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EmrR Is a Negative Regulator of the *Escherichia coli* Multidrug Resistance Pump EmrAB

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The *emrAB* locus of *Escherichia coli* encodes a multidrug resistance pump that protects the cell from several chemically unrelated antimicrobial agents, e.g., the protonophores carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and tetrachlorosalicyl anilide and the antibiotics nalidixic acid and thiolactomycin. The *mprA* gene is located immediately upstream of this locus and was shown to be a repressor of microcin biosynthesis (I. del Castillo, J. M. Gomez, and F. Moreno, J. Bacteriol. 173:3924–3929, 1991). There is a putative transcriptional terminator sequence between the *mprA* and *emrA* genes. To locate the *emr* promoter, single-copy *lacZ* operon fusions containing different regions of the *emr* locus were made. Only fusions containing the *mprA* promoter region were expressed. *mprA* is thus the first gene of the operon, and we propose that it be renamed *emrR*. Overproduction of the EmrR protein (with a multicopy vector containing the cloned *emrR* gene) suppressed transcription of the *emr* locus. A mutation in the *emrR* gene led to overexpression of the EmrAB pump and increased resistance to antimicrobial agents. CCCP, nalidixic acid, and a number of other structurally unrelated chemicals induced expression of the *emr* genes, and the induction required EmrR. We conclude that *emrRAB* genes constitute an operon and that EmrR serves as a negative regulator of this operon. Some of the chemicals that induce the pump serve as its substrates, suggesting that their extrusion is the natural function of the pump.

It has become clear in recent years that bacteria possess pumps of low specificity that can extrude structurally unrelated amphipathic toxins. These have been termed multidrug resistance pumps (MDRs). The MDRs are widely distributed, and their presence in pathogens such as *Staphylococcus aureus* (9, 10, 15, 16) and *Pseudomonas aeruginosa* (31) poses a serious threat to public health.

Three families of bacterial MDRs are known (13, 27). The simplest one is the Smr (staphylococcal multidrug resistance) family of four membrane domain proteins which are by far the smallest known translocases of any kind. Discovered originally in *S. aureus*, Smr-encoding genes are carried on broad-host-range plasmids (29) and are found in many bacteria including *Escherichia coli* (9, 10, 15, 16). The Smrs extrude membrane-permeable cations such as ethidium bromide and tetraphenylphosphonium. Another family is made up of the Acr (acridine) pumps that were originally thought to be components of the outer membrane. The Acr pumps are in the same family with the metal efflux translocases of *Alcaligenes* spp. and have the broadest substrate spectrum, protecting from sodium dodecyl sulfate, acriflavine, and a number of antibiotics such as novobiocin and rifampin (20, 31, 38). The third family of MDRs constitutes the large major facilitator family (6, 22) and includes the QacA pump of *S. aureus* involved in the extrusion of quaternary ammonium compounds used as antiseptics (34, 36) and the *Bacillus* multidrug resistance (Bmr) pump extruding, among other compounds, ethidium bromide, puromycin, and chloramphenicol (26); this family also includes the well-studied tetracycline/H^+ antiporter (12).

The EmrAB pump of *E. coli* was found in an effort to identify the genetic basis of *E. coli* adaptation to protonophores which uncouple oxidative phosphorylation (18). It was shown that *emrAB* expression conferred resistance to such structurally unrelated compounds as protonophores (carbonyl cyanide *m*-chlorophenylhydrazone [CCCP] and tetrachlorosalicylanilide [TCS]), 2-chlorophenylhydrazine, and some antibiotics (nalidixic acid and thiolactomycin). EmrAB consists of a translocase proper, the EmrB protein which is a member of the major facilitator family, and the EmrA protein, belonging to the membrane fusion family, that might have the function of organizing an efflux pathway for EmrB substrates across both the inner and the outer membranes (6, 18). The addition of CCCP to *E. coli* growing under conditions of oxidative phosphorylation causes a marked decrease in growth rate, but the growth recovers within 1 to 2 h (25). This suggested that expression of *emrAB* can be induced by CCCP. We report here that the *emrAB* transcription initiates from the regulatory region of a previously described gene, *mprA* (4), that the expression of *emrAB* can be induced by several structurally unrelated compounds, and that *mprA* encodes a repressor of the pump synthesis. Some of the chemicals that induce this operon serve as substrates for the pump. Because *mprA* is part of the *emrAB* operon, we propose that it be renamed *emrR*.

An additional promoter for the *emrB* gene is located in the *emrA* coding sequence. This promoter is independent of EmrR and will be the subject of another communication.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth media. The *E. coli* strains used in this study are listed in Table 1; plasmids are schematically shown in Fig. 1 and 3. Luria-Bertani (LB) medium was used throughout. MacConkey agar supplemented with 1% lactose was used for screening the *lacZ* fusions. When necessary, ampicillin, tetracycline, or kanamycin (Sigma, St. Louis, Mo.) was added at a concentration of 100, 15, or 50 μg/ml, respectively. Generalized transductions

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TABLE 1. *E. coli* strains used in this study

Strain	Genotype or description	Reference or source
AMS6	K-12 Δ*lacU169*	Our laboratory collection
JC7623	*thr-1 ara-14 leuB6* Δ(*gpt-pro*) *lacY1 sbcC tsx-33 supE galK sbcB hisG recB recC rpsL31 xyl-5 mtl-1 argE3 thi-1*	37
CDM5	*metB1 relA1 spoT1 gyrA216* λ^r λ^- F^- Tlm^r	11
OLS101	AMS6 *emrR*::Km	This study
OLS102	AMS6 *emrA*::Km	This study
OLS103	AMS6 *emrB*::Km	This study
MLM63	AMS6 λRS45 [Φ(*emrRA-lacZ*)]	This study
MLM632	MLM63 *emrR*::Km	This study
MLM634	AMS6 λRS45 [Φ(Δ*emrR*)*A-lacZ*]	This study
MLM635	MLM634 *emrR*::Km	This study
MLM636	MLM63 *emrA*::Km	This study
MLM637	MLM63 *emrB*::Km	This study
MLB69	AMS6 λRS45 [Φ(*emrRAB-lacZ*)]	This study
MLB694	AMS6 λRS45 [Φ(Δ*emrR*)*AB-lacZ*]	This study
MLB695	MLB694 *emrR*::Km	This study
MLB29	AMS6 λRS45 [Φ(*emrB-lacZ*)]	This study

with P1*vir* were performed as described by Miller (24). β-Galactosidase assays were performed with chlorophenol red-β-D-galactopyranoside (CPRG) as described previously (19). CPRG provides an approximately 10-fold-higher sensitivity than does *o*-nitrophenyl-β-D-galactopyranoside (ONPG). The activity was calculated by Miller's formula but with CPRG optical density measurements and is expressed in arbitrary units (24).

Molecular biology techniques. Chromosomal DNA for the PCR amplification was isolated as described by Liu and Turnbough (17). Total cellular RNA for primer extension and plasmid DNA were isolated with the RNeasy total RNA kit or mini- or midiplasmid kits (Qiagen Inc., Chatsworth, Calif.). Restriction endonuclease digestion and ligation were carried out according to the manufacturer's protocols (New England Biolabs, Beverly, Mass.). Plasmids were introduced into bacteria by electroporation (21). Restriction fragments were purified from agarose gels with a Geneclean II kit, purchased from Bio 101, La Jolla, Calif. Dideoxy sequencing was performed with the Sequenase version 2.0 DNA sequencing kit (United States Biochemicals, Cleveland, Ohio). A GeneAmp kit from Perkin-Elmer Cetus was used for PCR amplification of DNA.

Primer extension experiments were performed as previously described (21). RNA was isolated either from overnight cultures or from log-phase cultures grown in LB medium. Primer 5′ TGATAAGGAAAATCTTCGTGGCGGCTG complementary to 148 to 171 of the *emrR* sequence (numeration is by the method of Moreno and coworkers [5]) was used to localize the promoter upstream of *emrR*. This and other primers were purchased from Operon Technologies, Inc. (Alameda, Calif.) or from Ana-Gen Technologies, Inc. (Sunnyvale, Calif.).

Construction of *emr-lacZ* single-copy fusion strains. Single-copy *emr-lacZ* operon fusions were generated with the system of Simons et al. (35). The region of interest was first cloned upstream of the promoterless *lacZ* gene in multicopy plasmid pRS415. If the resulting plasmid produced a higher level of β-galactosidase activity compared with that of pRS415 itself, it was recombined into phage λRS45, which was used to lysogenize AMS6 to yield a fusion strain. PCR was used to generate DNA fragments of various lengths. Primers were flanked by the appropriate restriction sites (*Eco*RI and *Bam*HI) to permit directional cloning. The forward primers were as follows (see Fig. 1): MLM23, (+603) GCGGAATTCGGCAAGCAGGTCGGCTCA; MLM03, (+456) GCGGAATTCCGAGCAAATCACCCGCAA; MLM43, (+308) GCGGAATTCGTCGTGAAAGCGATAACG; MLM53, (+81) GCGGAATTCTTCTGACTCGTCTTTGCA; and MLM63, (−155) GCGGAATTCCATCCCAACACTGCTTAG. The numbers in parentheses are the positions relative to the translational start site of *emrR*. Reverse primer GCGGGATCCGTGAGAAGGAGGAGCAGA was complementary to bases 66 to 83 downstream of the translational start site of *emrA* and was the same for the above-listed strains. The fusion MLM67 was obtained with the same forward primer as for the fusion MLM63 and with reverse primer CCCGGATCCCTGGATTTTTGAGCGAGA, complementary to bases 8 to 25 downstream of the TAA stop codon of *emrR*. To construct the MLB69 fusion, the following two primers were used: the forward (*Eco*RI) primer was the same as for construction of the MLM63 fusion; the reverse (*Bam*HI) primer, GGGGGATCCGTGTTATCGGCCCAGACTTT, was complementary to bases 48 to 65 downstream of the *emrB* translational start site. The fusions MLM634 and MLB694 were obtained by deleting the *Sph*I-*Xho*I fragment from the pMLM63 and MLB69 plasmids, respectively. The resulting fusions are thus deleted of *emrR*. To construct the MLB29 fusion, we used the same forward primer as for MLM23 and the same reverse primer as for MLB69.

Construction of p*emrR* and p*emrR plasmids.** The *emrR* gene was amplified from the chromosome of *E. coli* AMS6 by PCR and cloned into the *Eco*RI-*Bam*HI-digested plasmid pGEM3Z to yield plasmid p*emrR*. Primers used for this PCR amplification were the same as for construction of the MLM63 fusion. These primers were also used to amplify *mprA* (*emrR*) from the chromosome of the Tlm^r strain CDM5 (11). The PCR product was cloned into the *Eco*RI-*Bam*HI-digested plasmid pUC18 to yield p*emrR**.

Construction of the *emrR*, *emrA*, and *emrB* null mutants. The null mutants were constructed by the method of Winans et al. (37), replacing the wild-type *emr* genes by *emr* genes inactivated by insertion of a kanamycin resistance gene. For *emrR* inactivation, the insertion was into the unique *Xho*I site of the plasmid p*emrR*; for that of the genes *emrA* and *emrB*, the insertions were into the *Nru*I or *Pst*I site, respectively, of the plasmid p*emr* 2.1 (18). The source of the kanamycin cassette was plasmid pUC4K (Pharmacia). Disruption and loss of function were confirmed as follows: for *emrR*, by the inability of the plasmid bearing the disrupted gene to inhibit β-galactosidase activity of the *emr-lacZ* fusions (see Results), and for *emrA* and *emrB*, by the inability of the appropriate plasmids to confer CCCP resistance. The plasmids were digested with *Eco*RI and *Bam*HI, and the appropriate fragment was eluted from the agarose gel and used to transform the strain JC7623. Kanamycin-resistant transformants were selected and tested for sensitivity to ampicillin. DNA from several Km^r Ap^s colonies was isolated and tested for gene disruption by PCR. P1 transduction was used to transfer *emr*::Km insertions into the appropriate genetic backgrounds.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper have been entered in the NCBI database under accession number U19993.

RESULTS

The genes for the multidrug resistance pump EmrAB form an operon with the upstream gene *emrR*. To identify the promoter of the *emrAB* locus, different-length fragments upstream of the *emrA* gene were cloned in the transcriptional fusion vector pRS415. The productive fusions were integrated into λ phage which were used to lysogenize *E. coli* AMS6 so as to study regulation in single-copy fusions.

A putative transcriptional terminator is present at nucleotide +602 in relation to the *emrR* translational start site (5), i.e., between the *emrR* and *emrA* genes (Fig. 1). We therefore expected to find a promoter in the 56-nucleotide-long DNA segment between these genes. However, a strain with plasmid pMLM23 containing the fusion to this segment had the same basal level of β-galactosidase activity as the pRS415-containing strain. This indicated the absence of a promoter immediately upstream of *emrA*. To test the possibility that the promoter was present in the coding region of the upstream *emrR* gene, fusions containing different lengths of this region were tested. However, even fusion pMLM53, which contains almost the entire coding region of *emrR*, was not productive (Fig. 1).

The results suggested that *emrA* was transcribed from the *emrR* promoter. Testing this hypothesis required construction of fusions containing the *emrR* regulatory region. Since only a

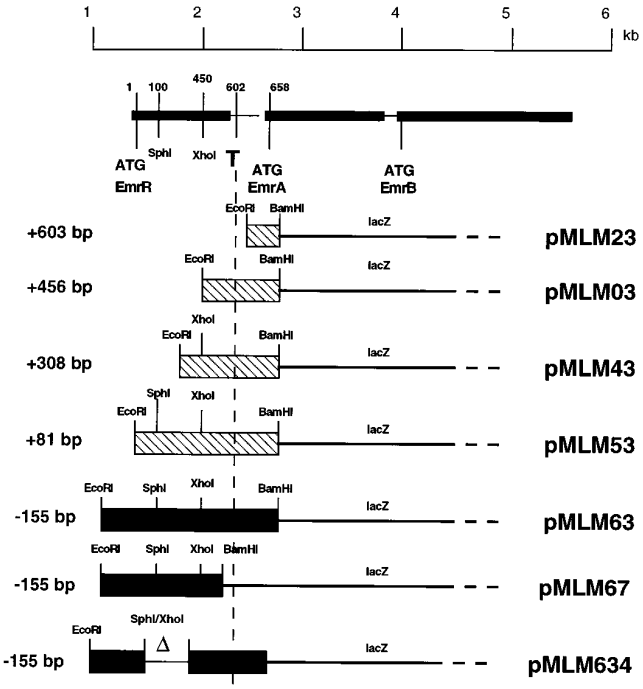

FIG. 1. Schematic representation of the cloned *emr-lacZ* fusions. The various fusions were constructed in the transcriptional vector pRS415 (35). *emr* sequences are shown as black and striped boxes for productive and nonproductive fusions, respectively. Numbers are given relative to the *emrR* translational start site and represent the 5′ end of the DNA fragment in the individual fusions. The dashed vertical line shows the position of the terminator.

short fragment (98 bp) of this sequence was known, a 250-bp portion of this region was sequenced in order to design a primer for PCR amplification of a longer upstream segment. This enabled us to construct the plasmid pMLM63, which contains 155 bp upstream of the *emrR* translational start site fused to the *lacZ* gene (Fig. 1). There was significant expression of β-galactosidase activity in this fusion, indicating that the *emrA* gene requires the *emrR* promoter for expression. For convenience, we will refer to this promoter as P*emr*. Strain MLM63 was constructed by incorporating the fusion from pMLM63 in *E. coli* AMS6 through λ lysogenization and was used to study *emrRA-lacZ* expression in single-copy fusions.

The transcriptional start site of the *emrRAB* operon was determined by primer extension analysis. One major extension product corresponding to 23 bp upstream of the ATG start codon of the *emrR* gene was found (Fig. 2). The −10 and −35 regions of P*emr* possess a typical σ^{70} promoter sequence; *emrR* is thus the first gene of the operon encoding the multidrug resistance pump EmrAB.

The *emr* operon contains an internal terminator. We sought direct evidence for the functionality of the putative terminator sequence downstream of *emrR*. Thus, we compared the β-galactosidase activity of *emr-lacZ* fusions in which the *lacZ* gene was fused either upstream (strain MLM67) or downstream (strain MLM63) of the terminator sequence. MLM67 showed a two- to threefold-higher activity than did MLM63 (Table 2), indicating that the terminator sequence is functional, albeit weakly functional.

The *emrR* gene product is a negative regulator of the multidrug resistance pump. The *emrR* (*mprA*) gene was originally cloned as a multicopy suppressor of microcin B and C production (4). Overexpression of *mprA* (*emrR*) decreased transcription of the microcin B-encoding gene, *mcb*, as determined with an *mcbA-lacZ* fusion, while *mprA* deletion caused increased transcription (5). The same study suggested that the *mprA* (*emrR*) gene is autoregulated, since *mprA-lacZ* expression was decreased in the presence of a multicopy plasmid bearing the *mprA* gene.

EmrR could regulate the EmrAB pump in a similar manner, and we therefore investigated the effect of *emrR* overexpression and inactivation on *emr* gene expression. A multicopy plasmid carrying *emrR* (p*emrR*) was introduced into strain MLM63 (*emrRA-lacZ*); β-galactosidase activity was decreased eightfold by the presence of this plasmid (Table 2). The MLM63 strain contains two copies of the *emrR* gene, one in the chromosomal *emr* locus and the other on the *emrRA-lacZ* fusion. Inactivation of either copy produced a 7- to 8-fold increase in *emr* expression (MLM63 [*emrR*] or MLM634 [$emrR^+$] versus MLM63 [$emrR^+$] [Table 2]); inactivation of both led to a 40- to 50-fold increase in expression (MLM634 [*emrR*] versus MLM63 [$emrR^+$]). Introduction of the p*emrR* plasmid resulted in strong inhibition of expression. The amount of the primer extension product, which reflected the amount of P*emr*-dependent transcript, was markedly increased when RNA isolated from *emrR*-lacking strain AMS6 *emrR*::Km was used as the template for the extension reaction (Fig. 2B). These results indicate that *emrR* acts as a repressor of the *emr* locus.

The *emrB* gene has an additional promoter. *emrA* and *emrB*, being part of the same operon, should ordinarily be affected to the same degree by EmrR. In an *emrRAB-lacZ* fusion (MLB69 [Fig. 3]), inactivation of both copies of *emrR* produced a 20-fold increase in β-galactosidase activity (MLB694 [*emrR*] versus MLB69 [$emrR^+$] [Table 2]), showing that EmrR indeed had a strong effect on the last gene of the operon. At the same time, the basal level of β-galactosidase production of an *emrRAB-lacZ* fusion (strain MLB69) was higher than that of an *emrRA-lacZ* fusion (MLM63 [Fig. 1]). Further, EmrR overexpression caused a less pronounced effect on *emrRAB-lacZ* compared with the *emrRA-lacZ* fusion. This suggested regulation of *emrB* by an additional promoter. Indeed, a fusion (MLB29) containing an upstream fragment of *emrB*, but lacking the P*emr* promoter, was productive (Fig. 3), confirming the presence of the additional promoter. As expected, this *emrB-lacZ* fusion was not affected by EmrR (Table 2). This promoter will be the subject of a future communication.

The *emrRAB* operon can be induced by structurally unrelated drugs. When CCCP, TCS, or nalidixic acid was added to the exponentially growing culture of the MLM63 fusion strain, β-galactosidase activity increased 1.5- to 3-fold, indicating induction of P*emr* (Fig. 4). Maximal induction occurred at concentrations that inhibited cell growth. However, induction does not appear to result from such growth inhibition, since thiolactomycin inhibited growth but yet had no effect on induction.

Salicylate and other weak acids as well as 2,4-dinitrophenol have been shown to induce multiple antibiotic resistance in *E. coli* that is controlled primarily by the *marRAB* operon (3). Interestingly, EmrR is homologous to MarR, and we therefore tested whether salicylate and 2,4-dinitrophenol would also induce expression of the *emr* locus. These compounds induced (at the same concentrations at which they induce multiple antibiotic resistance) the MLM63 fusion strain by six- to eightfold (Fig. 4). As expected from these results, the amount of the P*emr*-dependent transcript also increased after salicylate addition (Fig. 2A). Many of the drugs mentioned above are weak acids. However, ethidium bromide, which is an amphiphilic cation, also induced the fusion in strain MLM63 (Fig. 4), showing that truly unrelated substances can be inducers.

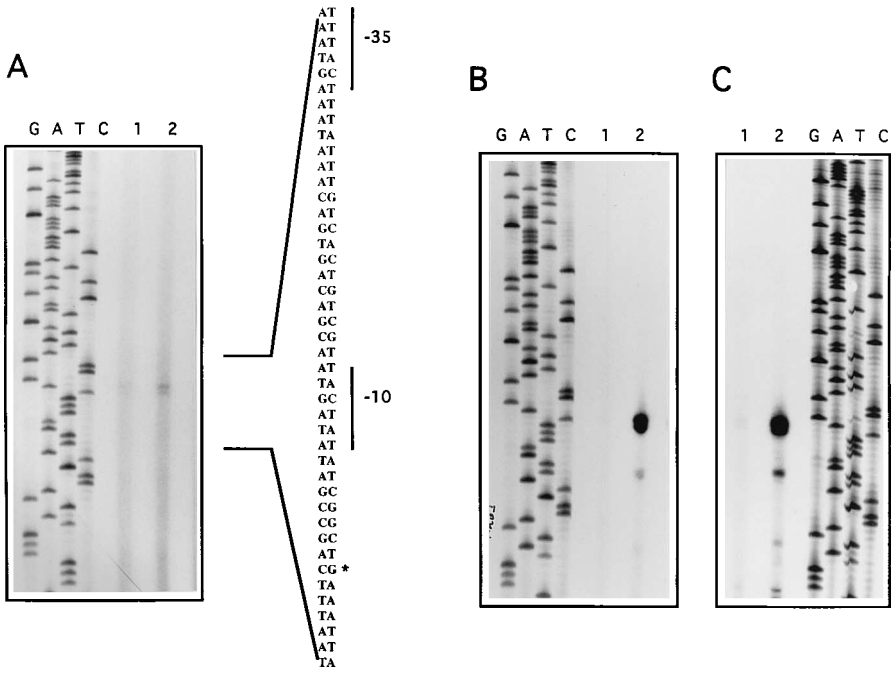

FIG. 2. Localization of the *emrRAB* transcriptional start site by primer extension. A primer complementary to *emrR* mRNA bases +148 to 171 relative to the *emrR* translational start site was used for both primer extension and sequencing reactions. (A) RNA was isolated from exponentially growing AMS6 cells incubated without or with 5 mM salicylate for 60 min. The extension products are shown in lanes 1 and 2, respectively. The transcriptional start site is indicated by the asterisk. The −10 (TACTAT) and −35 (TTTACT) regions are indicated by vertical lines. (B) Extension product of RNA isolated from an overnight culture of strain OLS101 (derived by an *emrR*::Km mutation in strain AMS6) (lane 2); lane 1 indicates that the same amount of RNA isolated from an overnight culture of AMS6 gives no extension product. (C) Extension product of RNA isolated from an overnight culture of strain CDM5, a Tlm^r derivative of strain UB1005 (lane 2); lane 1 indicates that the same amount of RNA isolated from an overnight culture of UB1005 gives almost no extension product. Minor bands are probably artifacts of the reverse transcription reaction.

Induction of P*emr* activity by all inducers was abolished in strain OLS101, which does not have the *emrR* gene, while *emrA* or *emrB* mutations had no effect (data not shown). This indicates that the inducers act through *emrR* to activate transcription. It is noteworthy in this connection that overexpression of MarR or MarA, or mutations in the cognate genes, did not affect *emr* expression (data not shown).

In the multidrug resistance phenomenon, exposure to one chemical or drug often results in the development of resistance to unrelated drugs. This was true also of the EmrAB pump. Thus, cells of the AMS6 strain grown in the presence of 2.5 mM salicylate exhibited increased CCCP resistance compared with that in the unexposed control cultures; the adapted cells possessed a more than threefold-higher growth rate in the presence of CCCP compared with that of the unadapted cells (Fig. 5).

EmrR and the nature of the thiolactomycin resistance mutation. An interesting mutation conferring resistance to the antibiotic thiolactomycin (Tlm^r) has recently been described and mapped to the *emr* locus (7). This mutation also increased resistance to CCCP (data not shown), as well as to the quantity of P*emr*-dependent transcript (Fig. 2C). The increase in the amount of the message could either be due to a promoter mutation or due to a mutation in the repressor. To distinguish between these possibilities, a complementation with the wild-type *emrR* locus was performed. The plasmid p*emrR* complemented the Tlm^r mutation in strain CDM5 and conferred hypersensitivity to CCCP (Fig. 6). Thus, the mutation is likely to be in the structural portion of the *emrR* gene.

To localize the Tlm^r mutation, this locus was cloned from strain CDM5 into plasmid pUC18, yielding the plasmid p*emrR**. The entire sequence of the *emrR** gene from the

TABLE 2. Effects of *emrR* on the expression of *emr-lacZ* fusions[a]

Strain	Fusion	β-Galactosidase activity with the following *emrR* status of the host strain:		
		emrR$^+$	*emrR*	p*emrR*
MLM63	*emrRA-lacZ*	690 ± 84	5,120 ± 362	83 ± 11
MLM67	*emrR-lacZ*	1,583 ± 176	ND	ND
MLM634	*emr*(Δ*R*)*A-lacZ*[b]	4,900 ± 388	23,170 ± 1,112	148 ± 20
MLB69	*emrRAB-lacZ*	1,450 ± 174	ND	518 ± 94
MLB694	*emr*(Δ*R*)*AB-lacZ*	5,225 ± 471	24,080 ± 1,926	724 ± 99
MLB29	*emrB-lacZ*	712 ± 68	780 ± 72	823 ± 107

[a] Samples were removed from cultures grown overnight in LB medium (37°C) with aeration and assayed for β-galactosidase activity with CPRG. ND, not determined. Results are given in arbitrary units.

[b] The deletion is in the structural region of the *emrR* gene.

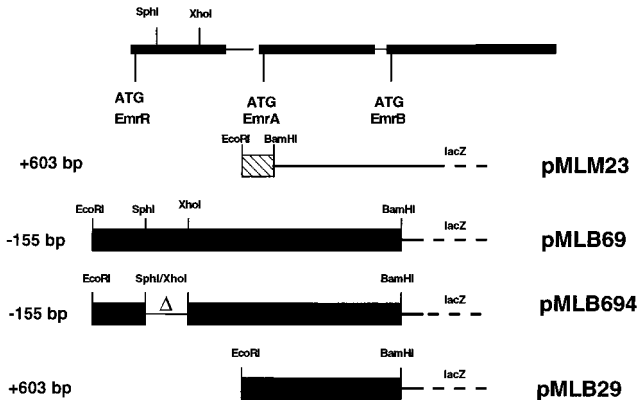

FIG. 3. Schematic representation of the *emrRAB-lacZ* and *emrB-lacZ* fusions. *emrRAB-lacZ* fusions have the same 5′ end as the productive *emrRA-lacZ* fusions presented in Fig. 1. The *emrB-lacZ* fusion MLB29 has the 5′ end located in the structural region of the *emrA* gene.

plasmid p*emrR** was determined and compared with the sequence of the wild-type *emrR* gene (Fig. 7). A single mutation, insertion of an additional C at position 479 (relative to *emrR* translational start site), was found in the structural region; this was confirmed by direct sequencing of the PCR product. The mutation produces a frameshift starting from amino acid residue 160 of the EmrR protein. The frameshift creates a 36-amino-acid-long extension of the open reading frame (Fig. 7). Since no other change in the regulatory or structural part of the *emrR* gene was found, we conclude that this single frameshift mutation is the cause of multiple drug resistance of strain CDM5.

DISCUSSION

Our original analysis indicated that *emrA* and *emrB* genes make up a two-gene operon. The *mprA* (*emrR*) gene that is adjacent to *emrA* has a typical putative rho-independent terminator and was ascribed the function of regulating expression of microcin synthesis (4). The use of *lacZ* fusions to systematically search for the promoter of the operon led to the unexpected finding that *emrR* was actually a part of the *emrAB* operon. Cloned portions of the *emrA* gene containing the DNA between the putative transcriptional terminator and the *emrA* translational start did not give productive fusions when

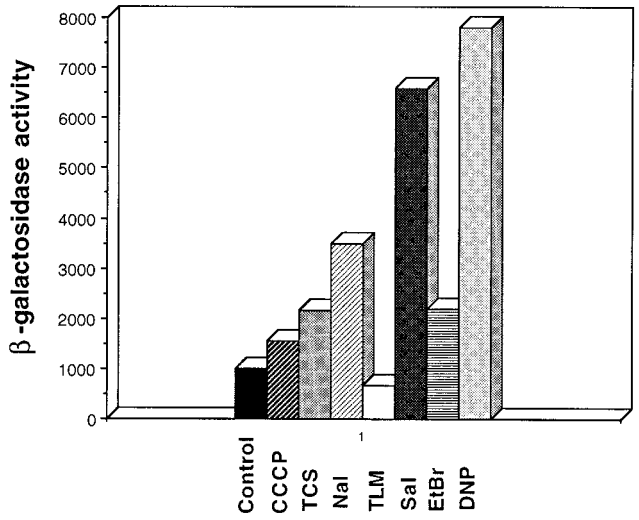

FIG. 4. Induction of *emrRA-lacZ* fusion strains by different drugs. The various drugs, CCCP (10 μM), TCS (10 μM), nalidixic acid (Nal; 160 μM), thiolactomycin (TLM; 10 to 400 μM), ethidium bromide (250 μM), salicylic acid (Sal; 5 mM), and 2,4-dinitrophenol (DNP; 0.5 mM), were added to an exponentially growing culture of MLM63 in LB medium. β-Galactosidase activity was measured 60 min later. The control bar shows β-galactosidase activity at time zero without any additions.

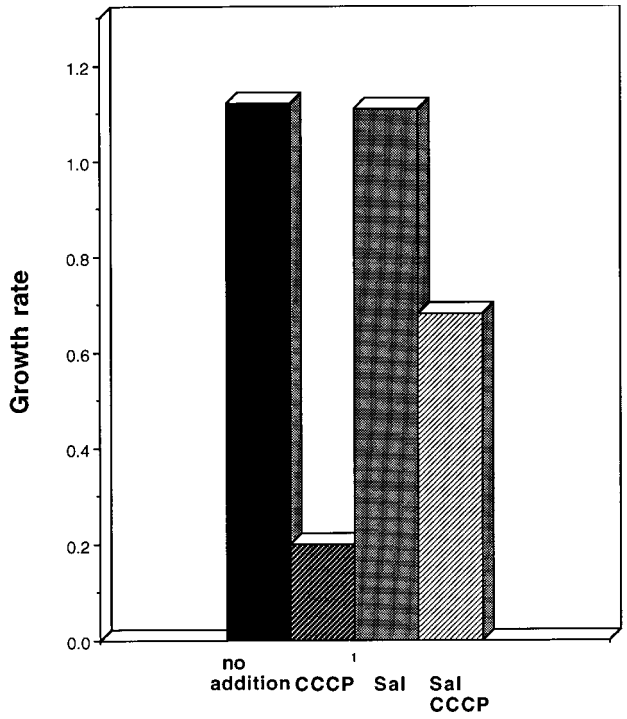

FIG. 5. Effects of preincubation with salicylate on CCCP sensitivity. Two cultures of the wild-type strain AMS6 were grown for 1.5 h in LB medium with or without salicylate (Sal; 2.5 mM). Both cultures were split in two, and 25 μM CCCP was added to one of the aliquots of each culture. Growth rates given are for 1.5 h after the addition of CCCP.

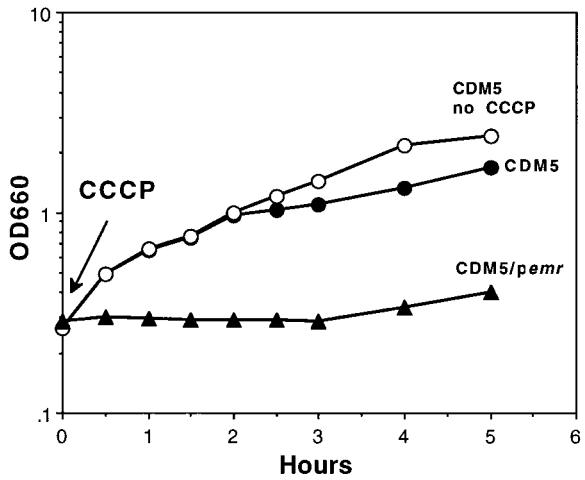

FIG. 6. Effects of EmrR on CCCP sensitivity of a Tlm^r mutant. The various strains were grown in LB medium in the presence of 25 μM CCCP, which was added at time zero. Tested were strain CDM5 Tlm^r *emrR** (11), strain CDM5/p*emrR*, and strain CDM5 without CCCP addition. OD660, optical density at 660 nm.

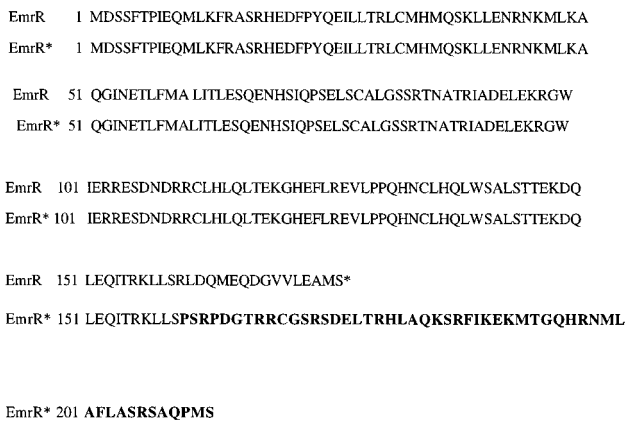

FIG. 7. Sequence comparison of EmrR and EmrR* proteins. EmrR* is the result of a frameshift mutation due to the insertion of a C at position 479 relative to the *emrR* translational start site. The two proteins share 160 identical amino acids at the N terminus. EmrR* is 36 amino acid residues longer than EmrR. The mutated part in the EmrR* protein is highlighted in boldface letters.

linked to a promoterless *lacZ* gene, indicating the absence of a promoter in this region. However, when the upstream region was extended to include the *emrR* gene and its putative promoter region, a productive *lacZ* fusion resulted. Primer extension analysis confirmed that the transcriptional start site of *emrA* is indeed upstream of the *emrR* gene. As discussed in the introduction, we have therefore renamed *mprA* as *emrR*. The terminator downstream of *emrR* appeared to be functional, inhibiting transcription of the downstream genes two- to threefold. This mild inhibition suggests that an antiterminator mechanism is involved.

An additional promoter for the *emrB* gene was discovered. This promoter is located in the structural part of the *emrA* gene. Expression from this promoter is specifically increased when *E. coli* enters the stationary phase (19a). The function of this additional promoter remains to be explored.

Our data further show that the EmrR protein acts as a negative regulator of the MDR pump. Placing *emrR* on a multicopy plasmid strongly inhibited the expression of a chromosomal *emr-lacZ* fusion, and conversely, an *emrR* null mutation caused a 40-fold increase in the activity of the fusion. The amount of *emr* message produced paralleled the results of fusion expression experiments. Overexpression of *emrR* increased the sensitivity of cells to CCCP, while a mutation in the repressor increased resistance to CCCP and the antibiotic thiolactomycin. Having the pump genes and the negative repressor of these genes as part of the same operon probably ensures a rapid and strong remedial response by the cell to exposure to deleterious drugs.

EmrR is homologous to a family of regulatory proteins. These proteins include a 17-kDa protein of unknown function (36% homology and 62% similarity) that is the product of the last gene of the *prs* operon which is necessary for expression of adhesion pili in uropathogenic *E. coli* (23); PecS, controlling pectinase, cellulase, and blue pigment production in *Erwinia chrysanthemi* (32); SlyA, which is involved in the production of salmolysin (14); MarR, a repressor of the *E. coli mar* locus conferring multiple antibiotic resistance (2, 8); HpcR, which regulates an inducible chromosomal gene cluster in *E. coli* that encodes homoprotocatechuate-degrading activity (33); and Hpr, which is involved in the regulation of protease production in *B. subtilis* (30). None of these proteins, like EmrR, has known DNA-binding motifs (28). We are now investigating whether the transcription repression caused by EmrR is due to direct binding to the promoter region. Sequence similarity has recently been found upstream of the promoter regions of P*emr* and P*mcb* (24a), strengthening the possibility that EmrR could directly bind to these promoters.

The EmrR repressor may serve as a relay mechanism for the signal(s) that controls the pump. Multiple drugs may directly bind to EmrR and induce MDR expression, a possibility now under investigation. It is noteworthy in this connection that a transcriptional activator of the Bmr multidrug resistance pump from *B. subtilis* has recently been found to directly bind a number of hydrophobic cations (that are substrates of the pump), which in turn leads to its interaction with the regulatory region of the *bmr* gene (1). The Bmr pump belongs to the same major facilitator family as EmrB, but BmrR is unrelated to EmrR, being a member of the Mer (mercury resistance) group of transcriptional activators. The fact that several structurally unrelated chemicals act as inducers as well as substrates for the EmrAB pump suggests that the physiological role of the translocase is indeed the extrusion of multiple drugs.

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