. Miller
TN2016	HfrK19 *leuBCD39, ara-7, zxx888*::Tn*10*	C. G. Miller
TN2369	*proAB47/*F'_{128} pro^+, lac^+, *zzf-1831*::Tn*10* Δtet (TT10423)	J. Roth
TN2370	*proAB47/*F'_{128}, pro^+, lac^+, *zzf-1834*::Tn*10* Δkan (TT10426)	J. Roth
TN2371	*proAB47/*F'_{128}, pro^+, lac^+, *zzf-1837*::Tn*10* Δcam (TT10605)	J. Roth
TN2372	LT2/pNK972 (TT10427)	J. Roth
TN2540	*metE551, metA22, hisC47*(Am), *trpB2, ilv-452, rpsL120, fla*-66, *xyl*-404, *galE496, hsdL6*(r^-m^+) *hsdSA29*(r^-m^+) (DB2546)	R. Maurer
TR3681	*his-3050, nag*-1	J. Roth
TT2342	*zbf99*::Tn*10*, *supE*(su2), *hisC527, leu-414*	J. Roth
TT12897	*pyrA2414*::MudJ	J. Roth

the N medium of Nelson and Kennedy (Nelson and Kennedy, 1971), supplemented with 0.4% glucose, 0.1% casamino acids and 50 μM $MgSO_4$. For minimal agar plates, the addition of $MgSO_4$ was omitted.

Quantification of Co^{2+} *resistance in the* cor *strains*

Growth of the isogenic series of corB/corC/corD strains was monitored in minimal-glucose medium containing 0.1% casamino acids, 50 μM $MgSO_4$ and different concentrations of $CoCl_2$. Cultures were initially inoculated with 2×10^7 cells that had been washed and resuspended in 0.85% NaCl. Resistance was then determined by growth of the strain, following a 16 h incubation at 37°C. *S. typhimurium* strains LT2 and MM199 were grown in parallel to assess growth inhibition by Co^{2+} for both wild-type and *corA* cells, respectively. The minimal inhibitory concentration of Co^{2+} was that concentration which completely prevented growth.

Genetic techniques

Transductions were carried out using a mutant (*HT*105/1*int*201) of the high-frequency, generalized transducing bacteriophage, P22. Hfr conjugation experiments were performed as described by Miller (Miller, 1972), by the direct plating of 2×10^8 cells of the Hfr donor strain and 2×10^8 cells of an appropriate F^- auxotrophic recipient strain onto minimal-glucose plates. Prototrophic recombinants were isolated after 36 h of incubation at 37°C. The isolation of a number of *S. typhimurium* strains carrying randomly spaced Tn*10*Δtet chromosomal insertions and their use for genetic mapping has been previously described (Kukral *et al.*, 1989).

Isolation of Co^{2+}*-resistance insertion mutations*

Random transposition of the mini-Tn*10* derivatives Tn*10*Δtet, Tn*10*Δkan, and Tn*10*Δcam (Way *et al.*, 1984) was achieved by transducing a recipient strain that harbours the transposase helper plasmid, pNK972 (TN2372), to antibiotic resistance with P22 lysates of TN2369, TN2370, and TN2371, as described (Davis *et al., 1980*). *Approximately 10 000–12 000 resistant* colonies were pooled in each case and a P22 transducing lysate prepared on the pools. These P22 lysates were used to transduce a suitable recipient strain (LT2), lacking the helper plasmid. This secondary pool of stable insertions was then used for selecting Co^{2+}-resistant mutants.

Selection of Co^{2+}*-resistant mutants*

Mutations conferring resistance to Co^{2+} were selected on minimal-glucose plates containing 80–150 μM $CoCl_2$. Cobalt resistance was also assessed on minimal-glucose plates by radial streaking of colonies around a 6-mm-diameter filter-paper disc impregnated with 10 μl of 100 mM $CoCl_2$. Sensitivity of strains to cobalt was determined by lack of growth within the area immediately surrounding the disc. A streak of a *corA* strain typically grows to the edge of the paper disc, while a streak of a wild-type strain grows no closer than 2 cm. The *corB, corC,* and *corD* mutations identified in this paper had an intermediate level of Co^{2+} resistance and grew to within about 1 cm of the disk. Auxotrophic requirements were identified by radial streaking around a filter-paper disc impregnated with 1 μmol of the appropriate amino acid.

Cation transport assay

Uptake and kinetic analysis of $^{57}Co^{2+}$ or $^{63}Ni^{2+}$ and the efflux of $^{28}Mg^{2+}$ were determined as previously described (Hmiel *et al.*, 1986; Grubbs *et al.*, 1989). For influx assays, overnight cultures were grown in N-minimal medium containing 1 mM Mg^{2+}. A 1:50 dilution was made into fresh N-minimal medium containing 1 mM Mg^{2+} and cells grown to an OD_{600nm} of 2–4 before being washed twice in N-minimal medium without added Mg^{2+}. Cells were finally resuspended in the same medium without Mg^{2+} at an OD_{600nm} of about 2.0 for use in transport assays. The strains used to quantify cation uptake via the CorA system also harboured wild-type alleles of *mgtA* and *mgtB* and thus carried functional MgtA and MgtB Mg^{2+} transport systems. However, as Co^{2+} is not taken up by either the MgtA or the MgtB system, uptake of $^{57}Co^{2+}$ is a measure of the CorA transport system only. Moreover, since

transcription of *mgtA* and *mgtB* and thus expression of the MgtA and MgtB transport systems are severely repressed by growth in high extracellular Mg^{2+} concentrations (Snavely *et al.*, 1989b; 1991), more than 99% of $^{63}Ni^{2+}$ uptake under these growth conditions occurs via the CorA system.

Acknowledgements

We thank Jane Florer and Sue Gura for assistance with the transport studies and numerous discussions, Jennifer Banks, Jessica Galazka and Shauna Trower for technical assistance with the mapping studies, and Dr J. W. Foster for sharing unpublished data. M.G. is a recipient of a postdoctoral fellowship from the Northeast Ohio Affiliate, American Heart Association. This work was supported by U.S.P.H.S. grants GM26340 and GM39447.

References

Bakker, E.P., Booth, I.R., Dinnbier, V., Epstein, W., and Gajewski, A. (1987) Evidence for multiple K^+ export systems in *Escherichia coli*. *J Bacteriol* **169:** 3743–3749.

Bossemeyer, D., Borchard, A., Dosch, D.C., Helmer, G.C., Epstein, W., Booth, I.R., and Bakker, E.P. (1989) K^+-transport protein TrkA of *Escherichia coli* is a peripheral membrane protein that requires other *trk* gene products for attachment to the cytoplasmic membrane. *J Biol Chem* **264:** 16403–16410.

Davis, R.W., Botstein, D., and Roth, J.R. (1980) *Advanced Bacterial Genetics*. Cold Spring Harbour, New York: Cold Spring Harbor Laboratory Press.

Dosch, D.C., Helmer, G.C., Sutton, S.H., Salvacion, F.F., and Epstein, W. (1991) Genetics analysis of potassium transport loci in *Escherichia coli*: evidence for three constitutive systems mediating uptake of potassium. *J Bacteriol* **173:** 687–696.

Grubbs, R.D., Snavely, M.D., Hmiel, S.P., and Maguire, M.E. (1989) Magnesium transport in eukaryotic and prokaryotic cells using magnesium-28 ion. *Meth Enzymol* **173:** 546–563.

Hmiel, S.P., Snavely, M.D., Miller, C.G., and Maguire, M.E. (1986) Magnesium transport in *Salmonella typhimurium*: characterization of magnesium influx and cloning of a transport gene. *J Bacteriol* **168:** 1444–1450.

Hmiel, S.P., Snavely, M.D., Florer, J.B., Maguire, M.E., and Miller, C.G. (1989) Magnesium transport in *Salmonella typhimurium*: genetic characterization and cloning of three magnesium transport loci. *J Bacteriol* **171:** 4742–4751.

Kukral, A.M., Strauch, K.L., Maurer, R.A., and Miller, C.G. (1989) Genetic analysis in *Salmonella typhimurium* with a small collection of randomly spaced insertions of transposon Tn*10*Δ. *J Bacteriol* **1987:** 1787–1793.

Lusk, J.E., and Kennedy, E.P. (1969) Magnesium transport in *Escherichia coli*. *J Bacteriol* **244:** 1653–1655.

Meury, J., and Robin, A. (1990) Glutathione-gated K^+ channels of *Escherichia coli* carry out K^+ efflux controlled by the redox state of the cell. *Arch Microbiol* **154:** 475–482.

Miller, J.H. (1972) *Experiments in Bacterial Genetics*. Cold Spring Harbor New York: Cold Spring Harbor Laboratory Press.

Nelson, D.L., and Kennedy, E.P. (1971) Magnesium transport in *Escherichia coli*. Inhibition by cobaltous ion. *J Biol Chem* **246:** 3042–3049.

Park, M.H., Wong, B.B., and Lusk, J.E. (1976) Mutants in three genes affecting transport of magnesium in *Escherichia coli*: physiology and genetics. *J Bacteriol* **126:** 1096–1103.

Silver, S. (1969) Active transport of magnesium in *Escherichia coli*. *Proc Natl Acad Sci USA* **62:** 764–771.

Silver, S., and Clark, D. (1971) Magnesium transport in Escherichia coli. Interference by manganese with magnesium metabolism. *J Biol Chem* **246:** 569–576.

Snavely, M.D., Florer, J.B., Miller, C.G., and Maguire, M.E. (1989a) Magnesium transport in *Salmonella typhimurium*: expression of cloned genes for three distinct Mg^{2+} transport systems. *J Bacteriol* **171:** 4752–4760.

Snavely, M.D., Florer, J.B., Miller, C.G., and Maguire, M.E. (1989b) Magnesium transport in *Salmonella typhimurium*: $^{28}Mg^{2+}$ transport by the CorA, MgtA, and MgtB systems. *J Bacteriol* **171:** 4761–4766.

Snavely, M.D., Gravina, S., Cheung, T., Miller, C.G., and Maguire, M.E. (1991) Magnesium transport in *Salmonella typhimurium*: regulation of *mgtA* and *mgtB* expression. *J Biol Chem* **266:** 824–829.

Walderhaug, M.O., Dosch, D.C., and Epstein, W. (1987) Potassium transport in bacteria. In *Ion Transport in Prokaryotes*. Rosen, B.P., and Silver, S. (eds). San Diego: Academic Press, pp. 85–130.

Way, J.C., Davis, M.A., Morisato, D., Roberts, D.E., and Kleckner, N. (1984) New Tn*10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. *Gene* **32:** 369–379.