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Distribution of the CorA Mg^{2+} Transport System in Gram-Negative Bacteria

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The CorA Mg^{2+} transport system is the dominant constitutive uptake mechanism in *Salmonella typhimurium* and *Escherichia coli*. Southern blot hybridization and PCR techniques were used to screen a panel of 18 additional gram-negative bacterial species for *corA* homologs. Virtually all strains tested positive for the presence of *corA*. Thus, *corA* appears to be ubiquitous within gram-negative bacteria and is likely their major Mg^{2+} influx system.

Salmonella typhimurium contains three Mg^{2+} transport systems, CorA, MgtA, and MgtB (3, 4, 13, 14). MgtA and MgtB belong to the P-type ATPase superfamily (8, 14, 15), and their expression is normally repressed (11, 14). In contrast, CorA is constitutively expressed and is the dominant Mg^{2+} influx system under laboratory growth conditions (13, 14). The *corA* locus encodes a single membrane protein of 37 kDa whose N-terminal region of about 235 amino acids forms a single periplasmic domain and whose C-terminal region of 80 residues has only three membrane-spanning regions (12). CorA lacks homology to any currently known protein.

CorA also mediates influx of Co^{2+} and Ni^{2+} (13), and consequently *corA* mutations confer Co^{2+} resistance, a phenotype also described in *Rhodobacter capsulatus* and *Bacillus subtilis* (10–13). This suggests that CorA might form the major Mg^{2+} transport system of the eubacteria (7, 13, 16), a hypothesis that we have addressed with Southern blot hybridization and PCR techniques.

Strains used are listed in Table 1. Culture conditions were the same as those described in standard references (1, 5), and all media were from Difco. Enzymes were from Life Technologies, Inc. (Gibco-BRL, Gaithersburg, Md.), Stratagene (La Jolla, Calif.), and U.S. Biochemicals Inc. (Cleveland, Ohio). PCR reagents were obtained from Perkin-Elmer (Norwalk, Conn.), and synthetic oligodeoxynucleotide primers were obtained from Oligos Etc. (Wilsonville, Ore.).

Genomic DNA was prepared as described by Ausubel et al. (2), and plasmid DNA was prepared with the Wizard mini prep DNA isolation system (Promega, Madison, Wis.). A probe consisting of bp 32 to 938 of the *corA* coding sequence was prepared by PCR from plasmid pRS117 (12) and labeled with biotin-14-dATP with a Bioprime labeling kit (Gibco-BRL). For Southern hybridizations, agarose-purified fragments of genomic DNA (10 μg) digested to completion with *Eco*RI were transferred to neutrally charged nylon membranes (Schleicher and Schuell, Keene, N.H.) and covalently bound by UV cross-linking at 12,000 μJ/cm^2 (2). Membranes were preincubated in hybridization solution (9) containing 50% (vol/vol) formamide for 4 h at 42°C. Biotinylated probe was denatured by boiling and was added to hybridization buffer containing 5% (wt/vol) dextran sulfate, 50% (vol/vol) formamide, and 20 mM sodium phosphate buffer (pH 6.5) before overnight incubation at 42°C. Low-stringency washes were conducted at 42°C, while high-stringency washes were conducted at 60°C. Detection of the biotinylated probe involved strepta-

TABLE 1. Summary of genomic Southern hybridization and PCR analyses for the presence of *corA*

Division and organism[a]	Hybridization stringency[b]	PCR analysis result[b]	
		PCR	Southern hybridization
γ Division			
S. typhimurium	High	+	+
Escherichia coli	High	+	+
Enterobacter aerogenes	High	+	+
Citrobacter freundii	High	+	+
Klebsiella pneumoniae	High	+	+
Serratia marcescens[c]	High	+	+
Yersinia enterocolitica	High	+	+
Alteromonas haloplanktis	High	+	+
Providencia stuartii	High	+	+
Pseudomonas aeruginosa	High	+	+
Proteus vulgaris	High	+	+
β Division			
Alcaligenes faecalis	High	+	+
Neisseria flavescens	None	NT	NT
N. sicca[c]	NT	+	NT
δ/ε Division			
Helicobacter pylori	Low	+	+
Campylobacter jejuni	None	+	+
Myxococcus xanthus	None	+	+
α Division			
Rhizobium meliloti	None	NT	NT
R. capsulatus[c]	Low	+	+
Paracoccus denitrificans	Low	+	+
Streptococcus faecalis	NT	+	+

[a] *S. typhimurium* and *Escherichia coli* were from this laboratory. *R. capsulatus* and *Paracoccus denitrificans* were provided by J. Gott, *Helicobacter pylori* was provided by S. Czinn and T. Blanchard, *Alteromonas haloplanktis* was obtained from the American Type Culture Collection, and *Yersinia enterocolitica* 8081c was provided by R. Jeter. A DNA sample from *Rhizobium meliloti* was provided by J. Gott, and *Myxococcus xanthus* DNA was provided by R. Maurer and T. Cardaman. The remaining strains were obtained from the Texas Tech University culture collection.

[b] NT, not tested; High, Southern hybridization to genomic DNA positive at high stringency (60°C); Low, Southern hybridization to genomic DNA positive at low stringency (42°C); None, Southern hybridization to genomic DNA negative; +, PCR product of appropriate size observed which hybridized to the *corA102* probe.

[c] Partial sequence was obtained from the 900-bp PCR product and was homologous to that of *S. typhimurium corA*.

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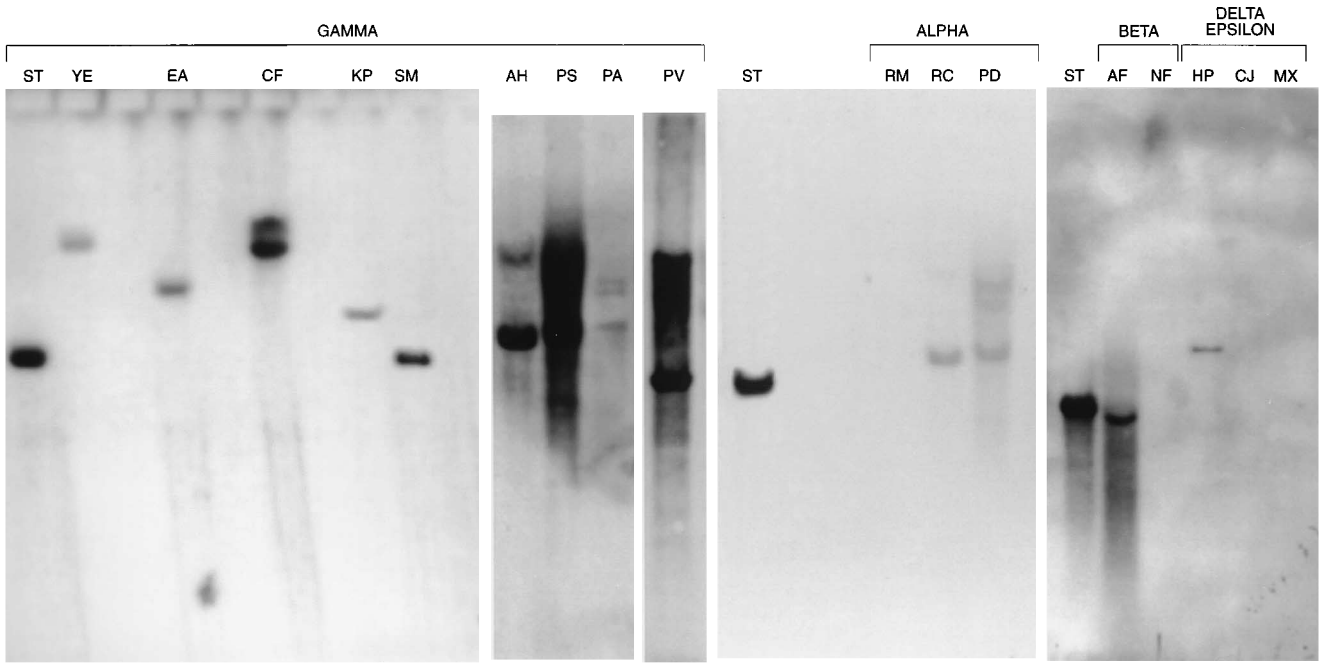

FIG. 1. CorA distribution in the γ division of the gram-negative bacteria. DNA preparation, Southern hybridizations, and data analysis were performed as described in the text and elsewhere (3, 12). Abbreviations for bacterial strains are as follows: ST, *S. typhimurium*; YE, *Yersinia enterocolitica*; EA, *Enterobacter aerogenes*; CF, *Citrobacter freundii*; KP, *Klebsiella pneumoniae*; SM, *Serratia marcescens*; AH, *Alteromonas haloplanktis*; PS, *Providencia stuartii*; PA, *Pseudomonas aeruginosa*; PV, *Proteus vulgaris*; RM, *Rhizobium meliloti*; RC, *R. capsulatus*; PD, *Paracoccus denitrificans*; AF, *Alcaligenes faecalis*; NF, *Neisseria flavescens*; HP, *Helicobacter pylori*; CJ, *Campylobacter jejuni*; and MX, *Myxococcus xanthus*. Hybridization bands were detected after high- (60°C) or low (42°C)-stringency washes as noted in Table 1. The blots shown are from three different experiments representative of at least five blots with each species. Additional *S. typhimurium* bands shown were positive controls in the specific experiments depicted.

vidin-conjugated alkaline phosphatase and the Photogene nucleic acid detection system (Gibco-BRL).

Genomic DNA (0.25 to 1.0 μg) was amplified by PCR by the Perkin-Elmer Gene-Amp protocol with the addition of 5% (vol/vol) formamide, 4.5 μg of nuclease-free bovine serum albumin, and primers (1.0 μM each). Reaction mixtures were overlaid with 2 drops of mineral oil, incubated for 10 min at 95°C, followed by 2 min at 45°C, and then subjected to 40 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min in a Perkin-Elmer DNA Thermal Cycler. *S. typhimurium* genomic DNA was the positive control, and bacteriophage λ DNA, no template DNA, and *S. typhimurium* genomic DNA without primers were negative controls. No amplification products were observed in any negative control reaction. Gels were scanned at 300 dots per inch on a Hewlett-Packard Scanjet IIcx scanner. Contrast of the digitized data was adjusted with Adobe Photoshop, version 2.5.

Molecular and physiological comparisons and especially sequence analysis of rRNA have led to the grouping of the gram-negative bacteria into several divisions. The bacterial species in this study were selected from the α, β, γ, and δ/ε phylogenetic divisions of gram-negative bacteria as described by Woese and colleagues (7, 16). Within the γ division, the *corA102* probe hybridized under high-stringency conditions to genomic DNA from all 11 species examined (Fig. 1 and Table 1). In the β division, *Alcaligenes faecalis* genomic DNA hybridized to the *corA102* probe at high stringency but *Neisseria flavescens* and *Neisseria sicca* failed to do so. In the δ/ε group, *Helicobacter pylori* hybridized at low stringency but neither *Campylobacter jejuni* nor *Myxococcus xanthus* hybridized. Similarly, in the α division, both *R. capsulatus* and *Paracoccus denitrificans* hybridized to the probe at low stringency but no hybridization was seen with DNA from *Rhizobium meliloti*. Overall, these data suggest that a *corA* homolog is common in the γ division and represented widely throughout the gram-negative bacteria.

The ability to detect a homologous gene by DNA hybridization techniques is inherently dependent upon the detection limit of the label, the effective concentration of the target sequence, and a variety of factors affecting the quality of the genomic DNA (6). A negative result might therefore be methodological in origin rather than a reflection of the absence of the *corA* allele. Consequently, we used PCR to amplify sequences homologous to *corA* which were then confirmed by Southern blot hybridization.

Primers complementary to the *S. typhimurium corA* allele and located near the termini of the coding region were tested for the ability to amplify products from genomic DNA templates. The resulting product should contain 95% of the *corA* structural gene. All 18 organisms tested produced a PCR product of approximately 900 bp (Fig. 2; Table 1). No amplification products were observed in reactions using bacteriophage λ DNA as the template or in control reactions containing no DNA template or without primers. To confirm that the PCR products were *corA* homologs, gels containing the PCR products were tested by Southern blot hybridization against the *corA102* probe. In all cases, hybridization to the band at about 900 bp was observed. In some species, other amplification products also hybridized, particularly a band of about 600 bp, suggesting that these bands also may contain a portion of the *corA* sequence. This result is likely explained by the physical properties of the primers, whose predicted annealing temperatures differ by about 30°C, a situation which lends itself to mispriming. As a control, a second set of primers, predicted to

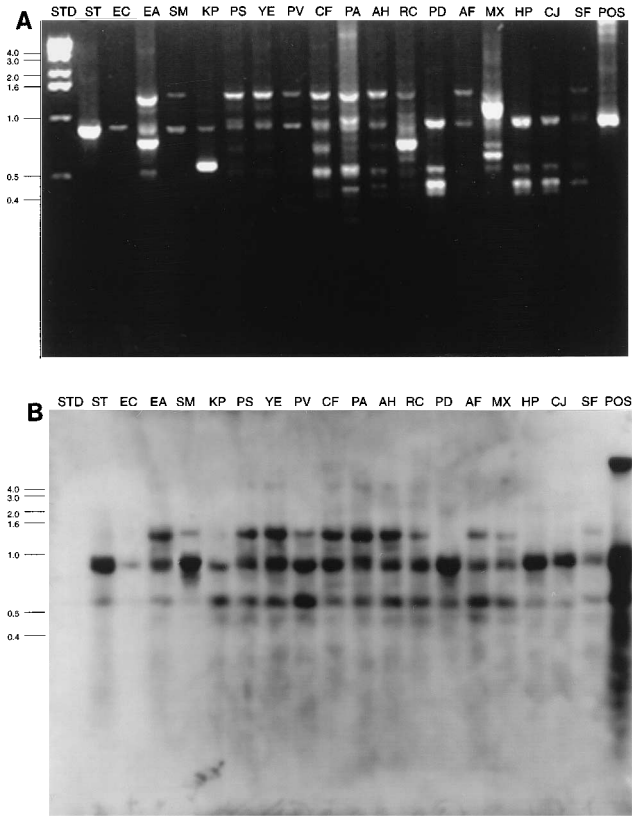

FIG. 2. PCR products from various bacterial species obtained with *corA* primers. (A) PCRs from genomic DNA from each species were run as described in the text and visualized with ethidium bromide. Abbreviations used are those described in the legend to Fig. 1, with the following additions: STD, molecular weight standards; EC, *Escherichia coli*; SF, the gram-positive bacterium *Streptococcus faecalis*; and POS, the *corA102* probe DNA used as a positive control. (B) Southern hybridization was performed as described in the text with the *corA102* probe on the same gel shown in panel A. Abbreviations used are those described in the legend to Fig. 1.

give a band of about 820 bp within the coding region and with identical annealing temperatures, was used. These primers were able to amplify a product of the correct size from six species in various divisions; in addition, the bands at about 820 bp in these reactions hybridized to the *corA102* probe (data not shown). A few species, however, did not yield products with this second primer pair, suggesting sequence divergence between species. Finally, to validate further the PCR data, partial sequence was obtained from the 900- and 600-bp PCR products from *Serratia marcescens* (γ), *N. sicca* (β), and *R. capsulatus* (α). In each case, the DNA sequence confirmed that the bands were *corA* homologs. In *S. marcescens*, for example, the amino acid and nucleotide sequences were about 65% and 85% identical, respectively, over the region examined.

The data presented here indicate that a *corA*-like sequence is present in each of the species tested. The conditions of relatively high stringency in the genomic hybridization experiments, the presence of products of the appropriate length resulting from all PCR regimens, the ability of two distinct sets of primers to elicit PCR products of the predicted size, the ability of the PCR products to hybridize under conditions of high stringency with the *S. typhimurium corA102* probe, and the partial sequence data all indicate strongly that the sequences detected represent true *corA* homologs. Thus, we conclude that *corA* is essentially ubiquitous in the gram-negative bacteria.

The species tested represent bacteria from a variety of classes and environments. For example, while most of the bacteria tested live in an environment relatively low in Mg^{2+}, *Alteromonas haloplanktis* is a marine organism that grows in a medium containing 50 mM Mg^{2+}. Additional research (unpublished data) indicates the presence of *corA* homologs in several gram-positive species, including *Streptococcus faecalis* (Table 1) and *Bacillus thuringiensis*. Phenotypic evidence of a CorA-like Mg^{2+} uptake system in *Bacillus subtilis* has also been adduced (10). Consequently, it seems probable that CorA is present in all eubacteria and that it forms their dominant system for Mg^{2+} influx.

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