Regulation of the nfsA Gene in *Escherichia coli* by SoxS

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Nitrosubstituted compounds have been used for many years in the health industry as antimicrobial agents (43) and more recently have been examined for use in enzyme-prodrug cancer chemotherapies (3). In addition, a large number of nitroaromatic and nitroheterocyclic derivatives are potent mutagens and suspected human carcinogens, and therefore their presence in environmental samples is of considerable health concern (33, 50, 51). The mutagenic and antimicrobial activity of these compounds is mediated by short-lived intermediates formed during their reduction by nitroreductases (31, 32). Two types of nitroreductases have been identified in Escherichia coli (6, 10, 32). Oxygen-insensitive nitroreductases are flavoproteins that mediate the transfer of two electrons from NAD(P)H to the nitro moiety of nitroaromatic compounds (10); biologically active intermediates produced through this pathway include nitroso and hydroxylamine derivatives that are further reduced to yield biologically inactive products (32). Oxygen-sensitive nitroreductases mediate single-electron transfers, producing nitro-anion free radicals which, in the presence of oxygen, can be rapidly reoxidized in a futile redox cycle through which superoxide is generated (37).

Two oxygen-insensitive nitroreductases, NfsA and NfsB, have been characterized in E. coli (10, 30, 54, 56, 57). NfsA is the major oxygen-insensitive nitroreductase and uses NADPH as an electron source, while NfsB is a minor nitroreductase that can use either NADH or NADPH as a source of reducing equivalents (10, 56, 57). The nfsA gene (mdaA) is located at 19.2 min on the E. coli chromosome (56), upstream of the rimK gene (9) that encodes a protein responsible for posttranslational modification of the S6 ribosomal protein (20), and downstream from ybjC, a small open reading frame (ORF) of unknown function (9). A role for NfsA in the bacterial oxidative stress response has been suggested by a recent study demonstrating that nfsA is upregulated by the redox cycling compound, paraquat, in a soxRS-dependent manner, suggesting that it is part of the soxRS regulon (27). A macroarray study demonstrating that nfsA, rimK, and ybjC are all members of the closely related marRAB regulon suggests that these genes may form an operon (7).

The soxRS regulon includes at least 15 genes that are upregulated in response to superoxide formed by redox-cycling compounds such as paraquat (8, 15, 24, 39, 52). The regulon includes several genes whose products play a direct role in responding to oxidative stress, including manganese superoxide dismutase (soxA), exonuclease IV (nfo), glucose 6-phosphate dehydrogenase (zwf) (15), fumarase C (fumC) (25), and NADPH ferredoxin reductase (fpr) (26). Upregulation of these genes occurs in a two-step process in which the SoxR protein acts both as a sensory protein to detect elevated levels of superoxide within the cell and as a positive regulator of soxS transcription (2, 15, 16, 53). The SoxR protein contains two binuclear iron-sulfur clusters [2Fe-2S] that remain in the reduced state under normal (nonstress) conditions. In the presence of elevated levels of superoxide, the SoxR [2Fe-2S] clusters become oxidized (16). While both the oxidized and reduced forms of SoxR bind equally well to the promoter sequence of soxS, only the oxidized form activates transcription of soxS, presumably by enhancing open-complex formation by RNA polymerase (12). The SoxS protein, in turn, is a positive regulator of the members of the soxRS regulon (2, 22).

The SoxS protein is a member of the XylS/AraC family of transcription activators that includes two other proteins, MarA and Rob, that share sequence identity (41 and 55%, respectively) with SoxS. The marRAB locus was first described as a multiple-antibiotic-resistance locus in E. coli responsible for increased bacterial resistance to a wide range of unrelated antibiotics and organic solvents after exposure to compounds such as sodium salicylate (1). Over 40 genes in E. coli, including 9 of the 15 genes upregulated by SoxS, are upregulated by the MarA protein (7). The Rob protein is constitutively expressed and binds to the right arm of the E. coli origin of chromosome replication (oriC), but its function is still un-
The to require interaction between SoxS and C-terminal domain of moters include fumC, and ribA zwf has been shown to be independent of sodA. fumC SoxS activation of the class II promoters /H11002,tolC and class II promoters and the oriC proteins (4, 19, 23, 29, 38). to various degrees, by the three closely related regulatory pro-
ered part of the same Sox/Mar/Rob regulon which is regulated, strongly suggesting that the genes involved should be consid-
overlap in the genes regulated by each of these three proteins, known (46). Many studies have demonstrated a considerable
involvement in SoxS/MarA/Rob binding, indicated by a bent arrow and /H11001
below the DNA sequence, depending on their orientation. Restriction sites are in boldface, and mismatches to DNA sequence are in lowercase.

FIG. 1. nfsA regulatory region. The nucleotide sequence of the region upstream of nfsA is presented with the numbering based on the TSS indicated by a bent arrow and +1 above the sequence and in a large, bold font within the sequence. The −35 and −10 promoter sequences associated with the TSS are indicated by lines above the sequence. Primer sequences used for PCR and primer extension are indicated above or below the DNA sequence, depending on their orientation. Restriction sites are in boldface, and mismatches to DNA sequence are in lowercase.

In this study, we examined regulation of nfsA gene expression by the redox-cycling agent paraquat. We have identified both the transcription start site (TSS) and the SoxS binding site of the nfsA gene and have demonstrated that SoxS regulates both nfsA and the small ybjC gene directly upstream of nfsA in a coordinated manner. From the location of the box, it would appear that the ybjC-nfsA promoter is a class I SoxS promoter in which SoxS binds 10 bp upstream of the −35 site in the forward orientation comparable to the zwf promoter.

MATERIALS AND METHODS

Bacterial strains and plasmids. Cloning was conducted in E. coli DH5a [sugA44 lacY169 (deoR lacZAM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1] (BRL). β-Galactosidase assays were conducted in E. coli S90 [para (λdeco pro) ster thi] (36). His-SoxS protein was prepared from E. coli HB310 [W3110 Δ(argF-lac)U169] carrying pBADHis-SoxS, a plasmid that expresses a SoxS protein tagged with six histidine residues at the amino terminus (55). All cultures were grown at 37°C in Luria broth supplemented with 30 µg of ampicillin per ml where necessary. Plasmid pMP28.5 is a pUC18 derivative carrying a lacZ gene lacking both transcriptional and translational signals (40). Construction of lacZ fusions. Regions upstream of the nfsA gene were amplified by PCR using primers depicted in Fig. 1. PCRs contained 10 mM Tris-HCl (pH 9), 50 mM KCl, 1% Triton X-100, 2 mM MgCl2, 0.2 mM deoxynucleoside triphosphate (dNTP), 0.3 mM primers, and 2 µl of Taq polymerase (BRL) and consisted of a 1-min, 94°C predwell, followed by 25 cycles of 30 s at 94°C, 30 s at 56°C, and finally a 5-min postdwell at 72°C. The PCR products were purified by Wizard column (Promega) prior to restriction digestion. PCR products are referred to by the primer set used in their amplification. Plasmids pH3X1, pH 2X1, and pS4X1 were constructed by cloning H3X1, H2X1, and S4X1 PCR products cut with XhoI and HindIII or SphI into plasmid pMP28.5 cut with the same enzymes. Plasmids pH3X3 and pS4X3 were
constructed by replacing the HindIII-XhoI or SphI-XhoI fragment of pHX1 or pS4X3 with the H3X3 or S4X3 PCR products. Plasmid pHX1 was constructed by recircularizing pH2X1 after it had been digested with XhoI and HindIII and the ends were made blunt with Klenow and dNTPs. Plasmid pH3B1 was constructed by cloning the H3B1 PCR product cut with HindIII and BamHI into pMP28.5 cut with the same enzymes. Recombinant plasmids were confirmed by gel electrophoresis, and the junctions were verified by dideoxy sequencing with Sequenase as previously described (54).

Lac assays. Assays for β-galactosidase activity were conducted according to the method of Miller (35). Cultures were grown at 37°C to an optical density at 600 nm (OD600) of 0.2, split into two tubes, and paraquat (0.2 mM) was added. Duplicate 1-ml samples of these cells were pelleted and washed with Z buffer (60 mM Na2HPO4, pH 7.0, 40 mM NaH2PO4, H2O, 10 mM KCl, 1 mM MgSO4, 50 mM β-mercaptoethanol). The cells were resuspended in 100 μl of Z buffer and permeabilized by vortexing for 1 min with 5 μl of acetone-toluene (1:9 [vol:vol]). Aliquots (5 to 50 μl) of permeabilized cells were diluted to a volume of 1 ml with Z buffer. After 5 min at 37°C, reactions were initiated by the addition of 200 μl of ONPG (o-nitrophenyl-β-D-galactopyranoside; 4 mg/ml) and allowed to proceed for 5 to 20 min until a yellow color was visible. Reactions were stopped with 500 μl of 1 M Na2CO3, and the OD405 and OD630 values were determined. The activity in Miller units was calculated by using the following formula: activity = 1,000 × (OD405 – (1.72 × OD630))/(OD405 × time [min] × volume of cells [ml]).

Primers. Total RNA for primer extension experiments was extracted as described by Rothmel et al. (42) from a 50-ml culture of DH5α/pH3X1 grown at 37°C for 5 h. The culture was induced for the last hour of incubation by the addition of paraquat (0.2 mM). PRX primer (see Fig. 1) was end labeled with [γ-32P]ATP by using T4 DNA polymerase, and 1.7 pmol was annealed to 12 μg of RNA in a 10-μl reaction containing 4 μl of RNasin (Boehringer Mannheim) by heating to 80°C for 2 min, followed by incubation at 50°C for 99 min. After this, the primer was extended in a reaction mixture containing 50 mM Tris-HCl (pH 8.3), 10 mM dithiothreitol, 30 mM KCl, 8 mM MgCl2, 25 mM dNTP, and 25 U of Moloney murine leukemia virus reverse transcriptase by incubation at 42°C for 99 min. The reaction was stopped with the addition of 10 μl of sequencing loading buffer. Primer extension reaction mixtures were boiled for 3 min, and a 3-μl portion was run on an 8% denaturing polyacrylamide gel together with mixtures from sequencing reactions carried out with pHX31 plasmid DNA template and PRX primer.

Purification of His6-SoxS protein. Purification of His-tagged SoxS protein was conducted according to the method of Wood et al. (55) by Ni-nitrotriacetic acid (NTA) affinity chromatography under denaturing conditions. Briefly, an 85-ml culture of HB301/pBADHis6-SoxS was grown at 37°C to an OD600 of 0.6 to 0.7 and induced with 0.02% arabinose for a further 4 h at 37°C. The cells were pelleted and resuspended in 1 ml of solubilization buffer (4 mM guanidine-HCl; 20 mM Tris, 190 mM glycine, 0.1 M EDTA [pH 8.3]) (14) at 80 V for 2 to 4 h at 4°C. Gels were dried and autoradiographed.

In vitro transcription-translation. Total RNA for primer extension experiments was extracted as described by Wood et al. (55) by using DEPC-treated miniprep. RNA was purified by precipitation with 200 μl of acetone-toluene (1:9 [vol:vol]) and then washed once with 1 ml of distilled H2O and 3 times with 2 ml of solvent rinsing buffer. The His6-SoxS protein was eluted with four washes of 200 μl of elution buffer (4 mM guanidine-HCl; 20 mM NaH2PO4, pH 5.5; 300 mM NaCl; 40% glycerol; 300 mM imidazole). The eluted protein was diluted to 0.5 mg/ml in elution buffer and renatured by dialysis (20 mM NaH2PO4, pH 5.5; 300 mM NaCl; 40% glycerol). The protein concentration was determined by the Bradford method using bovine serum albumin as standard. A total of 2.6 mg of protein was purified from the 85-ml culture. The purity was estimated to be >95%.

Gel shift mobility assays. DNA for binding assays was generated by PCR as described above. In each PCR, one primer was end labeled with [γ-32P]ATP and T4 polynucleotide kinase. Binding assays were conducted in 10-μl reaction mixtures containing 20 mM NaCl, 50 mM KCl, 5 mM Tris, 2.4 mM HEPES (pH 7.9), 0.4 mg of bovine serum albumin/ml, 18% glycerol (14), 40 fmol of labeled DNA, and 12.5 to 50 ng of His6-SoxS protein. Binding was allowed to proceed for 30 min at 37°C. Reaction mixtures were chilled 10 min before being loaded on a prerun, 5% nondenaturing polyacrylamide gel and then run against TGE buffer (25 mM Tris, 190 mM glycine, 0.1 M EDTA [pH 8.3]) (14) at 80 V for 2 to 4 h at 4°C. gels were dried and autoradiographed.

FIG. 2. Primer extension analysis to determine the transcription start site of nfsA and ybjC. The primary extension product (large arrow) was generated from RNA isolated from paraquat-induced E. coli carrying pHX1 by using the PRX primer. Secondary primer extension products are indicated by small arrows. The sequence shown to the left corresponds to the upper DNA strand depicted in Fig. 1 and is complementary to the sequence ladder (GATC) generated by dideoxy sequencing of pHX1 by using the PRX primer. The transcription start site (+1) is in boldface and is indicated by a bent arrow. The −10 sequence is indicated.

RESULTS

Identification of the transcription start site of nfsA by primer extension. The sequence directly upstream of the nfsA coding region contains the ybjC coding region such that the 3′ end of ybjC overlaps 17 bp of the 5′ end of nfsA (Fig. 1). Based on the nucleotide sequence, a putative promoter had been identified 226 bases upstream of the nfsA start codon within the ybjC coding region (56). Primer extension was conducted to determine whether this promoter sequence is associated with the TSS. As seen in Fig. 2, the strongest primer extension product observed corresponds to a point 292 bp upstream of the nfsA start codon rather than to the putative promoter at
within the fainter primer extension products, corresponding to start sites of nfsA form an operon. In addition to the primary TSS, several sequence (nt graph, including one corresponding to the putative promoter the TSS (see Fig. 1). This putative promoter is upstream of both 35 promoter sequences are located upstream of the primary /H11002 units) of E. coli is the fold increase in activity of paraquat-induced strains relative to uninduced strains.

226 bases upstream. Sequences consistent with E. coli –10 and –35 promoter sequences are located upstream of the primary TSS (see Fig. 1). This putative promoter is upstream of both the ybjC and the nfsA coding regions, suggesting that ybjC and nfsA form an operon. In addition to the primary TSS, several fainter primer extension products, corresponding to start sites within the ybjC coding region, were visible on the autoradiograph, including one corresponding to the putative promoter sequence (nt +40 to +67 in Fig. 1) previously identified from the nucleotide sequence.

Expression of lacZ fusions. To confirm the activity of the promoter associated with the TSS identified by primer extension, sequences upstream of nfsA were cloned into plasmid pMP28.5 to create lacZ fusions in which the ninth codon of the lacZ gene is fused in frame with the second codon of nfsA (Fig. 3A). The expression of β-galactosidase from the lacZ fusions was measured in the Lac E. coli strain S90C (Fig. 3B).

The nfsA-lacZ fusion, pH3X1, which carries the entire nfsA upstream region (nt –74 to +297), demonstrated a level of β-galactosidase activity 200 times the background level observed with pHXΔ, which carries only the nfsA ribosomal binding site and start codon (nt +281 to +297). Furthermore, the activity of the pH3X1 fusion increased an additional 3.6-fold in the presence of paraquat, indicating that this construct contains not only the promoter sequence of nfsA but also the sequences necessary for activation by SoxS. Deletion of the distal portion of the upstream region (nt –74 to +3) in pH2X1 resulted in a 20-fold decrease in β-galactosidase activity relative to pH3X1, confirming the activity of the promoter identified by primer extension. The 10-fold increase in activity of this construct over pHXΔ may be due to weak promoter activities within the ybjC coding region associated with the weak primer extension products observed in Fig. 2. It should be noted, however, that any weak promoter activity within the ybjC coding region is not inducible by paraquat.

As seen in a comparison of the results with pH3X1 and pH3X3, deletion of most of the ybjC coding region (nt +84 to +280) had little effect on the expression or induction of β-galactosidase. Construct pS4X3 is identical to pH3X3 except for a deletion of 38 bp directly upstream of the promoter identified by primer extension. While β-galactosidase activity increased over threefold in the presence of paraquat in strains carrying pH3X3, no such increase was observed in strains carrying pS4X3. Lack of induction in pS4X3 places the SoxS activation site either overlapping or just upstream of the ybjC-nfsA promoter identified by primer extension. Higher basal levels of β-galactosidase activity observed with pH3X3 over pS4X3 in the absence of paraquat may reflect a small stimulatory effect of basal levels of SoxS, MarA, or Rob at the intact binding site of pH3X3.

Placement of the nfsA promoter upstream of the ybjC coding region suggests that this ORF is transcribed in vivo. In order to test whether this ORF is translated in vivo, a ybjC-lacZ fusion, pH3B1, was constructed in which lacZ is fused to the 20th codon of ybjC such that the fusion protein is translated solely from the putative ybjC translation signals. The β-galactosidase...
activity obtained with this ybjC-lacZ fusion was ca. 100 times greater than the background level, demonstrating that YbjC can be expressed in vivo. Furthermore, as would be expected for a gene transcribed from the same promoter as nfsA, the activity of the ybjC-lacZ fusion increased more than threefold in the presence of paraquat, indicating that ybjC and nfsA constitute a functional operon that is inducible by paraquat.

**Gel shift mobility assays.** Examination of the nucleotide sequence upstream of the ybjC-nfsA promoter identified two overlapping sequences (nt /H11002 66 to /H11002 47 and nt /H11002 50 to /H11002 31), each sharing sequence identity at 17 of 20 bases of the soxbox consensus sequence AYNGCACNNWNNRYYAAAYN (28). Both of these sequences are in the forward orientation. However, one is 10 bp upstream of the /H11002 35 sequence comparable to the Class I zwf promoter, while the other overlaps the /H11002 35 sequence in a manner consistent with class II promoters (14, 28).

Gel shift mobility assays with His-tagged SoxS protein were conducted with radiolabeled PCR DNA fragments generated with the primers depicted in Fig. 1. It should be noted that, while the sequences carried by the PCR fragments are similar to those cloned into the lacZ fusion constructs, the latter are slightly smaller (by 6 to 10 bp) due to cleavage of the ends at restriction sites in the PCR primers prior to cloning. The upstream region of nfsA extending from nt –84 to +307 is contained on the H3X1 PCR fragment. Gel shift mobility assays with H3X1 confirmed that His6-SoxS does indeed bind to the region upstream of nfsA (Fig. 4). Gel shift assays with two smaller fragments representing either half of this region demonstrated that the right portion carried by fragment B2X1 (nt +73 to +307) was not able to bind the His6-SoxS protein and that binding was restricted to the left portion present in fragment H3X3 (nt –84 to +91). The binding site was further localized to the 89 left-most nucleotides based on the binding of the protein to PCR fragment H3X2 that carries nt –84 to +5. More telling are the results obtained with the S4X3 fragment which, lacks 38 nt from the left-most side (nt –84 to –48). Deletion of these 38 bases eliminated binding to the S4X3 fragment and clearly places all or part of the His6-SoxS binding site within the sequence immediately upstream or overlapping the –35 promoter sequence. These results are consistent with the induction results obtained with the lacZ fusions.

Based on the nucleotide sequence, a third putative soxbox containing 17 of 20 bases of the soxbox consensus sequence was identified within the ybjC coding region (nt +21 to +40) in the forward orientation, overlapping one of the weak putative promoters in a manner consistent with a class II promoter. Lack of binding of His6-Sox to S4X3 clearly demonstrates that this putative soxbox is not functional, a finding consistent with the lack of induction of the pS4X3 or pH2X1 nfsA-lacZ fusions by paraquat.

**DNase I protection studies.** DNase I footprinting assays were conducted to determine which of the two putative soxboxes identified by gel shift experiments is involved in SoxS binding. As seen in Fig. 5, His6-SoxS protects the sequence extending from nt –65 to –46 which corresponds to the up-
stream soxbox sequence. From these results, we can conclude that the ybjC-nfsA promoter is a class I promoter in which the soxbox lies 10 bases upstream of the −35 site in the forward orientation in a manner comparable to the class I zwf promoter (14, 28).

**DISCUSSION**

The results of primer extension and lacZ fusion experiments presented in this study place the nfsA promoter upstream of both the nfsA and ybjC coding regions rather than within the ybjC coding region as predicted from the nucleotide sequence alone (56). Gel shift and footprinting analysis identified the regulatory sequence associated with this promoter that is responsible for SoxS-dependent induction of NfsA by paraquat (27). Coregulation of nfsA and ybjC from the same SoxS-dependent promoter confirms an earlier suggestion that these two genes are part of an operon, consisting of ybjC, nfsA, rimK, and b0853, that is upregulated by MarA (7). Inclusion of rimK and b0853 in this operon would indicate that the putative transcription termination sequence identified between nfsA and rimK is not functional (56). Further evidence for the inclusion of rimK in this operon can be taken from two earlier studies. Analysis of transcription products of rimK identified a transcript carrying both rimK and the nfsA upstream region (20). Similarly, two-dimensional gel analysis of proteins in E. coli identified an increase in the size of the small subunit ribosomal protein S6 concurrent with paraquat induction (15). The size increase was attributed to the addition of two to six glutamic acid residues to the carboxyl terminus of the S6 ribosomal protein by the rimK gene product (20).

Examination of sequence data available in the unfinished microbial genome NCBI database indicated that the order of the ybjC-nfsA-rimK genes found in E. coli is conserved in closely related enteric bacteria Klebsiella pneumoniae, Salmonella enterica serovar Typhi, Salmonella enterica serovar Enteritidis, and Salmonella enterica serovar Typhimurium. Another member of the Enterobacteriaceae with available sequence data is Yersinia pestis. Interestingly, although Y. pestis does not encode either an nfsA or a rimK homologue, it does retain a ybjC homologue upstream of a gene that encodes a protein with sequence similarity to the homologue upstream of a gene that encodes a protein with bacteriaceae since no similar proteins have been identified (9). The YbjC homologue appears to be unique to the Entero-Mar/Rob binding sites (28) by molecular information theory (44, 45, 55) and from the protein-DNA interaction sites identified from the crystal structure of the MarA (41) and Rob (21) proteins complexed with the mar and micF promoter sequences, respectively. The crystal structure for SoxS-DNA interaction has not yet been determined.

In the functional ybjC-nfsA soxbox, mismatches to the soxbox consensus sequence lie at positions 1 (G), 7 (T), and 19 (A). Adenosine is conserved at position 1 in 15 of the 16 known Sox/Mar/Rob binding sites and hence contributes high information content. However, this first nucleotide has not been shown to have any direct interaction with the MarA or Rob proteins, and systematic replacement of it with any other nucleotide has been reported to have no effect on activity in micF, fpr or zwf promoters (28). The pyrimidine at position 19 of the second domain of the soxbox consensus sequence has low sequence information content, and there is no evidence of interaction between it and either MarA or Rob. The third mismatch in the ybjC-nfsA soxbox is nucleotide T7, which lies within the highly conserved GCAC sequence. Both Gln45 of MarA and Gln39 of Rob make van der Waals contacts with the complement of C7 in the mar and micF soxboxes; however, a T is found at this position in three of the four other soxboxes with mismatches at this position (28), suggesting that such a change is tolerated. Thus, the three mismatches found in the functional ybjC-nfsA soxbox appear to be either unimportant, as in G1 and A19 or, in the case of T7, represents a change found in other functional soxboxes. The second, nonfunctional, soxbox contains mismatches at positions 2 (A), 6 (T), and 7 (A) of the soxbox consensus sequence. While the A2 is in a position that is not highly conserved and does not interact directly with either the MarA or Rob protein, the T6 and A7 mismatches lie within the GCAC domain at positions that have been shown to be protein contact sites. The complement of A6 of the mar soxbox forms van der Waals interactions with both Gln45 and Trp42 residues of MarA, while the complement of C7 interacts with Gln45 of MarA. Similar interactions are found in the Rob-micF complex. Only one known soxbox contains a T at position 6, and none contain an A at position 7. No functional soxbox contains two mismatches within this domain, and it is probably the combined effect of these two adjacent bases that prevents interaction with the protein.

The ybjC-nfsA promoter is the second class I SoxS-dependent promoter, after the zwf promoter, in which the soxbox is in the forward orientation (relative to the promoter). In the other six class I promoters characterized to date, the soxbox is in the reverse orientation 14 to 16 bases (fpr, fldA, mar, poxB) or 26 or 27 bases (acrAB, riba) upstream of the −35 site (28, 55). A study by Wood et al. (55) has clearly demonstrated the correlation between the orientation and the distance of the soxbox from the −35 sequence in class I SoxS-dependent promoters. They observed a complete loss of induction with either the fpr (reverse orientation, 15 bp upstream) or the zwf (forward orientation, 7 bp upstream) soxbox if the spacing from the promoter was maintained but the orientation was switched to that found in the other promoter, or if the orientation was conserved but the position was changed. It has been proposed that, in class I promoters, a change in the orientation of the soxbox from reverse to forward at distances closer to the −35 sequence may facilitate interaction of SoxS with αCTD (28, 55). Thus, the forward orientation of the ybjC-nfsA soxbox is...
might arise during oxidative stress. In this way, Grx1 has been
den bonds, Grx1 may also play an important role in the
addition to its role in the reduction of ribonucleotide reductase
ship between the physiological roles of the gene products. In
tory sequences is intriguing, since there is no obvious relation-
different oxidative-stress regulons possess overlapping regula-
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