SoxRS-Regulated Expression and Genetic Analysis of the yggX Gene of Escherichia coli

Pablo J. Pomposiello,1,* Anastasia Koutsolioutsou,2 Daniel Carrasco,1 and Bruce Demple2

Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003,1 and Department of Cancer Cell Biology, Harvard School of Public Health, Boston, Massachusetts 022152

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Genomic studies with bacteria have identified redox-responsive genes without known roles in counteracting oxidative damage. Previous transcriptional profiling showed that expression of one such gene, yggX, was activated by superoxide stress in Escherichia coli. Here we show that this activation could be mimicked by artificial expression of the regulatory protein SoxS. Northern analysis confirmed the transcriptional activation of yggX by oxidative stress or SoxS expression but not in response to the related MarA or Rob proteins. Northern analysis showed that mltC, which codes for a peptidoglycan hydrolase and is positioned immediately downstream of yggX, was also regulated by oxidative stress or ectopic expression of SoxS. Purified SoxS protein bound to the predicted yggX promoter region, between positions 223 and 163 upstream from the yggX translational start site. Within this region, a 20-bp sequence was found to be necessary for oxidative stress-mediated activation of yggX transcription. A yggX deletion strain was hypersensitive to the redox-cycling agent paraquat, and a plasmid expressing YggX complemented the sensitivity of the deletion strain. Under exposure to paraquat, the yggX deletion strain showed a deficiency in aconitase activity compared to the isogenic wild-type strain, while expression of YggX from a multicopy plasmid increased the aconitase levels above those of the wild-type strain. These results demonstrate the direct regulation of the yggX gene by the redox-sensing SoxRS system and provide further evidence for the involvement of yggX in protection of iron-sulfur proteins against oxidative damage.

Cellular responses to environmental stress involve concerted changes in the expression of multiple genes. Recently developed genomic techniques such as transcriptional profiling have allowed the identification of hundreds of stress-responsive genes, including many lacking a known function or significant homology to genes with known functions. In particular, the genetic response of Escherichia coli to oxidative stress includes dozens of such genes (39, 52). The observation that some of these uncharacterized genes are under the control of known oxido-responsive signal transduction systems suggests that they might have direct roles in the antioxidant response (39, 52).

The yggX gene of E. coli was identified as an open reading frame with a predicted 11-kDa protein product during genomic sequencing (7), and further protein expression studies demonstrated that the gene codes for a small and abundant protein (29, 49). Sequence comparisons revealed that yggX homologs are conserved through gram-negative bacteria and that the structural organization of three neighboring genes is conserved (15). Moreover, the yggX gene is transcribed as part of a complex operon that may direct several transcripts containing different combinations of structural genes (Fig. 1). The first report on YggX function showed that overexpression of the Salmonella enterica YggX protein complements the thiamine dependence of a gshA (glutathione-deficient) Salmonella strain, probably by restoring the function of thiamine-synthetic enzymes affected by increased oxidant levels (16).

Overexpression of YggX also enhances Salmonella’s resistance to oxidants, reduces oxidant-induced mutagenesis, and restores the aconitase deficiency of a gshA mutant. Hence, Gralnick and Downs have proposed that YggX protects FeS clusters in biosynthetic enzymes from oxidative damage (16). Recently, the same authors have shown that YggX decreases chelatable iron in solution and protects DNA from iron-mediated oxidative damage (16a).

Transcriptional profiling experiments (39) showed that yggX is activated as part of E. coli’s response to superoxide stress under the control of SoxRS, a well-characterized signal transduction system (see reference 41 for a review). In the SoxRS regulatory cascade, SoxR senses oxidative stress in the cytoplasm via the oxidation state of its Fe-S cluster (24, 50). When oxidized, SoxR activates the transcription of soxS, and the resulting SoxS protein activates genes that collectively help to avoid or repair the damage caused by oxidants (13, 18, 19). SoxS binds as a monomer to sites resembling the asymmetric, degenerate consensus AYnGCACnWmRYYAAYn (where n is any base, Y is T or C, W is A or T, and R is A or G) (33).

SoxS-activated genes with known functions include sodA (Mn superoxide dismutase), accA (aconitase A), fpr (ferredoxin oxidoreductase), and fur (Fe-binding transcriptional repressor).

In this report we show that the transcriptional activation of the E. coli yggX gene under oxidative stress is mediated directly by SoxS and provide further evidence for its role in the cellular defense against oxidation.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** The bacterial strains and plasmids used in this study are described in Table 1.
activity. Fragments of DNA containing the predicted yggX promoter region were amplified by PCR with primers that introduced convenient BamHI and EcoRI sites in the reaction products. The PCR products were cloned as BamHI-EcoRI fragments into the lacZ reporter vector pRJ800, a derivative of pRS416 (ColEl replication; Amp r; multiple cloning site from pUC18). Typical PCR mixtures included 2.5 U of Taq polymerase (courtesy of K. Nueslein) in ThermoPol buffer (New England Biolabs) plus 50 pmol of specific primers designed to introduce convenient restriction sites (OpoI), 0.2 mM deoxynucleoside triphosphates (New England Biolabs), and 100 ng of chromosomal DNA from strain GC4468. The E. coli strain GC4468 was transformed to ampicillin resistance with the resulting amplified DNA fragments into the lacZ fusion constructs. For β-galactosidase activity assays, overnight cultures were diluted in 15 ml of fresh Luria-Bertani broth in 125 ml Erlenmeyer flasks and were grown at 37°C with strong aeration (250 rpm). After 2 h (optical density at 600 nm of approximately 0.5), the cultures were exposed to 250 μM paraquat (PQ) for 30 min, and cells were assayed as described by Miller (37).

In vitro binding of SoxS by gel mobility shift assay. In vitro binding of SoxS protein to DNA targets was done as described previously (28). Essentially, purified SoxS protein was diluted in a buffer containing 10 mM sodium acetate (pH 5.0), 75 mM sodium chloride, and 1 mM diethiothreitol and incubated with end-labeled PCR fragments. Typical binding reaction mixtures contained 10 mM Tris-HCl (pH 8.0), 75 mM potassium chloride, 2 mM dithiothreitol, 10 fmol of 32P-end-labeled PCR product, 1 pmol of randomized 32-mer oligonucleotide, and different amounts of purified SoxS protein as indicated, in a total volume of 20 μl. The binding reaction mixtures were incubated at 20°C for 20 min and run in 5% polyacrylamide gels (20 mM Tris-HCl [pH 8.0], 3 mM sodium acetate, and 1 mM EDTA) at 200 V for 3 to 4 h. Gels were dried and visualized by autoradiography.

Northern blot analysis. Probes for specific genes were generated by PCR amplification with chromosomal DNA from strain GC4468 as the template and gene-specific primers obtained from Sigma-Genosys. Typically, PCR amplifications were done with 30 cycles of annealing at 60°C (45 s), elongation at 72°C (1 min), and denaturation at 94°C (30 s). The PCR products were resolved by electrophoresis in 1.25% agarose gels, recovered by excision from the gel, and purified with Qiagen DNA-binding microspin columns (Qiagen). The DNA fragments were labeled by using Klenow DNA polymerase fragment, random-primed with Qiaquick DNA-binding microspin columns (Qiagen). The DNA fragments were labeled by using Klenow DNA polymerase fragment, random-primed with Qiaquick DNA-binding microspin columns (Qiagen). The DNA fragments were labeled with 32P-end-labeled PCR products and electrophoresis in agarose gels were performed by using well-established protocols (3).

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mRNA isolated from *E. coli* cultures. Strain GC4468 (wild type) was exposed to increasing concentrations of PQ (0, 25, 250, and 500 μM) and from strain DJ901/pJP115 exposed to increasing concentrations of IPTG (0, 0.125, 0.500, 1, and 2 mM). The positions and lengths in bases of RNA molecular size standards are indicated. (B) Northern blot analysis of *mltC* transcription. The membrane used for panel A was stripped and reprobed with a radioactively labeled PCR fragment containing the complete *mltC* coding region. The positions and lengths in bases of RNA molecular size standards are indicated. (C) Loading control. A duplicate gel was stained with ethidium bromide and visualized under UV light. (D) Quantitation of *yggX* signal. The radioactive intensity for the ~400-nt bands in the Northern blot was measured by phosphorimaging. The values shown are the relative activation ratios for the treatments, normalized to the signal intensity in the untreated sample.

FIG. 2. Expression of *yggX* and *mltC* under oxidative stress. (A) Northern blot analysis of *yggX* transcription. A PCR fragment containing the complete coding sequence of *yggX* was radioactively labeled and hybridized with total RNAs from strain GC4468 (wild type) exposed to increasing concentrations of PQ (0, 25, 250, and 500 μM) and from strain DJ901/pJP115 exposed to increasing concentrations of IPTG (0, 0.125, 0.500, 1, and 2 mM). The positions and lengths in bases of RNA molecular size standards are indicated. (B) Northern blot analysis of *mltC* transcription. The membrane used for panel A was stripped and reprobed with a radioactively labeled PCR fragment containing the complete *mltC* coding region. The positions and lengths in bases of RNA molecular size standards are indicated. (C) Loading control. A duplicate gel was stained with ethidium bromide and visualized under UV light. (D) Quantitation of *yggX* signal. The radioactive intensity for the ~400-nt bands in the Northern blot was measured by phosphorimaging. The values shown are the relative activation ratios for the treatments, normalized to the signal intensity in the untreated sample.

mRNA isolated from *E. coli* cultures. Strain GC4468 (wild type) was exposed to increasing concentrations of PQ, a redox-cycling agent that generates intracellular superoxide in a reaction dependent on NADPH and at least three diaphorases (30, 31). Strain DJ901/pJP115 (*ΔsoxRS/pSoxS*) was exposed to increasing concentrations of IPTG (isopropyl-β-D-thiogalactopyranoside), an artificial inducer of the *soxS* gene under the control of the *lacZ* promoter in pJP115 (39). A PCR product spanning the whole *yggX* coding sequence was radioactively labeled and used as a probe for *yggX* expression (Fig. 2A). The autoradiogram of this Northern blot showed two transcripts. The smaller transcript comigrated with a 400-nucleotide (nt)
molecular size marker, consistent with the size of the yggX coding region and the known promoter structure (15). The steady-state level of this transcript was elevated in a dose-dependent manner by either treatment with PQ or expression of SoxS. The relative abundance of this ~400-nt transcript was measured by phosphorimaging (Fig. 2D). A larger transcript comigrated with a 1,520-nt molecular size marker and was activated by either treatment with PQ or expression of SoxS.

The size of the larger transcript was consistent with either a mutY-yggX message starting from one of the promoters upstream of mutY or, alternatively, a yggX-mltC message starting at the promoter upstream of yggX (Fig. 1). To distinguish between these two possibilities, the filter from the previous experiment was stripped and reprobed with a PCR fragment spanning the complete mltC coding region (Fig. 2B). A single transcript was detected, and its size was estimated at ~1.5 kb. The steady-state level of this transcript containing mltC was increased by exposure to PQ or expression of SoxS. Due to the low levels of the basal mltC signal, reliable quantitation by phosphorimaging was difficult to obtain. However, we estimated that induction of mltC is similar in ratio to the yggX induction (data not shown).

In contrast, when the mutY coding region was used as probe, a ~1.5-kb transcript was detected, but this transcript did not show any activation by either treatment with PQ or expression of SoxS (data not shown). This lack of activation of mutY by PQ was consistent with the observations of Gifford et al. (14). Finally, probing the activity of the nupG gene with a PCR product spanning the nupG coding region revealed a single transcript of the expected size (~1 kb), which showed no variation with PQ treatment or SoxS expression (data not shown). These results suggest that the transcription of yggX and mltC, but not that of mutY or nupG, is activated by oxidative stress and that ectopic expression of the SoxS protein is sufficient to evoke this activation. Additionally, the yggX gene can be transcribed alone or as a transcript including both yggX and mltC.

**Purified SoxS binds to the yggX promoter.** The observation that SoxS regulates the yggX-mltC promoter was inconsistent with a previous suggestion that SoxS binding sites are absent from this regulatory region (15). To rule out an indirect effect of SoxS on the activation of yggX, we mapped the putative SoxS binding site by constructing a set of nested deletions of the yggX promoter region (Fig. 3A). These deletion fragments were fused to a lacZ reporter gene, and β-galactosidase activity was measured for strains carrying each construct, both untreated and in the presence of 250 μM PQ. A lacZ fusion containing the 223 bp upstream from the yggX translational start site was regulated by oxidative stress. Conversely, a fragment containing the 163 bp upstream from the yggX translational start site was not regulated. In vitro binding experiments with purified components showed that SoxS formed a complex with the 223-bp fragment of the yggX promoter (Fig. 3B). In contrast, SoxS failed to form any detectable complex with the smaller, 163-bp fragment (Fig. 3B). Sequence analysis revealed a match for an inverted SoxS binding site (5′-TAGGCACAA TATCTAAGTG-3′) between positions 190 and 171 upstream from the yggX translational start site. To confirm the position of the SoxS binding site, two additional deletions of the yggX promoter were constructed and fused to lacZ. The lacZ fusion containing the 193 bp upstream from the yggX translational start site and harboring the proposed SoxS binding site was regulated by oxidative stress (Fig. 3A). Conversely, a fragment containing the 173 bp upstream from the yggX translational start site and lacking 17 out of the 20 bases of the putative SoxS binding site was not regulated (Fig. 3A). These in vitro binding and in vivo regulation results show that SoxS is directly involved in the transcriptional regulation of the yggX gene.

**The SoxS paralogs MarA and Rob are not sufficient for activation of yggX.** The SoxS protein is a member of the AraC-XylA family of transcriptional activators (12). The closest SoxS paralogs are MarA, a protein involved in multiple-antibiotic resistance (1), and Rob, an abundant protein of uncertain physiological relevance (6, 46). MarA and Rob can activate several of the SoxS-activated genes, since both MarA and Rob recognize the same DNA sequence for binding (26, 27). To test whether either MarA or Rob could activate the transcription of yggX, we measured the relative level of yggX mRNA in cells in which the expression of MarA or Rob could be selectively activated. This selective expression of MarA and Rob was achieved by using strains MB102 (ΔmarA) (39) and MB101 (ΔrobA) (6). Both strains are GC4468 derivatives transformed with plasmids that allow, respectively, the expression of MarA or Rob under the control of IPTG-regulated promoters. As shown before, the levels of yggX mRNA were increased after treatment with PQ or expression of SoxS (Fig. 4A). In contrast, the level of yggX transcript was unaffected by the expression of MarA or Rob (Fig. 4A). As a positive control for transcriptional activation by MarA and Rob, the membrane from the former Northern blot was stripped and rehybridized with a labeled PCR product containing the inaA coding region. The inaA gene is activated by SoxS, MarA, and Rob, with MarA being the strongest activator (34, 43). This experiment confirmed the transcriptional activity of artificially expressed MarA and Rob proteins (Fig. 4B) and verified that they do not activate yggX expression.

A yggX deletion mutant is hypersensitive to PQ. It was reasonable to believe that the induction of yggX expression was indicative of a potential role for this gene in the resistance to oxidative stress. To test this putative role of yggX, we built strain PP139, a ΔyggX derivative of strain GC4468 (see Materials and Methods). Overnight cultures of both wild-type and ΔyggX strains were diluted into fresh medium in the presence or absence of 50 μM PQ and grown with strong aeration (Fig. 5). Both strains grew equally in the absence of PQ (Fig. 5A), but the ΔyggX strain grew poorly in medium containing PQ (Fig. 5B). The hypersensitivity of the ΔyggX strain was also measured in solid medium by using gradient plates (8), and this method was used to assay the growth of the yggX mutant exposed to various oxidative stress agents. This experiment showed that a ΔyggX strain was not only more sensitive to PQ than the isogenic wild-type strain but also more sensitive than a ΔsoxRS strain (Table 2). Interestingly, other superoxide-producing drugs, such as menadione and phenazine methosulfate, had a small or no effect on the growth of PP139 on plates. This observation was unexpected, since both menadione and phenazine methosulfate produce substantial oxidative stress through the generation of intracellular superoxide. The organic peroxide tert-butyl-hydroperoxide had only a modest relative effect on the growth of PP139.
As shown before, the \( \text{yggX} \) and \( \text{mltC} \) genes are apparently cotranscribed, and therefore, the hypersensitive phenotype of \( \text{a} \text{yggX} \) mutant could result from polar effects on \( \text{mltC} \) expression. To test this possibility, we cloned the \( \text{yggX} \) gene under the control of the \( \text{araBAD} \) promoter by using the pBAD28 expression vector (23). By using the resulting plasmid, pJP133, the expression of \( \text{yggX} \) could be activated by the addition of arabinose. Strain PP139 (\( \text{H9004} \text{yggX} \)) was transformed to Ampr with either vector pBAD28 or pJP133, grown overnight, and diluted into fresh medium supplemented with arabinose in the presence or absence of PQ. In the absence of PQ, all three strains (wild type, \( \text{H9004} \text{yggX} \text{pBAD28} \), and \( \text{H9004} \text{yggX} \text{pJP133} \)) grew similarly (Fig. 5A). Alternatively, in the presence of PQ, strain PP139/ pBAD28 grew poorly compared with the wild-type strain GC4468. This growth defect was complemented by plasmid pJP133, since strain PP139/pJP133 grew as well as the wild-type strain (Fig. 5B). These results show that expression of \( \text{yggX} \) was sufficient to complement the PQ-dependent growth defects of the \( \Delta \text{yggX} \) strain and therefore that the hypersensitive phenotype of PP139 was not due to polar effects.

**Deletion of \( \text{yggX} \) results in decreased aconitase activity under superoxide stress.** Recent work has shown that overexpression of YggX complements the deficiency in aconitase activity displayed by \( \text{S. enterica gshA} \) mutants (16). This observation has been interpreted as evidence for a role of YggX in protecting or repairing oxidative damage of the Fe-S clusters that form the active site of aconitase. To test this putative protective role, we measured aconitase activity in wild-type and \( \text{H9004} \text{yggX} \) strains growing under oxidative stress (Fig. 6). In the absence of stress, the aconitase activities of the wild-type and \( \Delta \text{yggX} \) strains were not significantly different, and the expression of \( \text{yggX} \) from plasmid pJP133 did not increase aconitase activity. Conversely, under oxidative stress induced by PQ, the \( \Delta \text{yggX} \) strain showed a significant decrease in aconitase activity compared to the wild-type strain. Expression of YggX from plasmid pJP133 restored this activity and increased it to a level
higher than that in the wild type (Fig. 6). These results showed that the product of the \textit{yggX} gene is necessary to maintain high levels of aconitase under oxidative stress.

**DISCUSSION**

The \textit{yggX} gene of \textit{E. coli} codes for a predicted 91-residue, 11-kDa polypeptide of unknown in vivo function. Transcriptional profiling has suggested that the expression of \textit{yggX} is activated by superoxide stress as part of the SoxRS regulon (39). The results presented here confirmed and extended the role of the SoxRS system in regulating not only \textit{yggX} but also the downstream gene \textit{mltC}. Our results are consistent with the genetic structure of the \textit{mutY-yggX-mltC-nupG} region proposed by Gifford and Wallace (15) (Fig. 1). The coregulation of \textit{yggX} and \textit{mltC} (Fig. 2) was consistent with SoxS-mediated activation of the proximal promoter upstream of the \textit{yggX} coding region, with a termination point between \textit{yggX} and \textit{mltC} that allows readthrough. The binding of purified SoxS to the \textit{yggX} promoter strongly suggests that the PQ- and SoxS-mediated activation of \textit{yggX} is the result of direct interaction between promoter and SoxS. A sequence necessary for PQ-mediated transcriptional induction of \textit{yggX} was mapped to a 20-bp region between positions 173 and 193 upstream the \textit{yggX} translational start site. This 20-bp region contained 17 bp of a partial match for a putative SoxS binding site. This putative SoxS binding sequence diverges substantially from the \textit{mar-sox-rob} consensus (33), with only seven matches out of 14 nonrandom positions.

Additionally, and despite similarities in the structures of their DNA binding domains and cognate DNA binding sites, the SoxS homologs MarA and Rob did not activate in vivo transcription of \textit{yggX} under the conditions tested (Fig. 4). This result was somewhat surprising, since it has been repeatedly

### Table 2. Sensitivity to oxidants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Avg growth (cm) on gradient plates with(^a)</th>
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<tbody>
<tr>
<td></td>
<td>No addition</td>
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<tr>
<td>GC4468 (wild type)</td>
<td>8.0</td>
</tr>
<tr>
<td>DJ901 (\textit{ΔsoxRS})</td>
<td>8.0</td>
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<tr>
<td>PP139 (\textit{ΔyggX})</td>
<td>8.0</td>
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\(^a\) The measurements were done in duplicate and repeated at least twice with independent cultures. Values from a representative experiment are shown. The amount or maximal concentration of each agent was as follows: PQ, 2 mM; menadione (MD), 7 mg; phenazine methosulfate (PM), 1 mg; tert-butyl hydroperoxide (TBP), 1 mM.
shown that regulatory cross talk exists between these proteins and SoxS (1, 17, 21, 27, 35). In this context, the different degree of activation of the \textit{inaA} gene by the three homologous proteins (Fig. 4) is noteworthy but not surprising, since such differences have been reported for other genes. For example, SoxS appears to be a better activator of \textit{fumC} than is MarA, which is in turn more potent than Rob (2, 34). A growing body of evidence suggests that, although overlapping, the MarA, Rob, and SoxS proteins have different regulatory targets. For example, the \textit{nfo} gene, coding for endonuclease IV, is activated by SoxS but not by MarA or Rob (17). Similarly, the \textit{gshB} gene, coding for a glutathione synthase, is activated by MarA but not by SoxS or Rob (5, 39). Recently, Barbosa and Levy showed that the \textit{nfrB} gene is regulated preferentially by MarA, which binds to a site that diverges substantially from the Mar-Sox-Rob consensus, with eight mismatches out of the 14 nonrandom positions (4). Hence, divergence from the Mar-Sox-Rob box consensus sequence might determine differential regulation of a promoter by these transcription factors. It is tempting to hypothesize that the sequence divergence from the consensus in the putative SoxS binding site at the \textit{yggX} promoter and the lack of regulation by the SoxS homologs MarA and Rob are related phenomena.

Recently, a function in antioxidant defense was proposed for the \textit{yggX} gene of \textit{S. enterica}, as a mediator of iron transactions between uptake and Fe-S cluster synthesis and/or repair (16a). The results presented here are consistent with this hypothesis, since growth of a \textit{\Delta yggX} strain was deficient in minimal medium under oxidative stress. Expression of YggX from a plasmid was sufficient to complement this sensitivity, which shows that the phenotype was not due to polar effects. Additionally, a \textit{\Delta yggX} strain showed deficient aconitase activity under oxidative stress, while expression of YggX in that same mutant enhanced aconitase activity above wild-type levels. Interestingly, \textit{acnA}, the gene coding for one of the two \textit{E. coli} aconitases, aconitase A, is also a member of the SoxRS regulon. It has been proposed that the SoxS-mediated induction of aconitase A replaces the labile activity of aconitase B, helping the cell in the adaptation to oxidative stress (22). Moreover, it has been estimated that under PQ-elicited oxidative stress, more than 95% of aconitase activity is provided by aconitase A (22). We can therefore propose a model in which the transcriptional activation of both \textit{acnA} and \textit{yggX} results in the maintenance of high levels of aconitase activity under oxidative stress. Moreover, the level of YggX appears to be limiting for aconitase activity under PQ-induced oxidative stress, since expression of YggX from a multicopy plasmid enhances aconitase activity beyond the wild-type levels. Interestingly, a recent report showed that despite its in vivo stability, the activity of purified aconitase A is destroyed in vitro by superoxide (48). The resistance of aconitase A to oxidation was restored in vitro by addition of cell extract. The protecting agent was sensitive to boiling but not to dialysis or RNase and could not restore the activity of damaged aconitase (48). It is tempting to speculate that the soluble factor that protects aconitase A in vitro from oxidative damage is YggX. We are currently addressing this hypothesis experimentally.

The significance of the SoxS-mediated activation of \textit{mhc} is
not clear. MltC is one of three E. coli lytic transglycosylases found in the outer membrane, where the enzyme modulates the turnover of the murein sacculus during cell growth (10, 11, 32). The products of murein lysis are transported into the cytoplasm and broken into peptide and disaccharide components by the amidaase AmpD (25). The peptides are recycled into the cell wall as UDP-N-acetylmuramic acid peptide intermediates (36). The disaccharides are cleaved by the β-glucosaminidase NagZ, but the metabolic fate of the released 1,6-anhydromuramic acid is not known (20). Since E. coli cells under superoxide stress showed a global activation of genes involved in sugar transport (39), it is possible that the activation of mltC contributes to increase the intracellular sugar pools by degrading the peptidoglycan.

The possibility of stress-regulated Fe-S cluster protection or repair is certainly consistent with the present models of global antioxidant response. Furthermore, the transcriptional activation of yggX might not be the sole example. Recently, a study of global regulation in response to oxidative stress (52) showed the induction of genes of the isc region, which is known to be involved in Fe-S cluster assembly (38, 44, 45, 51). Interestingly, this transcriptional activation was independent of both YoxR and SoxRS. Therefore, stress-mediated activation of genes related to Fe-S cluster dynamics could involve multiple operons and pathways.

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