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Repressor Mutations in the *marRAB* Operon That Activate Oxidative Stress Genes and Multiple Antibiotic Resistance in *Escherichia coli*

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Resistance to multiple antibiotics and certain oxidative stress compounds was conferred by three independently selected mutations (*marR1*, *soxQ1*, and *cfxB1*) that mapped to 34 min on the *Escherichia coli* chromosome. Mutations at this locus can activate the *marRAB* operon, in which *marR* encodes a putative repressor of *mar* transcription and *marA* encodes a putative transcriptional activator of defense genes against antibiotics and oxidants. Overexpression of the wild-type MarR protein reversed the phenotypes (antibiotic resistance and increased antioxidant enzyme synthesis) of all three mutants. DNA sequence analysis showed that, like *marR1*, the other two mutations were alterations of *marR*: a 285-bp deletion in *cfxB1* and a GC→AT transition at codon 70 (Ala→Thr) in *soxQ1*. All three mutations cause increased amounts of *mar*-specific RNA, which supports the hypothesis that MarR has a repressor function in the expression of the *marRAB* operon. The level of *mar* RNA was further induced by tetracycline in both the *marR1* and *soxQ1* strains but not in the *cfxB1* deletion mutant. In the *cfxB1* strain, the level of expression of a truncated RNA, with or without tetracycline exposure, was the same as the fully induced level in the other two mutants. Overproduction of MarR in the *cfxB1* strain repressed the transcription of the truncated RNA and restored transcriptional inducibility by tetracycline. Thus, induction of the *marRAB* operon results from the relief of the repression exerted by MarR. The *marRAB* operon evidently activates both antibiotic resistance and oxidative stress genes.

The adaptive defense of *Escherichia coli* against oxidative stress imposed by superoxide-generating agents involves the coordinate regulation of at least 10 promoters controlled by the *soxRS* locus (1, 10, 15, 31). Previous work has shown that, independently of *soxRS*, the *soxQ1* and *cfxB1* mutations cause transcriptional activation of some of the *soxRS* regulon genes and the induction of seven other proteins not influenced by *soxRS* or elevated by oxidative stress (14). Both *soxQ1* and *cfxB1*, together with the previously identified *marR1*, map to the 34-min locus on the *E. coli* chromosome (6, 13, 14, 19), which has been implicated in chromosome-mediated multiple antibiotic resistance (*mar*) (6, 13, 16). The *soxQ1*- and the *cfxB1*-dependent phenotypes do not depend on the *soxRS* locus, and gene induction by *soxRS* in response to redox stress does not depend on *mar* (14). It has been proposed that the *mar* locus controls some oxidative stress proteins as part of a regulon that responds to one or more environmental stress signals (14). The responsive nature of this putative new regulon is illustrated by the fact that the *mar* transcript is induced by at least two different antibiotics, tetracycline and chloramphenicol (16), as well as by salicylate and related compounds (7).

The *mar* region has been cloned (16) and sequenced to reveal an operon composed of three putative structural genes (*marR*, *marA*, and *marB*) (6). The *marA* gene encodes a predicted 13-kDa polypeptide strongly similar to the SoxS protein (42% identical, 65% similar), which activates the *soxRS* regulon genes (1). The predicted sequences of the polypeptides encoded by the *marR* and *marB* genes did not show significant identity to known or translated protein sequences (6).

The *marR1* mutation is positioned in the *marR* gene (6). Five other independent Mar mutants were shown to contain a mutation either in *marR* or in the putative operator region (*marO*) of the operon. Since all six mutations lead to transcriptional activation of the operon, it has been suggested that the MarR protein is a repressor of the *marRAB* operon and acts at *marO* (6).

The present work shows that the *soxQ1* and *cfxB1* mutations are located in the *marR* gene. These mutations increase the level of the *marRAB* transcript, which can be repressed in *trans* by multicopy *marR*. This repression reverses both antibiotic and oxidant resistances of *soxQ1* and *cfxB1* strains but does not prevent antibiotic-induced expression of the *mar* transcript.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The *E. coli* strains and plasmids used in this study are listed in Table 1. Permanent bacterial stocks were stored frozen at −80°C in 20% glycerol, and working stocks were maintained on LB agar (24) at 4°C for up to 2 weeks. Cells were grown at 37°C with shaking at 150 rpm in LB broth (24) unless otherwise indicated.

Antibiotic susceptibility. The sensitivities of various strains to antibiotics were determined by measuring bacterial growth on gradient plates prepared as previously described (9), except that each plate contained 60 ml of LB agar (30 ml in top and bottom layers), and the medium was supplemented with 0.1 mg of ampicillin per ml for plasmid-bearing strains. Cell culture

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TABLE 1. Strains and plasmids

Strain or plasmid	Relevant properties[a]	Reference
Strains		
EN226-8	Same as KL16; *cfxB1*	19
GC4468	F⁻ Δ(*lac*)*U169 rpsL*	15
JHC1069	Same as GC4468; *cfxB1 zdd-2207*::Tn*10*Km	14
JHC1072	Same as GC4468; *soxQ1 zdd-2207*::Tn*10*Km	14
JHC1096	Same as GC4468; *zdd-239*::Tn*9 del*1738 (Δ*mar*)	14
JHC1113	Same as GC4468; *marR1 zdd-2207*::Tn*10*Km	14
JTG102	F⁻ *metB btuB*::Tn*10 thr-1 leuB6 his-4 proA2 lacY1 galK2 ara-14 xyl-5 mtl-1 rpsL tsx-33 supE44 thi-1*	15
JTG320	Same as JTG102; *soxQ1*	14
JTG1078	Same as GC4468; *soxR105 zjc-2204*::Tn*10*Km	15
KL16	Hfr *thi-1 relA spoT1*	19
Plasmids		
pUC18	Multicopy vector, Amp^r	28
pMarR(WT)	pUC18 carrying *marO*$^+$ and *marR*$^+$ on an 818-bp fragment	6

[a] Tn*10*Km designates a minitransposon derivative of Tn*10* which encodes resistance to kanamycin.

and plating were performed as previously described (1, 14, 15). Confluent growth along the gradient was scored after 18 to 24 h at 37°C.

Cell extracts and enzyme assays. Cells grown in LB broth to the mid-logarithmic phase (optical density at 600 nm, 0.2 to 0.4) were centrifuged, washed with ice-cold M9 salts (24), and frozen as pellets at −80°C. Cell pellets were thawed for 1 to 3 h on ice, suspended in buffer containing 50 mM Tris · HCl (pH 7.5)–0.2 M NaCl, and lysed in a French pressure cell by two passes at 10,000 lb/in^2. Cell debris was removed by centrifugation at 10,000 × *g* for 45 min, and the cleared supernatants were collected and stored on ice for up to 16 h or frozen at −80°C. Protein concentrations were estimated according to the method of Bradford (4).

Superoxide dismutase (SOD) activity was assayed in nondenaturing 7.5% polyacrylamide slab gels (17) by the method of Beauchamp and Fridovich (3). Glucose-6-phosphate dehydrogenase activity was monitored by measuring NADPH production at 340 nm (20). Fumarase C activity was determined as described by Hill and Bradshaw (18).

DNA manipulation and sequencing. Two *marRA*-specific oligonucleotides containing 5′ additions that specify restriction sites (a, 5′-CCGAATTCGTCGACAACGCTAGCCTTGCATCGCATT-3′, with nucleotides 1311 to 1329 underlined; b, 5′-CCCGGGATCCAAGCTTCATGATTGCCTCAGTGACGTTG-3′, with nucleotides 2318 to 2295 underlined) were synthesized to be used as primers in a PCR (28) with chromosomal DNA as the template. The numbering system refers to the previously published nucleotide sequence of the *mar* region (6). The PCR products were purified in agarose gels by using the GeneClean II Kit (Bio 101, La Jolla, Calif.) and used as templates for thermal cycle dideoxy sequencing with the CircumVent DNA Sequencing Kit (New England Biolabs Inc., Beverly, Mass.) according to the supplier's protocol for the incorporation of α-^{35}S-dATP, except that the length of the cycles was increased to 180 s.

RNA extraction and Northern (RNA) blot analysis. Overnight cultures in LB broth at 37°C were diluted 250-fold into 25 ml of fresh medium and incubated at 150 rpm and 30°C until the cell density reached an optical density at 650 nm of 0.3. Where indicated, tetracycline was added to a final concentration of 2 μg/ml and the incubation of all the cultures was continued for 60 min before the cells were harvested. The RNA was extracted from the cells obtained from 12.5 ml of culture, in duplicate for each strain and treatment, by using a modification of the hot-phenol extraction method previously described by Emory and Belasco (11). Briefly, samples were rapidly cooled to 0°C, and the cells were pelleted and suspended in 125 μl of ice-cold 0.3 M sucrose–0.01 M sodium acetate (pH 4.5). After addition of 125 μl of 2% sodium dodecyl sulfate–0.01 M sodium acetate (pH 4.5), the cell suspension was heated for 3 min at 70°C and extracted three times for 3 min each at 70°C with 250 μl of hot phenol previously equilibrated with unbuffered water. The RNA was ethanol precipitated and stored at −80°C in 20 mM sodium phosphate (pH 6.5)–1 mM EDTA. The concentration of total RNA in the samples was determined spectrophotometrically (28). RNA samples (30 μg) were denatured and separated on formaldehyde-agarose gels and then transferred overnight to positively charged nylon membranes (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) (28). Following transfer, the membrane was air dried and the RNA was fixed to it by exposure to UV light at 254 nm for 3 min in a Stratalinker 2400 UV cross-linker (Stratagene, La Jolla, Calif.). The probes for the *marRAB* RNA were a 1,008-bp DNA fragment containing *marR* and *marA* (positions 1311 to 2318) and a 511-bp fragment containing part of *marA* and the complete *marB* gene (nucleotides 2043 to 2553). Both probes were labeled by random priming (28) with digoxigenin-dUTP (Boehringer Mannheim Biochemicals). The hybridization of the labeled probe to the membrane-bound RNA and the washing and detection of the labeled DNA were carried out by using the Genius Luminescent system from Boehringer Mannheim Biochemicals according to the manufacturer's specifications. The RNA bands revealed by Northern blotting were quantified by densitometry scanning of the exposed film, using a BioImage system (Millipore).

RESULTS

Complementation of *soxQ1* and *cfxB1* phenotypes by *marR*. Although the *soxQ1*, *cfxB1*, and *marR1* mutations were mapped to the same chromosomal region, the differences in the detailed phenotypes of strains bearing these mutations left open the possibility that they might reside in different genes. This question was addressed by testing the effect of the putative *mar* repressor gene, *marR*, on cells bearing the various mutations. The introduction of a multicopy plasmid containing the *marR*$^+$ gene [pMarR(WT)] into the *cfxB1* and *soxQ1* strains diminished their resistance to chloramphenicol and tetracycline (Fig. 1). Although the plasmid reduced antibiotic resistance in the *soxQ1* strain to wild-type levels, significant

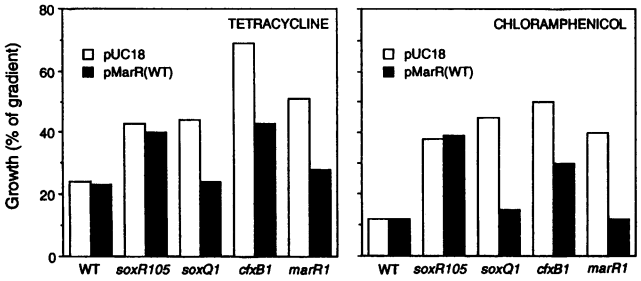

FIG. 1. Effect of the overexpression of MarR on antibiotic resistance. The sensitivities of strains bearing pUC18 or pMarR(WT) to tetracycline (0.25 mg per plate) and chloramphenicol (0.9 mg per plate) were scored in gradient plates (see Materials and Methods). The entire experiment was repeated at least three times; values from a representative experiment are shown. WT, wild type.

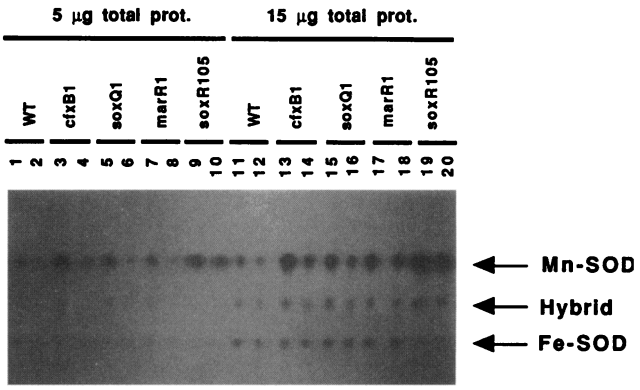

FIG. 2. SOD activity gel. WT, GC4468; *cfxB1*, JHC1069; *soxQ1*, JHC1072; *marR1*, JHC1113; *soxR105*, JTG1078. Lanes 1 to 10 and 11 to 20 were loaded with different amounts of extract protein, as indicated, and the gel was processed to reveal SOD activity (see Materials and Methods). Odd-numbered lanes correspond to pUC18-bearing cells, and even-numbered lanes correspond to pMarR(WT)-bearing cells. Hybrid, heteromeric protein with subunits from both Mn-SOD and Fe-SOD. The entire experiment was repeated twice with identical results. A negative image of the gel is shown.

resistance remained in the *cfxB1* strain containing pMarR (WT). As expected, the antibiotic resistance of a *marR1* strain was reversed to wild-type levels. In contrast, the elevated antibiotic resistance of a *soxRS*-constitutive strain was virtually unaffected.

Two of the antioxidant defense genes activated by *soxQ1* and *cfxB1* mutations are *sodA* (encoding Mn-containing SOD [Mn-SOD]) and *zwf* (which encodes glucose-6-phosphate dehydrogenase) (14). Another enzyme that shows modestly elevated levels in *soxQ1*, *cfxB1*, and *marR1* strains is fumarase C (Table 2). This fumarase of *E. coli* was previously shown to be under the control of *soxRS* (23). The enhanced expression of all three enzymes (Mn-SOD, glucose-6-phosphate dehydrogenase and fumarase C) was diminished nearly to wild-type levels by the introduction of pMarR(WT) into *soxQ1*, *cfxB1*, and *marR1* mutants but was without effect in the *soxRS*-constitutive strain (Table 2 and Fig. 2). Thus, all three mutations mapping to the *mar* locus are also suppressed by the same putative repressor in multiple copies.

Sequence analysis of the *soxQ1* and *cfxB1* mutants. The suppression of the *soxQ1*- and *cfxB1*-dependent phenotypes still left open the possibility that these mutations might be located in a gene other than *marR*, perhaps in the putative activator gene *marA*. We therefore determined the DNA sequence of the *mar* operon in the *soxQ1* and *cfxB1* mutant strains. To this end, a 1,008-bp fragment containing both the *marR* and *marA* genes was amplified by PCR and directly sequenced on both strands. The sequences for both mutant DNAs were determined with duplicate PCR products and with DNA from both the original mutant strains (JTG320 and EN226-8) and transductants that retain the *soxQ1* and *cfxB1* phenotypes (JHC1072 and JHC1069) (Table 1). Consistent DNA sequences were found in each case. The sequencing results (Fig. 3) showed that the *cfxB1* mutation is a 285-bp deletion into the *marR* gene. This deletion eliminates part of the putative operator region of the *mar* operon as well as the first 85 codons and part of the 86th codon of *marR*. Presumably, strains with the *cfxB1* mutation lack the MarR protein entirely. The *soxQ1* mutation is a GC→AT transition that changes alanine to threonine at amino acid position 70 of MarR. This base change is consistent with the mutational specificity of the alkylating agent *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (24), the mutagen used to induce the *soxQ1* mutation (14). The lower antibiotic resistances and antioxidant enzyme levels of *soxQ1* strains compared with *cfxB1* strains (5, 14) (Fig. 1 and 2 and Table 2) are consistent with the retention of some activity in the mutant MarR protein encoded by *soxQ1*. In no case was a mutation found in the *marA* gene.

TABLE 2. Enzyme levels in strains transformed with pUC18 and pMarR(WT) plasmids[a]

Strain	Plasmid	Enzyme activity (U/mg of protein)	
		G6PD	Fumarase C[b]
GC4468 (WT)	pUC18	0.25	4.1
	pMarR(WT)	0.25	4.0
JHC1072 (*soxQ1*)	pUC18	0.53	9.6
	pMarR(WT)	0.24	4.0
JHC1069 (*cfxB1*)	pUC18	0.85	14.4
	pMarR(WT)	0.31	4.8
JHC1113 (*marR1*)	pUC18	0.49	8.7
	pMarR(WT)	0.25	4.4
JTG1078 (*soxR105*)	pUC18	0.61	55
	pMarR(WT)	0.56	55

[a] Extracts were made from cells grown in LB medium plus ampicillin. The entire experiment was repeated at least twice; a representative result is shown. WT, wild type.

[b] Stable fumarase activity was assayed as described by Hill and Bradshaw (18).

Expression of the *mar* region in *soxQ1* and *cfxB1* mutants. According to the hypothesis that proposes a repressor role for MarR (6), the increased level of antioxidant enzymes and the enhanced antibiotic resistance observed in the *soxQ1* and the *cfxB1* mutants would be due to increased transcription of the *mar* operon and therefore to elevated levels of the putative activator protein MarA in these strains.

We therefore studied the levels of *mar* mRNA in the *cfxB1* and *soxQ1* strains. Total cellular RNA was subjected to electrophoresis, blotted to a nylon membrane, and hybridized to a 1,008-bp DNA probe comprising both the *marR* and *marA* genes (Fig. 4). The results showed an increased expression of an ~1.2-kb transcript in both the *soxQ1* and *marR1* mutants. In some experiments a smaller hybridizing RNA was also observed (data not shown), as reported earlier (6). Densitometric analysis of the ~1.2-kb band indicated an increase in the level of this transcript of approximately eightfold in the *soxQ1* and

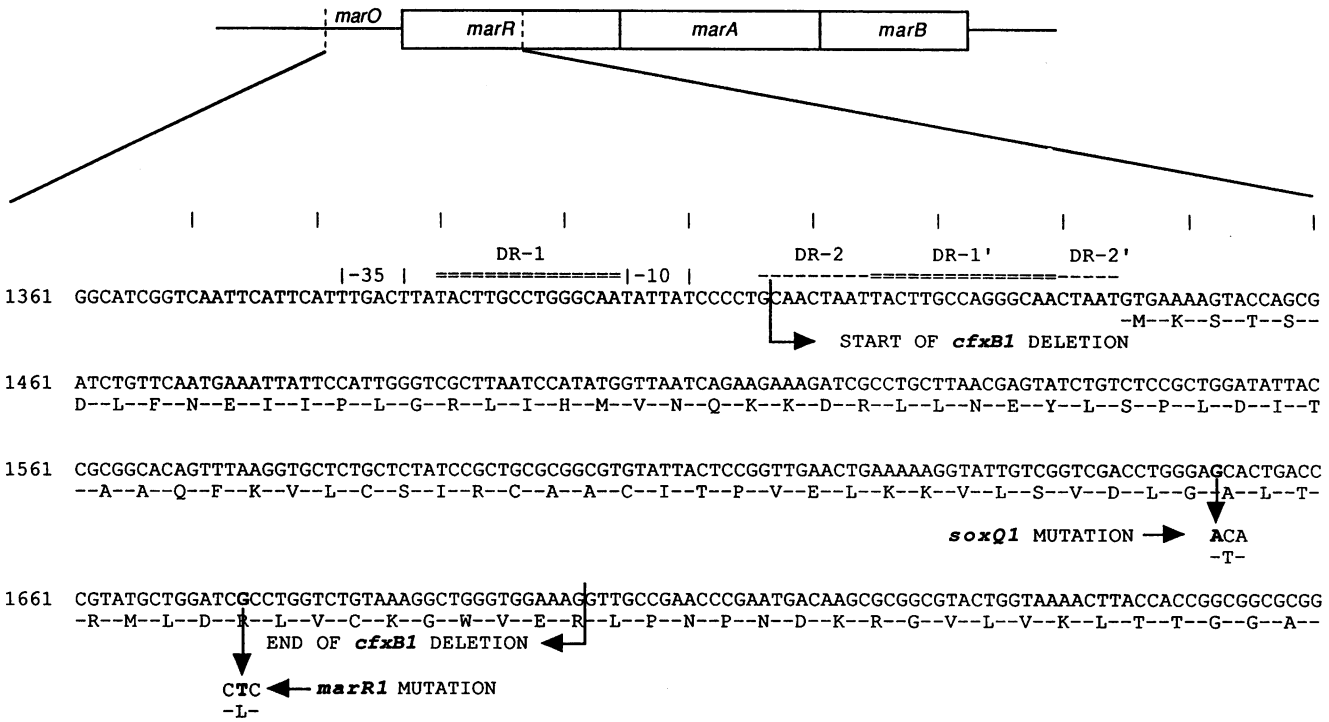

FIG. 3. Nucleotide sequence changes in the *cfxB1* and *soxQ1* mutations. The upper diagram shows the relative positions of the *mar* operator (*marO*) and the open reading frames encoding the proteins of the *marRAB* operon. The DNA region affected by the mutations is shown in detail below, together with the first 105 amino acids of the MarR protein. The direct repeat elements DR-1 and DR-1′ (double dashed lines) and DR-2 and DR-2′ (single dashed lines) are indicated above the sequence. The proposed −10 and −35 promoter sites (6) are also shown. Nucleotide numbers refer to the previously published sequence for the *mar* region (6), except that the initiator codon has been reassigned to a GTG as shown (29).

marR1 strains compared with wild-type cells. The apparent size of the transcript in the *cfxB1* mutant was ~0.8 kb, about the size expected to result from the 285-bp deletion found in this allele. The level of *mar* mRNA in the *cfxB1* mutant was ~40-fold higher than that detected in the corresponding wild-type strain.

Given the previously reported induction of the *mar* transcript by antibiotics (6, 16), we analyzed the inducibility of *mar* expression in the *cfxB1* and *soxQ1* mutant strains after treatment with tetracycline (Fig. 4). This antibiotic caused a significant increase in the amount of *mar* transcript in both the *soxQ1* and *marR1* strains. As expected, the wild-type *mar* transcript was also inducible by tetracycline, but to a lower final level than that measured for tetracycline-treated *soxQ1* and *marR1* strains (Fig. 4). In contrast, in the *cfxB1* strain the already-elevated level of *mar* RNA was not detectably increased by the tetracycline treatment (Fig. 4).

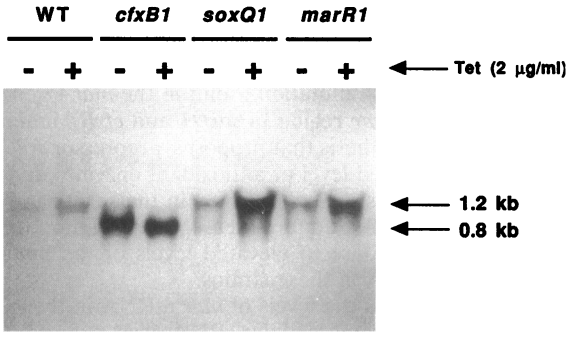

FIG. 4. Northern blotting analysis of *mar* transcript levels in wild-type and mutant strains. Tetracycline (Tet) was added as indicated to a final concentration of 2 μg/ml 1 h before extraction. Total RNA (30 μg per lane) was subjected to electrophoresis in an agarose-formaldehyde gel, blotted onto a nylon membrane, and hybridized to the 1,008-bp *marRA* probe (see Materials and Methods). The relative amounts of RNA loaded in each lane were estimated by visualization of the rRNA by ethidium bromide staining. WT, GC4468; *cfxB1*, JHC1069; *soxQ1*, JHC1072; *marR1*, JHC1113.

The lack of inducibility of the *cfxB1* mutant could be due either to the lack of MarR repressor or to the partial deletion of the *marO* operator region (Fig. 3). To distinguish between these two possibilities, we studied the effect that the overexpression of MarR from pMarR(WT) had on the inducibility of the *cfxB1* strain by tetracycline (Fig. 5). The introduction of pMarR(WT) into the *cfxB1* mutant strain caused a marked decrease (about 50-fold) in the level of the truncated *mar* transcript. This repressed amount of *mar* transcript was then inducible approximately fourfold by tetracycline in the *cfxB1* strain containing pMarR(WT) (Fig. 5). The control plasmid (pUC18) was without effect in the *cfxB1* strain.

DISCUSSION

Three independently isolated mutations (*marR1*, *soxQ1*, and *cfxB1*) that map to the *mar* locus have been shown here to alter the same gene, *marR*. All three mutations have pleiotropic effects on antibiotic resistance and expression of oxidative stress genes that are partially or completely reversed by the $marR^+$ gene on a multicopy plasmid. All three mutations also cause increased expression of the *mar* operon mRNA, which

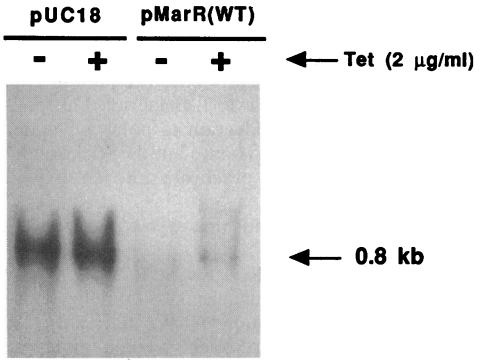

FIG. 5. Effect of pMarR(WT) on *mar* expression in a *cfxB1* mutant. Cells and RNA samples were processed as described in the legend to Fig. 4, except that the nylon membrane was hybridized with the 511-bp *marAB* probe (see Materials and Methods). *cfxB1*, JHC1069.

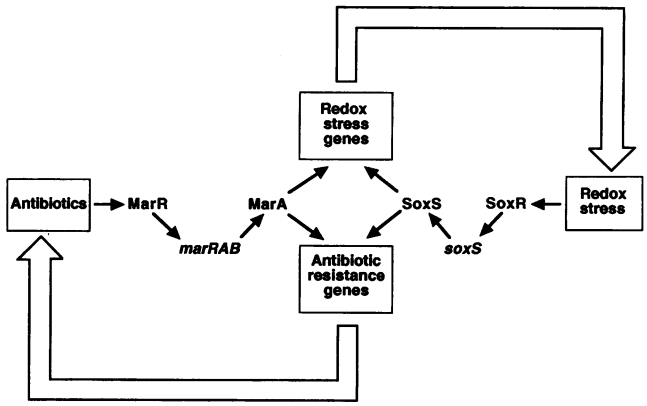

FIG. 6. Homeostatic nature of the overlapping *mar* and *soxRS* responses. The small arrows indicate signal transduction and gene regulation steps. The large open arrows indicate alleviation of the various physiological effects by induced gene products (see text).

suggests that they exert regulatory effects through a common mechanism, derepression of *mar* transcription.

Previous experiments suggested a repressor role for the product of *marR*, the 5′-most gene of the *marRAB* operon (6). This hypothesis is supported by the data presented here, and particularly so in the case of *cfxB1*, a deletion that evidently eliminates the MarR protein. Thus, the *mar* transcript appears to be fully derepressed in bacteria with the *cfxB1* mutation. In contrast, the *soxQ1* and *marR1* alleles evidently encode repressor proteins that retain some function, because induction by tetracycline was required for the *mar* transcript in these strains to reach a level similar to that seen in the *cfxB1* strain with or without tetracycline.

The loss of tetracycline responsiveness caused by the *cfxB1* mutation might also arise from the deletion of part of the *mar* operator region (Fig. 3). However, the pMarR(WT) plasmid was able to suppress both the *cfxB1* mutant phenotypes and the high constitutive expression of the truncated *mar* transcript. The overproduction of MarR restored the inducibility of the *cfxB1* transcript by tetracycline (Fig. 5). These results collectively support the model in which induction of the *marRAB* operon results from the relief of the repression exerted by MarR. The residual antibiotic resistance of the *cfxB1* strain containing pMarR(WT) might be due to an effect on the level of the proposed transcript leftward from *marO* (6) or to another mutation in this strain. The incomplete suppression of Mn-SOD by pMarR(WT) (Fig. 2) may be due to limited MarR expression from this construct, which employs the *mar* promoter and so would remain subject to autoregulation.

The gene expression triggered by the mutations in the *marR* repressor gene shows considerable overlap with that of the genes controlled by the redox-responsive *soxRS* regulon (10, 14, 15). Antibiotic resistance in these cases depends in part on the elevated synthesis of *micF* RNA (5, 8), an antisense transcript that destabilizes the *ompF* mRNA (2) encoding a major porin of the outer membrane (25). Several oxidative stress genes are also transcriptionally activated by both the *mar* and the *soxRS* systems: *sodA* and *zwf*, as noted above, as well as the *soi17/19* locus (21) and the genes encoding two other, unidentified oxidative stress proteins (14, 15). Here we have extended this overlap to include the fumarase C gene (23).

This overlap between the *marRAB*- and the *soxRS*-controlled genes may have a physical basis embodied in the similarity between the SoxS protein, the proximal activator of the *soxRS* regulon (1, 10, 26), and MarA, the product of the second gene of the *mar* operon (6). The elevated expression of the SoxS protein alone is sufficient to activate its target genes of the *soxRS* regulon in the absence of a redox stress signal (1). Recent experiments similarly indicate that elevated MarA synthesis (via a multicopy plasmid containing *marA*) is also sufficient to switch on *mar*-dependent antibiotic resistance in the absence of an inducing signal (12). The work presented here and previously (14) indicates that elevated MarA expression also directly activates some oxidative stress genes.

The mechanism by which such small (13-kDa) proteins as SoxS and MarA activate gene expression is unknown. Recent evidence indicates that SoxS protein interacts directly with its target promoters (e.g., *micF*, *sodA*, *zwf*, and *nfo*) (22). The similar predicted helix-turn-helix motifs of SoxS and MarA, which differ by a single amino acid residue in the second helix (6), might mediate protein binding to the same or overlapping sites in the responsive promoters. Of interest in this vein is the recent identification of a protein that binds to the right arm of the replication origin *oriC*, called Rob (30), which contains a predicted helix-turn-helix motif with the second helix identical to that of MarA (6). This unexpected similarity could indicate an undiscovered overlap between oxidative or antibiotic stress and the control of DNA replication in *E. coli*. Thus, it would be of great interest to understand the interaction of multiple regulators such as SoxS, MarA, and perhaps Rob at single sites that could respond differentially to various environmental signals.

A final question concerns the overlap between oxidative stress functions and antibiotic resistance genes that is embodied in the *soxRS* and *mar* systems (Fig. 6). The known inducers of *soxRS* exert redox or free radical stress in *E. coli* but switch on both oxidative stress genes and antibiotic resistance mechanisms. The inducers of *mar* expression are more broadly based and include both antibiotics (tetracycline and chloramphenicol) and redox-cycling agents (29), as well as the weak acid salicylate (7). The probable location of *inaR* mutations in *mar* (27, 32) adds other weak acids to this list. Perhaps the toxicity of all these agents includes a more substantial component of redox stress than had been assumed, and this stress could be counterbalanced by activation of some oxidative stress genes (5, 14). Similarly, the agents that activate *soxRS* may damage cellular components that are also targets for antibiotics, with protection provided by the activation of antibiotic resistance genes.

ACKNOWLEDGMENTS

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REFERENCES

1. **Amábile-Cuevas, C. F., and B. Demple.** 1991. Molecular characterization of the *soxRS* genes of *Escherichia coli*: two genes control a superoxide stress regulon. Nucleic Acids Res. **19:**4479–4484.
2. **Andersen, J., and N. Delihas.** 1990. *micF* RNA binds to the 5′ end of *ompF* RNA and to a protein from *Escherichia coli*. Biochemistry **29:**9249–9256.
3. **Beauchamp, C., and I. Fridovich.** 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. **44:**276–287.
4. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
5. **Chou, J. H., J. T. Greenberg, and B. Demple.** 1993. Posttranscriptional repression of *Escherichia coli* OmpF protein in response to redox stress: positive control of the *micF* antisense RNA by the *soxRS* locus. J. Bacteriol. **175:**1026–1031.
6. **Cohen, S. P., H. Hächler, and S. B. Levy.** 1993. Genetic and functional analysis of the multiple antibiotic resistance (*mar*) locus in *Escherichia coli*. J. Bacteriol. **175:**1484–1492.
7. **Cohen, S. P., S. B. Levy, J. Foulds, and J. L. Rosner.** 1993. Salicylate induction of antibiotic resistance in *Escherichia coli*: activation of the *mar* operon and a *mar*-independent pathway. J. Bacteriol. **175:**7856–7862.
8. **Cohen, S. P., L. M. McMurry, and S. B. Levy.** 1988. *marA* locus causes decreased expression of OmpF porin in multiple-antibiotic-resistant (Mar) mutants of *Escherichia coli*. J. Bacteriol. **170:**5416–5422.
9. **Cunningham, R. P., S. Saporito, S. G. Spitzer, and B. Weiss.** 1986. Endonuclease IV (*nfo*) mutant of *Escherichia coli*. J. Bacteriol. **168:**1120–1127.
10. **Demple, B.** 1991. Regulation of bacterial oxidative stress genes. Annu. Rev. Genet. **25:**315–337.
11. **Emory, S. A., and J. G. Belasco.** 1990. The *ompA* 5′ untranslated RNA segment functions in *Escherichia coli* as a growth-rate-regulated mRNA stabilizer whose activity is unrelated to translational efficiency. J. Bacteriol. **172:**4472–4481.
12. **Gambino, L., S. J. Gracheck, and P. F. Miller.** 1993. Overexpression of the MarA positive regulator is sufficient to confer multiple antibiotic resistance in *Escherichia coli*. J. Bacteriol. **175:**2888–2894.
13. **George, A. M., and S. B. Levy.** 1983. Gene in the major cotransduction gap of the *Escherichia coli* K-12 linkage map required for the expression of chromosomal resistance to tetracycline and other antibiotics. J. Bacteriol. **155:**541–548.
14. **Greenberg, J. T., J. H. Chou, P. Monach, and B. Demple.** 1991. Activation of oxidative stress genes by mutations at the *soxQ/cfxB/marA* locus of *Escherichia coli*. J. Bacteriol. **173:**4433–4439.
15. **Greenberg, J. T., P. Monach, J. H. Chou, P. D. Josephy, and B. Demple.** 1990. Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**6181–6185.
16. **Hächler, H., S. P. Cohen, and S. B. Levy.** 1991. *marA*, a regulated locus which controls expression of chromosomal multiple antibiotic resistance in *Escherichia coli*. J. Bacteriol. **173:**5532–5538.
17. **Hames, B. D.** 1981. An introduction to polyacrylamide gel electrophoresis, p. 1–91. *In* B. D. Hames and D. Rickwood (ed.), Gel electrophoresis of proteins, a practical approach. IRL Press Ltd., London.
18. **Hill, R. L., and R. H. Bradshaw.** 1969. Fumarase. Methods Enzymol. **13:**91–99.
19. **Hooper, D. C., J. S. Wolfson, K. S. Souza, E. Y. Ng, G. L. McHugh, and M. N. Swartz.** 1989. Mechanisms of quinolone resistance in *Escherichia coli*: characterization of *nfxB* and *cfxB*, two mutant resistance loci decreasing norfloxacin accumulation. Antimicrob. Agents Chemother. **33:**283–290.
20. **Kao, S. M., and H. M. Hassan.** 1985. Biochemical characterization of a paraquat-tolerant mutant of *Escherichia coli*. J. Biol. Chem. **260:**10478–10481.
21. **Kogoma, T., S. B. Farr, K. M. Joyce, and D. O. Natvig.** 1988. Isolation of gene fusions (*soi::lacZ*) inducible by oxidative stress in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **85:**4799–4803.
22. **Li, Z., and B. Demple.** Unpublished data.
23. **Liochev, S. I., and I. Fridovich.** 1992. Fumarase C, the stable fumarase of *Escherichia coli*, is controlled by the *soxRS* regulon. Proc. Natl. Acad. Sci. USA **89:**5892–5896.
24. **Miller, J. H.** 1992. A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
25. **Mizuno, T., M.-Y. Chou, and M. Inouye.** 1984. A unique mechanism regulating gene expression: translational inhibition by a complementary RNA transcript (micRNA). Proc. Natl. Acad. Sci. USA **81:**1966–1970.
26. **Nunoshiba, T., E. Hidalgo, C. F. Amábile Cuevas, and B. Demple.** 1992. Two-stage control of an oxidative stress regulon: the *Escherichia coli* SoxR protein triggers redox-inducible expression of the *soxS* regulatory gene. J. Bacteriol. **174:**6054–6060.
27. **Rosner, J. L., and J. L. Slonczewski.** 1993. Multidrug resistance specified by *inaR*, a weak-acid regulator in *Escherichia coli*, abstr. H258. Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993. American Society for Microbiology, Washington, D.C.
28. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
29. **Seoane, A., and S. B. Levy.** 1993. Transcriptional activation of the multiple antibiotic resistance (*mar*) operon in *Escherichia coli*, abstr. A24. Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993. American Society for Microbiology, Washington, D.C.
30. **Skarstad, K., B. Thöny, D. S. Hwang, and A. Kornberg.** 1993. A novel binding protein of the origin of the *Escherichia coli* chromosome. J. Biol. Chem. **268:**5365–5370.
31. **Tsaneva, I. R., and B. Weiss.** 1990. *soxR*, a locus governing a superoxide response regulon in *Escherichia coli* K-12. J. Bacteriol. **172:**4197–4205.
32. **White, S., F. E. Tuttle, D. Blankenhorn, D. C. Dosch, and J. L. Slonczewski.** 1992. pH dependence and gene structure of *inaA* in *Escherichia coli*. J. Bacteriol. **174:**1537–1543.