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Genetics

Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*

(oxygen radicals/adaptive responses/DNA repair/antibiotic resistance/*soxR*)

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ABSTRACT **_Escherichia coli_ responds to superoxide-generating agents by inducing ≈40 proteins. We have identified a genetic locus, _soxR_ (superoxide response), that positively regulates 9 of these proteins during superoxide stress. Induction under _soxR_ control is at the transcriptional level, as shown with _lac_ fusions to five paraquat-inducible promoters. Members of the _soxR_ regulon include at least three proteins with demonstrable antioxidant roles: Mn-containing superoxide dismutase (which destroys superoxide radicals), endonuclease IV (which repairs radical-induced damages in DNA), and glucose-6-phosphate dehydrogenase (which produces NADPH). Induction of the _soxR_ regulon also leads to diminished levels of the major outer membrane protein OmpF and alteration of the small-subunit ribosomal protein S6. These latter changes confer resistance to a variety of antibiotics. The _soxR_ regulon may thus operate as an inducible defense against xenobiotics in general.**

Both prokaryotic and eukaryotic cells gain adaptive advantages by responding to various environmental stresses (1–3). The responses to oxidative stress (3) are of special interest because reactive oxygen species are generated by both environmental agents (4) and normal aerobic metabolism (5–7). The reactive forms of oxygen include the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH), the latter capable of damaging nearly any biological macromolecule. A specific adaptive response to H_2O_2 in *Escherichia coli* (8) is partly controlled by the *oxyR* gene, which regulates 9 of the >35 polypeptides synthesized in response to H_2O_2 (3, 9).

More recently we (3) and others (10) identified a stress response that is activated by superoxide-generating agents and comprises a set of ≈40 polypeptides distinct from those induced by H_2O_2. Compounds that produce intracellular superoxide include the naphthoquinone menadione (MD) and the aromatic quaternary amine paraquat (PQ). These agents mediate the transfer of electrons from NADPH or NADH to O_2, generating a flux of $O_2^{\cdot-}$ in a process called redox cycling (4). Some of the genes induced by redox-cycling agents might be members of a regulon that responds specifically to increased $O_2^{\cdot-}$, rather than to other effects of such agents (such as NADPH depletion). For example, some PQ-inducible genes or proteins can also be induced by growth in high (hyperbaric) levels of oxygen (11, 12) or by normal oxygenation of superoxide dismutase-deficient *E. coli* (refs. 10 and 13; unpublished data).

The gene(s) controlling the response to superoxide has not been described. We sought to identify such regulatory genes by isolating mutant strains with increased resistance to redox-cycling agents. Such resistance might be mediated by constitutive increases in proteins that normally require induction. We report here the identification in this way of a regulon in *E. coli* that includes nine polypeptides induced in response to MD or PQ. A newly identified locus, *soxR*, is required for the transcriptional induction of the regulon genes.

MATERIALS AND METHODS

Strains. The following strains were obtained for use in this study: AB1186 (*uvrA6*), from G. Walker (Massachusetts Institute of Technology); AQ4517 (*soi17*::*lac*), AQ4519 (*soi19*::*lac*), and AQ4528 (*soi28*::*lac*), from S. Farr (Harvard School of Public Health); E107 ($dnaB107_{ts}$), M2508 (*melA7*), N3055 (*uvrA277*::Tn*10*), and TST1 (*malE52*::Tn*10*), all from B. Bachmann (*E. coli* Genetic Stock Center, Yale University); GC4468 (Δ*lac4169*) and QC909 [GC4468 Φ(*sodA*::MudPR13)25Φ(*sodB*::*kan*)Δ1-2], from D. Touati (University of Paris); JTG102 (*btuB*::Tn*10*), from Greenberg and Demple (7); MV1608 (*aidC8*::Mud*lac*), from M. Volkert (University of Massachusetts Medical School). Lysogens harboring λB11 (*zwf*::*lac bla*; from R. E. Wolf, Jr., University of Maryland, Baltimore County) were selected on LB agar containing ampicillin (100 μg/ml). Frozen stocks were stored in 20% (vol/vol) glycerol at −80°C, and working stocks were stored on solid medium at 4°C for up to 2 weeks.

Toxicity Measurements. Resistance to various agents was measured with fresh overnight cultures in LB broth (14) using gradient plates as described (15). Resistance was scored as confluent growth along the gradient and is expressed as a percentage of the maximum distance (80 mm) after 18–24 hr at 37°C.

Two-Dimensional Gel Analysis. Labeling of bacterial proteins with [^{35}S]methionine (1072 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear), extract preparation, and electrophoresis of samples were performed as described (3); nonequilibrium gels were run as described by Phillips (16).

Cell Extracts, Enzyme Assays, and Enzyme Activity Gels. Cell extracts were made as described (3, 7) unless specified otherwise. Where indicated, bacteria were treated with 1.45 mM MD or 1.3 mM PQ for 45 min of growth prior to processing. Superoxide dismutase activity gels (17) used nondenaturing 7.5% acrylamide slab gels (18). Endonuclease IV assays (19) and activity gels (20) were done as described. Glucose-6-phosphate dehydrogenase (G6PD) was assayed by monitoring the production of NADPH at 340 nm (21). β-Galactosidase was assayed in cells permeabilized with SDS/chloroform (14).

Isolation of MD-Resistant (MD^R) Bacteria. JTG102 was mutagenized with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (5 μg/ml) for 10 min as described (7), yielding 60% survival, and stored frozen. The cultures were thawed, and 0.1-ml aliquots

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Abbreviations: MD, menadione; PQ, paraquat; G6PD, glucose-6-phosphate dehydrogenase.

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were diluted into 5 ml of K medium (7). After 80 min at 37°C, 0.1-ml samples were plated on MD plates [supplemented minimal A agar (7, 14) containing MD (1.4 mg/ml) (the bisulfite adduct)]. After 24 hr, colonies were patched onto LB plates. MD^R isolates arose only from mutagenized JTG102 at a frequency of 1–5 × 10^{-4}. Fifty colonies from each of five independent pools were stabbed into LB plates containing 25% glycerol, incubated 24 hr at 37°C, and frozen initially at −30°C.

Spontaneous MD^R mutants were isolated from strain GC4468 by spreading 5 × 10^7 cells on an MD plate. After 24 hr at 37°C the resulting lawn was replica plated onto an LB plate containing chloramphenicol (7.5 μg/ml). After 24 hr at 37°C the five colonies visible were restreaked for analysis. Two representative MD^R (*soxR*^{c}) mutants (JTG319, *soxR101*, derived from JTG102; and JTG936, *soxR105*, derived from GC4468) were chosen for extensive characterization.

Isolation of Minitransposons Linked to *soxR*^{c} Mutations. Strain GC4468 was mutagenized by the "lambda hop" procedure using a kanamycin-resistant (Kan^R) derivative of Tn*10* [element 9 of Way *et al.* (22)], hereafter called Tn*10*km. $P1_{vir}$ grown on the Kan^R cells was used to transduce (14) strain JTG319 (*soxR101*), and the Kan^R colonies were replica plated onto LB plates with and without chloramphenicol (7.5 μg/ml). $P1_{vir}$ grown on individual chloramphenicol-sensitive (Cml^S) derivatives was then used to transduce JTG319 to Kan^R while retaining the *soxR101* marker (scored as Cml^R), yielding an insertion (*zjc2206*::Tn*10*km) that was 40% linked to *soxR101*. Alternatively, $P1_{vir}$ grown on the Tn*10*km-mutagenized GC4468 was used to transduce N3055 (*uvrA*::Tn*10*) to Kan^R Uvr^+ [tetracycline-sensitive (Tet^S)]. One insertion (*zjc2204*::Tn*10*km) was 90% cotransducible with *soxR105*. The *soxR*^{c} alleles were cotransduced with the Tn*10*km insertions by selection for Kan^R colonies and screening for either Cml^R (5–7.5 μg/ml) or nalidixic acid resistance (Nal^R) (6 μg/ml).

Isolation of *soxR* Deletions. Strain JTG936 (*soxR105*) containing the *zjc2204*::Tn*10*km insertion was mutagenized with Tn*10* [element 1 of Way *et al.* (22)]. $P1_{vir}$ grown on the pooled Tet^R derivatives was used to transduce JTG936 to Kan^R and Tet^R, yielding *zjc2205*::Tn*10* >90% linked to *zjc2204*::Tn*10*km (strain JTG1066). Strain M2508 was transduced to Kan^R Tet^R Cml^R (*soxR105*) with P1 lysates of JTG1066, to give strain DJ1001. Of 400 Tet^S derivatives (23) of DJ1001 initially characterized, 9 had lost resistance to chloramphenicol, of which 8 retained Kan^R. The loss of Kan^R is evidence for a Tn*10*-mediated deletion; since both the Kan^R and the Kan^S strains exhibited identical phenotypes, all of these strains apparently harbor deletions of *soxR*. A *soxR* deletion (Δ*soxR901*) was transferred to strain JTG936 by cotransduction with *zjc2204*::Tn*10*km; 20/20 Kan^R transductants lost both Nal^R and MD^R. One of these latter transductants (DJ901) was chosen for further characterization.

RESULTS

Mutants with Constitutive Increases in Nine Superoxide-Inducible Proteins. *E. coli* mutants selected for resistance to MD displayed increased resistance to a range of oxidants (Table 1), including the redox-cycling agents plumbagin and phenazine methosulfate, and the organic peroxide *t*-butyl hydroperoxide. Although PQ also generates intracellular superoxide (4), these MD^R mutants were not abnormally resistant to PQ (Table 1). We also noticed that the MD^R mutants showed increased resistance to the antibiotic chloramphenicol (Table 1) and we exploited that phenotype in genetic studies (see below).

These MD^R mutants define a co-regulated group of superoxide-inducible proteins. The most abundant class of mutants exhibited elevated levels of nine polypeptides, as determined by two-dimensional PAGE (Fig. 1). These same 9 proteins are among the ≈40 proteins induced strongly in wild-type *E. coli* by redox-cycling agents (Fig. 1; refs. 3 and 10).

Table 1. Sensitivity to oxidants and antibiotics

Strain	% growth on gradient plates					
	MD	PB	tBH	PQ	PM	Cml
JTG102	56	75	69	34	47	32
JTG319 (*soxR101*)	100	100	100	34	63	75
GC4468	53	ND	69	37	79	21
JTG936 (*soxR105*)	70	ND	85	36	95	100
DJ901 (Δ*soxR*)	40	ND	ND	34	5	16

The measurements were repeated multiple times with independent cultures; values from a representative experiment are shown. Amounts of the agents were (weight or volume per plate) as follows: MD, 90 mg of the bisulfite adduct (JTG102, JTG319) or 7 mg of nonderivatized MD in dimethyl sulfoxide (GC4468, JTG936, and DJ901); PB (plumbagin), 2 mg (dissolved in 95% ethanol); PQ, 12 mg (JTG102, JTG319) or 10 mg (GC4468, JTG936, DJ901); tBH (*t*-butyl hydroperoxide), 30 μl of 0.78 M stock; PM (phenazine methosulfate), 1 mg; Cml (chloramphenicol), 350 μg. ND, not determined.

A comparison of these two-dimensional gels with published data (3, 10, 24) served to identify many of the proteins increased in MD^R strains (see legend to Fig. 1). These include polypeptides that correspond to the Mn-containing superoxide dismutase (SodA), G6PD, the DNA repair enzyme endonuclease IV, the ribosomal protein S6C, and the gene products controlled by the PQ-inducible loci *soi17*/*soi19* and *soi28* (12). These co-regulated proteins are encoded by genes dispersed in the chromosome (12, 26, 27) and thus constitute a regulon. We have termed these MD^R strains *soxR*-constitutive mutants (*soxR*^{c}; for superoxide response).

***soxR*^{c} Mutants Have Elevated Levels of Oxidative Defense Enzymes.** The enhanced intensities of the endonuclease IV, G6PD, and SodA polypeptides seen on two-dimensional PAGE were paralleled by increases in the respective enzyme activities in extracts. G6PD activity was elevated ≈3-fold and endonuclease IV was increased 6- to 8-fold over wild-type levels in the *soxR*^{c} mutants (Table 2). Activity gels showed that the SodA, but not the Fe-containing (SodB), superoxide dismutase band was intensified in the *soxR*^{c} mutants (Fig. 2*A*). On DNA repair activity gels (20), the endonuclease IV band was specifically enhanced in the *soxR*^{c} mutants (Fig. 2*B*).

Consistent with the two-dimensional PAGE results, none of the mutants examined had elevated catalase or glutathione reductase activity (data not shown). This indicated that the *soxR*^{c} mutations do not affect the *katG*, *katE*, or *katF* genes (29), or the *gor* (28) or *oxyR* (9) genes (see below).

Genetic Mapping. The *soxR*^{c} mutations were linked to minitransposons to facilitate genetic analysis. Hfr mapping of a transposon (*zjc2206*::Tn*10*km) 40% linked to the *soxR101* mutation localized the insertion to 90–100 min. P1-mediated transductions further localized the *soxR101* mutation to 92.2 min, between the *uvrA* and *aidC* genes (Fig. 3); five other *soxR*^{c} alleles all mapped to the same locus. The *soxR*^{c} phenotypes (elevated enzyme levels, enhanced resistance to oxidants and antibiotics, and two-dimensional PAGE patterns) were retained upon cotransduction with *zjc2206*::Tn*10*km of five different *soxR*^{c} alleles (data not shown).

***soxR* Is a Positive Regulator.** Deletions mediated from a transposon (*zjc2205*::Tn*10*) tightly linked to *soxR105* abolished induction by MD or PQ of only the nine *soxR*^{c}-controlled proteins, as determined by two-dimensional PAGE (data not shown). Thus, *soxR* acts in a positive fashion. The inducibility of the SodA, endonuclease IV, and G6PD enzymes was restored in a Δ*soxR* strain carrying a cosmid that includes the region around minute 92 of the *E. coli* chromosome (E4398; ref. 31). However, the Δ*soxR* strains retained a limited ability to increase the activities of both SodA (Fig. 2*A*) and endonuclease IV (Table 2), but not

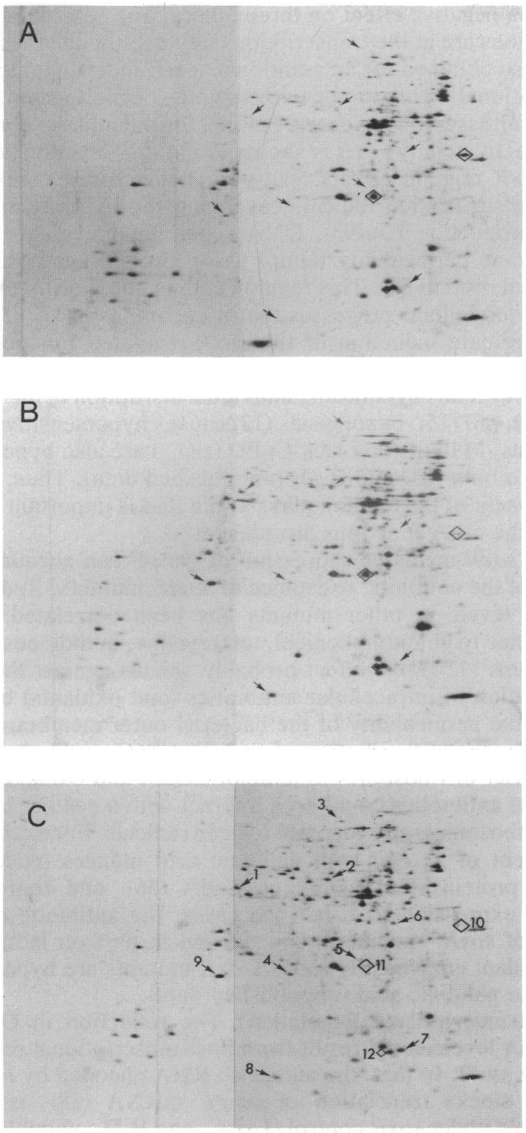

FIG. 1. Two-dimensional PAGE analysis of *soxR*^{c}-encoded proteins. (*A*) Wild type (JTG102) untreated. (*B*) Wild type treated with MD. (*C*) Untreated *soxR101* (JTG319). These panels are composite pictures showing the pH range from 3 to 10. →, Proteins increased in *soxR*^{c} strains; ◇, proteins decreased in *soxR*^{c} mutants. Only the *soxR*-controlled spots are indicated in *B*, although many other proteins are induced by MD (3). The identities of certain protein spots were determined by comparison with published data or with gels of the indicated strains. Spots 1 and 3, *soi17/soi19* dependent and *soi28* dependent, respectively (10). Spot 2, G6PD, analysis of a mutant (DF102) with 20-fold elevated G6PD (41). Spot 5, endonuclease IV [see Greenberg and Demple (3)]. Spots 7, 12, and 9, ribosomal proteins S6C and S6A, and SodA protein, respectively (24). Spot 10, OmpF, analysis of a *ompF*::*lac* mutant (MH513; ref. 25). Note the following features: spot 7 is inducible by PQ (but poorly by MD) in wild-type *E. coli* (3); the decreased intensity of spot 11 is not evident in this figure but was seen in other experiments with MD (ref. 3; data not shown); spot 12 was decreased after treatment of bacteria with PQ (data not shown).

G6PD (Table 2), upon treatment with MD. The *oxyR*-regulated proteins were induced normally in the Δ*soxR* background (data not shown).

Expression of Inducible Gene Fusions. Strains containing *soxR*$^+$, *soxR*^{c}, or Δ*soxR* alleles and fusions of the *lac* operon (containing the *lacZ* ribosome-binding site) to the *sodA*, *zwf*, *soi17*, *soi19*, or *soi28* promoters were examined for β-galactosidase expression in the presence or absence of PQ

Table 2. Enzyme levels in *soxR* mutants

Strain	Enzyme activity, unit(s)/mg	
	Endo IV	G6PD
GC4468	10	0.10
GC4468 + MD	28	0.20
JTG936 (*soxR105*)	86	0.30
DJ901 (Δ*soxR*)	6.2	0.14
DJ901 + MD	17	0.07

Strains were grown in LB medium; the lysis buffer contained 5 mM 2-mercaptoethanol and 10% glycerol. The entire experiment was repeated three times; data from a typical experiment are shown. Endonuclease IV (Endo IV) assays contained 3′-phosphoglycolaldehyde (19).

(Fig. 4; data not shown). Transcription of all five fusions was activated by PQ in the *soxR*$^+$ but not in the Δ*soxR* strains, confirming the positive role of *soxR*.

The *soxR*^{c} mutations also activated transcription of the fusions even in the absence of PQ, and expression was further PQ inducible (Fig. 4). Parallel increases (up to 2-fold) of the enzymes SodA (Fig. 2*A*), endonuclease IV, and G6PD (data not shown) were also seen after treatment of *soxR*^{c} strains with MD or PQ. The *soxR* locus may also be involved in the basal expression of *soi28*::*lac*, since a Δ*soxR* strain expressed only 5–25% of the β-galactosidase seen in its *soxR*$^+$ counterpart (Fig. 4).

Pleiotropic Effects of *soxR* Mutations. The *soxR*^{c} mutants exhibited phenotypes that appeared unrelated to oxidative stress. One such characteristic was elevated resistance to chloramphenicol (Table 1) and other drugs, including nali-

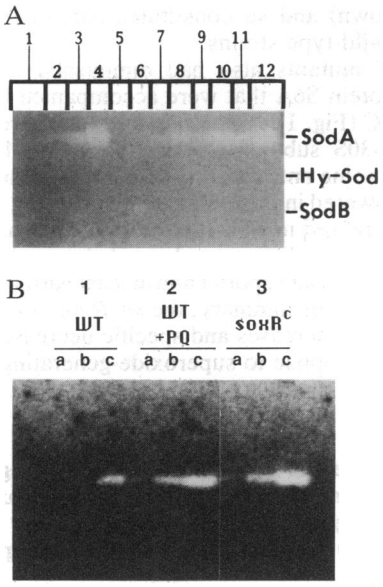

FIG. 2. Enzyme activity gels. (*A*) Superoxide dismutase activity gel. Lanes: 1 and 2, untreated GC4468; 3 and 4, MD-treated GC4468; 5 and 6, untreated DJ901 (Δ*soxR*); 7 and 8, MD-treated DJ901; 9 and 10, untreated JTG936 (*soxR105*); 11 and 12, MD-treated JTG936. Odd-numbered lanes had 10 μg of protein loaded, even-numbered lanes had 30 μg of protein. SodA is the product of the *sodA* gene; SodB is the product of the *sodB* gene; Hy-Sod is a hybrid protein containing both SodA and SodB (17). The SodA band was abolished in all strains (data not shown) by introduction of the Φ(*sodA*::MudPR13)*25* mutation (34). (*B*) Endonuclease IV activity gel. (*1*) Wild type (WT) (JTG102). (*2*) Wild type treated with PQ. (*3*) *soxR*^{c} (JTG319). Lanes a, b, and c were loaded with, respectively, 0.5, 2, and 10 μg of extract protein. The endonuclease IV band was abolished in all strains (not shown) by introduction of the *nfo*::*kan* mutation (15).

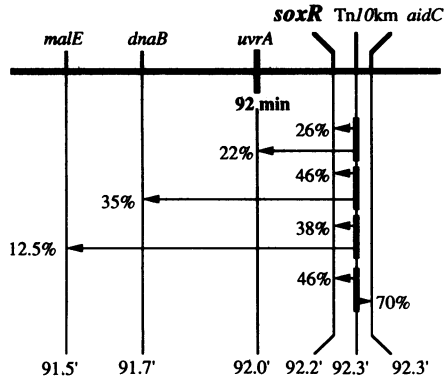

FIG. 3. Transduction mapping of the *soxR* locus. The percentages represent the degree of cotransduction observed in different crosses as indicated by the arrows; the number at the bottom of each line is the map position to the nearest 0.1 min. Tn*10*km is the *zjc2206*::Tn*10*km insertion. The donor was JTG319 *zjc2206*::Tn*10*km, selecting Kan^R and scoring the *soxR101* allele by resistance to chloramphenicol (7.5 μg/ml). The numbers of transductants scored were as follows (recipient; phenotype scored; total transductants): AB1886 (*uvrA6*; UV resistance; 200); E107 (*dnaB*; growth at 42°C; 220); TST1 (*malE*; red colonies on MacConkey/maltose agar; 200); MV1608 [*aidC*::Mud*lac*(30); loss of ampicillin resistance; 56].

dixic acid, ampicillin, tetracycline, and bleomycin (unpublished data). Such a multiple antibiotic resistance can be partially explained by lowered levels of the OmpF porin in these mutants (Fig. 1; see ref. 32). This reduction was confirmed independently by a >3-fold reduced ability of *soxR^c* strains to adsorb the OmpF-specific phage K20 (data not shown). The amount of OmpF protein was also lowered upon treatment of *soxR$^+$* bacteria with MD (Fig. 1) and PQ (data not shown) and so constitutes part of the inducible response in wild-type strains.

The *soxR^c* mutants also had diminished levels of the ribosomal protein S6A that were accompanied by increases in protein S6C (Fig. 1). These two species are related forms of the same 30S subunit polypeptide whose levels affect resistance to some antibiotics (33). All three proteins whose levels were lowered in the mutants (S6A, OmpF, and spot 11) were also decreased in wild-type cells in response to MD or PQ (Fig. 1). These decreases depended completely on the *soxR* locus for S6A and spot 11 and at least partially for OmpF (data not shown). In summary, the *soxR* locus is responsible for both specific increases and specific decreases in protein expression in response to superoxide-generating agents.

DISCUSSION

We have identified an *E. coli* oxidative stress regulon that is controlled by a previously undescribed locus, *soxR*, and that is activated by agents that generate intracellular superoxide. The *soxR* gene(s) is a positive regulator of nine proteins and exerts a negative effect on three others. The *soxR*-mediated inductions are at the transcriptional level, although evidence was also obtained for an additional, *soxR*-independent posttranslational activation of two enzymes of the regulon.

A Multilayered Response. A model for the multilevel resistance activated by *soxR* is shown in Fig. 5. The induction of the *soxR* regulon affects many biochemical processes, including superoxide radical scavenging (SodA protein), energy production (G6PD), DNA repair (endonuclease IV), membrane permeability (OmpF), and protein synthesis (ribosomal protein S6). This regulon is thus noteworthy for the variety of cellular processes that it encompasses.

Coordinate induction of the *soxR*-regulated functions is apparently important for resistance to oxidative stress. Consistent with this hypothesis, individual disruption of the *sodA* (26, 34), *nfo* (15), or *soi* genes (12) causes hypersensitivity to oxidants. Mutants that lack G6PD (*zwf*$^-$) are also hypersensitive to both MD and H_2O_2 (unpublished data). Thus, each component of the regulon plays a role that is important even when the other functions are present.

The *soxR*-mediated repression of OmpF can account for some of the antibiotic resistance of *soxR^c* mutants. Reduced OmpF levels in other mutants has been correlated with resistance to chloramphenicol, tetracycline, quinolones, and β-lactams (32). This effect probably shields against the accumulation of intracellular antibiotics (and oxidants) by altering the permeability of the bacterial outer membrane. In contrast, the redistribution of S6A into S6C could alter the sensitivity of ribosomes to antibiotics (33) and oxidants.

Some antibiotics could both interact with a cellular target (e.g., ribosomes) and generate oxygen radicals. For example, treatment of *E. coli* with nalidixic acid induces oxidative stress proteins (35), including SodA (36), and represses OmpF expression (37). In such cases, the antibiotic resistance of *soxR^c* mutants might depend in part on inducible antioxidant enzymes. Indeed, Δ*soxR* mutants are hypersensitive to nalidixic acid (unpublished data).

Posttranscriptional Regulation? The reduction in OmpF and S6A levels could result from posttranscriptional regulation by *soxR*. In fact, the antisense RNA encoded by *micF*, which blocks translation of *ompF* mRNA (38), is PQ-inducible under *soxR* control (J.H.C. and B.D., unpublished data). Such a repression mechanism also applies to *marA* mutants, which exhibit multiple antibiotic resistance that depends largely on the *micF*-mediated repression of OmpF synthesis (32).

A different kind of posttranscriptional control might operate on the ribosomal protein S6. The S6C form (which accumulates when the *soxR* regulon is activated) contains additional C-terminal glutamic acid residues compared to S6A, evidently added by a specific enzyme (33). The *soxR* locus might induce this processing enzyme in response to superoxide.

***soxR* Is One Facet of Multiple Controls.** Anoxic growth decreases the activities of SodA (11) and of endonuclease IV

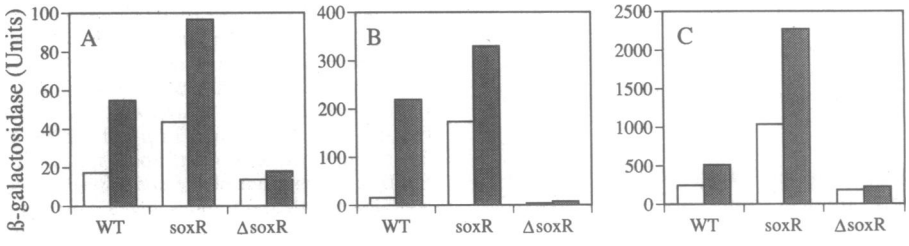

FIG. 4. β-Galactosidase activity from *lac* fusions to superoxide-inducible genes. Saturated cultures were diluted 100-fold into LB medium containing 1% glucose at 30°C (*soi*::*lac* strains) or 37°C (*zwf*::*lac* strains). When the cultures reached OD_{600} = 0.1, 40 μM PQ was added where indicated. (*A*) *soi19*::*lac* strains; wild type (WT) is AQ4519. (*B*) *soi28*::*lac* strains; WT is AQ4528. (*C*) *zwf*::*lac* strains; WT is GC4468 *zwf*::*lac*. Open bars, untreated samples; stippled bars, PQ-treated samples. soxR refers to the constitutive mutations *soxR101* (*A* and *B*) and *soxR105* (*C*). Note that the *soi28* fusion was expressed at subnormal levels in the Δ*soxR* background (4.3 units) compared to the wild type (16.8 units).

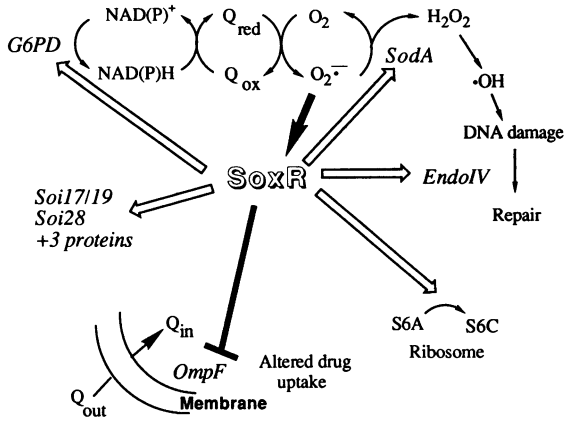

FIG. 5. The *soxR* regulon as a multilevel response to oxidative stress. Boldface arrow indicates triggering of the regulon by superoxide. Open arrows point to functions activated under *soxR* control, while the bar toward OmpF indicates inhibition of expression. *EndoIV*, endonuclease IV; Q, a redox-cycling drug such as MD. Redox-cycling agents (Q_{out}) enter the cell (Q_{in}) in the oxidized form (Q_{ox}) and are reduced (to Q_{red}) enzymatically by NAD(P)H-dependent diaphorases (4). Nonenzymatic oxidation of Q_{red} by O_2 generates $O_2^{\cdot-}$. The inductions of SodA and G6PD mitigate two direct effects of redox cycling: respectively, the production of superoxide and the depletion of NAD(P)H. The effect on OmpF might act at an even earlier step by preventing drug uptake. The modified distribution of one ribosomal component (S6) could alter an antibiotic or oxidation target. The induction of a DNA repair enzyme (endonuclease IV) enhances the removal of potentially toxic or mutagenic radical damages from DNA. The biochemical functions of many of the *soxR*-regulated proteins are unknown [the Soi proteins and spots 4, 6, and 8 (see Fig. 1)]. Therefore, the *soxR* regulon probably intervenes in additional biochemical processes not listed here.

and G6PD (unpublished data). It seems unlikely that *soxR* is involved in this regulation. The Δ*soxR* mutants express wild-type levels of SodA, endonuclease IV, and G6PD when grown aerobically, while the *soxR*^{c} mutants maintained the same relative elevation of all three activities when grown anoxically (unpublished data). It is possible that *soxR* exerts a negative function anaerobically and a positive one in response to redox-cycling agents. However, only with the *soi28* gene might *soxR* affect expression in the absence of redox cycling agents.

We have identified mutations at a locus distinct from *soxR* (named *soxQ*) that transcriptionally elevate the levels of SodA, G6PD, Soi17/19, and Soi28 (unpublished data). In contrast to *soxR*, *soxQ* is not required for the induction of these enzymes by PQ. Consequently, the *soxQ* locus could be a regulator that overlaps partially with *soxR* but responds to a different signal, such as aerobiosis.

The mechanism of the *soxR*-independent induction (by PQ and MD) of SodA and endonuclease IV is unknown, but it apparently occurs posttranslationally. Perhaps these proteins are activated by insertion of the appropriate transition metal cofactors, as has been shown in a different context for SodA (39).

Function of *soxR*. The *soxR* regulon requires both a detector of superoxide (receptor) and a transcriptional regulator (transducer) specific for *sodA*, *nfo*, etc. Since we have not found superoxide-responsive regulators of the regulon other than *soxR*, this locus might encode either a single protein with combined functions, or a receptor/transducer pair. We have recently cloned the *soxR* locus by using a *uvrA*-containing plasmid (40) to complement all the phenotypes of the Δ*soxR* mutants. A detailed analysis of the *soxR* region and gene products will guide our understanding of the biochemical mechanism by which the *soxR* regulon is activated in response to a free radical, superoxide.

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