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Activation of Oxidative Stress Genes by Mutations at the *soxQ/cfxB/marA* Locus of *Escherichia coli*

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Exposure of *Escherichia coli* to superoxide-generating drugs, such as menadione or paraquat, uniquely induces ~40 proteins, nine of which are under the positive control of the *soxR* locus (at min 92). We report here that certain mutations at a separate locus that we have named *soxQ* (at min 34) confer some of the phenotypes seen in *soxR*-constitutive strains, including resistance to menadione. A previously known mutation called *cfxB*, identified through antibiotic resistance, is likely an allele of *soxQ*. The *soxQ1* and *cfxB* mutations cause transcriptional activation of the genes that encode Mn-containing superoxide dismutase, glucose 6-phosphate dehydrogenase, and the *soi-17/19::lac* and *soi-28::lac* fusions. These genes are also activated by *soxR*, but the *soxQ1* and *cfxB* mutations increase the synthesis of seven other proteins not influenced by *soxR*. Moreover, the *soxQ1*- and *cfxB*-dependent phenotypes do not depend on the *soxR* gene, and gene induction by *soxR* in response to redox stress does not depend on the *soxQ* locus. As well as increasing cellular resistance to some oxidants, the *soxQ1* and *cfxB* mutations confer elevated resistance to various antibiotics, probably via diminished expression of outer membrane protein OmpF. The *marA1* multiple-antibiotic resistance mutation (also at min 34) behaves like a weak allele of *soxQ* but probably resides in a nearby gene that, with *soxQ*, is part of a regulatory complex. We propose that *soxQ* helps control some oxidative stress proteins as part of another regulon that responds to an unknown environmental signal.

Aerobic metabolism produces reactive byproducts in the form of superoxide ($O_2^{-\cdot}$) and hydrogen peroxide (10, 12). These two species can conspire to generate hydroxyl radicals ($^{\cdot}OH$) (15), which can damage any biological macromolecule (20). Such active oxygen species are also produced intracellularly by various environmental agents, notably, redox-cycling drugs, which divert electrons from NADPH to O_2 to generate a flux of $O_2^{-\cdot}$ (24).

The stress generated by redox-cycling compounds, such as the naphthoquinone menadione (MD) or the aromatic quaternary amine paraquat (PQ), induces a total of ~80 proteins in *Escherichia coli* (13, 33) and represses three others (14). About half of these proteins are also induced by H_2O_2, which indicates that they are triggered indirectly by redox-cycling agents, through the H_2O_2 formed from dismutation of $O_2^{-\cdot}$ (13). Nine of the proteins induced by MD or PQ, but not by H_2O_2, are members of a regulon that is under the positive transcriptional control of a locus called *soxR* (14, 32).

The activating signal for *soxR* may be excess $O_2^{-\cdot}$, because other conditions that increase this radical (e.g., exposure to hyperbaric oxygen or aerobic growth of superoxide dismutase-deficient bacteria) induce at least some elements of the regulon (5, 31, 33). Some of the *soxR* regulon proteins have known antioxidant functions: Mn-containing superoxide dismutase (SodA) scavenges superoxide, endonuclease IV repairs oxidative sugar damages in DNA (26), and glucose 6-phosphate dehydrogenase (G6PD) provides NADPH for enzymes such as glutathione reductase (24) and alkyl hydroperoxidase (22). The *soxR* locus also acts to repress expression of outer membrane porin OmpF and increase the C-terminal glutamate modification of ribosomal protein S6 (14). These changes may both limit the accumulation of intracellular toxins and alter cellular targets for oxidative or other damage. The specific functions of the *soxR*-controlled *soi-17*, *soi-19*, and *soi-28* genes are not known, but their disruption by Mu d(*lac*) insertions does render *E. coli* hypersensitive to PQ (25).

Mutants with constitutive alleles of *soxR* [*soxR*(Con)] have elevated resistance to oxidants and many antibiotics (5a, 14). While analyzing putative *soxR*(Con) mutants, we discovered one strain whose mutation mapped to a locus distinct from *soxR*, which we have named *soxQ*. Genetic mapping experiments and analysis of the patterns of protein and gene expression indicate that the antibiotic-resistant *cfxB* mutant (19) probably harbors an allele of *soxQ*. We also show that the *soxQ1*- and *cfxB*-dependent phenotypes are independent of the *soxR* locus.

MATERIALS AND METHODS

Strains and media. The strains used in this study are listed in Table 1 (see below for specific constructions). Permanent bacterial stocks were stored frozen at −80°C in 20% glycerol, and working stocks were maintained on Luria-Bertani (LB) agar at 4°C for up to 2 weeks. Cells in liquid culture were grown at 37°C with shaking at 200 rpm in LB broth (27), K medium (12), or supplemented M9 medium (13), unless otherwise indicated. The solid medium for plates was LB broth supplemented with 1.5% agar.

Isolation of the *soxQ1* mutant. The *soxQ1* mutant was identified in a pool of mutagenized bacteria from which *soxR*-constitutive mutants were also obtained (14). Briefly, strain JTG102 was mutagenized with 5 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml and stored at −80°C (14).

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TABLE 1. Bacterial strains

Strain	Genotype	Source or reference
AG102	*marA1 argE3 thi-1 rpsL xyl mtl galK supE44* Δ(*gal-uvrB*)	6
AQ4517	Same as GC4468; Mu dX*soi-17*::*lacZ*	25
AQ4519	Same as GC4468; Mu dX*soi-19*::*lacZ*	25
AQ4528	Same as GC4468; Mu dX*soi-28*::*lacZ*	25
DJ901	Same as GC4468; Δ(*soxR–zjc-2205*) *zjc-2204*::Tn*10*km	14
EN226-8	Same as KL16; *cfxB*	19
GC4468	F⁻ Δ(*lac*)*4169 rpsL*	4
JHC1050	Same as JTG320; Δ(*soxR zjc-2205*) *zjc-2204*::Tn*10*km	This study
JHC1052	Same as EN226-8; Δ(*soxR zjc-2205*) *zjc-2204*::Tn*10*km	This study
JHC1068	Same as GC4468; *cfxB zdd-2207*::Tn*10*km	This study
JHC1071	Same as GC4468; *soxQ1 zdd-2207*::Tn*10*km	This study
JHC1090	Same as GC4468; *zdd-230*::Tn*9 zde-234*::Tn*10*	This study
JHC1092	Same as GC4468; Δ(*soxR zjc-2205*) *zjc-2204*::Tn*10*km	This study
JHC1096	Same as GC4468; *zdd-239*::Tn*9* del*1738* from PLK1738 (Δ*soxQ*)	This study
JHC1098	Same as JHC1096; Δ(*soxR zjc-2205*) *zjc-2204*::Tn*10*km	This study
JHC1113	Same as GC4468; *marA1 zdd-2207*::Tn*10*km	This study
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*	13
JTG320	Same as JTG102; *soxQ1*	This study
JTG1052	Same as *GC4468*; *soxR101 zjc-2204*::Tn*10*km	14
JTG1078	Same as GC4468; *soxR105 zjc-2204*::Tn*10*km	14
KL16	Hfr *thi-1 relA spoT1*	19
PK1268	*zdd-239*::Tn*9 zde-234*::Tn*10*	2
PLK1738	Same as PLK1268; del*1738* from *zde-234*::Tn*10* (Δ*soxQ*)	17
QC909	Same as GC4468; Φ(*sodA*::Mu dPR13)*25* Φ(*sodB*::*kan*)Δ*1-2*	4

Thawed samples were diluted in K medium, cultured for 80 min at 37°C, spread on plates containing 1.4 mg of MD (the bisulfite adduct) per ml, and incubated as previously described (14).

Toxicity measurements. The toxicity of oxidants and antibiotics was routinely determined by measuring bacterial growth on gradient plates prepared as previously described (8), except that each plate contained 60 ml of solid medium. Growth along the gradient was scored after 18 to 24 h at 37°C.

Two-dimensional gel analysis. Growth and labeling of bacteria with [^{35}S]methionine (1,072 Ci/mmol; New England Nuclear), preparation of extracts, and electrophoresis of samples were performed as described previously (13).

Cell extracts and enzyme assays. Cells grown in LB broth to the mid-log phase (optical density at 600 nm, 0.2 to 0.4) were centrifuged, washed with ice-cold M9 salts (27), 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) and 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 determined as previously described (3).

Superoxide dismutase activity was assayed in nondenaturing 7.5% polyacrylamide slab gels (16) by the method of Beauchamp and Fridovich (1). Endonuclease IV was assayed in extracts by monitoring the liberation of ^{32}P-labeled phosphoglycolaldehyde from a synthetic poly(dA-dT) substrate as previously described (26). G6PD activity was monitored by measuring NADPH production at 340 nm as previously described (23). β-Galactosidase activity in whole cells grown in LB broth (to an optical density at 600 nm of ~0.2) containing 1% glucose at 30 or 37°C was assayed after permeabilizing the bacteria with sodium dodecyl sulfate and chloroform (27).

Strain constructions and genetic mapping. For strain constructions, transducing bacteriophage P1 *vir* was used (27). Genetic mapping was facilitated by isolation of minitransposons linked to the *soxQ1* and *cfxB* mutations. Strain GC4468 was randomly mutagenized (14) with a minitransposon derivative of Tn*10* that confers kanamycin resistance (Kan^r; element 9 of Way et al. [34]), hereafter called Tn*10*km. P1 *vir* grown on mutagenized GC4468 was used to transduce strains JTG320 (*soxQ1*) and EN226-8 (*cfxB*) to Kan^r. The Kan^r colonies were replica plated onto LB agar with or without 7.5 μg of chloramphenicol per ml. P1 *vir* was grown on the Cm^s isolates and used to transduce strains JTG320 and EN226-8 to Kan^r while either retaining the *soxQ1* and *cfxB* alleles (scored as Cm^r) or gaining the wild-type alleles (scored as Cm^s). Three separate linked insertions were obtained in this way: *zdd-2207*::Tn*10*km (40% linked to *cfxB*), *zdd-2208*::Tn*10*km (80% linked to *cfxB*), and *zdd-2209*::Tn*10*km (30% linked to *soxQ1*). The *soxQ1* and *cfxB* alleles were cotransduced with the minitransposons by selecting for Kan^r and screening for Cm^r (5 to 7.5 μg/ml) or resistance to nalidixic acid (6 μg/ml).

Minitransposon *zdd-2207*::Tn*10*km was transferred into strains bearing the *soxQ1*, *cfxB*, or *marA1* allele (JTG320, EN226-8, and AG102, respectively). The resulting strains were then used as transduction donors for GC4468, with selection for Kan^r and screening for Cm^r (to score the presence of *soxQ1*, *cfxB*, or *marA1*), to yield strains JHC1071, JHC1068, and JHC1113. These strains were used together with a *soxQ*$^+$ strain (JHC1090) containing transposons *zdd-230*::Tn*9* and *zde-234*::Tn*10* from strain PLK1268 (2) to map the *zdd-2207*::Tn*10*km, *soxQ1*, *cfxB*, or *marA1* allele (see Fig. 1 and its legend). JHC1071, JHC1068, and JHC1113 were also used as transduction donors to transfer the *soxQ1*, *cfxB*, and *marA1* alleles into other strains by cotransduction with *zdd-2207*::Tn*10*km with selection for Kan^r and screening for Cm^r.

The Δ*soxQ* strain (JHC1096) was constructed as follows.

TABLE 2. Resistance to oxidants and antibiotics[a]

Strain	Growth (% of gradient)				
	MD	PM	Bleomycin	Chloram-phenicol	Nalidixic acid
JTG102 (wild type)	45	61	23	17	39
JTG320 (*soxQ1*)	72	100	25	39	69
GC4468 (wild type)	60	75	37	15	39
JTG1052 (*soxR101*)	69	76	55	32	52
JHC1092 (Δ*soxR*)	59	23	27	21	29
JHC1071 (*soxQ1*)	71	99	37	60	55
JHC1068 (*cfxB*)	76	81	100	80	64
JHC1113 (*marA1*)	71	99	99	61	61

[a] The measurements were repeated at least three times; values from a representative experiment are shown. Amounts (weight or volume of drug per plate; see Materials and Methods) of the drugs were as follows: MD (menadione), 8 mg dissolved in 2 ml of dimethyl sulfoxide; PM (phenazine methosulfate), 1.8 mg; bleomycin, 175 μg; chloramphenicol, 900 μg; nalidixic acid, 600 μg.

The *zde-234*::Tn*10* marker (2) was transduced from PLK1268 into GC4468, and the resulting Tet^r strain was transduced to Cm^r by using P1 *vir* grown on PLK1738, scoring for *zdd-230*::Tn*9*, linked to del*1738* (Δ*soxQ*), a 39-kb deletion (17). Screening of these Cm^r transductants for Tet^s yielded JHC1096 (Δ*soxQ*). The Δ*soxR* derivatives were constructed by using P1 *vir* grown on DJ901 (*zjc-2204*::Tn*10*km Δ(*soxR-zjc-2205*) (14) to cotransduce Kan^r and Δ*soxR* (>95% linkage; 14) in various strains. The isolates selected for study were named JHC1050 (*soxQ1* Δ*soxR*), JHC1052 (*cfxB* Δ*soxR*), JHC1092 (Δ*soxR*), and JHC1098 (Δ*soxR* Δ*soxQ*).

The various fusion-bearing strains were constructed as follows. Lysogens carrying λDR52 (*zwf*::*lac bla*; from R. E. Wolf, Jr., University of Maryland, Baltimore County; previously called λB11) were prepared as previously described (14). Strains bearing the Φ(*sodA*::Mu dPR13)*25* fusion were constructed by transducing *metB* strains to Met$^+$ and screening for high-level Cm^r (34 μg/ml) by using a P1 *vir* lysate grown on strain QC909 [*met*$^+$ Φ(*sodA*::Mu dPR13)*25*; see reference 4]. The *soi-19*::*lac* and *soi-28*::*lac* strains were constructed by cotransduction into either AQ4519 or AQ4528 of *zjc-2204*::Tn*10*km *soxR105* from strain JTG1078 (14), *zdd-2207*::Tn*10*km *soxQ1* from strain JHC1071, or *zdd-2207*::Tn*10*km *cfxB* from strain JHC1068. In each case, Kan^r was selected and the indicated allele was scored as Cm^r (7.5 μg/ml).

RESULTS

Oxidant and antibiotic resistance via mutations in the *soxQ/cfxB/marA* region. The *soxR* locus was identified by analysis of *E. coli* mutants selected for increased resistance to MD. The MD^r phenotype of *soxR*(Con) mutants is due to increased expression of superoxide stress proteins in the absence of inducing agents (14). One MD^r mutant (JTG320) shared many phenotypes with the *soxR*(Con) strains but contained a mutation that was genetically unlinked to *soxR* (<1% cotransducible with *zjc-2204*::Tn*10*km; reference 14). We termed JTG320 a *soxQ1* strain to distinguish it from the *soxR*(Con) strains. Like *soxR*(Con) strains, JTG320 had increased resistance to the redox-cycling agents MD and plumbagin and to the oxidant *t*-butyl hydroperoxide (Table 2 and data not shown). Neither JTG320 nor the *soxR*(Con) strains exhibited extra resistance to H_2O_2 or PQ (data not shown). Also in common with the *soxR*(Con) strains, the *soxQ1* mutant exhibited increased resistance to the antibiotics chloramphenicol, nalidixic acid (Table 2), tetracycline, and ampicillin (5a). Unlike the *soxR*(Con) strains (14), the *soxQ1* strains did not show extra resistance to bleomycin.

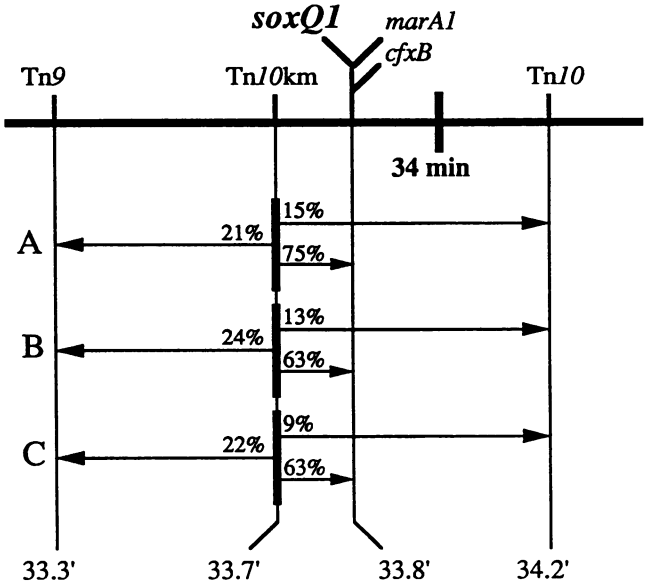

FIG. 1. Genetic map of the *soxQ/cfxB/marA* region. The percent linkages observed in different crosses (A, B, and C) are shown above the arrows; 150 transductants were scored in each cross shown here. Two additional mapping experiments, which yielded results consistent with the data shown, were completed. The number at the bottom of each line is the estimated map position to the nearest 0.1 min. Tn*9* is *zdd-230*::Tn*9*; Tn*10*km is *zdd-2207*::Tn*10*km; Tn*10* is *zde-234*::Tn*10*. The donors were as follows: A, JHC1071 (*soxQ1 zdd-2207*::Tn*10*km); B, JHC1068 (*cfxB zdd-2207*::Tn*10*km); C, JHC1113 (*marA1*, *zdd-2207*::Tn*10*km). The recipient was JHC1090 (*soxQ*$^+$ *zdd-230*::Tn*9 zde-234*::Tn*10*). Transductants were scored for Nal^r (8 μg/ml for *soxQ1*, *cfxB*, or *marA1*), loss of Tet^r (15 μg/ml for *zde-234*::Tn*10*), and loss of Cm^r (34 μg/ml for *zdd-230*::Tn*9*). The positions of *zdd-230*::Tn*9* and *zde-234*::Tn*10* are as given on the map of Bitner and Kuempel (2).

It seemed possible that the broad antibiotic resistance exhibited by JTG320 might be due to mutations at the known drug resistance locus *cmlA* (29), *marA* (6, 11), *ompF* (9), or *cfxB* (18, 19). Both the *cfxB* and *marA1* strains had increased resistance to MD (Table 2), as did a *cmlA* strain (data not shown). Both the *cfxB* and *marA1* strains also exhibited resistance to bleomycin, although that property was not seen with the *soxQ1* mutation (Table 2). Nevertheless, preliminary experiments indicated that the *cfxB* strain, but not the *cmlA* or *marA1* strain, showed extensive similarity to the *soxQ1* strain with respect to the elevated enzyme and polypeptide levels (see below). Because the *cfxB* and *soxQ1* strains seemed to share so many characteristics, we chose to focus most of our analysis on these two strains.

The Cm^r phenotype conferred by *soxQ1* was lost in >98% of transductants that received a *marA*::Tn*5* allele (11), as was reported previously for *cfxB* (19). However, since *marA* mutations also affect antibiotic resistance (6, 11), these experiments were not conclusive for establishing the position of *soxQ1* relative to *marA*. Large-scale transduction mapping experiments using transposon insertion markers showed that the *soxQ1*, *cfxB*, and *marA1* mutations all map to 33.8 min (Fig. 1).

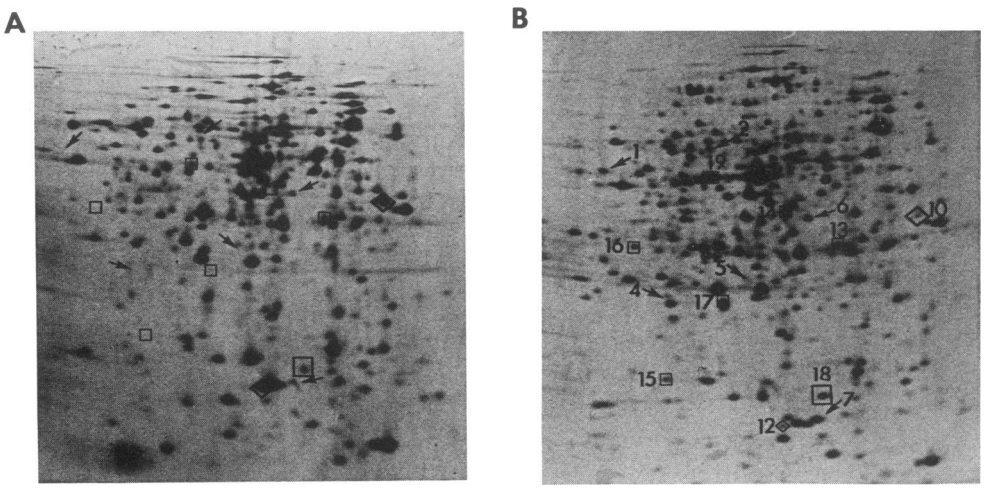

FIG. 2. 2DPAGE analysis of a *soxQ1* strain. Panels: A, *soxQ*$^+$ (JTG102); B, *soxQ1* (JTG320). Symbols: →, proteins increased in *soxR*(Con) bacteria; ◇, proteins decreased in *soxR*(Con) bacteria; □, proteins elevated only in the *soxQ1* mutant. The numbering system of Greenberg et al. (14) is used here. Only spots that overlap between *soxR*(Con) and *soxQ1* mutants (no. 1, 2, 4 to 7, 10, and 12) or are *soxQ1* specific (no. 13 to 19) are indicated. The identities of some of the proteins are shown in Table 2.

Mutations in *soxQ* affect a subset of *soxR* regulon proteins. Analysis by two-dimensional polyacrylamide gel electrophoresis (2DPAGE) showed that *soxQ1* mutant strain JTG320 synthesized six of the nine *soxR* regulon polypeptides (14) at increased rates: SodA, G6PD, endonuclease IV, ribosomal protein S6C, the *soi-17/19*-controlled protein, and the spot 4 protein (Fig. 2 and Table 3). Three other proteins are controlled negatively by the *soxR* regulon (14), and two of these, outer membrane porin OmpF and ribosomal subunit protein S6A (Fig. 2 and Table 3), were diminished in JTG320. The *soxQ1* strain also had increased expression of seven additional polypeptides that are not elevated in the *soxR*(Con) strains or induced by MD or PQ in wild-type *E. coli* (spots 13 to 19; Fig. 2 and Table 3). These latter proteins were not further identified. The *cfxB* mutant (EN226-8) had a 2DPAGE pattern that was very similar to that of JTG320, except that endonuclease IV protein was not increased in EN226-8 (summarized in Table 3).

The increased synthesis of the SodA and G6PD polypeptides in the *soxQ1* and *cfxB* mutants was paralleled by increases in the enzymatic activities of SodA on activity gels (Fig. 3) and G6PD in crude extracts (Table 4). Although endonuclease IV levels were increased in the original *soxQ1* strain JTG320 (Table 4), this elevation was lost when the *soxQ1* mutation was transduced into strain GC4468 (Table 4). The transduced *soxQ1* allele still specified increased levels of all of the other proteins mentioned above (Table 4 and data not shown) and conferred the oxidant and antibiotic resistances (Table 2) characteristic of the original *soxQ1* strain. JTG320 may therefore harbor a promoter mutation in the endonuclease IV structural gene (*nfo*) in addition to the *soxQ1* allele, but this point was not further examined.

Transcriptional activation via the *soxQ1* and *cfxB* mutations. The *soxQ1* and *cfxB* alleles gave increased expression, relative to *soxQ*$^+$ *cfxB*$^+$ strains, of *lac* fusions to the *soi19*, *soi-28*, *zwf*, and *sodA* promoters in the absence of MD or PQ (Fig. 4). All of these fusions in both the *soxQ1* and *cfxB* backgrounds showed modest additional induction (<twofold) in response to PQ (data not shown). Since each of the fusions examined here includes the *lacZ* gene with its own ribosome-binding site, the increased β-galactosidase expres-

TABLE 3. Summary of 2DPAGE analysis of *soxQ* mutants compared with *soxR*(Con) mutants[a]

Spot[b]	Treatment or allele				Characteristic or protein (gene)
	Wt + Ox.	*soxR*(Con)	*soxQ1*	*cfxB*	
1	+	+	+	+	*soi-17/19* dependent
2	+	+	+	+	G6PD (*zwf*)
3	+	+	0	0	*soi-28* dependent
4	+	+	+	+	
5	+	+	+	0	Endonuclease IV (*nfo*)
6	+	+	+	+	
7	+	+	+	+	S6C (*rpsF*)
8	+	+	0	0	
9[c]	+	+	+	NR	SodA (*sodA*)
10	−	−	−	−	OmpF (*ompF*)
11	−	−	0	0	
12	−	−	−	−	S6A (*rpsF*)
13	0	0	+	+	
14	0	0	+	+	
15	0	0	+	+	
16	0	0	+	+	
17	0	0	+	+	
18	0	0	+	+	
19	0	0	+	+	

[a] The data shown were confirmed with multiple independent gels and cell samples in each case. Plus and minus signs indicate, respectively, increased and decreased abundance of a polypeptide by at least twofold relative to the wild-type (wt) level, as estimated from the visual inspection of spot intensities. Other symbols and abbreviations: Ox., MD or PQ; 0, no change in intensity; NR, not resolved on the gels; S6A and S6C, forms of ribosomal small-subunit protein S6. For determination of the identities of spots, see Greenberg et al. (14). Data for the wt induced with MD or PQ and the *soxR*(Con) mutant are from Greenberg and Demple (13) and Greenberg et al. (14).

[b] The spot numbers refer to polypeptides indicated by Greenberg et al. (14) and in Fig. 1.

[c] This spot was seen on the basic side of gels, with a pH range from 3 to 10 (data not shown; see Greenberg et al. [14]), and hence is not visible in Fig. 1.

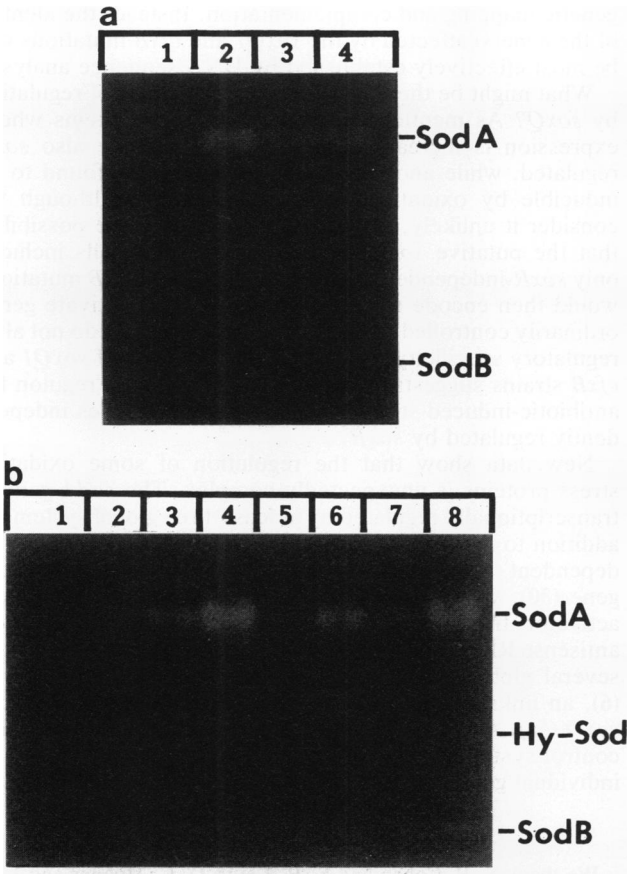

FIG. 3. Superoxide dismutase activity gels. Lanes in panel A: 1, untreated JTG102; 2, MD-treated JTG102; 3, JTG320 (*soxQ1*); 4, JHC1050 (*soxQ1* Δ*soxR*). All lanes in panel A were loaded with 30 μg of protein. Lanes in panel B: 1 and 2, untreated KL16; 3 and 4, MD-treated KL16; 5 and 6, untreated EN226-8 (*cfxB*); 7 and 8, JHC1052 (*cfxB* Δ*soxR*). Odd-numbered lanes were loaded with 10 μg of protein, and even-numbered lanes were loaded with 30 μg.

sion mediated by the *soxQ1* and *cfxB* mutations probably indicates transcriptional activation. However, selective increases in mRNA stability caused by *soxQ1* or *cfxB* cannot be ruled out.

Independence of *soxQ* and *soxR*. The phenotypes of the

TABLE 4. Enzyme levels in *soxQ* and *cfxB* strains[a]

Strain (genotype)	Enzyme activity (U/mg)	
	Endonuclease IV	G6PD
JTG102 (wild type)	7.5	0.22
JTG320 (*soxQ1*)	30	0.48
JHC1050 (*soxQ1* Δ*soxR*)	32	0.45
KL16 (wild type)	5.1	0.26
EN226-6 (*cfxB*)	7.5	1.40
JHC1052 (*cfxB* Δ*soxR*)	9.0	1.90
GC4468 (wild type)	6.0	0.23
JHC1068 (*cfxB*)	9.0	1.20
JHC1071 (*soxQ1*)	6.0	0.84

[a] Extracts were made from cells grown in LB medium. The entire experiment was repeated at least twice.

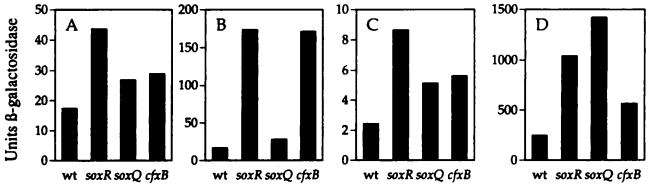

FIG. 4. β-Galactosidase activity from *lac* fusions to the promoters of *soi-19*, *soi-28*, *sodA*, and *zwf*. Saturated cultures were diluted 100-fold into LB broth containing 1% glucose and grown at 30°C (*soi* fusion strains) or 37°C (*zwf*::*lac* and *sodA*::*lac* strains) to an optical density at 600 nm of ~0.2. (A) *soi-19*::*lac* strains; the wild type (wt) was AQ4519. (B) *soi-28*::*lac* strains; the wt was AQ4528. (C) *sodA*::*lac* strains; the wt was QC909. (D) *zwf*::*lac* strains; the wt was GC4468/λDR52. For panels A to C, *soxR* represents the *soxR101* allele; for panel D, it represents the *soxR105* allele in strain JTG1078. *soxQ* stands for the *soxQ1* allele. The twofold higher expression of *soi-28*::*lac* in the *soxQ1* strain compared with the *sox*$^+$ strain shown here was seen consistently. The *sodA*::*lac* strains were cultured with shaking at only 50 rpm in this experiment to avoid induction of the fusion caused by vigorous aeration in these superoxide dismutase-deficient strains (4, 31).

soxQ1 and *cfxB* strains did not depend on the presence on an intact *soxR* locus. Strains bearing a Δ*soxR* mutation combined with either the *soxQ1* or the *cfxB* mutation still had elevated levels of active SodA (Fig. 3) and G6PD (Table 4). The *soxQ1* Δ*soxR* and *cfxB* Δ*soxR* double mutants also retained the characteristic 2DPAGE patterns and oxidant and antibiotic resistances of their *soxR*$^+$ counterparts (data not shown). The endonuclease IV and G6PD activities were slightly higher in the *cfxB* Δ*soxR* strain than in the *cfxB* *soxR*$^+$ strain (Table 4), but we did not test whether this effect depends on the genetic background. One explanation for this increased expression is that the *soxQ*-*cfxB* and *soxR* products may compete for the same or overlapping sites near these genes, so that the competition is relieved by elimination of *soxR*.

Induction of the *soxR* regulon by redox-cycling agents was independent of the *soxQ* locus. A deletion that must remove the *soxQ* region (nearly all of the DNA from *zde-234*::Tn*10* to *zdd-230*::Tn*9* was deleted, i.e., ~39 kb; reference 17) did not eliminate the inducibility of the SodA (data not shown), endonuclease IV, or G6PD enzyme by PQ (Table 5). This observation also rules out a critical role for the *marA* gene product(s) in induction of these enzymes by redox-cycling agents.

DISCUSSION

Previous work identified two loci in *E. coli* that control the induction of two separate oxidative stress regulons: *soxR* (14, 32) and *oxyR* (13). We have described here two other mutations, *soxQ1* and *cfxB*, that increased the synthesis of several proteins already known to be controlled by *soxR*. These increases are apparently mediated transcriptionally. However, the *soxQ1* and *cfxB* mutations also elevate the synthesis of seven additional proteins not previously associated with *soxR*, *oxyR*, or oxidative stress. The *soxQ1* and *cfxB* alleles map to a locus (at min 34) distant from *soxR* (min 92). The *soxR*-mediated gene inductions occur independently of the *soxQ1*/*cfxB* locus. Conversely, the phenotypes conferred by the *soxQ1* and *cfxB* mutations are independent of the *soxR* locus, although it is still possible that the function of the *soxQ*$^+$ gene depends on *soxR*. These findings

TABLE 5. Induction of enzyme levels in *soxR* and *soxQ* deletion strains[a]

Strain (genotype) and treatment	Enzyme activity (U/mg)	
	Endonuclease IV	G6PD
GC4468	12.6	0.11
GC4468 + PQ	72	0.57
JHC1092 (Δ*soxR*)	8.8	0.13
JHC1092 + PQ	14.2	0.12
JHC1096 (Δ*soxQ*)	10.5	0.11
JHC1096 + PQ	98	0.75
JHC1098 (Δ*soxR* Δ*soxQ*)	6.3	0.10
JHC1098 + PQ	14.5	0.15

[a] Extracts were made from cells grown and treated in LB medium. PQ treatment was with 1.0 mM PQ for 45 min. The experiment was also repeated twice with a 0.1 mM PQ treatment, which gave qualitatively the same results. The residual PQ inducibility of endonuclease IV activity in Δ*soxR* strains has been observed previously (14). Strains harboring Δ*soxQ* lack 39 kb of DNA because of excision of *zde-234*::Tn*10* (17).

together indicate an unexpected complexity in the regulation of some oxidative stress proteins and suggest that some elements of the *soxR* regulon are also members of another coregulated group.

The *soxQ1* and *cfxB* mutations were isolated by using distinct selections (resistance to a superoxide-generating agent and resistance to a quinolone antibiotic, respectively) but give rise to similar cellular and biochemical phenotypes, expressed as elevated cellular resistance to various oxidants and antibiotics. The nearby *marA1* mutation also confers multiple antibiotic resistance (11). Indeed, all three mutations (*marA1*, *soxQ1*, and *cfxB*) enhance bacterial resistance to a range of antibiotics via diminished expression of the OmpF outer membrane protein, which is mediated posttranscriptionally by *micF* antisense RNA (5a, 6).

This overlap raises the question of whether all three mutations affect the same gene. Strains with the *soxQ1* or *cfxB* mutation had few phenotypic differences and many common characteristics. This provides strong, although still circumstantial, evidence that *soxQ1* and *cfxB* are allelic and affect a gene that we named *soxQ*. The pattern of protein expression in a *marA1* strain mimics what might be expected for strains with a weak allele of *soxQ*: initial 2DPAGE analysis indicated small increases in spots 4, 13, and 14 and decreases in spots 10 and 12, and the activities of G6PD and SodA were elevated about twofold compared with those of *marA*$^+$ strains (unpublished data). The *marA1* mutation could therefore be allelic with *soxQ1*. However, recent molecular data suggest an alternative explanation.

The *marA* region has recently been cloned and sequenced, revealing a potentially complex operon of at least two genes (15a). One possibility is that the *marA1* mutation affects one of these genes (*marA*), while *soxQ1* and *cfxB* affect the other (*soxQ*). Indeed, the *marA1* mutation increases transcription of the operon (15b), while *soxQ1* does not (15a). Thus, mutations in *marA* may affect expression of the *soxQ* gene, and mutations in the *soxQ* gene may affect its ability to activate other genes (e.g., *sodA*, *zwf*, *soi*, and *micF*). Such a situation would account for the multiple antibiotic resistance conferred by all three mutations, as well as the differences in protein expression between the *soxQ1* and *cfxB* mutant strains and the *marA1* mutant strains. A regulatory locus of this complexity would be difficult to analyze by conventional genetic mapping and complementation. Instead, the identity of the gene(s) affected by the *soxQ1* and *cfxB* mutations will be most effectively established by DNA sequence analysis.

What might be the physiological significance of regulation by *soxQ*? As mentioned above, six of the proteins whose expression is increased by *soxQ1* or *cfxB* are also *soxR* regulated, while another seven have not been found to be inducible by oxidative or heat stress (13). Although we consider it unlikely, we have not eliminated the possibility that the putative *soxQ* regulon in wild-type cells includes only *soxR*-independent genes; the *soxQ1* and *cfxB* mutations would then encode renegade regulators that activate genes ordinarily controlled by *soxR*. If these mutations do not alter regulatory specificity, the antibiotic resistance of *soxQ1* and *cfxB* strains suggests that *soxQ* could control a regulon for antibiotic-induced stress that includes some genes independently regulated by *soxR*.

New data show that the regulation of some oxidative stress proteins is unexpectedly complex. The *sodA* gene is transcriptionally regulated by at least three global systems in addition to *soxQ*: *fur* and *arcA* encode, respectively, Fe^{2+}-dependent and anaerobiosis-dependent repressors of the gene (30), while the *soxR* locus specifies a transcriptional activator triggered by redox stress (14, 32). The *micF* antisense RNA that destabilizes OmpF mRNA is also part of several global regulons, including *envZ/ompR* (28), *marA* (6), an unknown heat-induced regulator (7), and *soxR* and *soxQ* (5a). A crucial goal is to determine how these various control systems interact to tune the levels of expression of individual genes to a variety of environmental conditions.

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