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The *Salmonella typhimurium mar* Locus: Molecular and Genetic Analyses and Assessment of Its Role in Virulence

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The *marRAB* operon is a regulatory locus that controls multiple drug resistance in *Escherichia coli*. *marA* encodes a positive regulator of the antibiotic resistance response, acting by altering the expression of unlinked genes. *marR* encodes a repressor of *marRAB* transcription and controls the production of MarA in response to environmental signals. A molecular and genetic study of the homologous operon in *Salmonella typhimurium* was undertaken, and the role of *marA* in virulence in a murine model was assessed. Expression of *E. coli marA* ($marA_{Ec}$) present on a multicopy plasmid in *S. typhimurium* resulted in a multiple antibiotic resistance (Mar) phenotype, suggesting that a similar regulon exists in this organism. A genomic plasmid library containing *S. typhimurium* chromosomal sequences was introduced into an *E. coli* strain that was deleted for the *mar* locus and contained a single-copy *marR'-'lacZ* translational fusion. Plasmid clones that contained both *S. typhimurium marR* ($marR_{St}$) and *marA* ($marA_{St}$) genes were identified as those that were capable of repressing expression of the fusion and which resulted in a Mar phenotype. The predicted amino acid sequences of $MarR_{St}$, $MarA_{St}$, and $MarB_{St}$ were 91, 86, and 42% identical, respectively, to the same genes from *E. coli*, while the operator/promoter region of the operon was 86% identical to the same 98-nucleotide-upstream region in *E. coli*. The *marRAB* transcriptional start sites for both organisms were determined by primer extension, and a $marRAB_{St}$ transcript of ~1.1 kb was identified by Northern blot analysis. Its accumulation was shown to be inducible by sodium salicylate. Open reading frames flanking the *marRAB* operon were also conserved. An *S. typhimurium marA* disruption strain was constructed by an allelic exchange method and compared to the wild-type strain for virulence in a murine BALB/c infection model. No effect on virulence was noted. The endogenous *S. typhimurium* plasmid that is associated with virulence played no role in *marA*-mediated multiple antibiotic resistance. Taken together, the data show that the *S. typhimurium mar* locus is structurally and functionally similar to $marRAB_{Ec}$ and that a lesion in $marA_{St}$ has no effect on *S. typhimurium* virulence for BALB/c mice.

In *Escherichia coli*, resistance to diverse classes of antibiotics is associated with mutations at the multiple antibiotic resistance (*mar*) locus located at 34 min on the *E. coli* genetic map (21). *mar* mutants display decreased susceptibility to a number of antibiotics, including chloramphenicol, tetracycline, β-lactams, and quinolones (14, 20). Apparently, reduced accumulation accounts, at least in part, for this resistance, through activation of efflux pumps coupled with decreased synthesis of outer membrane porins (14, 15, 20, 34, 38). Mutations contributing to high-level quinolone resistance in *E. coli* include those located at *mar* (29), and a multiple antibiotic resistance (Mar) phenotype has been observed in clinical quinolone-resistant gram-negative organisms (28), suggesting that nonspecific resistance systems may play a role in the outcome of antibiotic therapy for this class of compounds.

Genes encoded at the *mar* locus that are involved in multidrug resistance have been identified. A 1.1-kbp *marRAB* operon regulates multiple antibiotic resistance (12, 19, 36) by controlling expression of at least 10 unlinked genes (22, 44, 48, 51), two of which, *acrA* and *acrB*, are absolutely required for resistance (44). The *marRAB* operon consists of (i) an operator-promoter region (*marO*) from which divergent transcripts are generated; (ii) *marR*, encoding a repressor of the *marRAB* operon (12, 50, 53); (iii) *marA*, encoding a positive transcriptional regulator of unlinked genes whose modulation affects antibiotic resistance (12, 15, 19, 21, 22, 30); and (iv) *marB*, a gene of unknown function. Previously characterized *mar* mutants contain mutations in either *marR* or *marO*, which disrupt autorepression and lead to increased expression of *marA* and, consequently, activation of the regulon (2, 12, 19, 36).

The *marRAB* operon is induced by a variety of chemical agents, including tetracycline, chloramphenicol, menadione, phenazine methosulfate, paraquat, plumbagin, dinitrophenol, carbonyl cyanide *m*-chlorophenylhydrazone, benzoate, acetaminophen, and sodium salicylate (SAL) (12, 13, 25, 50). Among these, induction of *marRAB* by SAL has been most studied. *E. coli* grown in the presence of SAL displays a condition of phenotypic antibiotic resistance (Par) that is similar to the Mar phenotype of *mar* mutants (47). Since SAL binds purified MarR repressor and enhances dissociation of MarR-*marO* complexes in vitro (37), it is thought that, in vivo, repression is relieved and activation of *marRAB* expression results from the direct interaction of SAL with the repression complex.

Since there are few informative mutations in the *E. coli marR* ($marR_{Ec}$) and $marA_{Ec}$ genes, comparing these genes with functionally related homologs could provide additional information about how these genes and their products carry out their roles in the *mar* system. Toward this end, we have determined, by genetic and molecular methods, the extent to which the *Salmonella typhimurium mar* locus is related to its counterpart in *E. coli.*

We also addressed the question of whether *marA* is a virulence factor for animal infection. A number of observations

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype or phenotype	Source or reference
Strains		
E. coli		
MC4100	F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR thi*	T. Silhavy (9)
B318	MC4100 *zdd-230*::Tn*9* Cm^r Δ1738 (Δ*mar*) Φ(*marR′-′lacZ*)	53
DPWC	*supE42* Δ*recA* [*Sst*II-*Eco*RI] *srl*::Tn*10*-[Tet^s], F$^+$::γδ	Gold Biotechnologies (52)
DH5α	*endA hsdR17 supE44 thi-1* λ$^-$ *recA1 gyrA96 relA1* ϕ80d*lacZ*ΔM15	Gibco-BRL
S. typhimurium		
LB5010	LT2 derivative; *hsdLT*(r$^-$ m$^+$) *hsdSA*(r$^-$ m$^+$) *hsdSB*(r$^-$ m$^+$) *metA22 metE551 trpD2 leu val rpsL120 galE*	J. Vandenbosch (8)
CR6600	Strain TML, mouse virulent	J. Vandenbosch (31)
CR8500	FIRN biotype, mouse virulent	J. Vandenbosch (31)
χ3181	pStSR101 100-kb plasmid, mouse virulent	P. Gulig (24)
PD188	χ3181 *marA*::*kan* (pStSR101)	This study
χ3306	χ3181 *gyrA1816* (pStSR101), mouse virulent	P. Gulig (24)
PD483	χ3306 *marA*::*kan* (pStSR101)	This study
χ3337	χ3306 plasmid cured	P. Gulig (24)
PD485	χ3337 *marA*::*kan*, plasmid cured	This study
Plasmids		
pMOB	Ap^r; smaller derivative of pUC vectors for γδ transposon mutagenesis	Gold Biotechnologies (52)
pUC4K	Ap^r Km^r; vector containing kanamycin resistance gene cassette	Pharmacia Biotech Inc.
p144	Ap^r; $marR_{St}$ insert in pBluescript SK from χ3181 plasmid library	This study
p145	Ap^r; $marRAB_{St}$ insert in pBluescript SK from χ3181 plasmid library	This study
p146	Ap^r; $marR_{St}$ insert in pBluescript SK from χ3181 plasmid library	This study
p147	Ap^r; $marRAB_{St}'$ insert in pBluescript SK from χ3181 plasmid library	This study
p151	Ap^r; $marR_{St}$ insert in pBluescript SK from χ3181 plasmid library	This study
p197	Ap^r; χ3181 $marRAB_{St}$ insert from p147 in pMOB	This study
p9	Ap^r; 2.2-kbp *E. coli marAB* insert in pBR322; *marAB* expressed from the tetracycline promoter	19
pBR322	Ap^r Tc^r; cloning vector	6
pBluescript SK$^-$	Ap^r; cloning vector	Stratagene
pIB276	Ap^r; pUC18 containing *Bacillus subtilis sacB*	I. Blomfield (5)
pIB307	Cm^r; temperature-sensitive vector	I. Blomfield (5)
pSacts	Cm^r; counter selectable temperature-sensitive vector for allelic exchange	This study
p162	Ap^r; 1.4-kbp kanamycin resistance DNA cassette from pUC4 subcloned into *Age*I site of p147	This study
p185	Cm^r Km^r; p162 *marA*::*kan Xba*I-*Hin*dIII fragment cloned in pSacts	This study

suggested that this was possible. First, MarR is a member of a family of phenolic-sensing regulatory proteins, some of which play a role in virulence (54). Two examples are SlyA, which is necessary for *S. typhimurium* infection of BALB/c mice (33), and PecS, and *Erwinia chrysanthemi* regulatory protein controlling extracellular enzymes involved in soft rot disease of plants (46). Second, in the absence of an inducing agent, *marRAB* is weakly expressed when *E. coli* is cultured on standard laboratory media (12, 13, 25, 39, 50, 53). As the identity of the true inducer of *marRAB* is not known, it is conceivable that it is present at some stage of infection. Third, it is thought that one physiological role for the AcrAB efflux pump is to protect bacteria from bile salts; such detergents are located in the intestinal tract and are toxic to some bacteria (34). Since expression of the genes encoding this pump is increased in *mar* mutants (34), it is possible that up-regulation of *acrAB* mediated by the *mar* locus in vivo results in enhanced protection from bile salts. Thus, a role for *marA* in virulence was investigated.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and general reagents. Bacterial strains and plasmids used in this study as well as their relevant properties are listed in Table 1. Cultures were routinely grown in LB medium (1% Bacto Tryptone, 0.5% Bacto Yeast Extract, 0.5% NaCl [Difco, Detroit, Mich.]) at 37°C unless otherwise noted. Also used was lactose-MacConkey agar medium (Difco). Antibiotics (Sigma, St. Louis, Mo.) were added to selective media at the following final concentrations (unless otherwise noted): ampicillin, 50 μg/ml; kanamycin, 40 μg/ml; and chloramphenicol, 20 μg/ml. Enoxacin (40), a fluoroquinolone, was obtained from an in-house synthesis. SAL was from Sigma and when used was added to a final concentration of 2.5 mM. [α-^{32}P]dATP was from Amersham (Arlington Heights, Ill.).

General molecular biology techniques. All molecular biology methods were performed following established protocols (4) unless otherwise noted. Restriction enzymes were purchased from Boehringer Mannheim (Indianapolis, Ind.) and were used as recommended by the manufacturer. Dephosphorylation of DNA was accomplished by using calf intestinal alkaline phosphatase (Gibco-BRL, Gaithersburg, Md.). DNA was routinely introduced into bacteria by transformation using the TSS method (11) unless otherwise noted. PCR amplifications of DNA were accomplished by using a Perkin-Elmer Cetus 9600 thermocycler and GeneAmp PCR reagent kit, both from Perkin-Elmer (Foster City, Calif.). Custom oligonucleotides were commercially prepared (Gibco-BRL). Southern hybridization analysis was performed under stringent conditions (4), using the Genius system (Boehringer Mannheim). The *marRAB* probe used for Southern analysis was a 0.7-kb *Sal*I-*Pvu*II DNA fragment from p9 (corresponds to nucleotides 1641 to 2341 of the published *E. coli marRAB* sequence [12]). It contains the 3′ half of *E. coli marR*, the entire *marA*, and 27 nucleotides of the 5′ of *marB*. The probe was hybridized to *Bam*HI-digested plasmid DNA following electrophoresis on a 0.8% agarose gel and transfer to a Nytran membrane (Schleicher & Schuell, Keene, N.H.).

Genomic library construction. The pBluescript SK$^-$ *S. typhimurium* χ3181 plasmid library was constructed as follows. A partial *Sau*3A digest of chromosomal DNA was electrophoresed through a 0.8% agarose–Tris-borate-EDTA gel. A gel slice containing 3- to 8-kbp DNA fragments was excised, and the DNA purified by using a Qiaex gel extraction kit (Qiagen, Chatsworth, Calif.). This DNA was ligated to *Bam*HI-digested and dephosphorylated pBluescript SK$^-$ plasmid DNA and transformed into library-efficiency *E. coli* DH5α competent cells (GIBCO-Life Technologies Inc., Gaithersburg, Md.) as instructed by the

manufacturer. Ampicillin-resistant transformants were selected. More than 3,000 independent colonies were combined, and plasmid DNA was purified from the pool and stored at −20°C.

γδ transposon mutagenesis. Cloned DNA containing presumptive *marR* and *marA* was mutagenized by the procedure of Strathmann et al. (52), using a commercially available Tn1000 kit (Gold Biotechnologies, Inc., St. Louis, Mo.). The 2.5-kbp *Sal*I-*Xba*I DNA segment from p147 was subcloned into the similarly digested pMOB, yielding p197. This plasmid was subjected to insertion mutagenesis. B318 (Table 1) was used as the recipient in the required conjugation event in order to directly determine the genetic locations of γδ transposon insertions. The physical locations and orientations of γδ insertions in p197 were determined relative to the multiple cloning site (MCS) by PCR, using one of the two γδ orientation-specific primers (G186 and G187; supplied by Gold Biotechnologies) and the plasmid-specific T7 primer located adjacent to the MCS (AATACGA CTCACTATAG). The G186 primer corresponds to positions 5923 to 5946 in γδ; the G187 primer corresponds to positions 62 to 38 in γδ. γδ coordinates are based on the nucleotide sequence determined by Broom et al. (7 [GenBank accession no. X60200]).

DNA sequence analysis and computer methods. The dideoxy-chain termination method of Sanger (49) was employed, using a Sequenase version 2.0 kit (U.S. Biochemicals, Cleveland, Ohio). Also used were a PRIZM Ready Reaction Dyedeoxy Terminator cycle sequencing kit and a PRIZM Sequenase Terminator double-stranded DNA sequencing kit (Perkin-Elmer) in conjunction with an Applied Biosystems 373 automated DNA sequencing system (Perkin-Elmer). Analyses of nucleotide and amino acid sequences were performed on a VAX computer (Digital Equipment Corp., Marlborough, Mass.), using the GCG (17) package (Genetics Computer Group Madison, Madison, Wis.), BLAST protein database search (1) through the Internet, and MacVector software (International Biotechnologies Inc., New Haven, Conn.) operating on a Macintosh computer. Estimation of conserved amino acid residues was based on the following groupings: YWF, QNED, MILV, PAGST, and HKR.

Northern (RNA hybridization) analysis. Total RNA from logarithmic-phase cultures grown in LB medium was prepared by the method of Chomczynski and Sacchi (10), using the TRIZOL reagent (GIBCO-Life Technologies) as specified by the manufacturer. SAL-treated cells were prepared by addition of SAL (final concentration of 2.5 mM) to the culture suspension 1 h prior to harvesting RNA. [α-^{32}P]dATP-labeled probes for Northern blots were prepared from PCR-generated DNA fragments that served as templates in reactions using the Random-Primers DNA labeling system (GIBCO-Life Technologies). (Some primers used for PCR amplification contained mismatched bases at their 5′ ends, which were included to introduce restriction sites for separate subcloning experiments.) The *E. coli marRA* probe was prepared by PCR amplification of genomic DNA from MC4100, using the following pair of primers: PM74 (CATTGGGTCGCTTAA TCC), which corresponds to nucleotides 1482 to 1499 of the published *E. coli mar* sequence (12), and PM85 (GCCTCAGTGACGTTGTCACGT), located from nucleotides 2309 to 2289 (12). The *E. coli marC* probe was prepared similarly; a 0.7-kbp DNA fragment corresponding to nucleotides 547 to 1239 was obtained by using primers MCS34 (CCACCCATGGTAGATTTGTTTAAAGC AAT), located from nucleotides 1239 to 1211 (12), and MCS35 (CGAGGATC CGAAAGGCCCATTCGGGCCTTT), located from nucleotides 547 to 567 (12). *S. typhimurium* X3181 probes were prepared differently. PCR amplification of *mar* locus sequences was carried out by using specific γδ-mutagenized derivatives of p197 as templates and a pair of DNA primers, one specific for *mar* sequences and the other corresponding to the left end of γδ (G187 [GTATTA TAATCAATAAGTTATACC]). For the *marORA* probe, PCR of plasmid template p197::γδ#33 (see Fig. 2 for the location of insertion 33) with primers MCS1 (GGCGGGAAACAGCATTTTCATGGT) and G187 resulted in a 0.9-kbp fragment containing sequences from positions 999 to 1871 (see Fig. 2). For the ORF221 probe, PCR amplification of template p197::γδ#66 (see Fig. 2, insertion 66) with primers MCS36 (CCATGGTGGATTTGTTTAAAGCGATT) and G187 yielded the predicted 0.65-kbp segment corresponding to nucleotides 64 to 722. The PCR products were then used to generate radioactively labeled probes as described above. The two *S. typhimurium* DNA probes thus contain 64 bp of γδ DNA sequence due to the use of oligonucleotide G187 in their syntheses. Significantly, no common secondary bands were detected by either probe in Northern blot analysis of *Salmonella* RNA, indicating that the additional γδ sequences do not generate spurious background signals. Northern blot analysis involved hybridization of probes under stringent conditions to RNA that was fractionated by agarose-formaldehyde electrophoresis and immobilized on Nytran by standard procedures (4).

Primer extension. RNA (30 μg) from SAL-treated cells was used in a primer extension reaction (4) with oligonucleotides described below and avian myeloblastosis virus reverse transcriptase (GIBCO-Life Technologies). The oligonucleotide primer for *E. coli* was MCS32PE (GATACTCGTTAAGCAGGCGAT CTTTCTTCTGATT), corresponding to nucleotides 1541 to 1508 of the *E. coli* sequence (12); the 5′ end of this primer is located 96 bp downstream of the *marR* GTG start codon. For *S. typhimurium*, the primer used was MCS30PE (GCGG GGATAAATAGTTATTTAACAGGCGATCTTT), corresponding to nucleotides 1090 to 1057 in Fig. 1, the 5′ end of which lies 115 bp downstream of the putative *marR* TTG start codon. Oligonucleotides were end labeled by using [γ-^{32}P]ATP (Amersham) and T4 polynucleotide kinase (Boehringer Mannheim) as described previously (4).

Construction of *S. typhimurium* χ3181 *marA*::*kan*. An *S. typhimurium marA*::*kan* strain was produced by allelic exchange using pSacts, a temperature-sensitive, counterselectable plasmid derivative of pSC101 whose construction is described below. p147 (Table 1), carrying the $marR_{St}$ and $marA_{St}$ genes, was digested with *Age*I, which cleaves in the middle of *marA*. This site was blunt ended with T4 DNA polymerase and ligated to the agarose-gel purified 1.6-kbp *Pst*I fragment of pUC4K, containing a kanamycin resistance determinant. This produced plasmid p162. pSacts was constructed by ligation of a *Bam*HI-*Xho*I DNA fragment of pIB276 (5), containing *sacB*, with the *Bam*HI- and *Sal*I-digested pIB307 (5) (both restriction sites located in the MCS). pSacts was then digested with *Pst*I and blunt ended with T4 DNA polymerase. The *Xba*I-*Hin*dIII fragment from p162 containing *marA*::*kan* was similarly rendered blunt ended and ligated to pSacts, resulting in plasmid p185. This plasmid was introduced first into *S. typhimurium* LT2 and subsequently into χ3181 by standard transformation protocols. Allelic exchange of *marA*::*kan* form p185 into the chromosome was accomplished by a modification of the methods of Hamilton et al. (26) and Blomfield et al. (5). χ3181(p185) was grown at 30°C to stationary phase, and dilutions were plated out at 44°C on prewarmed LB agar containing kanamycin and chloramphenicol. After overnight incubation, surviving colonies were presumed to have the plasmid integrated in the chromosome. Independent colonies were inoculated into LB medium containing kanamycin and incubated at 30°C overnight; they were then diluted 1:50 in LB medium and grown 6 h at 44°C and finally plated onto prewarmed (to 44°C) solid medium containing 1% tryptone, 0.5% yeast extract, 6% sucrose, and kanamycin. Sucrose-resistant survivors that were kanamycin resistant and chloramphenicol sensitive were presumed to be cured of the plasmid. Successful allelic exchange was verified by PCR amplification, using a pair of oligonucleotide primers that flank *marA*, MCS1 (see above) and MS93 (CAACGACCAGGGCAAGACCTGCAT), whose sequences correspond to nucleotides 1871 to 1848 and 1321 to 1344, respectively, in Fig. 2. The χ3181 *marA*::*kan* strain was designated PD188.

Antibacterial susceptibility testing. Comparison of antibiotic susceptibility levels was performed by using gradient plates as described previously (19). Results are expressed as percent growth across the gradient.

Virulence studies. Female, 31- to 53-day-old BALB/c mice (Charles River, Bar Harbor, Maine) were used for animal experiments. Inoculation was done as described by Gulig and Curtiss (24). Briefly, mice were starved for 6 h prior to oral inoculation with 50 μl of 10% sodium bicarbonate and 500 μl of bacteria suspended in saline containing 0.1% gelatin. The 50% lethal dose (LD_{50}) was determined by the method of Reed and Muench (45), using four to five mice per bacterial challenge dose.

Nucleotide sequence accession number. The GenBank accession number for the nucleotide sequence shown in Fig. 2 is U54468.

RESULTS

Analysis of phenotypic antibiotic resistance in *S. typhimurium*. SAL induces Par in *E. coli* (47), in part by activation of the *marRAB* operon. Representative *S. typhimurium* strains, including the avirulent LT2 derivative LB5010 along with the mouse-virulent strains χ3181, CR8500, and CR6600, were assessed for a SAL-inducible Mar phenotype. SAL was found to induce Par in *S. typhimurium*. In the presence of 2.5 mM SAL, all strains displayed increased resistance to two distinct antibiotics, chloramphenicol and enoxacin (Table 2). In addition, the mouse-virulent strains were at least threefold more resistant than was the avirulent LB5010. Since *E. coli* and *S. typhimurium* are closely related, we wondered if $marA_{Ec}$, when introduced on a multicopy plasmid into *S. typhimurium*, could promote increased resistance to these same antibiotics, as has been observed in *E. coli*. As shown in Table 2, plasmid p9 containing $marA_{Ec}$ expressed from the pBR322 tetracycline promoter (19) induced antibiotic resistance in all *S. typhimurium* strains, indicating that a MarA-regulatable resistance system exists in this species. Interestingly, the mouse-virulent strains containing p9 showed a greater increase in antibiotic resistance than did the *E. coli* and *S. typhimurium* laboratory strains.

Cloning of the *S. typhimurium mar* operon by complementation. The ability of $marA_{Ec}$ to function in *S. typhimurium* suggested that *marA*, and very likely *marR*, from either organism would be functional in both genera. Based on that assumption, a previously described (54) genetic approach, was used in the cloning of the *S. typhimurium* χ3181 *marRAB* genes. Briefly, *E. coli* B318 contains the Δ1738 deletion (27), which

TABLE 2. Induction of antibiotic resistance in *E. coli* and *S. typhimurium* by SAL and plasmid p9 expressing *marA*

Strain	Relevant feature[b]	Plasmid[c]	Growth (% of gradient)[a]			
			Chloramphenicol (0–50 μg/ml)		Enoxacin (0–0.6 μg/ml)	
			−SAL	+SAL	−SAL	+SAL
E. coli						
MC4100	Standard lab strain	pBR322	<5	18	<10	61
MC4100	Standard lab strain	p9	65		83	
S. typhimurium						
LB5010	Standard lab strain	pBR322	<5	<5	30	77
LB5010	Standard lab strain	p9	60		>95	
χ3181	Mouse virulent	pBR322	<5	46	65	>95
χ3181	Mouse virulent	p9	>95		>95	
CR8500	Mouse virulent	pBR322	<5	57	34	>95
CR8500	Mouse virulent	p9	>95		>95	
CR6600	Mouse virulent	pBR322	<5	62	35	>95
CR6600	Mouse virulent	p9	>95		>95	

[a] Extent of growth across a gradient of chloramphenicol or enoxacin.
[b] Demonstration of mouse virulence for the strains used was from Gulig and Curtiss (24) and Jones et al. (31).
[c] Transformants containing pBR322 have antibiotic resistance patterns identical to that of the plasmidless parent.

removes ~39 kb of DNA including *marRAB*; it is also lysogenic for a recombinant lambda phage containing a *marR'-'lacZ* protein fusion under the control of the *mar* operator/promoter region. Expression of the fusion is constitutive (Lac^+) due to the absence of a source of MarR repressor. Introduction of a plasmid containing *marR* results in repression of the *mar* operon and a Lac^- phenotype, identified as white colonies on lactose-MacConkey indicator medium. The presence of *marA* on the same plasmid confers increased antibiotic resistance and restores SAL-inducible antibiotic resistance. Accordingly, B318 was used as a reporter strain for the detection of plasmids carrying the *S. typhimurium marR* and *marA* genes. A plasmid library consisting of random χ3181 chromosomal DNA was introduced into this strain, and nine white (Lac^-) colonies were obtained. Plasmid DNA was isolated from the nine transformants and analyzed by Southern blot analysis for the presence of *mar* sequences, using an *E. coli marRAB* probe. Five clones gave positive signals (data not shown). Of the five, only two (p145 and p147) also restored SAL-inducible antibiotic resistance in B318. Both B318(p145) and B318(p147) exhibited an almost fourfold increase in intrinsic enoxacin resistance over that of the plasmidless control strain B318. In addition, SAL-induced resistance to enoxacin was more than threefold higher than in the control. Taken together, these results suggested that we had isolated five recombinant plasmids that contained $marR_{St}$, and that two of these (p145 and p147) also had a functional $marA_{St}$ gene.

Genetic analysis of the *S. typhimurium mar* locus. γδ transposon mutagenesis was used to identify those sequences contained within the p147 DNA insert fragment that encoded the $marR_{St}$ and $marA_{St}$ activities (52). As described in Materials and Methods, the 2.5-kb DNA insert fragment from p147 was subcloned into pMOB yielding p197, and this plasmid was subsequently mutagenized. We identified 96 plasmids that contained transposon insertions spanning the entire cloned DNA insert in p197, and their locations were mapped relative to the MCS. By monitoring the Lac and Par phenotypes of B318 transformants carrying the γδ-mutagenized plasmids, complementation units for the presumptive $marR_{St}$ and $marA_{St}$ genes were identified. Insertions in an ~400-bp region (located from bp 1002 to 1392 on the linear map in Fig. 1) abolished the ability of p197 to complement the *mar* deletion in B318 for both repression of the *marR'-'lacZ* fusion as well as stimulation of antibiotic resistance. A second set of insertions that were clustered in an adjacent 400-bp segment disrupted antibiotic resistance enhancement without affecting repression of the *marR'-'lacZ* fusion. These could be interpreted, by analogy to the *E. coli* system, as evidence for the second cluster of insertions lying in $marA_{St}$ and the first residing in $marR_{St}$ or adjacent promoter regions, with a polar effect on expression of the downstream $marA_{St}$ gene. All other insertions had no effect on either phenotype.

DNA sequence analysis of the *S. typhimurium mar* locus. The DNA sequences of both strands of portions of the DNA inserts from p197 and p145 were determined (Fig. 2). Five ORFs were identified in the 2,337-nucleotide sequence. They are homologous to the *E. coli marRAB* genes and flanking ORFs described by Cohen et al. (12). The products of ORF144, ORF129, and ORF71 have significant amino acid sequence similarity to *E. coli* MarR, MarA, and MarB, respectively, indicating that these ORFs are the $marRAB_{St}$ genes. Specifically, the product of ORF144 shows 91% amino acid sequence identity to $MarR_{Ec}$ and is the same length. ORF129 begins 13 nucleotides downstream from the stop codon of ORF144, and the predicted amino acid sequence of its product is 94% identical to $MarA_{Ec}$. Predicted helix-turn-helix DNA-binding motifs found in both proteins are identical. ORF71, located 28 nucleotides downstream of ORF129, could encode a protein with 42% amino acid sequence identity (64% similarity when including conserved residues) to $MarB_{Ec}$. In protein database searches, the only significant match to this ORF was $MarB_{Ec}$, suggesting that ORF71 is the $marB_{St}$ gene. A stretch of 98

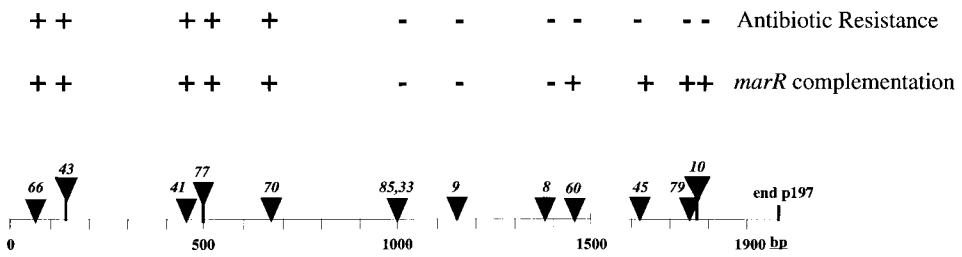

FIG. 1. Locations and associated phenotypes of γδ-transposon insertions in plasmid p197. Base pair coordinates correspond to those in Fig. 1 and are shown by the scale underneath. One end of the cloned *Salmonella* DNA fragment of p197 is indicated. The locations of γδ transposon insertions in p197 are indicated by numbered triangles. Symbols for antibiotic resistance: −, insertions that result in a loss of antibiotic resistance compared to the control B318(p197); +, those that have no effect. Symbols for *marR* complementation: −, insertions that result in a failure to repress the *marR'-'lacZ* fusion in strain B318 compared with the control p197 plasmid; +, those that have no effect.

nucleotide residues located immediately upstream from the $marR_{St}$ coding region (ORF144) is 86% identical with the $marRAB_{Ec}$ operator/promoter region. Within this region, a putative σ^{70} promoter was identified as well as imperfect direct repeats (DR1 and DR1′) that differ by only one nucleotide in DR1′ from similar elements found in the *E. coli* sequence. These direct repeats are the proposed MarR-binding regions in the *E. coli mar* promoter (37). A marbox, a proposed MarA-binding site (35), was found upstream of the putative −35 promoter element; it is 93% identical to its *E. coli* counterpart.

ORF221 and ORFA flank the $marRAB_{St}$ region on either side and are similar to ORFs that neighbor the $marRAB_{Ec}$ operon. ORF221 is located 258 nucleotides upstream of an oriented opposite ORF144 ($marR_{St}$). ORF221 could encode a protein that has 91% amino acid sequence identity with MarC (21a) from *E. coli* (originally proposed to encode two adjacent ORFs, ORF64 and ORF157 [12]) and is similar in location (211 nucleotides upstream) and orientation to $marR_{Ec}$. Database searches also identified an *E. coli* ORF of unknown function located upstream of *adhE* (32) whose product exhibits 74% amino acid sequence similarity to that encoded by ORF221. ORF221 encodes a predicted basic (pI = 9) protein with a hydrophobicity profile that suggests the presence of six membrane-spanning domains (data not shown).

Located 188 nucleotides downstream of ORF71($marB_{St}$) is the C-terminal end of a putative ORF, designated ORFA. Translation of this sequence generates a polypeptide that has 70% residue identity to the C-terminal region of *E. coli* ORF266, which is located downstream of $marB_{Ec}$. Thus, sequence comparison with the *E. coli mar* locus suggests that the $marRAB_{St}$ operon, as well as its surrounding regions, is organized similarly.

The conclusions from the sequence data for $marR_{St}$ and $marA_{St}$ are corroborated by the genetic data described earlier. The γδ insertions that identified the *marR* complementation unit in p197 (insertions 85, 33, 9, and 8) are located in ORF144; those presumed to identify *marA* (insertions 60, 45, 79, and 10) are found in ORF129 (Fig. 2). Clear roles for ORF221 and $marB_{St}$ in either antibiotic resistance or *mar* regulation did not emerge from this analysis. For example, γδ insertions 66, 43, 41, 77, and 70, which are located in ORF221 (Fig. 2), had no effect on the resistance phenotype or regulation of *marR′-′lacZ* (Fig. 1). Truncation of *marB* did not affect either antibiotic resistance or repression of the *marR′-′lacZ* fusion. Sequence analysis of the p147 DNA showed that only two-thirds of *marB* is present on this clone whereas p145 contains the entire *marB* gene (data not shown). Nonetheless, B318 containing either plasmid had the same antibiotic resistance phenotype and reporter fusion expression patterns.

Expression of *marRAB* and ORF221 by Northern analysis. Since SAL induces *marRAB* transcription in *E. coli* (13, 39), we investigated whether this is also true for *S. typhimurium*. Total RNA from *S. typhimurium* χ3181 and *E. coli* MC4100 grown in the absence and presence of SAL was analyzed by Northern blot analysis, using probes specific for *marRA* and ORF221 (Fig. 3). With the $marRA_{St}$ probe, a ~1.1-kbp band was identified from *S. typhimurium* RNA (data not shown). This size is consistent with the predicted size of 1.1 kbp of the $marRAB_{St}$ operon from the putative initiation codon of $MarR_{St}$ to a rho-independent-like stem-loop structure located downstream of $marB_{St}$ (residues 985 to 2091 in Fig. 2). When RNA from the SAL-treated culture was examined, the intensity of this band increased greatly (Fig. 3d). By comparison, a ~1.0-kbp $marRAB_{Ec}$-specific band, identified by a specific *E. coli* probe, was also clearly more intense in the RNA sample obtained from SAL-treated cells than in that obtained from untreated cells (Fig. 3b). The data indicate that SAL induces the transcription of *marRAB* in both species.

In addition, expression of the *S. typhimurium* ORF221 transcript was also induced by SAL, although to a much lesser extent than was *marRAB*. With the *S. typhimurium* ORF221 probe, bands at ~0.7, 1.0, and 1.2 kb increased in intensity in the presence of SAL, indicating some accumulation of mRNAs derived from the ORF221 region (Fig. 3c). By contrast, no marked effect of SAL treatment on induction of $marC_{Ec}$ mRNA was observed (Fig. 3a).

Transcriptional start site of the *marRAB* promoter. To more firmly establish our assignments of promoter regions, the transcriptional start sites for both the *S. typhimurium* and *E. coli marRAB* operons were determined by primer extension analysis. In *E. coli*, a major extension product terminated 27 bp upstream of the GTG start site of *marR*, located at nucleotide 1418 of the sequence reported by Cohen et al. (12) (Fig. 4A, lane 1). Major extension products terminating 29 and 30 bp upstream of the $marR_{St}$ TTG start codon are shown in Fig. 4B, lane 3. These findings indicate that the putative −10 and −35 regions identified by sequence analysis for the *E. coli* (12) and *S. typhimurium* (Fig. 2) *marRAB* operons are the likely promoter elements for both transcription units.

Construction of *S. typhimurium marA* mutant strains: Mar phenotype and virulence studies. An *S. typhimurium* χ3181 *marA*::*kan* strain, PD188, was constructed by the allelic exchange method of Hamilton et al. (26; see Materials and Methods) by using p185, a temperature-sensitive vector containing a counterselectable marker and the *marA*::*kan* allele. Successful allelic exchange was verified by PCR of the allele. Subsequently, the Mar phenotype of this strain was determined. A reduction in SAL-inducible multiple antibiotic resistance was observed (Table 3), as evidenced by increased susceptibility to ampicillin, chloramphenicol, and tetracycline compared to the isogenic $marA^+$ parent strain. These results are similar to those reported for *E. coli* Δ*mar* strains (13, 53). Hypersensitivity to chloramphenicol and tetracycline in this strain in the absence of SAL was not observed.

To determine if *marA* plays an essential role in *S. typhimurium* virulence, per oral LD_{50}s in BALB/c mice for both $marA^+$ and *marA*::*kan* strains were determined. The results of these studies showed the LD_{50}s for both strains to be 60 CFU by the method of Reed and Muench (45). Thus, the absence of a functional *marA* does not diminish *S. typhimurium* virulence in an orally administered infection of BALB/c mice.

The *S. typhimurium* virulence plasmid plays no role in *marA*-mediated antibiotic resistance. Since both SAL treatment as well as a $marA_{Ec}$-overproducing plasmid induce a more robust antibiotic resistance response in *S. typhimurium* virulent strains than in *E. coli* MC4100, it seemed plausible that the *S. typhimurium* virulence plasmid, pStSR101, was responsible for this difference. Therefore, the effectiveness of *marA* in promoting multiple antibiotic resistance was assessed in isogenic strains that either contained (χ3306) or were cured (χ3337) of the 100-kbp virulence plasmid. The *marA*::*kan* allele was recombined, by homologous recombination, into both strains as described in Materials and Methods, and all four strains were assayed for sensitivity to several antibiotics. As shown in Table 3, the presence of the virulence plasmid did not affect any of the antibiotic resistance phenotypes in all strains tested.

DISCUSSION

A previous study by Cohen et al. (16) showed that *marA*-like sequences could be detected in *S. typhimurium* by Southern blot analysis. In this study, we confirm and extend this obser-

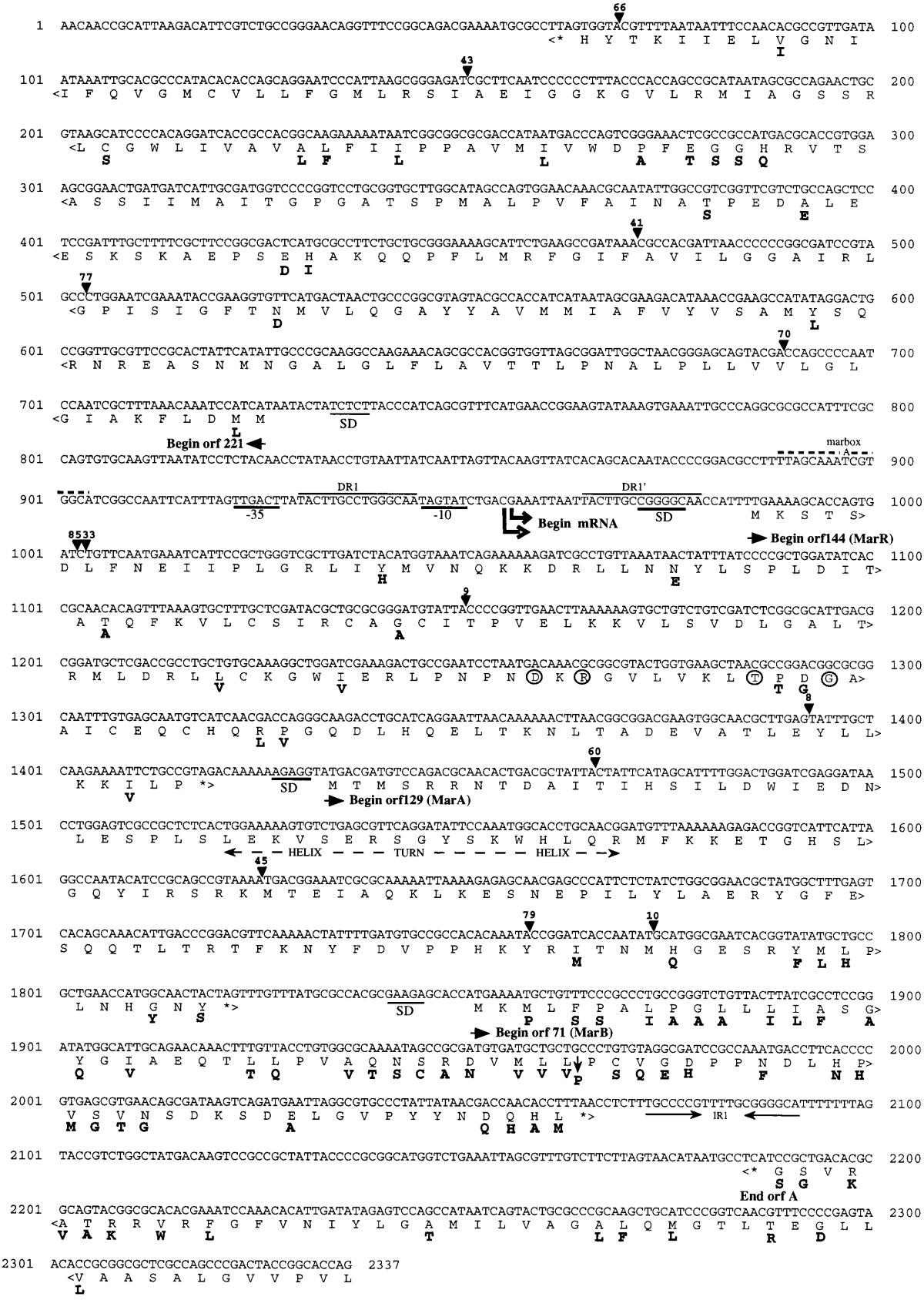
Begin orf 221
marbox
DR1
DR1'
-35
-10
SD
Begin mRNA
Begin orf144 (MarR)
Begin orf129 (MarA)
HELIX
TURN
HELIX
Begin orf 71 (MarB)
IR1
End orf A

FIG. 2. Nucleotide sequence of the *S. typhimurium* χ3181 *mar* locus. Potential ORFs are shown translated below the nucleotide sequence. One-letter amino acid symbols are centered under the nucleotide triplets. All amino acid residues are identical to those in the homologous ORFs of the *E. coli mar* locus determined by Cohen et al. (12), except for those indicated in boldface, in which case a different amino acid is present in *E. coli*. The homologous ORFs are as follows: ORF221 and *E. coli marC*, ORF144 and *E. coli marR*, ORF129 and *E. coli marA*, ORF71 and *E. coli marB*, and ORFA and *E. coli* ORF266. Potential ribosome-binding sites (SD), promoter elements (−35 and −10), imperfect direct repeats (DR1 and DR1′; nomenclature of Cohen et al. [12]), and the marbox (35) are indicated. The *marRAB* operon transcriptional start site is also indicated, as is the helix-turn-helix motif of MarA. Circled residues in MarR are those that are completely conserved among 14 of 15 members of the MarR family (39a). A putative rho-independent transcription termination motif downstream from *marB* is identified as IR1. Numbered triangles indicate the specific locations of the γδ transposon insertions shown in Fig. 2. The downward arrow pointing to a P in *marB* shows the location of an additional proline codon in the *E. coli* gene.

vation to show that *S. typhimurium* encodes a *marRAB* operon that is structurally and functionally almost identical to that identified in *E. coli*. Transposon insertions in $marR_{St}$ were polar for $marA_{St}$, and no promoter sequences are apparent upstream from the coding regions of $marA_{St}$ or $marB_{St}$. Moreover, the SAL-inducible *marRA*-specific transcript of 1.1 kbp (determined by Northern analysis) extending from the mRNA start site upstream of *marR* (identified by primer extension) is able to accommodate the three *mar* ORFs. Thus, as previously shown for *E. coli* (12, 13, 53), the $marR_{St}$, $marA_{St}$, and $marB_{St}$ genes are organized in an operon.

The high degree of homology of $MarA_{St}$ to $MarA_{Ec}$ explains why both are functionally interchangeable between organisms. When present on a multicopy plasmid, *marA* from either organism was able to confer a Mar phenotype in the heterologous background. The two proteins have identical helix-turn-helix DNA-binding motifs, and it follows that the target promoter sites recognized by these proteins are likely to be conserved between the two organisms. Analogous to what was previously reported for *E. coli* (13, 53), *S. typhimurium marA* strains are attenuated for SAL-inducible antibiotic resistance in comparison to wild-type strains (Table 3); however, such attenuation is not complete. Thus, SAL induces Par by both *mar*-dependent and *mar*-independent pathways, as was previously reported for *E. coli* (13). Interestingly, the phenotypic effect of a *marA* mutation appears to differ among particular enteric strains: *E. coli* AG100 *marA*::Tn*5* strains are hypersensitive to tetracycline and chloramphenicol compared to the wild type (56), while *E. coli* MC4100 *marA*::Tn*10kan* and the *S. typhimurium* χ3337, χ3306, and χ3181 *marA*::*kan* strains are only slightly or not at all hypersensitive, respectively, compared to their isogenic parental strains (Fig. 3). Although antibiotic resistance phenotypes were generally stronger in the virulent *S. typhimurium* strains, we found that the MarA target sites necessary for antibiotic resistance are located exclusively on the chromosome. They were not present on the *S. typhimurium* virulence plasmid, since a plasmidless strain showed a Mar phenotype identical to a wild-type strain (Fig. 3). Taken together, the data show that $MarA_{St}$ is almost identical to its *E. coli* counterpart in structure and function. In this light, it is interesting that the seven amino acid differences between $MarA_{St}$ and $MarA_{Ec}$ are clustered in the last 19 amino acids of the proteins. This finding suggests either that this portion of each protein plays no role in the activities of MarA assessed here

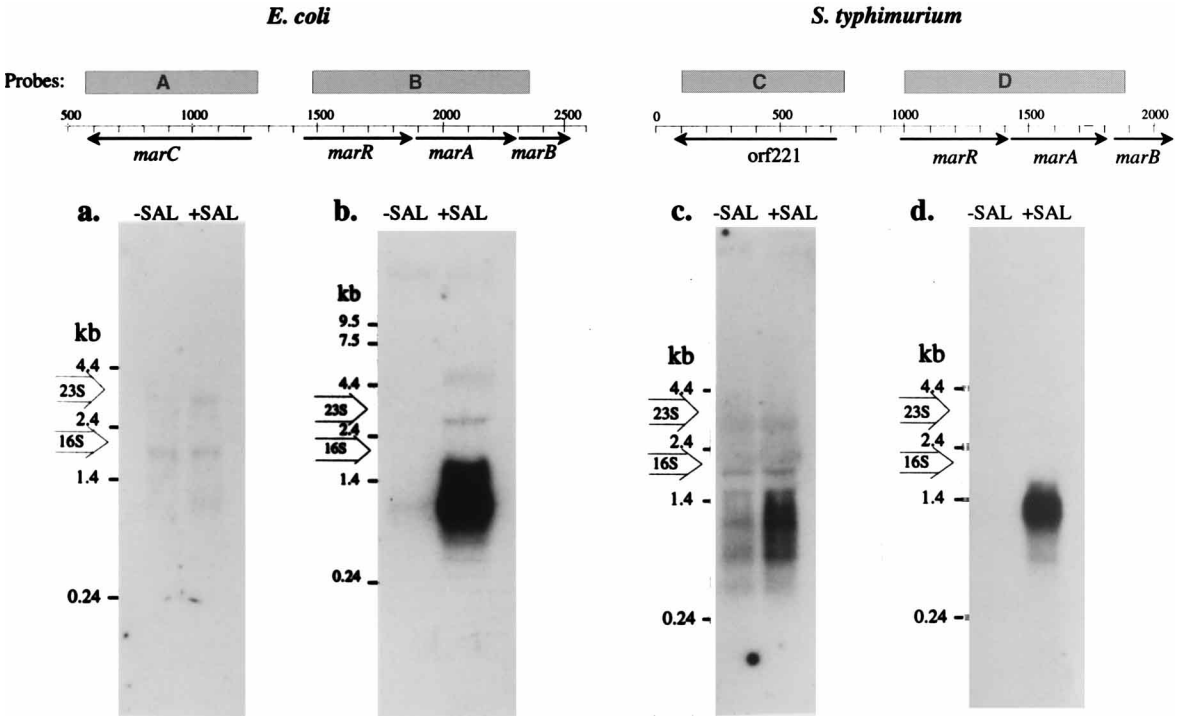

FIG. 3. Northern blot analysis of RNA transcripts from both the *E. coli* MC4100 and *S. typhimurium* χ3181 *marRAB* regions. (Top) Schematics are shown of the DNA sequence interval (base-pair numbered line) and ORFs (arrows) of the *E. coli* region, corresponding the numbering system of Cohen et al. (12), and that shown for *S. typhimurium* in Fig. 2. Probes used are indicated by rectangular boxes. (Bottom) Northern blots of RNA isolated from exponentially growing cells incubated without (−SAL) or with (+SAL) 2.5 mM SAL for 1 h and hybridized to the corresponding DNA probes indicated above. Locations of 16S and 23S rRNAs are indicated along with locations of RNA molecular size standards.

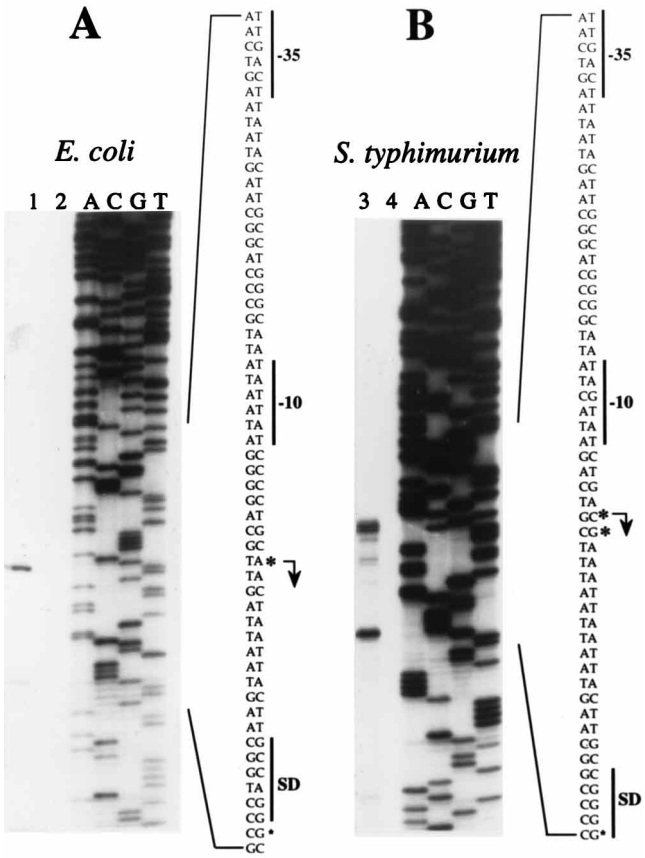

FIG. 4. Primer extension mapping of the 5′ end of *marRAB* transcripts for *E. coli* MC4100 (A) and *S. typhimurium* χ3181 (B). Primers complementary to *marRAB* mRNA and corresponding to nucleotides 1541 to 1508 for *E. coli* (12) and 1090 to 1057 for *S. typhimurium* (Fig. 2) were used for both sequencing and primer extension reactions. RNA was isolated from exponentially growing cells incubated with 2.5 mM SAL for 1 h prior to harvesting of RNA. The extension products are shown in lanes 1 and 3. Lanes 2 and 4 are negative control reactions using yeast RNA with the above-named primers. The −10 and −35 regions and ribosome binding sites (SD) are indicated by vertical lines; transcriptional start sites are indicated by broken arrows; asterisks indicate the locations of observed bands. The lower strong band in lane 3 is likely an artifact caused by a stable secondary structure in the *S. typhimurium* mRNA template that interferes with the progression of reverse transcriptase in the extension reactions.

(i.e., antibiotic resistance) or that it can tolerate significant alteration. However, γδ insertion 10, which lies in the 16th codon from the 3′ end of *marA*, abolishes the ability of that construct to restore inducible antibiotic resistance to the Δ*mar* strain. Presumably, the effect of that transposon insertion is either to produce an unstable MarA protein or to add at the C terminus of the protein γδ-encoded amino acids which are incompatible with MarA function.

$MarR_{St}$ was found to be functionally similar to $MarR_{Ec}$ because it repressed a *marR′-′lacZ* fusion in the heterologous *E. coli* background in a manner that could be antagonized by SAL. Moreover, this feature was the basis for cloning the *marRAB* locus. Although we did not directly assess $marR_{St}$ function in *S. typhimurium*, the ability of SAL to induce transcription of $marRAB_{St}$ suggests that $MarR_{St}$ is expressed and represses $marRAB_{St}$ transcription. This protein can also be considered the newest member of the MarR family of phenolic-binding regulatory proteins (54), of which there are now 16 (39a). An alignment of these sequences has revealed residues which are either completely or frequently conserved among members of this family. None of the 11 amino acid differences between $MarR_{St}$ and $MarR_{Ec}$ affects one of these conserved positions. Thus, these 11 substitutions collectively do not affect MarR function.

MarB has no known function in *E. coli*. In this regard, it is interesting that the divergence between the *E. coli* and *S. typhimurium marB* sequences is much greater (42% identical) than was observed for MarR and MarA (>91% identical). The significance of this finding is unclear, but it is consistent with the lack of any clear obligatory role for MarB in the activities analyzed here.

To put these comparisons into a larger context, the structures and organizations of neighboring sequences were also investigated. The $marRAB_{St}$ operon is flanked by ORFs homologous to those found at the same positions in *E. coli* (12, 21a). MarC from *E. coli* has 91% amino acid identity to *S. typhimurium* ORF221. White et al. (56) suggest that this locus is involved in multidrug resistance. In *mar* deletion strains, a plasmid containing DNA encoding this ORF along with *marRAB* increased resistance levels two- to threefold above those induced by plasmids expressing only *marA* and *marB* (56). By analogy, ORF221 may play a similar role. However, one observation made for ORF221 and its role in antibiotic resistance is noted here. The *E. coli* strain deleted for the *mar* locus and containing p197, encoding $ORF221_{St}$ and $marORA_{St}$, showed decreased susceptibility to enoxacin compared to a plasmidless strain. This level of resistance was unchanged when ORF221 was disrupted by the transposon γδ. Thus, in the heterologous system used here, we found no role for ORF221 in antibiotic resistance.

A 95-bp segment of the operator-promoter region beginning at the $marR_{St}$ TGT start codon and extending upstream is 86%

TABLE 3. Resistance phenotypes and LD_{50}s for *S. typhimurium* strains

Strain	Relevant property	Growth (% of gradient)[a]					
		Ampicillin		Chloramphenicol		Tetracycline	
		0–10 μg/ml	0–20 μg/ml + SAL	0–15 μg/ml	0–30 μg/ml + SAL	0–10 μg/ml	0–20 μg/ml + SAL
χ3181	pStSR100	<5	34	19	59	53	58
χ3181 *marA*	pStSR100 *marA*::*kan*	<5	<5	19	19	53	50
χ3306	pStSR100 *gyrA*	<5	38	22	63	53	59
χ3306 *marA*	pStSR100 *gyrA marA*::*kan*	<5	<5	19	22	52	50
χ3337	Plasmid-cured χ3306	<5	31	22	69	55	59
χ3337 *marA*	Plasmid-cured χ3306 *marA*::*kan*	<5	<5	29	28	50	47

[a] Note that the maximum concentration of antibiotic used in the presence of salicylate (+SAL) is twice that used in the absence of SAL. This allowed for determination of endpoints for most strains for challenges with these antibiotics.

identical at the nucleotide level to the same region in *E. coli* and contains nearly identical regions implicated in MarR and MarA binding (35, 37). To better compare the operator/promoter regions, we identified *marRAB* transcription start sites by primer extension, thereby empirically defining the promoter sites of both organisms. The start site for *E. coli* conforms to the expected site defined by putative −10 and −35 regions upstream of *marRAB*, originally identified by Cohen et al. (12). The *S. typhimurium* promoter sites are similarly situated (Fig. 2).

SAL-mediated induction of *marRAB* transcription appears identical in both organisms. By Northern blot analysis, SAL was shown to induce the accumulation of $marRAB_{St}$ mRNA (Fig. 3). Also, since treatment of *E. coli mar* mutants with tetracycline has been reported to elevate mRNA levels of both *marRAB* and the upstream *marC* (12), it was possible that treatment with SAL would do the same. While induction of the upstream transcript by SAL was more pronounced in *S. typhimurium* than in *E. coli*, where little effect was observed, this induction was much smaller than that of the *marRAB* transcript (Fig. 3). The similarity to *E. coli* of nucleotide sequences of the operator/promoter regions, Northern data on inducibility of the operon by SAL, and structural and functional similarities of MarR to its *E. coli* counterpart all suggests that regulated control of *marRAB* expression in *S. typhimurium* is nearly identical to that of *E. coli.*

When grown in the presence of SAL or when containing plasmids expressing $MarA_{Ec}$, *S. typhimurium* strains that are capable of infection in BALB/c mice were less susceptible to different classes of antibiotics than were less virulent *S. typhimurium* and *E. coli* strains (Table 2). This finding suggested a possible correlation between MarA-mediated antibiotic resistance and virulence potential. Therefore, the contribution of *marA* in virulence was assessed by using a well-established murine model of infection (24). However, the *S. typhimurium marA* strain did not differ from the wild-type control in the level of killing of BALB/c mice. We note that our LD_{50}s were much lower than those reported for an isogenic *gyrA 1816* strain (24); however, this difference does not affect the result since our two strains exhibited indistinguishable LD_{50}s. Thus, we could not implicate *marA* in *S. typhimurium* virulence.

If *marA* is involved in virulence, it is possible that its effect is masked by the activation of other overlapping regulons that engender a phenotypic antibiotic resistance. Some of the genes controlled by *marRAB* are also members of the redox-sensitive *soxRS* regulon (2, 22) in which constitutive mutations also confer a Mar phenotype (23, 39). The regulatory overlap is presumably due to the strong homology between the direct activators of both regulons, SoxS and MarA (12). The *soxRS* regulon protects the cell against both redox-cycling compounds (43, 55) and the nitric oxide free radical ($NO^{\cdot}$) (41, 42), as well as $NO^{\cdot}$-generating activated macrophages (41, 42). Thus, some genes common to both the *marRAB* and *soxRS* regulons are likely to be expressed in vivo, as a consequence of *soxRS* activation. *marRAB* may therefore be redundant when *S. typhimurium* is in contact with macrophages, where it is known to escape humoral defenses (18). In addition, recent evidence indicates that overexpression of another MarA homolog, Rob, also results in multiple antibiotic resistance (3), presumably by activating promoters common to the MarA regulon. Experiments designed to evaluate strains containing combinations of *marRAB*, *soxRS*, and *rob* mutations for attenuation of virulence may identify overlapping roles for these genes in pathogenesis.

Studies focusing on MarA-regulated intrinsic antibiotic resistance can now be conducted in two different organisms. Although the natural physiological role of the two operons is unclear (here the *Salmonella mar* operon is not implicated as a virulence factor), their close similarities suggest that an analysis of MarA target genes from both organisms may identify functions that collectively befit a concerted physiological response. Given the interchangeability of $marA_{Ec}$ and $marA_{St}$, it seems reasonable to consider these genes allelic. The functional similarities between $marR_{Ec}$ and $marR_{St}$ suggest that these genes can be compared similarly. Such observations should contribute to our understanding of how these interesting regulatory proteins carry out their specific roles in modulating intrinsic antibiotic resistance.

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