The MarR Repressor of the Multiple Antibiotic Resistance (*mar*) Operon in *Escherichia coli*: Prototypic Member of a Family of Bacterial Regulatory Proteins Involved in Sensing Phenolic Compounds

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ABSTRACT

Background: The *marR* gene of *Escherichia coli* encodes a repressor of the *marRAB* operon, a regulatory locus controlling multiple antibiotic resistance in this organism. Inactivation of *marR* results in increased expression of *marA*, which acts at several target genes in the cell leading to reduced antibiotic accumulation. Exposure of *E. coli* to sodium salicylate (SAL) induces *marRAB* operon transcription and antibiotic resistance. The mechanism by which SAL antagonizes MarR repressor activity is unclear.

Materials and Methods: Recombinant plasmid libraries were introduced into a reporter strain designed to identify cloned genes encoding MarR repressor activity. Computer analysis of sequence databases was also used to search for proteins related to MarR.

Results: A second *E. coli* gene, MprA, that exhibits MarR repressor activity was identified. Subsequent database searching revealed a family of 10 proteins from a variety of bacteria that share significant amino acid sequence similarity to MarR and MprA. At least four of these proteins are transcriptional repressors whose activity is antagonized by SAL or by phenolic agents structurally related to SAL.

Conclusions: The MarR family is identified as a group of regulatory factors whose activity is modulated in response to environmental signals in the form of phenolic compounds. Many of these agents are plant derived. Some of the MarR homologs appear more likely to control systems expressed in animal hosts, suggesting that phenolic sensing by bacteria is important in a variety of environments and in the regulation of numerous processes.

INTRODUCTION

In *Escherichia coli*, a simultaneous increase in resistance to a number of structurally unrelated antibiotics is associated with mutations at the multiple antibiotic resistance (*mar*) locus, located at 34′ on the *E. coli* chromosome (1). Susceptibility to a number of antibiotic classes is affected by this system, including tetracycline, chloramphenicol, β-lactams, and fluoroquinolones (2,3). Reduced drug accumulation, resulting from activation of efflux systems along with down-regulation of outer membrane porins, appears to be the primary resistance mechanism (2–5). A Mar phenotype has also been observed in a variety of gram-negative, quinolone resistant clinical isolates, suggesting that nonspecific multidrug resistance systems may play a role in the outcome of antibiotic therapy regimens with this class of antibiotics (6).

Recent efforts from several labs have helped clarify the identity and function of genes en-

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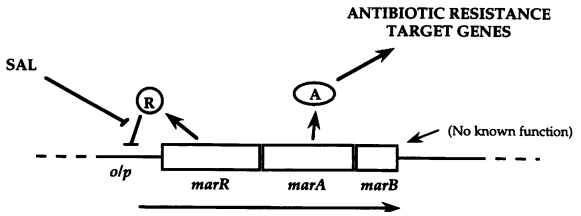

FIG. 1. Genetic organization of the *marRAB* operon

MarR is an autorepressor that normally maintains *mar* operon expression at a low level. In the presence of inducers such as salicylate (SAL), MarR repressor activity is antagonized, resulting in increased levels of MarA and, consequently, increased antibiotic resistance.

coded at the *mar* locus that play a role in the resistance scheme. These studies have drawn attention to a small operon encoding the *marRAB* genes as the critical element in this system (Fig. 1). This locus appears to be regulatory in nature. *marA* encodes a positive regulator of antibiotic resistance whose level of expression seems to be the critical determinant of antibiotic resistance. A Tn5 insertion in *marA* reverses the resistant phenotype of *mar* mutants (1), and overexpression of this gene confers resistance even in a strain containing a large deletion of the *mar* region of the chromosome (7). MarA shares significant amino acid sequence similarity to members of the AraC family of transcriptional activators (7,8), most notably SoxS, a positive regulator of the superoxide stress response whose induction also leads to a Mar phenotype (9,10).

Control of *marRAB* operon transcription, and thus of *marA* expression, is the role of the *marR* gene product. Mutations that result in increased antibiotic resistance are frequently located in *marR* (8,11), and *marR* is necessary to maintain *marRAB* operon expression at a low level (11,12). These findings indicate that MarR is a negative regulator of the *marRAB* operon, and imply that *marRAB* operon expression is inducible.

Support for this notion has recently emerged. A conditional antibiotic resistant phenotype similar to that associated with *mar* mutants can be elicited by culturing *E. coli* in the presence of the aromatic weak acid sodium salicylate (SAL) (13). Moreover, exposure to SAL results in the rapid induction of *marRAB* operon transcription and of expression of a *mar-lacZ* fusion, providing a partial explanation for the effects of this agent (14). Strains deleted for the *mar* locus are defective in SAL-inducible antibiotic resistance (14), and the only genes encoded at the *mar* locus that are required for the salicylate effect are *marR* and *marA* (12). A recent report indicated that a hybrid protein consisting of a fusion between maltose binding protein and MarR can bind the *marRAB* promoter region *in vitro*, and that this binding is antagonized by a variety of chemical agents including SAL (15). These observations suggest a simple model in which MarR directly represses *marRAB* operon transcription, and SAL antagonizes MarR repressor activity, presumably by dissociating MarR from the operator region (Fig. 1). Since exposure to acetylsalicylic acid (aspirin) can induce the same antibiotic resistance phenotype (13), an awareness of the importance of the *mar* locus during infection and antibiotic therapy seems warranted.

Thus, an understanding of the physiological signals governing MarR repressor activity would be very informative with respect to the control of intrinsic antibiotic resistance in enteric organisms. We report here the finding that MarR is related, both in primary amino acid sequence and in function, to a family of bacterial regulatory proteins that are involved in specific responses to environmental phenolic compounds. This discovery significantly expands the context in which the *mar* system should be considered and suggests that the *marRAB* operon is part of a larger stimulon that may be important in virulence and resistance processes in bacteria.

MATERIALS AND METHODS

Bacterial Strains and Plasmids

All strains described in this study are *E. coli* K12 derivatives of MC4100 (F^- *araD139* Δ(*lac*)*U169* *rpsL relA thi*) (16). Strain B318, used extensively herein, contains the Δ1738 deletion (17) which removes ~39 kbp of DNA from the *mar* region of the chromosome including the *marRAB* operon. It is also lysogenic for a recombinant lambda phage carrying a *marR-lacZ* protein fusion, in which *lacZ* is fused in frame after the 75th codon of *marR*, and expression of the fusion is under the control of the *marRAB* operon promoter (12). Expression of this fusion is constitutive in strain B318 due to the absence of a source of MarR repressor.

E. coli and *Salmonella typhimurium* plasmid libraries were constructed from size-selected fragments of *Sau*3A partial digests of chromosomal DNA from either strain W3110 (*E. coli*) or SR-11 (*S. typhimurium* [18]), cloned into the *Bam*HI site of pBR322 (for *E. coli* [19]) or pBluescript SK$^-$ (for *S. typhimurium*; Stratagene). p*marR* (12) consists of a 570 bp PCR fragment containing the *marRAB* operon operator/promoter region and all of *marR*, cloned into pBR322. The pS plasmids shown in Fig. 2 were constructed by subcloning the indicated restriction fragments of p*marD* (see text) into pBluescript SK$^-$ (Stratagene). p*mprA* was constructed by PCR synthesis of the *mprA* gene using primers based on the published sequence (20). The vector was pBluescript SK$^-$. The *mprA*::*cat* allele was constructed by first isolating the *cat* gene from λ1324 (21) by digestion of the phage DNA with *Bam*HI, converting the *Bam*HI sites into *Sph*I sites using linkers, and cloning the fragment into the *Sph*I site in *mprA* in plasmid pS6 (Fig. 2). This results in disruption of *mprA* after the 34th codon. The *mprA*::*cat* plasmid was linearized by digestion with *Pst*I and used to replace the normal *mprA* gene by linear transformation of a *recD*::Tn*10* strain (22). Subsequent strains containing the *mprA*::*cat* allele were constructed by P1 transduction. pEc17kd was constructed by PCR synthesis of a DNA fragment corresponding to the coding region of the 17kd gene from the *prs* locus (23) using chromosomal DNA from strain ECOR 55 (24) as a template. This fragment was then cloned into the IPTG-inducible expression plasmid pTrc*99A* (Pharmacia, Piscataway, NJ).

Reagents and Growth Conditions

Bacteria were propagated in LB media (25), which, when needed, was solidified with 1.5% Bacto-Agar (DIFCO, Detroit, MI). All antibiotics were from Sigma Chemical Co. (St. Louis, MO, U.S.A.) other reagents were from commercial sources and were of the highest purity available.

Other Genetic and Biochemical Methods

DNA hybridizations were performed following standard protocols (26). Probes were labeled with digoxigenin using the Genius kit (Boehringer-Mannheim, Indianapolis, IN) following the manufacturers instructions. For Southern hybridizations, DNA samples were resolved on agarose gels and transferred to Nytran membranes (Schleicher and Schuell, Keene, NH) by capillary transfer following protocols from the membrane supplier. The miniset blot of the ordered recombinant phage collection of Kohara (27) was from TaKaRa. β-Galactosidase assays were performed as described by Miller (25) using the chloroform-SDS method of cell permeabilization. Briefly, cultures were grown in L broth to an A_{600} = 0.3, at which time inducers were added to 2.5 mM. Incubation was continued for 1 hr, and β-galactosidase activity, expressed as Miller Units, was determined.

Computer Methods

Alignments of promoter regions shown in Fig. 3 were performed using MacVector version 4.5 software for the Macintosh (Eastman Kodak Co., Rochester, NY, U.S.A.). The computer searches that generated the sequences shown in Fig. 4 were performed using TFASTA (28) in the GCG package of sequence analysis programs (29). Initial searches used MarR or MprA as the query sequence. New matches found in these searches were then used as queries in additional searches. This iterative process was repeated until no new members were found. Alignment of the sequences was performed using the PILEUP program (29).

RESULTS

Identification of a Functional Homolog of *marR*

In the course of genetic studies on the regulation of *mar* operon transcription, we constructed an *E. coli* strain, B318, which proved useful for cloning *mar* homologs from related enteric organisms. B318 contains a 39-kbp deletion of the *mar* region of the chromosome including the *marRAB* operon, and also carries a λ phage containing a *marR-lacZ* translational fusion under the control of the *mar* operator/promoter region (12). Expression of this fusion is constitutively derepressed (Lac$^+$) in this strain due to the absence of MarR repressor. Introduction of a plasmid containing *marR* restores both repression of fusion expression and inducibility by SAL (12). The presence of *marA* on the same plasmid restores normal levels of SAL-inducible antibiotic resistance (14).

Our initial objective was to use B318 to clone *mar* sequences from virulent enteric organisms,

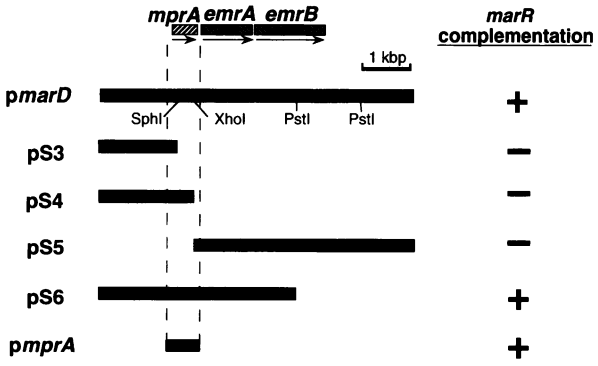

FIG. 2. Restriction map and complementation data for *marD* plasmids

The restriction map for p*marD* and the positions of the *mprA*, *emrA*, and *emrB* genes are shown at the top. p*marD* is pBluescript SK$^-$ (Stratagene) derivative containing a 6.0-kb fragment from a random *Sau*IIIA digest of chromosomal DNA of *E. coli* strain W3110. Specific subclones were generated as described in Materials and Methods. *marR* complementation indicates the ability of the specified plasmid to restore a Lac$^-$ phenotype on MacConkey-lactose media to strain B318.

TABLE 1. Regulation of *marR-lacZ* by MarR and MprA

Plasmid	Background[a]	β-Galactosidase Activity Following Induction with: No Addition	SAL[b]
None	*WT*	15	300
None	Δ*mar*	420	730
p*marR*	Δ*mar*	5	150
p*mprA*	Δ*mar*	10	250

[a]All experiments were performed in strain B318 except for the first entry, which was in an isogenic strain containing a wild-type *mar* locus.

[b]SAL, sodium salicylate.

and then construct deletion strains for the purpose of testing the importance of the *mar* system in various pathogenic models. When B318 was transformed with a plasmid library containing random *S. typhimurium* chromosomal DNA, nine white (Lac$^-$) transformants were obtained on MacConkey-lactose indicator plates. These were further tested for the presence of *marRAB* operon DNA by Southern blotting. Two classes of clone were identified. The first class (five transformants) contained sequences that hybridized with an *E. coli mar* probe, and included the *S. typhimurium marR* gene as judged by DNA sequence analysis. The remaining four transformants contained cloned DNA sequences that were not detected by the *E. coli mar* probe, but cross-hybridized with each other, indicating that they were derived from the same locus and possibly encoded a second *mar* repressor. This new *marR*-like activity was designated *marD*.

To determine if *E. coli* also had a *marD* gene the cloning strategy was repeated, this time using an *E. coli* plasmid library. White transformants were again identified and, as in the Salmonella experiment, the population was mixed. Fourteen of fifteen clones were shown by hybridization to carry *mar* sequences. The remaining isolate contained a cloned insert that cross-hybridized with the *S. typhimurium marD* fragment, indicating that this locus was also present in *E. coli*. The corresponding plasmid was designated p*marD* (Fig. 2).

To find the location of *marD* on the *E. coli* chromosome, p*marD* was used as a hybridization probe against the Kohara miniset blot (27). Positive hybridization signals localized the cloned sequence to the 52′ region of the chromosome. Comparison of the restriction map for this region of the chromosome with that of p*marD* indicated that three known genes, *mprA*, *emrA*, and *emrB*, were present within the 6 kbp cloned segment (Fig. 2). To localize *marD* repressor activity within this interval, deletion and subcloning experiments were performed. As is shown in Figure 2, this activity was associated with the *mprA* gene, previously identified as a repressor of genes involved in the biosynthesis of the peptide antibiotic microcin B17 (20,30).

One feature of MarR repressor activity is its antagonism by SAL (14). To determine if MprA-mediated repression of *marR-lacZ* was similarly affected, B318 derivatives carrying either *mprA* or *marR* plasmids were treated with SAL and assayed for β-galactosidase activity. As is shown in Table 1, SAL relieved repression mediated by either *marR* or *mprA*. These results indicate that *mprA*, when present on a multicopy plasmid, can functionally replace *marR* and restore regulation to a *marR-lacZ* fusion.

It seemed unlikely that *mprA* played a significant role in regulating *mar* gene expression in wild type cells, since a deletion of *marR* alone

results in high level derepression of a *marR-lacZ* fusion. To determine if *mprA* played a minor role in regulating *mar* gene expression, a chloramphenicol resistance determinant was inserted after the 34th codon of the cloned *mprA* gene, and the disrupted allele was recombined onto the chromosome in place of the normal gene (see Materials and Methods). In an otherwise wild-type strain (i.e., *marR*$^+$), the *mprA*::*cat* allele had no affect on *marR-lacZ* fusion expression. In addition, expression of this fusion in a strain containing a *marR*::Tn*10* allele was also unaffected by the introduction of *mprA*::*cat* (data not shown). Thus, *marR* and *mprA* are not redundant regulators in the *E. coli* strains used here. The complementation of a *marR* deficiency by *mprA* requires that the latter be present in multicopy.

In wild-type cells, MprA is known to repress expression from two specific promoters: that of the microcin B17 biosynthetic gene cluster (P_{mcbA})(30) and its own promoter (P_{mprA})(20). A recent report indicated that *mprA* expression is significantly induced following salicylate treatment (31). To confirm that this is the case in the strain background used here, we measured expression of a *mprA-lacZ* transcriptional fusion present in single copy on a λ prophage under conditions identical to those used to follow *marR-lacZ* fusion expression; namely, 1-hr exposure to 2.5 mM SAL using cells grown to mid-log prior to SAL addition. A 5-fold induction of fusion enzyme expression was consistently observed under these conditions (data not shown). Thus, SAL stimulates the expression of a normal cellular target of MprA.

MarR and MprA may thus act as sequence-specific repressors. As such, promoters controlled by these regulators may contain conserved sequences at appropriate positions with respect to known or suspected recognition sites for RNA polymerase (32). A comparison of the promoter regions for *mprA* (20) and *mcbA* (33) revealed such a sequence that was identical at 19 of 24 positions (Fig. 3A). In P_{mprA}, this element is located 5 bp upstream from the predicted −35 sequence. In contrast, this same sequence is found 77 bp upstream from the −35 element of a promoter known to direct the post-exponential expression of *mcbA* (33). It seemed possible that the increase in *mcbA* expression observed in an *mprA* mutant during exponential growth results from activation of a different promoter element than that used for postexponential expression. Consistent with this notion, good −35 and −10 elements could be found in the vicinity of the

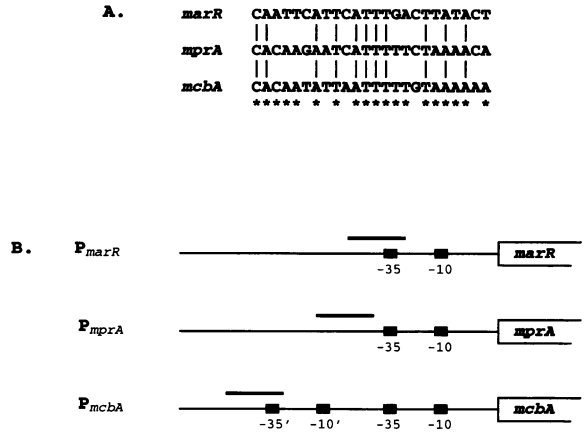

FIG. 3. Identification of a conserved sequence element among the promoters for *marR*, *mprA*, and *mcbA*

(A) Alignment of conserved sequences. Vertical lines indicate nucleotide positions that are identical among all three sequences. Asterisks beneath the *mcbA* sequence show residues that are identical in *mprA* and *mcbA*. (B) Positions of conserved elements in the promoter regions of *marR*, *mprA*, and *mcbA*. Conserved sequences aligned in Panel A are indicated by solid bars.

putative MprA recognition sequence (−35′ and −10′ in Fig. 3B). The −35′ was buried within the putative MprA recognition sequence.

Comparison of the promoter regions of *marR* (8) and *mprA* indicated that the region of greatest similarity corresponded to the conserved sequence found in the P_{mprA}/P_{mcbA} alignment. Figure 3A shows that the P_{marR} element is identical at 11 of 24 positions with the other two sequences. Moreover, the putative −35 sequence of P_{marR} is nested within this conserved element (Fig. 3B).

Identification of Other Bacterial Homologs of MarR

Previous computer-assisted searches had failed to identify any significant similarities between MarR and translated sequences in the available databases (8). However, an alignment of MarR and MprA showed that the two proteins share 28% identity at the amino acid sequence level. Inclusion of conservative amino acid replacements increased the similarity to 47%. Since the complementation results (Table 1) indicated that this level of similarity is significant and related to function, we reexamined the databases for evidence of other homologs, using the program

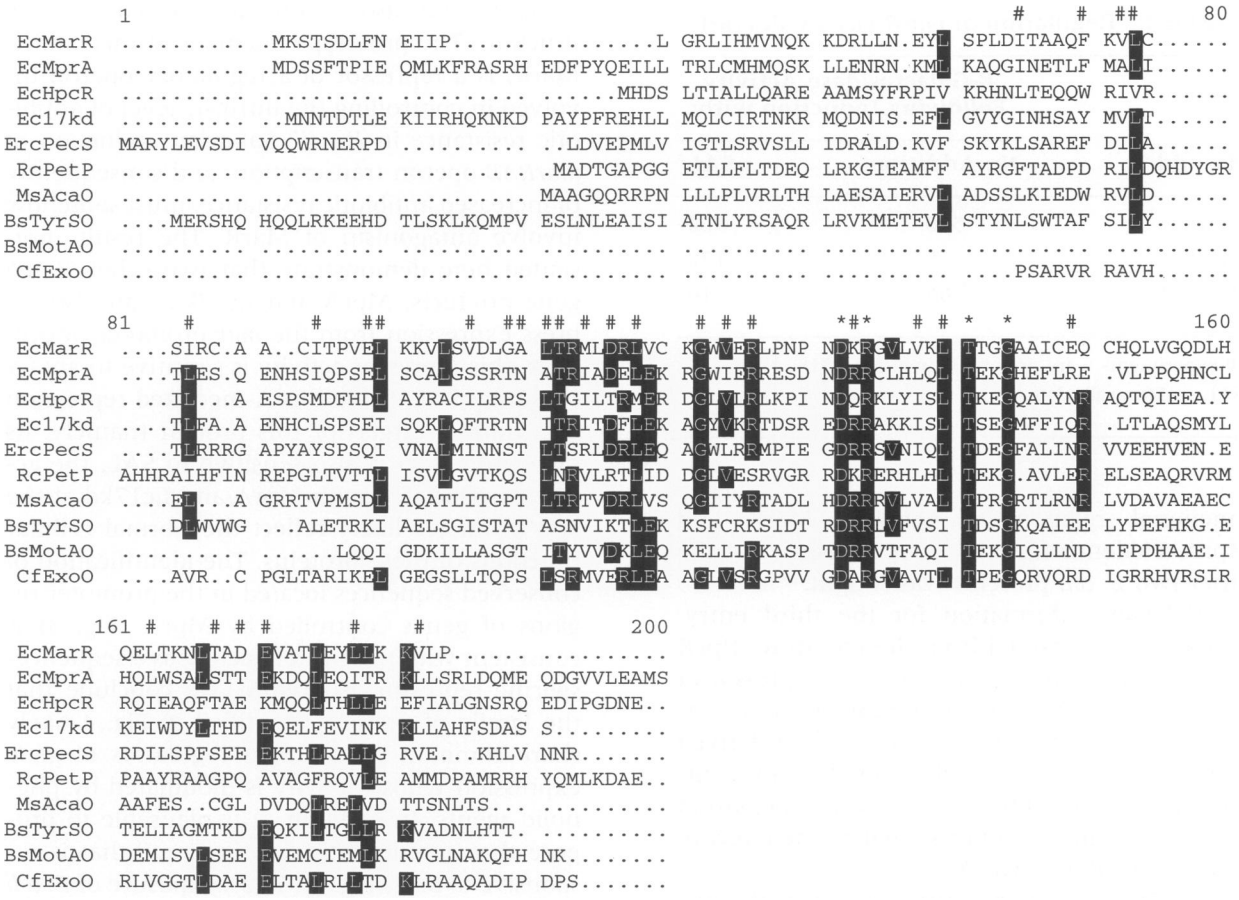

FIG. 4. Alignment of MarR and related proteins

Protein sequences shown were identified using TFASTA to search currently available GenEMBL and SwissProt databases. The alignment was generated using the PILEUP program in the GCG package (29). Entries are not listed in order of similarity, but rather reflect their bacterial origin as described in the text. Amino acids highlighted in black boxes are residues identical at that position for at least five of the proteins. *, the four residues that are identical in all ten sequences; #, a position in which a chemically conserved amino acid is present in at least 8 of the 10 entries, based on the following groupings of conserved amino acids: YWF, QNED, MILV, PAGST, HKR. Relevant information, GenEMBL accession numbers, and references, respectively, are as follows: EcMarR: MarR from *E. coli*, accession number M96235 (8); EcMprA: MprA from *E. coli*, X54141 (20); EcHpcR: HpcR from *E. coli* C strain, S56952 (34); Ec17kd: 17 kd ORF from *prs* gene cluster in pyelonephritic *E. coli* ECOR 55 strain, X62158 (23); ErcPecS: PecS from *Erwinia chrysanthemi*, X74409 (35); RcPetP: PetP from *Rhodobacter capsulatus*, Z12113 (36); MsAcaORF: ORF P1 in the entry for *Mycobacterium smegmatis* acetamidase gene, X57175 (37); BsTyrSORF: ORF3 in the *tyrS* sequence entry from *Bacillus subtilis*, X52480 (38); BsMotABORF: 3′ end of an ORF downstream from and convergently transcribed towards the *motAB* genes of *B. subtilis*, M77238 (39); CfExoORF: An ORF upstream of and divergently transcribed from the *Cellulomonas fimi* exogluconase gene, L11080 (40). This last open reading frame does not contain a normal initiation codon and may be derived from incorrect sequence information. Additional information on some of the entries is described in the text.

TFASTA (28). This analysis revealed eight additional proteins that were similarly related to MarR (Fig. 4). All of the sequences identified are of bacterial origin, with four entries from *E. coli* strains and the remainder from diverse species including gram-positive, gram-negative and mycobacteria. Except for the extreme amino terminal regions, conserved residues are found distributed throughout the entire length of the alignment. In addition, there is a cluster of highly conserved amino acids extending from residues 111–144 of the alignment that includes four invariant positions. A preliminary consensus sequence of DXRXXXXX(L/I)TXXG (residues 132–144) can be compiled that appears to be a signature for this family of proteins. Given the

TABLE 2. Regulation of *marR-lacZ* by Ec17kd

Plasmid	β-Galactosidase Activity Following Induction with:	
	No Addition	SAL[a]
None	530	960
p*marR*	5	170
pEc17kd	66	310

All experiments were performed in strain B318.
[a]SAL, sodium salicylate.

functional relationship between MarR and MprA, evidence for analogous properties of the other two *E. coli* proteins was sought.

Relevant information for the third entry, EcHpcR, was obtained from the literature. HpcR is found in *E. coli* C strains and is a repressor of adjacent genes involved in the catabolism of homoprotocatechuate (HPC) (34), a plant-derived phenolic that is structurally related to SAL. Importantly, HpcR repressor activity is antagonized by HPC (34) in a manner similar to the effect of SAL on MarR and MprA.

Ec17kd, the fourth entry shown, is the putative product of an open reading frame linked to the *pap/prs* cluster of genes associated with pilus formation in certain pyelonephritic *E. coli* strains. Although no function has been associated with this ORF as yet, its similarity to MprA has been noted previously (23). In order to determine if Ec17kd could restore MarR repressor activity to strain B318, the coding region for Ec17kd was synthesized by PCR and cloned into the IPTG-inducible expression plasmid pTrc*99A*. However, we found that even in the absence of induction with IPTG, the Ec17kd construct repressed *marR-lacZ* expression in strain B318 (Table 2), suggesting that leaky expression from the *trc* promoter produced sufficient 17kd protein to complement the *marR* deficiency in this strain. Furthermore, this repressor activity was antagonized by SAL. Thus, Ec17kd is also functionally related to MarR.

DISCUSSION

The findings presented in this study identify a new family of bacterial regulatory proteins related not only by primary amino acid sequence similarity but also, in the cases tested here, by function. The prototypic member of this family, MarR, is a repressor of a regulatory operon involved in controlling the intrinsic level of antibiotic resistance in *E. coli*. Salicylate induction of *marRAB* operon transcription, and consequently of increased antibiotic resistance, must somehow involve antagonism of MarR. The results presented here demonstrate that two other *E. coli* gene products, MprA and Ec17kd, can also repress expression from the *mar* promoter. Moreover, this repressor activity is sensitive to antagonism by SAL. Since MprA-mediated repression of a normal target for this protein (namely, its own promoter) is also sensitive to SAL, the activities associated with MprA and Ec17kd at the *mar* promoter likely reflect the normal cellular functions of these proteins. The identification of conserved sequences located in the promoter regions of genes controlled by MprA (Fig. 3) is consistent with this protein acting as a sequence-specific repressor. Therefore, we conclude that the family of proteins identified in Fig. 4 likely share common functions as regulators of gene expression whose activity is modulated by phenolic agents. In addition, it is plausable to propose that a stimulon exists in *E. coli* that is responsive to SAL (Fig. 5). Thus, exposure of *E. coli* strains to this agent would derepress the expression of genes controlled by MarR and its related repressors, and thus affect the activity of several independently regulated genetic pathways. Such a treatment would result in an increase in antibiotic resistance (MarR controlled) and microcin B17 antibiotic production (MprA controlled), along with the target genes controlled by Ec17kd, should the particular strain contain the *pap/prs* cluster. The proximity of *mprA* to the downstream *emrA* and *emrB* genes, which encode an efflux pump for xenobiotic agents (42), is similar to the physical arrangement of *marR* and *marA*, and raises the possibility that expression of the EmrAB pump is also controlled by MprA. The cellular target(s) for Ec17kd regulation is not known; however, its association with a virulence locus suggests that it may participate in a pathogenic function. Since the *mar* locus is known to be present in a large number of enteric species (43) and the experiments reported here indicate that *mprA* is also found in *S. typhimurium*, it seems reasonable to propose that the SAL stimulon is conserved among the enterics. SAL has also been reported to induce increased antibiotic resistance in *Pseudomonas cepacia*, sug-

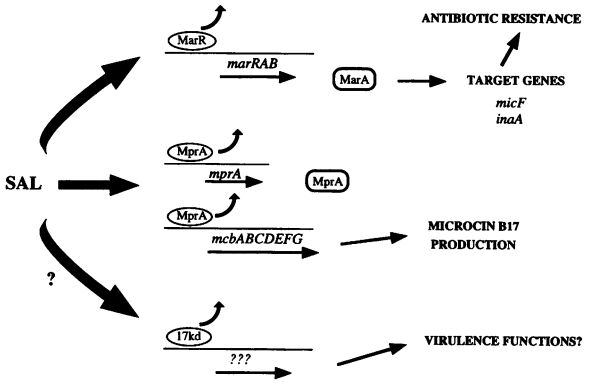

FIG. 5. The SAL stimulon

MarR and its relatives MprA and Ec17kd are transcriptional repressors acting at specific promoters. In the presence of SAL, the repressing activity of these proteins is antagonized, allowing transcription of the regulated operons. For the MarR and MprA pathways, the consequences of derepression are shown at the right. *micF* encodes an antisense mRNA that acts to repress production of the major outer membrane porin OmpF, and is induced in *mar* mutants (3). *inaA* is a weak acid-inducible gene whose expression is stimulated by MarA (41). For Ec17kd, the target(s) of repression have not been identified; the question mark next to the arrow leading from SAL to 17kd indicates that antagonism of 17kd-mediated repression of a normal target promoter has not been demonstrated. Other MarR homologs are predicted to carry out similar activities in controlling the expression of specific target genes.

gesting that drug resistance associated with sensing of this phenolic is widespread (44).

Although not specifically related to pathogenesis, the identification of HpcR as a member of this family may help clarify other issues regarding the mechanism of repressor antagonism. HpcR is known to regulate expression of a catabolic pathway that allows soil-dwelling *E. coli* C strains to metabolize homoprotocatechuate (HPC) (34,45). As HPC is thought to be the authentic inducer for the *hpc* system, the possibility that SAL is structurally related to the natural inducer of the *mar* system is raised. The observation that the *mar* and *hpc* systems have nothing in common other than their respective repressors and inducers is consistent with the concept of direct recognition of the inducer by the repressor, as has been suggested for MarR (15).

The remaining proteins listed in Fig. 4 are from bacteria other than *E. coli*. These include gram-positive and gram-negative species as well as mycobacteria, indicating that MarR family members are not restricted to enterics. The PecS protein from *Erwinnia chrysanthemi* (ErcPecS) is associated with the transcriptional control of pectate lyase and cellulase production, which are involved in invasion of plant tissues (35). *petP* from *Rhodobacter capsulatus* is part of a two-gene operon with a second putative transcriptional regulator *petR*, a genetic organization similar to that of *marR* and *marA* (36). The remaining proteins are inferred from open reading frames present in database entries, but for which no genetic information exists. However, since the non-*E. coli* members of this family exhibit similar levels of sequence conservation and include the same highly conserved residues as the *E. coli* proteins, a reasonable expectation is that they are also regulatory proteins and that their activity is affected by a phenolic compound. The target genes for this latter group remain to be identified, but the finding that they can be associated by sequence similarity with the MarR family should provide a means for their detection. It is worth noting that other as yet unidentified MarR family members may exist in *E. coli* and could have escaped detection in the cloning experiments described here, since the screen we employed demanded that the cloned gene product recognize and repress expression from the *marRAB* promoter. MarR homologs capable of binding SAL but that have different promoter specificities would not have been identified.

The organisms in which the gene products listed in Fig. 4 are found are largely free-living bacteria or plant pathogens. In addition, SAL is an important signaling molecule in plants (46). It thus seems likely that phenolic sensing is an important component of environmental surveillance by free-living bacteria, particularly when considering the variety of noxious aromatic agents produced by plant species (47). However, the association of Ec17kd with the MarR family suggests that phenolic sensing may play a role in animal infections as well, since the presence of the *pap* cluster with which the gene encoding Ec17kd is associated correlates strongly with the pyelonephritic capability of *E. coli* (23). Thus, MarR family members may also carry out important roles in phenolic sensing in animal hosts, and so might monitor either plant-derived compounds ingested by the host or host-synthesized phenolic metabolites. Such inducers might themselves be toxic compounds against which these organisms need to protect themselves; alternatively, they might serve as cues that the bacterium is in the intestinal environment, and could signal

the induction of protective (MarR, MprA) and colonization (Ec17kd) factors. Experiments directed towards determining the importance of specific MarR family members in these processes should be extremely informative in this regard.

Preliminary experiments indicate that there is some specificity with respect to the inducing agent. Syringaldehyde, a substituted phenolic that has been identified as an inducer of virulence gene expression in *Agrobacterium tumefaciens* (48), is also a potent inducer of the *mar* system (PFM, unpublished observations). Dinitrophenol has also been shown to efficiently stimulate *marRAB* operon transcription (14). In contrast, HPC is not an effective inducer of *mar* expression (PFM and MCS, unpublished observations). Moreover, while SAL is a good inducer of *mar* expression, salicyl alcohol is not (14). In this light, it is interesting to speculate on the role of the conserved amino acids shared by MarR family members (Fig. 4). These residues could be involved in phenolic binding, operator/promoter recognition, or protein-protein interactions (e.g. dimerization of repressor molecules, interaction with an accessory factor, etc). Since the MarR homologs are likely to exhibit different specificities for phenolics and promoters, conserved amino acids should be involved in interactions that are carried out in very similar ways among these repressors.

The identity of the natural inducers for most of the proteins in the MarR family remains to be determined. However, the finding that the repressor activities of MarR, MprA, and Ec17kd can be antagonized by SAL, a plant-derived phenolic, raises the possibility that plant products are the natural ligands for many, if not all, of these systems. For HpcR this is clearly the case. Moreover, syringaldehyde and related compounds were first identified as stimulants of virulence gene expression in *Agrobacterium tumefaciens* (48); similar signals may be recognized by MarR homologs from other plant pathogens and symbionts (e.g., ErcPecS, RcPetP, CfExoO) and may play a role in regulating virulence gene expression in these organisms. MarR and its relatives found in enteric bacteria such as *E. coli* (EcMprA, Ec17kd) might modulate gene expression in response to similar phenolics encountered in the gut of mammalian hosts as products of plant diets. In any case, the results reported here suggest that environmental surveillance of phenolic compounds is highly conserved in bacteria and may be important in a variety of signal transduction pathways.

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