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Oxidative stress sensing by the iron–sulfur cluster in the transcription factor, SoxR

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ABSTRACT

All bacteria are continuously exposed to environmental and/or endogenously active oxygen and nitrogen compounds and radicals. To reduce the deleterious effects of these reactive species, most bacteria have evolved specific sensor proteins that regulate the expression of enzymes that detoxify these species and repair proteins. Some bacterial transcriptional regulators containing an iron–sulfur cluster are involved in coordinating these physiological responses. Mechanistic and structural information can show how these regulators function, in particular, how chemical interactions at the cluster drive subsequent regulatory responses. The [2Fe–2S] transcription factor SoxR (**s**uper**ox**ide **r**esponse) functions as a bacterial sensor of oxidative stress and nitric oxide (NO). This review focuses on the mechanisms by which SoxR proteins respond to oxidative stress.

1. Introduction

Iron–sulfur proteins are ubiquitous in nature and play critical roles in a wide range of biological processes [1–5]. In most iron–sulfur proteins, the clusters function as redox centers in electron transfer reactions, although some have other functions including enzymatic reactions and metabolic regulation [6–8]. A group of bacterial iron–sulfur cluster proteins, which function as sensors of iron, dioxygen (O_2), and nitric oxide (NO), was described [9,10]. These clusters act as sensors of the environment, enabling organisms to adapt to prevailing conditions. This can range from responding to oxidative or nitrosative stress [11] to switching between anaerobic and aerobic respiratory pathways [12]. Within this group of redox-sensing transcription factors, SoxR (**s**uper**ox**ide **r**esponse) is unique in that, unlike the other proteins, which are regulated by the assembly/disassembly of their Fe–S clusters, SoxR activity is regulated by reversible one-electron oxidation–reduction of the [2Fe–2S] clusters [13–16]. This review will focus specifically on the mechanisms by which SoxR proteins respond to oxidative stress.

2. SoxR in *Escherichia coli*

The [2Fe–2S] cluster containing SoxR protein is a transcription factor that regulates the *soxRS* response to oxidative stress in bacteria [17,18]. NO also activates SoxR by direct nitrosylation of the [2Fe–2S] cluster [19]. In enteric bacteria, the target gene for SoxR is *soxS*, a transcription factor that promotes the production of various antioxidant proteins, including superoxide dismutase (SOD) [20], the outer-membrane drug effluxer TolC [21], and DNA repair-related endonuclease IV [22]. SoxR is a homodimer of 17 kDa subunits containing a [2Fe–2S] cluster essential for its transcriptional activation. Apo SoxR, the iron-free form of the protein, remains dimeric and retains its ability to bind to its target DNA in the *soxS* promoter ($K_D \sim 10^{-10}$ M) [23]. Similarly, mutation of any of the SoxR cysteine residues yields a stable dimeric protein that lacks detectable [2Fe–2S] clusters but still binds tightly to DNA [24]. Thus, the [2Fe–2S] clusters are not required to maintain the structure and DNA-binding affinity of SoxR, but are essential for activating the transcription of the *soxS* gene [14,24].

The [2Fe–2S] clusters of SoxR can undergo reversible one-electron reduction, similar to electron transfer proteins. Since the reduced form of SoxR ($SoxR_{red}$) is paramagnetic, its redox changes can be assessed quantitatively by electron paramagnetic resonance spectroscopy. Using this method, the [2Fe–2S] clusters in intact *E. coli* were found to be >95% $SoxR_{red}$ during aerobic growth [13,14]. Upon exposure of the cells to superoxide (O_2^-)-generating compounds such as paraquat and other redox-cycling compounds, the EPR signal disappeared rapidly, reappearing within a few minutes when the oxidative stress was withdrawn [15]. The in vivo transcription of *soxS* in intact cells was found to correlate with redox states ($[SoxR]_{ox}/[SoxR]_{red}$), as measured by EPR [15]. Therefore, SoxR senses oxidative stress using the redox states of the [2Fe–2S] cluster and regulates the transcription of the *soxS* gene by redox changes in SoxR. In vivo, oxidized SoxR is reduced rapidly and is maintained in its reduced state by specific enzymes [25,26].

2.1. SoxR in the MerR Family

SoxR is a member of the MerR family [17,18] of proteins which function as activators of transcription in response to a variety of stresses in

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the environment, including exposure to heavy metals [27–32], reactive oxygen species [17,18], and antimicrobial agents [33–38]. The regulated genes are required for metal efflux or detoxification or in some cases defense against oxidative stress or drug resistance [39]. The structures of MerR proteins share the same topology [29,33,35,39], with the structural diversity of their C-terminal domains allowing individual MerR family proteins to sense not only various metal ions but also oxidative stress through the [2Fe–2S] clusters and antimicrobial agents. The metal binding sites in the individual metal sensors of MerR proteins have evolved to sense a wide range of metals with high sensitivity and specificity [29,40]. For example, the copper-receptor site in CueR restricts the metal to a linear, two-coordinate geometry and uses helix-dipole and hydrogen-bonding interactions to enhance metal binding [29].

An unusual feature of the genes that are regulated by MerR family members is the 19 or 20 base pair separation between the -10 and -35 promoter elements, rather than the optimal 17-bp spacer observed within most bacteria promoters [41]. The unusual promoter structure of genes regulated by MerR proteins has led to a model of transcriptional regulation by these proteins, in which activation is achieved by DNA distortion and untwisting. The crystal structures of BmrR, MtaN and SoxR bound to their target promoters have provided structural evidence of DNA distortion during transcription activation [33,35,39]. These structures reveal that the promoters are bent sharply at the center with base pair breaking and sliding, resulting in the remodeling of the -35 and -10 promoter elements such that they are located on the same face of the DNA helix in a position optimized for RNA polymerase binding and ultimately transcriptional activation [42].

2.2. Overall structure of SoxR

The crystal structures of *E. coli* SoxR and its complex with the *soxS* promoter in the oxidized state have been solved at resolutions of 3.2 Å and 2.8 Å, respectively [39]. The SoxR protein consists of an N-terminal winged helix (α1–4) DNA-binding domain, a dimerization helix (α5) domain and a C-terminal sensor domain that contains the [2Fe–2S] clusters (Fig. 1). Dimerization of the α5 helix results in the formation of an antiparallel coiled coil, which stabilizes the SoxR dimer. The α3 and α4-helices within the DNA binding domain form hydrophobic contacts with the α5-helix in the same subunit. The Fe–S cluster-binding domain is further stabilized by interaction with the α3′, α4′ and α5′-helices of the other subunits. The overall architecture is similar to that of other MerR family proteins [29,33,35,39].

Upon binding of SoxR to DNA, both the DNA-binding and Fe–S cluster domains of SoxR undergo outward rotation, resulting in a widening of the distance between the α2- and α2′-helices. Changes in the α5-helix connecting both domains lead to changes in the relative positions of the dimerized helices [39]. Ultraviolet resonance Raman (UVRR) spectroscopy of Trp98 and Trp91 in SoxR have shown that these Trp residues undergo small environmental changes upon DNA binding [43], suggesting that the structure of the individual domains of free and DNA bound SoxR do not differ significantly. However, the redox potential of SoxR following DNA binding shifts from −290 mV [14] to +200 mV [44] versus NHE at pH 7, suggesting that the solvent-exposed environment and electronic structures of the [2Fe–2S] cluster in SoxR may be altered upon DNA binding.

In contrast, binding of the promoter DNA triggers changes in the UVRR spectroscopy of Tyr49 and Tyr31 in the DNA-binding domain. Model studies have shown that the intensity and frequency of these Tyr bands are influenced by the formation or cleavage of the hydrogen bond [45,46]. Upon DNA-binding, rotation of Tyr31 is accompanied by rearrangement of the hydrogen bond from Gln64 to the phosphate backbone of DNA. In contrast, DNA binding cleaves the hydrogen bond between Tyr49 and Arg47, with Arg47 forming a hydrogen bond with the phosphate backbone of DNA; this bond, may be functionally important in stabilizing the conformation of SoxR bound to DNA [39].

2.3. Environment of the [2Fe–2S] cluster of SoxR

The [2Fe–2S] cluster of SoxR involves four cysteine residues (Cys119, Cys122, Cys124, and Cys130) near the carboxyl terminus. It is interesting to note that one of the S atoms (S2) and two Fe atoms are nearly completely exposed to the solvent (Fig. 2). In contrast, the lower S

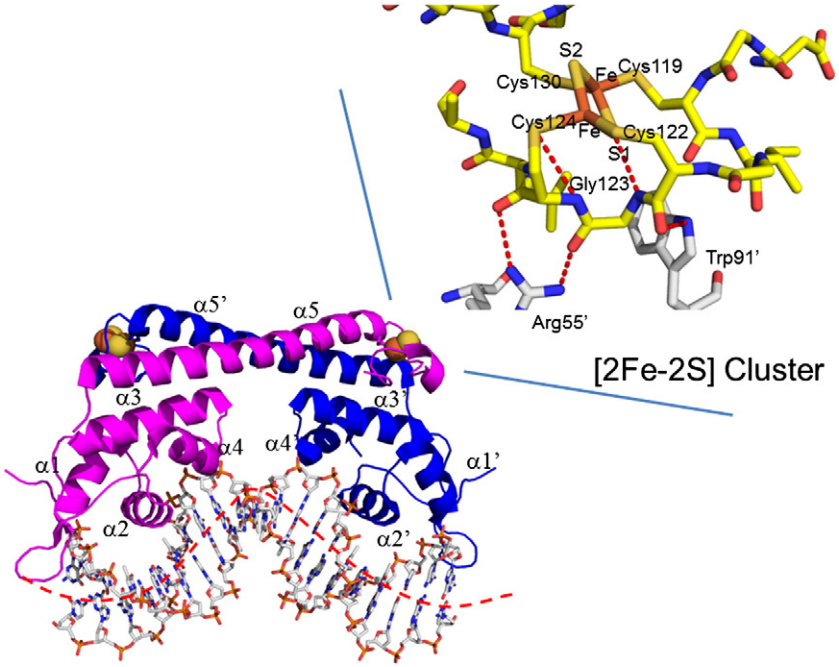

Fig. 1. Structure of SoxR–*soxs* promoter complex showing a significant bend (~65°). in the *sox* operator DNA. The two protomers of the SoxR homodimer are shown as ribbon structures and DNA fragment appears in a stick model. Inset: A close-up view of the [2Fe–2S] cluster is shown. Red dashed lines show hydrogen bonds. The structure was reproduced with PyMol using a structure from the Protein Data Bank (code 2ZHZ).

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atoms (S1) is hydrogen-bonded to the amide of Gly123 and makes van der Waals contacts with the amides of Cys124 and Leu125. The asymmetric environment of the [2Fe–2S] cluster may cause redox-dependent conformation changes of SoxR [39]. In other [2Fe–2S] proteins such as ferredoxin and putidaredoxin, the crystal structures show remarkably similar main chain folds and their [2Fe–2S] cluster geometries are very close to each other. The [2Fe–2S] cluster is shielded from the solvent by surrounding residues [47,48], with both sulfur atoms of the cluster forming similar hydrogen bonds with main chain amides. In contrast, the [2Fe–2S] cluster of SoxR is located on the surface of the molecule with the S2 atom and two Fe atoms fully exposed to the solvent. Exposure to solvent likely enables rapid electron transfer and interactions with various redox agents, suggesting that SoxR responds not only to O_2^- directly but to redox-cycling compounds [49–51]. Exposure to solvent also allows the rapid and direct nitrosylation of the [2Fe–2S] cluster [19].

An unusual feature of a CX_2CXCX_5C motif in the [2Fe–2S] cluster of SoxR is the presence of a single residue (Gly123) between the two internal Cys residues. Completely conserved Gly123 residue interacts with the lower sulfur atom (S1) of [2Fe–2S] forms. In addition, hydrogen bond interactions between the backbone carbonyl oxygens of Gly123 and Cys124 in the [2Fe–2S] binding loop and the NH1 and NH2 of Arg55′ in the α3′ helix from the opposite promoter may be important in driving conformational change coupled to DNA distortion.

The Nε1 atom of Trp91′, which forms a hydrogen bond with the backbone carbonyl of Cys119, may be important for the redox signaling of SoxR. UVRR spectra shows environmental or structural changes of Trp91 following reduction [43]. The environment around Trp91 is altered to become hydrophilic and changes in cation–π interactions between the [2Fe–2S] clusters and Trp91 are suggested. The relative orientations of the α3 and α4-helices in the DNA binding domain and the α5-helix in the dimerization domain depend on the redox state of SoxR.

2.4. Interactions between SoxR and promoter DNA

DNA in SoxR–DNA complexes is in a bent conformation with local untwisting, with a greater degree of bending (~65°) (Fig. 1) from the middle toward the major groove of the protein than observed for the BmrR– and MtaN–DNA complexes (~47–50°). SoxR recognizes the *soxS* promoter by its helix-turn-helix motif and wings, W1 and W2, in a fashion nearly identical to that of BmrR [33] and MtaN [35]. The bend in the DNA is centered at the central TpA base pair steps where the two central Ade1–Thy1′ and Thy1′–Ade1 base pairs of the *soxS* promoter form Watson–Crick base pairs. The rise, roll and twist of the central base steps between A1/T1′ and T1′/A1 of the *soxS* promoter are distorted with respect to B-form DNA. The distorted DNA conformation is stabilized by interactions between the bent phosphate backbone near the center of the DNA element and the SoxR residues Ser26, Tyr31, located in the helix-turn-helix motif, and Gln64 in the α3 helix (Fig. 3). In addition, the Cyt8 phosphate interacts with residues Arg47 and His29 in the helix-turn-helix motif and Arg41 in W1. Specifically, base-specific interactions at position 26 (Ser for SoxR, Glu for MerR, Lys for CueR) may play an important role for each individual MerR protein to recognize their own operator sequences.

In the absence of their target molecules, all MerR proteins are stable dimers that bind promoter DNA. However, it is not known whether the DNA bound to MerR proteins is distorted. No inducer-free structures, in their inactive forms, have been observed to date for any protein of the MerR family, including $[SoxR]_{red}$. Incorporation of pyrrolo-dC into the central part of the sequence in the CueR promoter provides an optical probe of local and global DNA conformation [52]. The fluorescence of pyrrolo-C in the CueR complex with duplex DNA is quenched in the absence of Cu^{1+}, whereas binding of Cu^{1+} to CueR increases the fluorescence intensity associated with the distortion of DNA. This finding suggests that the DNA bound to CueR in the absence of Cu^+ is in its B-form. Similarly the DNA bound to $[SoxR]_{red}$ may also exist in B-form, without distorting the duplex structure.

2.5. Propagation of structural changes from the [2Fe–2S] cluster to distorted DNA

Redox-induced structural changes in the [2Fe–2S] cluster of SoxR are communicated to the DNA-binding domain, leading to distortions in target promoter DNA. The SoxR–DNA structure suggests a plausible mechanism by which reversible oxidation of the $[2Fe–2S]^+$ cluster drives an interdomain reorganization required to allosterically induce DNA distortion upon oxidation. In the absence of a high-resolution structure of the transcriptionally inactive $[2Fe–2S]^+$ form of SoxR bound to DNA, however, these suggestions are speculative. According

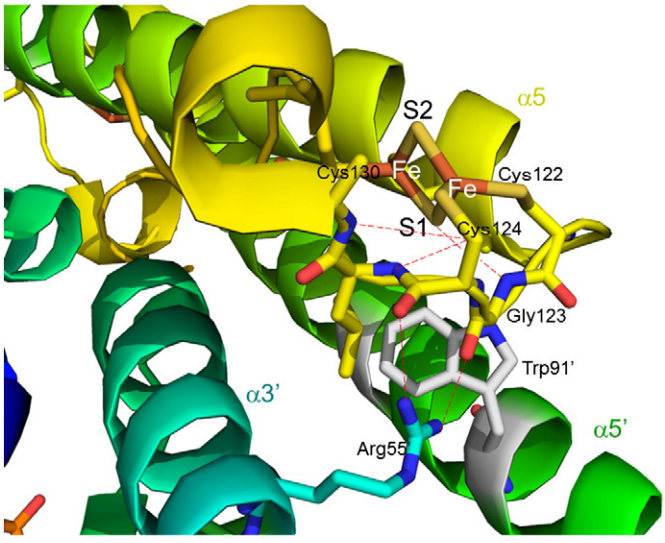

Fig. 2. Close-up region near the [2Fe–2S] cluster. The structure was reproduced with PyMol using a structure from the Protein Data Bank (code 2ZHZ).

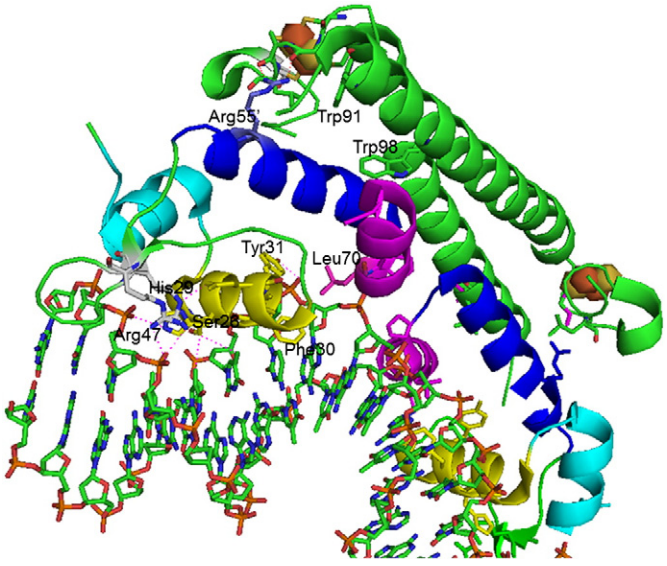

Fig. 3. SoxR–DNA interaction. SoxR–DNA half site interface. The structure was reproduced with PyMol using a structure from the Protein Data Bank (code 2ZHZ).

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to theoretical calculations, most of the added charge in the reduced [2Fe–2S] cluster is evenly distributed between the bridging and cysteinyl sulfur atoms [53]. Upon reduction, an additional negative charge on the S1 atom may attract main-chain amides and increase the charge repulsion with the carbonyl oxygen atom of Cys-119, possible resulting in conformational change. The resulting conformational changes affect the [2Fe–2S] cluster binding loop of Gly123, which subsequently changes the position of Arg55′ in the α3′ helix, thereby altering the DNA-binding domain. The above sequential events induce considerable changes in the Raman spectra of Trp91 [43]. An analogous set of events in the drug-bound BmR–DNA complex appears to be crucial for transcriptional activation, suggesting that these interactions may be common to all MerR family regulators [35].

2.6. Direct response to superoxide

SoxR is activated, directly or indirectly, by O_2^- [54–57], but the extent of its activation in response to O_2^- is not clear [58–60]. The *soxRS* response of *E. coli* was found to be directly activated by redox-cycling drugs rather than by O_2^- [60]. In contrast, using a pulse radiolysis method, we recently demonstrated that O_2^- reacts directly with $SoxR_{red}$ to form $SoxR_{ox}$ [61]. The reaction schemes after pulse radiolysis can be summarized as follows

$$e_{aq}^- + [2Fe\text{–}2S]^{2+} \rightarrow [2Fe\text{–}2S]^{+} \quad (1)$$

$$O_2^- + [2Fe\text{–}2S]^{+} + 2H^+ \rightarrow [2Fe\text{–}2S]^{2+} + H_2O_2. \quad (2)$$

Radiolytically generated hydrated electrons (e_{aq}^-) reduce the oxidized form of the [2Fe–2S] cluster of SoxR within 2 μs, with subsequent reoxidation of the [2Fe–2S] cluster occurring in milliseconds. The reaction sequence is reversible; thus iron–sulfur clusters are not irreversibly destroyed, since O_2 can completely oxidize the [2Fe–2S] cluster of $SoxR_{red}$ within a time scale of ~20 s. The addition of human Cu/Zn SOD inhibited this delayed oxidation in a concentration-dependent fashion (I_{50} = 1.0 μM), indicating that O_2^- oxidized the reduced form of SoxR directly. The second-order rate constant of Reaction (2) (5×10^8 M^{-1} s^{-1}) is similar to those of Mn-SOD (15–1.6 $\times$ 10^8 M^{-1} s^{-1}) [62] and Fe-SOD (3.3×10^8 M^{-1} s^{-1}) [63], suggesting that intracellularly generated O_2^- reacts with $SoxR_{red}$. The ability of O_2^- to activate SoxR in vivo, however, is unclear. The basal amount of SOD (~10^{-5} M) in aerobic cells would efficiently scavenge O_2^- to maintain non-toxic steady-state concentration of O_2^- [63,64]. However, high levels of SOD are often required to protect enzymes against O_2^-, which is frequently used as a killing agent in mammalian immune systems. Changes in O_2^- concentration may trigger pathways involved in host–pathogen interactions. Responses to O_2^- are partially organized around the reversible oxidation of the sensor, SoxR, enhancing the expression of the regulator, SoxS.

It is important to note that Reaction (2) in SoxR is the first direct observation of the reaction between an iron–sulfur protein and O_2^-. O_2^- has been reported to inactivate several iron–sulfur proteins with second-order rate constants ranging from 10^6 to 10^7 M^{-1} s^{-1} [65–68]. In each of these cases, the process was irreversible and was associated with concomitant cluster degradation. In contrast, it can be said that that Reaction (2) is specific to SoxR protein.

3. SoxR in *Pseudomonas aeruginosa*

SoxR homologs have been identified in 176 α-, β-, δ-, γ-Proteobacteria and Atinobacteria [69]. All of these homologs contain the SoxR-specific cysteine motif CI[G/Q]CGC[L/M][S/L]XXC required for binding of the [2Fe–2S] cluster. Unlike *E. coli* and related enteric bacteria, however, the majority of SoxR regulons in *P. aeruginosa* and *Streptomyces coelicolor* lack the genes typically involved in O_2^- resistance and detoxification [70]. Many of these bacteria produce redox-active pigments independent of oxidative stress [69]. *P. aeruginosa* SoxR was shown to be activated by the endogenous, redox-cycling antibiotic compound pyocyanin [50,69], which is the physiological signal for the upregulation of quorum-sensing controlled genes.

P. aeruginosa SoxR, a homolog with 62% sequence identity and 77% sequence similarity to *E. coli* SoxR [71], exhibited physical–chemical properties similar to that of *E. coli* protein, including the redox potential of the [2Fe–2S] cluster and visible absorption spectra [72]. Similar to *E. coli* SoxR, O_2^- reacts with $[2Fe\text{–}2S]^+$ of *P. aeruginosa* SoxR [61]. However, the sensitivity of the *E. coli soxRS* response to O_2^-, with a rate constant of 5×10^8 M^{-1} s^{-1}, is 10-fold higher than that of *P. aeruginosa* (rate constant 4×10^7 M^{-1} s^{-1}), suggesting that SoxR proteins play distinct regulatory roles in the activation of O_2^-. The difference between the two responses reflects the physiological function of SoxR-mediated gene regulation. Direct oxidation of *E. coli* SoxR by O_2^- induces SoxS, which in turn, activates genes involved in O_2^- stress protection and repair [73,74]. The SoxR regulon responds to metabolically generated O_2^-, adjusting SOD synthesis to control intracellular O_2^- levels. In contrast, *P. aeruginosa* SoxR is activated by the endogenous, redox-cycling antibiotic compound pyocyanin [50,69], the physiological signal for upregulating quorum-sensing-controlled gene expression. In addition, pyocyanin serves as an electron acceptor to balance the intracellular redox state [75]. Our pulse radiolysis data also support the hypothesis that O_2^- responsiveness, the major function of the *E. coli* SoxR regulon, is not the major function of the *P. aeruginosa* SoxR regulon.

What is the mechanism underlying SoxR's different sensitivities to O_2^-? The difference cannot be explained by the redox potential of the [2Fe–2S] clusters in the *E. coli* and *P. aeruginosa* SoxR [13,71]. O_2^- reduction to H_2O_2 is favored in the presence of a proton source, as is observed in the reaction of superoxide reductase [76–79]. Both proton and solvent may play important roles in the SoxR mechanism. An analysis of the crystal structure revealed that the [2Fe–2S] clusters of oxidized SoxR are solvent-exposed, creating an asymmetrically charged environment and a possible conformational change in SoxR. The unusual positioning of the metal ion on the surface of SoxR provides an active site with a readily available source of protons. Minor changes in the primary sequence may lead to the functional difference of SoxR proteins.

4. Distinct activation pathways for SoxR by NO

SoxR can also be activated by NO [19]. Exposure of cells to pure NO gas or macrophage-generated NO results in the SoxR-dependent activation of *soxS* expression [79,80]. Since the transcriptional activities of nitrosylated SoxR and oxidized SoxR are similar [13], the DNA present in the complex with nitrosylated SoxR may be in a bent conformation with local untwisting, similar to that observed in the complex with oxidized SoxR. However, the mechanisms of NO- and O_2^--meditated activation differ markedly. The redox regulation with SoxR is mediated by a shift in the redox equilibrium of SoxR [81,82], with transcription reversibly inhibited by reactions linked to NAD(P)H [23,24]. In contrast, the reaction between NO and $SoxR_{ox}$ is irreversible, with the concomitant formation of a protein-bound dinitrosyl–iron complex (DNIC). Enzymatic pathways, however, may eliminate the DNIC [13]. For example, cysteine desulfurase, together with L-cysteine, has been shown to efficiently repair the NO-modified form of the [2Fe–2S] cluster of ferredoxin [15,83–85]. These processes may therefore contribute to cellular defenses against nitrosative stress, especially since DNIC plays a physiologically important role in the storage and transport of NO [85].

5. Conclusion

The [2Fe–2S] transcription factor SoxR, a member of the MerR family, functions as a bacterial sensor of oxidative stress such as O_2^- and NO in *E. coli*. SoxR is activated by reversible one-electron oxidation of the [2Fe–2S] cluster. The structures reveal that redox-induced structural changes in the [2Fe–2S] cluster of SoxR are communicated to

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the DNA-binding domain, leading to distortion in the target promoter. The $[2Fe–2S]_{red}$ cluster of SoxR in *E. coli* is oxidized rapidly by O_2^-, suggesting that the direct reaction of O_2^- is an important mechanism for *E. coli* SoxR signaling. The sensitivity of *P. aeruginosa* SoxR response to O_2^- is 10-fold lower than that of *E. coli*, suggesting that SoxR proteins play distinct regulatory roles in the activation of O_2^-.

Abbreviations

SoxR	superoxide response;
$SoxR_{ox}$	oxidized SoxR;
$SoxR_{red}$	reduced SoxR;
UVRR	ultraviolet resonance Raman;
SOD	superoxide dismutase;
e_{aq}^-	hydrated electron;
DNIC	dinitrosyl–iron complex

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