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Molecular characterization of the *soxRS* genes of *Escherichia coli*: two genes control a superoxide stress regulon

Carlos F.Amábile-Cuevas and Bruce Demple*
Laboratory of Toxicology, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA

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ABSTRACT

The *soxR* locus of *Escherichia coli* K12 mediates transcriptional activation of a complex oxidative stress regulon in response to superoxide-generating (redox-cycling) agents. We have cloned the *soxR* locus, which is positioned near the *uvrA* gene at 92.2 min on the genetic map, by monitoring complementation of a Δ*soxR* mutation. Subclones from the *soxR* region in the Δ*soxR* strain simultaneously restored cellular resistance to the redox-cycling agent phenazine methosulfate and inducibility of at least two of the regulon proteins, glucose-6-phosphate dehydrogenase and endonuclease IV, by paraquat, another redox-cycling agent. DNA sequence analysis revealed the presence of two genes involved in activating the *soxR* regulon. These genes, named *soxR* and *soxS*, are arranged divergently with their 5′ ends separated by only 85 bp. The predicted 12.9-kDa SoxS protein is related to the AraC family of one-component gene regulators, but corresponds only to the putative DNA-binding regions of these proteins. The 17.1-kDa SoxR protein bears significant homology only to the MerR family of proteins including a predicted DNA-binding helix-turn-helix and a cluster of cysteine residues positioned similarly to those that regulate the activity of MerR in response to Hg^{2+}. This suggests that SoxR could be a metal-binding gene regulator that acts as the intracellular sensor for superoxide. SoxS is evidently the proximal activator of the regulon genes: antibiotic resistance and high-level expression of at least three of the regulon proteins was effected in vivo by the individual expression of SoxS, but not of SoxR, whether or not the cells were exposed to paraquat. These data, together with the recently reported paraquat-inducibility of the *soxS* gene (Wu, I., and Weiss, B. (1990) *J. Bacteriol.* 173, 2864 – 2871), indicate that SoxR and SoxS may constitute a novel type of two-component regulatory system in which the two proteins act sequentially to activate transcription of the various regulon genes in response to superoxide stress.

INTRODUCTION

Aerobic metabolism generates oxygen radicals as byproducts, including superoxide ($O_2^{-\cdot}$) (1). Moreover, certain xenobiotics, called redox-cycling compounds (e.g., paraquat (PQ)), divert molecular oxygen from its physiological pathway, mediating a one-electron reduction of O_2 to $O_2^{-\cdot}$ (2). Exposure of aerobically growing *Escherichia coli* to redox-cycling agents induces ~80 proteins (3, 4). Nine of these proteins are under the positive transcriptional control of a locus called *soxR* (5,6).

Some of the *soxR*-controlled proteins include Mn-containing superoxide dismutase (SOD), DNA repair endonuclease IV, glucose 6-phosphate dehydrogenase (G6PD), and the *soi17/19*-, and *soi28*-controlled proteins (5, 6). These proteins all play roles in defending *E. coli* against the toxicity of oxidative agents. Activation of the *soxR* regulon also provides broad cellular resistance to antibiotics, mediated at least in part by diminished synthesis of the outer membrane porin OmpF (5), which is achieved by increased production of the *micF* antisense RNA under *soxR* control (J.H. Chou and B.D., in preparation).

The induction of the *soxR* regulon by conditions that increase intracellular $O_2^{-\cdot}$, but not by other oxidants such as H_2O_2 (3), suggests the existence of a novel sensing system to trigger this response (5, 6). To characterize such a system, molecular analysis of the controlling elements is necessary. In this paper, we report the molecular cloning and characterization of the *soxR* locus. The functional *soxR* locus actually includes two genes, *soxR* and *soxS*, arranged head-to-head, as suggested previously (6). The predicted SoxR and SoxS proteins are each related to a distinct family of bacterial regulatory proteins that control responses to environmental change, and may act sequentially to activate the regulon.

MATERIAL AND METHODS

Strains and plasmids

The *E. coli* strains used were GC4468 (*soxR*$^+$), JTG936 (*soxR105*, a constitutive mutation) and DJ901 (Δ*soxR901*), all described by Greenberg et al. (5), and strain BL21(DE3), which bears a λ lysogen with the phage T7 gene 1 (7) was a gift of S.Tabor, Harvard Medical School. Plasmids pDR1998 and pDR2000 were the generous gifts of A.Sancar (University of

* To whom correspondence should be addressed

North Carolina, Chapel Hill). The Bluescript plasmid was obtained from Stratagene (La Jolla, CA). Plasmid pSE380, a *trc* promoter-containing expression vector that contains the *lacI*^{q} gene, was from Invitrogen (San Diego, CA). Other plasmids are described below. General procedures for DNA manipulations and cloning were as outlined by Sambrook et al.(8)

Toxicity measurements

Resistance to various agents was measured using gradient plates (9). Resistance was scored as confluent growth along the gradient, expressed as a percentage of the maximum possible growth (80 mm) after 18–24 h at 37°C.

Cell extracts and enzyme assays

Bacteria were grown in 50 ml LB broth (10) at 37°C until they reached OD_{600} ~0.4, PQ was added (where indicated) to 1 mM, and the incubation continued for 45 min. The cells were then harvested by centrifugation at 5,000×g for 10 min at 2°C, and washed with 1 ml M9 salts (10). Cell pellets were frozen at −80°C, thawed on ice for 1–2 h, and resuspended in lysis buffer (50 mM Tris, 0.2 M NaCl, pH 7.5). The cell suspension was mixed with 1 ml of 0.1 mm glass beads, and disrupted for 2 min using a Mini-bead beater (Biospec Products, Bartlesville, OK). Cell debris was removed by centrifugation at 10,000×g for 45 min at 2°C, and the cleared supernatant was kept on ice for immediate assay or stored frozen at −80°C. Protein concentrations were determined as described by Bradford (11).

Superoxide dismutase activity was measured in activity gels by the method of Beauchamp and Fridovich (12) using 7.5% polyacrylamide non-denaturing slab gels. Endonuclease IV was assayed as EDTA-resistant AP endonuclease activity (13, 14). G6PD activity was monitored by following the production of NADPH at 340 nm (15).

Subcloning and DNA sequencing

Plasmid pDR2000 was cut with *Cla*I, once in the insert sequence (16) and once within the pBR322 vector (8) and recircularized to yield pBD100 (Fig. 1). Excising a 1-kb EcoRV fragment, or a 1-kb SphI fragment from pBD100, and re-ligating, resulted in plasmids pCA261 and pCA262 respectively. Removing a 1.8-kb *Aat*II-*Eco*RI fragment from pCA262, end-filling and religating yielded pCA2710, while removing a 2-kb *Sma*I-*Eco*RI fragment resulted in pCA2711 (Fig. 1).

For DNA sequencing, the following plasmids were constructed. A 3.6-kb *Eco*RI-*Cla*I fragment from pBD100 was subcloned into plasmid pBluescript to generate pCA263. A 500-bp *Sph*I-*Cla*I fragment was then removed from pCA263, and blunt ends formed with S1 nuclease, followed by religation to form pCA271. Excision of a 2-kb *Kpn*I-*Eco*RI fragment from pCA271, end-filling and religation resulted in pCA273, while excision of a 2.5-kb *Hpa*I-*Eco*RI fragment, end-filling and religation yielded pCA274. Plasmids pCA273 and pCA274 were sequenced with the Sequenase kit (United States Biochemical, Cleveland, OH), using initially the SK and M13 reverse primers (Stratagene). As the sequence accumulated, three additional oligonucleotides were synthesized, which corresponded to nucleotides 122–136, 610–627, and 1028–1044 of the DNA sequence (EMBL Data Library, Accession number X59593). The entire DNA sequence was determined on both strands. Computer analysis of translated protein sequences was done with the TFASTA program (17).

DNA amplification and cloning

Four oligonucleotides (*a*, 5′-GGCG*AAGCTT*CGCAGGTGTT-TATGC-3′, nucleotides 274–297; *b*, 5′-CAGAT*GAATTC*AC-GAACTGAACAC-3′, nucleotides 705–682; *c*, 5′-GGGAGTA*GAATTC*CTCAAGTTAAC-3′, nucleotides 708–732; and *d*, 5′-CGTCGGGGG*AAGCTT*TCCTGTGTAC-C-3′, nucleotides 1296–1271) were synthesized to be used as primers in a polymerase chain reaction (PCR) (8). These primers contain 1–3 mismatches each, in order to generate *Hin*dIII sites with primers *a* and *d*, and *Eco*RI sites with primers *b* and *c*. Using primers *a-b* and *c-d*, the *soxS* and *soxR* ORFs were amplified separately. These PCR-fragments, 588 bp and 432 bp for *soxR* and *soxS* respectively, were ligated into *Eco*RI/*Hin*dIII-digested pBluescript to form plasmids pSOXR and pSOXS, respectively, and into *Eco*RI/*Hin*dIII-digested pSE380 to form plasmids pSXR and pSXS, respectively. Using primers *a-d*, the entire 1022-bp region was amplified and then ligated into *Hin*dIII-linearized pBluescript, resulting in pSOXRS. All three PCR products were sequenced to ensure that no replication errors were introduced during the amplification.

Protein expression

Plasmids pSOXR and pSOXS were transformed into BL21(DE3) strain (7). Isolates were then grown in M9 medium with 0.4% glucose at 37°C to OD_{600} ~ 0.6 and incubated 30 min with 1 mM IPTG (to induce the synthesis of T7 RNA polymerase). Rifampicin (200 μg/ml) was then added, and after an additional 30 min, 200 μl aliquots of the culture were pulse-labeled at 37°C with [^{35}S]-methionine (10 μCi for 3 min). The labeled cells were harvested, resuspended in 50 μl sample buffer (18), and boiled for 3 min. Samples were electrophoresed on a 15% SDS-polyacrylamide gel (19) and autoradiographed.

For the independent expression of SoxR and SoxS, plasmids pSXR and pSXS were transformed into strain DJ901 (Δ*soxR*). Isolates were then grown in LB broth with or without IPTG (1 mM added 2 h before harvesting) and/or PQ (1 mM added 45 min before harvesting), and extracted to determine enzyme levels as described above.

RESULTS

Cloning of the soxR locus. Transduction mapping experiments had localized the *soxR* locus to 92.2 min on the *E. coli* genetic map, close to the *uvrA* and *ssb* genes (20). Deletions in this region mediated by excision of transposon Tn*10* rendered the *soxR*-regulated genes transcriptionally uninducible by PQ (5).

Plasmids pDR1998 and pDR2000, which carry the *uvrA* gene on 9-kb *Eco*RI-*Eco*RI and 7-kb *Eco*RI-*Cla*I fragments, respectively (16), were tested for complementation of the Δ*soxR* phenotypes. Strain DJ901 (Δ*soxR901*) bearing these plasmids was scored for resistance to nalidixic acid and the redox-cycling drug phenazine methosulfate, agents to which Δ*soxR* strains are hypersensitive compared to *soxR*$^+$ bacteria (5), and for PQ-inducibility of G6PD, Mn-SOD and endonuclease IV activities. Plasmid pDR2000, but not pDR1998, complemented these phenotypes (data not shown).

A 6-kb *Cla*I fragment was excised from pDR2000 to yield plasmid pBD100, which also complements the Δ*soxR* phenotypes (Table 1). DJ901 bearing pBD100 exhibited higher induced levels of endonuclease IV and G6PD than did *soxR*$^+$ bacteria without the plasmid (data not shown), which suggests that the *soxR* gene product(s) are limiting for induction. Various derivatives of

pBD100 were then tested for complementation of Δ*soxR901* (Table 1). By this analysis, a 1.3-kb *Sph*I-*Aat*II fragment in pCA2710 was sufficient to restore drug resistance and enzyme inducibility to the Δ*soxR* strain (Table 1, Fig. 1). However, a deletion up to the *Sma*I site yielded a plasmid (pCA2711) with exceptional properties: DJ901 bearing pCA2711 had constitutively high levels of G6PD expression, exceptional resistance to phenazine methosulfate, only modest resistance to nalidixic acid, and marginal enzyme inducibility by PQ (Table 1). These results are consistent with those previously reported by Tsaneva and Weiss (6).

DNA sequence of the *soxR* genes

The DNA from pBD100 was subcloned into the Bluescript plasmid for DNA sequence analysis (see Methods). The DNA sequence corresponding to the insert in pCA2710 revealed two open reading frames (ORFs), in opposite orientations. The first ORF is encoded by the complement of nucleotides 665 to 341 and the second ORF is encoded by nucleotides 748 to 1215. The entire sequence has been deposited in the EMBL Data Library (accession number X59593) and is identical to that just reported by Wu and Weiss (21). The first ORF encodes a predicted protein of 107 amino acids and molecular weight 12,902. The second ORF encodes a predicted protein of 154 amino acids and molecular weight 17,139. To be consistent with the nomenclature of Tsaneva and Weiss (6), we have named these divergent genes *soxS* (encoding the 12.9-kDa protein) and *soxR* (encoding the 17.1-kDa protein).

Table 1. Complementation of Δ*soxR901* by recombinant plasmids

Plasmid	Drug resistance Nal	Drug resistance PMS	Activity (units/mg) G6PD −PQ	G6PD +PQ	EndoIV −PQ	EndoIV +PQ
pBR322	23	49	0.11	0.12	5.2	5.7
pBD100	59	65	0.09	0.42	8.4	49
pCA261	21	49	0.10	0.13	5.8	6.8
pCA262	54	64	0.09	0.33	9.6	63
pCA2710	54	61	0.07	0.56	4.9	58
pCA2711	34	91	1.39	1.77	135	102

Strain DJ901 (Δ*soxR901*) bearing the indicated plasmids was scored for resistance to the indicated agent as decribed in Methods; the numbers correspond to the percentage of the maximum possible growth exhibited by each strain. Nal, nalidixic acid, 0.4 mg per plate; PMS, phenazine methosulfate, 1.25 mg per plate. The enzyme activities in crude extracts were determined with and without paraquat (PQ) induction, as described in the text. The entire experiment was repeated three times; one of the repetitions is shown. EndoIV, endonuclease IV.

A PCR product encompassing just the soxS and *soxR* genes in *soxRS* (see Methods) was used to examine the structure of the *soxR* locus in wild-type (GC4468), constitutive (*soxR105*; JTG936) and deletion (Δ*soxR901*; DJ901) strains. Southern blot analysis revealed that the Δ*soxR901* deletion removes all detectable DNA that hybridizes to *soxS* and *soxR* (Fig. 2). This result shows clearly that neither gene is essential for laboratory growth of *E. coli* consistent with previous results (5, 6). The pattern for GC4468 was as expected for the DNA in this region (16, 22), and was identical to that for JTG936, the spontaneously-generated *soxR105* mutant (5). This result indicates that the constitutive mutation does not involve a large insertion or deletion at the *soxR* locus. Although a small deletion affecting *soxR* in JTG936 cannot be ruled out by this analysis, we note that the *Kpn*I site removed in the generation of the constitutive plasmid pCA2711 (Fig. 1) is retained in the *soxR105* strain.

Homology between SoxR, SoxS, and other proteins

The protein sequence comparison program TFASTA (17) was used to search for homology between the predicted SoxS and SoxR proteins, and various protein and translated DNA databases (NBRF and GenBank). This comparison revealed that SoxR is

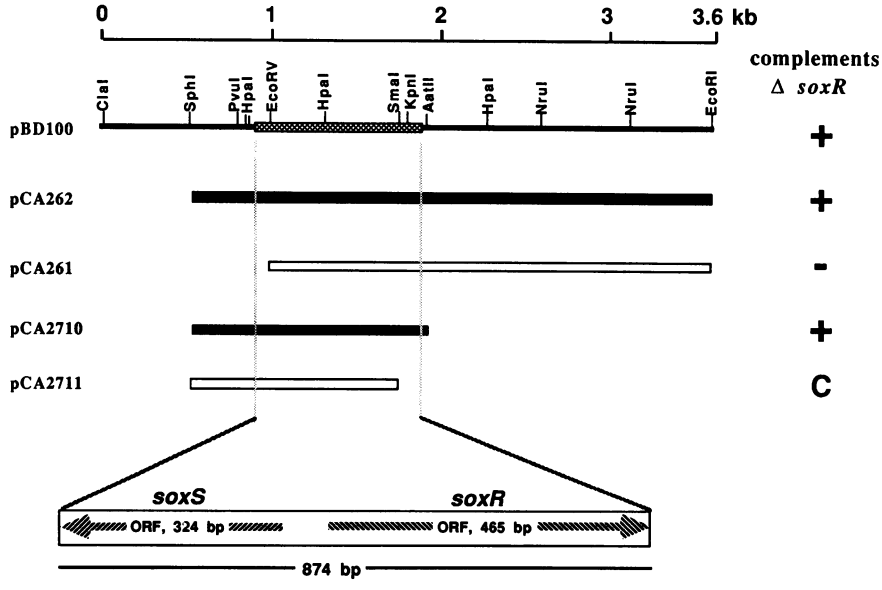

Figure 1. Physical and functional map of the *soxRS* region. The various bars represent subclones from pBD100 in plasmid pBR322; only the insert DNAs are shown. Filled bars correspond to plasmids that complement Δ*soxR901*, open bars to noncomplementing plasmids or one (pCA2711) with altered regulation. 'C' indicates constitutive expression. The hatched region in pBD100 corresponds to the functional locus, shown schematically at the bottom part with the positions and orientations of *soxR* and *soxS* indicated. Complementation of the Δ*soxR* phenotypes was tested by transforming each plasmid into strain DJ901 and measuring resistance to nalidixic acid and PMS, and the inducibility of G6PD and endonuclease IV by PQ (see Table 1).

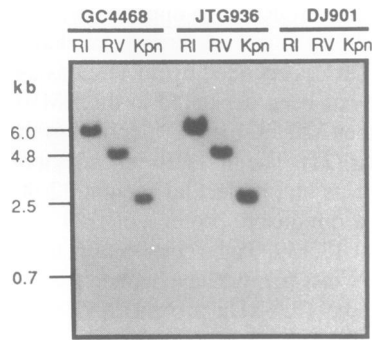

Figure 2. Physical analysis of Δ*soxR901* and *soxR105*. Chromosomal DNA from strains GC4468 (*soxR*$^+$), JTG936 (*soxR105*, a constitutive mutation) and DJ901 (Δ*soxR901*, a Tn*10*-mediated deletion) were digested with *Eco*RI (RI), *Eco*RV (RV) or *Kpn*I (Kpn), electrophoresed in a 1% agarose gel in TPE, and blotted onto a nitrocellulose filter (8). The insert DNA in plasmid pSOXRS was labeled by random hexamer priming and used to probe the blot at high stringency. The indicated sizes of the fragments are those expected from the physical map (6, 22) (Fig. 1). The 0.7-kb fragment produced by *Eco*RI digestion is faintly visible.

homologous to the MerR family of proteins, which are positive/negative regulators of the mercury resistance operons in transposon Tn*501* (23) and various plasmids (24) (Fig 3a). Most importantly, this homology includes two regions that are required for the function of the MerR protein of Tn*501* (25): a probable helix-turn-helix at residues 12−34 in SoxR, and a cluster of cysteines at residues 122, 124 and 130 in SoxR. The only other cysteine residue in SoxR is nearby, at position 119. The MerR protein of Tn*501* also contains a fourth cysteine residue, at position 82 (25).

The SoxS protein shows homology with the C-terminal regions of a family of regulatory proteins that includes AraC, the positive regulator of the arabinose operon in *E.coli* (26) and other gram-negative bacteria (27), and RhaS and RhaR, the positive regulators of the L-rhamnose operon in *E.coli* (28). SoxS is most similar to the transposon Tn*10*-encoded TetD protein (~50% identity, Fig.3b), whose function is unknown (29). The AraC family of gene regulators are DNA-binding proteins that contain probable helix-turn-helix DNA recognition sequences; such a sequence is also present in SoxS. This homology between SoxS and the AraC family has been identified by Wu and Weiss (21).

Individual expression of SoxR and SoxS

Protein expression plasmids for the individual production of the SoxS and SoxR proteins were constructed by PCR amplification of the respective genes, which were then placed behind T7 promoters in multicopy plasmids (see Methods). These plasmids were transformed into a strain carrying the phage T7 RNA polymerase gene (gene 1) in a lysogenized λ phage (7). Sequential treatment of these cells with IPTG (to induce the phage polymerase under *lac* operator control) and rifampicin (to suppress transcription of host genes) allowed labeling of the *soxS* and *soxR* gene products with [^{35}S] methionine. This analysis showed that *soxS* encodes a protein of ~13 kDa and *soxR* a protein of ~17 kDa (Fig. 4) as predicted from their DNA sequences, and consistent with a previous report (6).

When the PCR-amplified genes were positioned in multicopy plasmids behind *trc* promoters and separately transformed into a strain with the *lacI*^{Q1} mutation (10), SoxR comprised up to

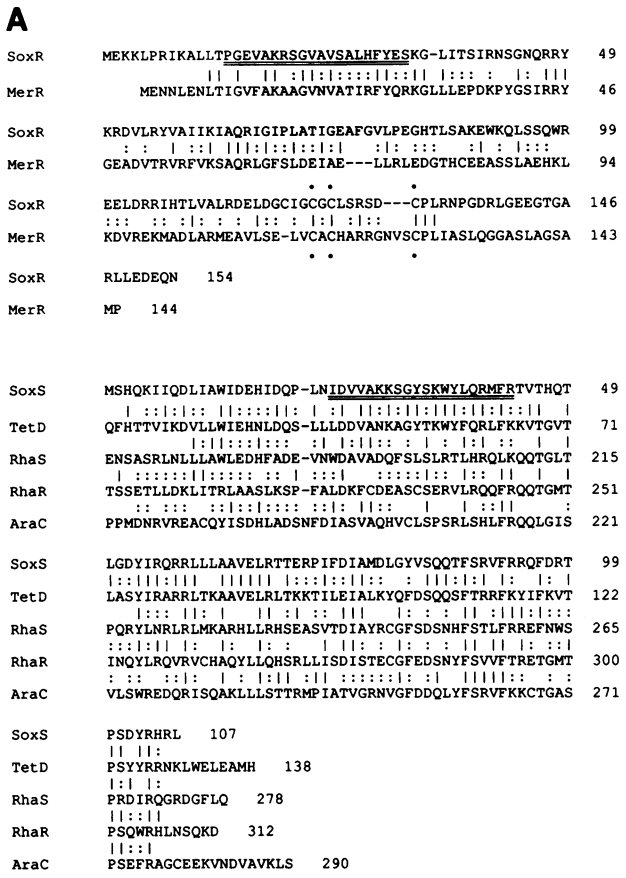

Figure 3. Protein sequences and homology. **(A)** The entire amino acid sequence of SoxR (upper) and SoxS (lower) is shown, along with their optimal alignment to the homologous regions of other polypeptides. A vertical line indicates identity, a colon a conservative replacement between amino acid residues in different sequences (17). The double underlining indicates putative helix-turn-helix motifs (36). The dots near individual cysteines indicate residues that are functional in MerR (33) and the corresponding residues in SoxR. **(B)** Location and extent of homology of SoxR and SoxS proteins with selected other regulatory proteins.

~3% and SoxS up to ~1% of the cell protein after induction with IPTG (data not shown). However, we were unable to obtain stable transformants of these plasmids in strain DJ901 (*lacI*$^+$).

Placement of the *soxR* and *soxS* genes behind the IPTG-

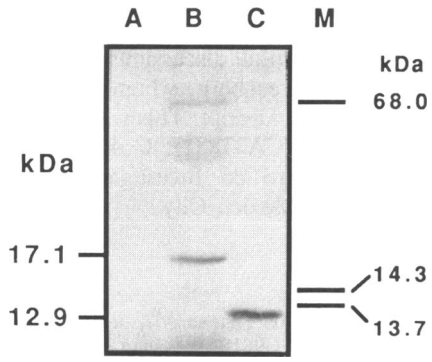

Figure 4. Expression of SoxR and SoxS. BL21(DE3) cells carrying (A) pBluescript, (B) pSOXR, and (C) pSOXS, were labeled with [^{35}S]-methionine and analysed by SDS-PAGE (see Methods). The marker proteins (M) were bovine serum albumin, lysozyme and pancreatic RNase.

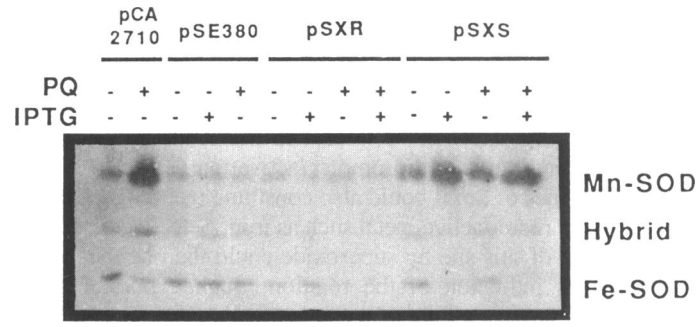

Figure 5. Activation of Mn-SOD expression by SoxS. DJ901 cells bearing pCA2710, pSE380, pSXR or pSXS were grown and treated with PQ and/or IPTG as indicated in the text. Extracts of these cells were analyzed for SOD activity: 30 μg of total protein was loaded in each lane. A negative image of the gel is shown. The entire experiment was repeated twice.

Table 2. Phenotype of cells individually expressing *soxRS* genes

Plasmid	Drug resistance Nal	PMS	Addition IPTG	PQ	Relative enzyme levels G6PD	EndoIV
pCA2710	53	39	−	−	1.5	1.3
			−	+	8.1	16.1
pSE380	37	24	−	−	≡1.0	≡1.0
			−	+	1.2	0.8
			+	+	1.0	1.0
pSXR	36	24	−	−	0.9	1.3
			+	−	0.8	1.0
			−	+	0.7	0.7
			+	+	0.9	1.3
pSXS	53	41	−	−	2.7	4.5
			+	−	11.0	16.2
			−	+	1.8	4.2
			+	+	11.9	28.0

Strain DJ901 carrying the indicated plasmids was scored for drug resistance as in Table 1. Enzyme levels are given relative to DJ901 carrying the vector plasmid (pSE380) and without IPTG or PQ treatment (≡1.0). 'Addition' indicates exposure to IPTG and/or PQ.

inducible *trc* promoter in plasmid pSE380 (which also contains the *lacI*^{Q}1 gene) yielded pSXR and pSXS, which produced stable transformants in DJ901. The resulting strains exhibited distinct phenotypes. The SXR plasmid showed no effect on the cellular sensitivity to nalidixic acid or PMS, or on the expression of Mn-containing SOD, G6PD or endonuclease IV (Table 2; Fig. 5). In contrast, expression of SoxS from pSXS provided wild-type resistance to nalidixic acid and PMS, and increased levels of Mn-containing SOD, G6PD and endonuclease IV (Table 2; Fig. 5). After induction of SoxS with IPTG, the activity levels of the three enzymes were equal to those obtained after PQ-induction of a strain with the complete *soxRS* locus (in plasmid pCA2710; Table 2; Fig. 5).

Treatment with PQ alone of the strains bearing pSXR and pSXS did not significantly increase production of the *soxRS*-regulated enzymes. A combined treatment with IPTG and PQ produced Mn-containing SOD and G6PD levels similar to those seen with IPTG alone (Table 2; Fig. 5). The combined IPTG and PQ treatment gave endonuclease IV expression ~70% higher than that seen with IPTG alone (Table 2). This difference may be due to the *soxRS*-independent increase in endonuclease IV activity sometimes detected after PQ treatment (5).

DISCUSSION

We have cloned the *soxR* locus of *E. coli* by functional complementation of a Δ*soxR* mutation. Complementation and DNA sequence analysis of this locus revealed the presence of two genes involved in activating the *soxR* regulon. These genes, named *soxR* and *soxS*, are arranged in opposite orientation, with their 5′ ends separated by 85 bp. The predicted 17.1-kDa SoxR protein bears significant homology only to the MerR family of proteins among known sequences, while the 12.9-kDa SoxS protein is related to the family of bacterial gene regulators exemplified by AraC. Except for the identification of homology between SoxR and the MerR family, the above observations have been made independently (6,21). Most importantly, expression of SoxS protein alone was sufficient to activate expression of at least three of the key regulon proteins, while SoxR alone was without effect.

The evidence presented here implicates both *soxR* and *soxS* in control of the *soxR* regulon: (i) a previously designated Δ*soxR* mutation actually eliminates both genes; (ii) even small deletions into the 3′ end of the *soxS* gene destroy induction of the regulon; (iii) a small deletion into the *soxR* gene gave rise to a constitutive phenotype; (iv) the overall resistance of *E. coli* to the redox-cycling agent phenazine methosulfate and induction by PQ of at least two active components of the regulon, G6PD and endonuclease IV, depend on the same functional region; (v) the predicted products of both genes bear resemblance to other known gene regulators; (vi) SoxS protein alone activates the regulon without superoxide stress. Points ii, iii and v above have also been demonstrated by Weiss and coworkers (6, 21).

Dual regulatory genes are known for many other bacterial multigene systems (30, 31), but those encode proteins unrelated to SoxR or SoxS. In those two-component regulatory systems, the sensor protein is usually a histidine autokinase (often a membrane protein) that transfers phosphate to the transducer (gene regulator) protein, which then activates transcription (30, 31). The predicted SoxR and SoxS proteins do not contain peptide segments related to any other kinase-like regions.

The possible mechanisms by which the two gene products act to control the *soxR* regulon are limited by the data presented here and by others (21). Such a system must incorporate both a sensor of the inducing signal and a transducer that activates the regulon genes. An attractive possibility is that SoxR constitutes the signal

receptor in this system. This conclusion is consistent with the protein's homology to virtually the entire length of MerR, including the metal-binding regulatory site. In MerR, three of the four cysteines (Fig. 3A) are involved in binding an atom of Hg^{2+} across the interface between two MerR monomers (32); this metal binding causes promoter activation (25).

The cysteines of SoxR could also constitute metal ligands, but which bind a redox-active metal such as iron. Selective oxidation or reduction of this site by superoxide could then be the signal that triggers induction of the regulon. Such a possibility is especially attractive in light of the demonstation that several iron-sulfur proteins are highly sensitive to redox inactivation by superoxide (33). Consistent with possible control exerted by the C-terminal region of SoxR, the small 3′ deletion into *soxR* in plasmid pCA2711, which generates a *soxR*-constitutive phenotype (Fig.1; ref.6), approaches but does not remove any of the cysteines of SoxR. However, we have not eliminated the possibility that this deletion generates a fusion protein that contains sequences specified by the vector downstream of *soxR*.

If SoxR is the sensor in this system, SoxS protein might mediate the activation of the regulon genes. Production of SoxS in the absence of SoxR (or of any superoxide-generating agent) was sufficient to induce strong expression of at least three *soxRS* regulon genes (Table 2; Fig.5). Wu and Weiss (21) recently demonstrated that the *soxS* transcript is inducible by PQ, although the possible dependence of this induction on *soxR* is unknown. A simple model would involve the sequential action of SoxR and SoxS: redox-activation of SoxR to switch on *soxS* expression, and the resulting increase in SoxS levels activating the regulon genes. If this is so, these two proteins would number among the smallest gene activators known. Such a model also predicts specific DNA binding by these proteins, which can be readily tested experimentally. We cannot yet eliminate the possibility that the SoxR and SoxS proteins act together to form a bipartite gene activator in wild-type bacteria. A redox-regulated, bipartite gene activator is already known in the case of the mammalian Jun-Fos heterodimer, although the physiological role of this redox control has not been established (34).

It is possible that the production of the SoxR and SoxS proteins is coordinated. The potential hairpin-forming sequence in the intergenic region (nucleotides 721−738) could operate at the DNA level or the RNA level to affect transcription, or in the mRNA to affect translation of the *soxR* message (this region overlaps the likely ribosome-binding site for *soxR*; ref. 21). Such symmetric sites can constitute binding sites for regulatory proteins. The work of Wu and Weiss (21) indicates that additional levels of control might act in the *soxRS* system: the *soxS* mRNA begins in the intergenic region, but the 5′ end of the *soxR* message originates ~165 bp within the *soxS* gene. The opposing transcription of these two genes and the potential for overlap between the transcripts could exert both transcriptional and post-transcriptional control.

A number of the *soxRS* regulon genes (but not *nfo*, the endonuclease IV structural gene) are also members of another coregulated group, transcriptionally controlled by the *soxQ* locus (35). Gene activation by *soxQ* is independent of the *soxRS* locus. Genes such as *sodA*, *zwf* and *micF* must therefore accomodate multiple controls in response to diverse environmental signals. The work presented here represents a key step toward revealing the mechanism by which one of these systems operates and will help clarify how such multiple controls are coordinated.

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