Transcription Factors That Defend Bacteria Against Reactive Oxygen Species

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Annu. Rev. Microbiol. 2015. 69:93–108

First published online as a Review in Advance on June 11, 2015

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi:
10.1146/annurev-micro-091014-104322

Keywords

hydrogen peroxide, superoxide, OxyR, PerR, SoxR, reactive oxygen species

Abstract

Bacteria live in a toxic world in which their competitors excrete hydrogen peroxide or superoxide-generating redox-cycling compounds. They protect themselves by activating regulons controlled by the OxyR, PerR, and SoxR transcription factors. OxyR and PerR sense peroxide when it oxidizes key thiolate or iron moieties, respectively; they then induce overlapping sets of proteins that defend their vulnerable metalloenzymes. An additional role for OxyR in detecting electrophilic compounds is possible. In some nonenteric bacteria, SoxR appears to control the synthesis and export of redox-cycling compounds, whereas in the enteric bacteria it defends the cell against the same agents. When these compounds oxidize its iron-sulfur cluster, SoxR induces proteins that exclude, excrete, or modify them. It also induces enzymes that defend the cell against the superoxide that such compounds make. Recent work has brought new insight into the biochemistry and physiology of these responses, and comparative studies have clarified their evolutionary histories.

Contents

INTRODUCTION

The most harmful effects of oxygen are mediated by its partially reduced forms—hydrogen peroxide (H_2O_2) and superoxide (O_2^-). These species continuously arise inside cells through the autoxidation of redox enzymes, and so aerobes maintain high titers of scavenging enzymes that hold these oxidants below the threshold of toxicity. But by the 1980s it was realized that a variety of natural circumstances can elevate these species above that threshold and that microbes universally maintain inducible defensive systems to counteract such events. Several such systems have now been dissected, and the goal of this review is to map out what is known and what key problems remain. The focus will be on the three transcription factors that have been most intensively examined: OxyR and PerR, which respond to H_2O_2 stress, and SoxR, which defrays the toxic effects of O_2^-.

DETECTING HYDROGEN PEROXIDE: OxyR AND PerR

The Threat of H_2O_2

H_2O_2 arises in natural habitats through reactions between sulfur and oxygen at oxic/anoxic interfaces, the photochemical reduction of oxygen by chromophores, and the redox cycling of pigments. More ominously, plants, animals, and certain bacteria excrete H_2O_2 to poison local microbes (5, 27, 79, 98). H_2O_2 is an ideal weapon. Because it is small and uncharged, it passively crosses membranes at rates similar to that of water, so it cannot be excluded by the targeted cell (97, 115). Once inside it disrupts multiple aspects of iron metabolism, thereby attacking a feature of life that is virtually universal.

The ways in which H_2O_2 damages cells have been identified in the model bacterium *Escherichia coli*. Exogenous H_2O_2 damages DNA, so it is mutagenic. This effect arises from Fenton chemistry, in which H_2O_2 reacts with the intracellular pool of unincorporated iron (42):

$$Fe^{2+} + H_2O_2 \rightarrow [FeO^{2+}] \rightarrow Fe^{3+} + OH^- + HO^{\cdot}.$$

The hydroxyl radical ($HO^{\cdot}$) reacts at diffusion-limited rates with most biomolecules. Some of the iron pool is loosely associated with DNA (94), and Fenton reactions on its surface produce the lesions that cause mutagenesis.

However, the most marked effect of H_2O_2 stress is to inactivate two families of iron-containing enzymes: nonredox mononuclear enzymes and [4Fe-4S]-containing dehydratases (2, 46, 103, 104). Both classes of enzymes employ a solvent-exposed reduced iron atom to bind and activate substrates, and in both cases incoming H_2O_2 can contact and oxidize the iron. The metal dissociates, and activity is lost. Such enzymes sit within the TCA cycle, the pentose phosphate pathway, and key biosynthetic pathways, so growth stops. As little as 0.5 μM H_2O_2 is sufficient to poison cells in this way, so the titers of scavenging enzymes are calibrated to keep the steady-state H_2O_2 concentration below this level. This goal becomes much harder when bacteria enter a habitat containing extracellular H_2O_2. The H_2O_2 quickly flows across membranes and into the cytoplasm. For that circumstance virtually all bacteria maintain inducible defensive regulons governed by either the OxyR or the PerR transcription factors.

Defensive Tactics: The OxyR Regulon

The OxyR protein is more widely distributed. It features a sensory cysteine residue that is directly oxidized by H_2O_2, thereby generating a disulfide bond that alters its binding to DNA (**Figure 1*a***) (4, 11). In some bacteria OxyR acts as a repressor, and its oxidation inactivates this function and stimulates gene expression (39, 40, 72, 73, 105). More commonly, OxyR acts as an activator protein that recruits RNA polymerase. In *E. coli* the oxidized protein stimulates the transcription of about two dozen genes that are well suited to address the problems that H_2O_2 causes (**Table 1**) (120). To drive down the H_2O_2 level, OxyR strongly induces both an NADH peroxidase (AhpCF) and a catalase (KatG) (44). A mini-ferritin, Dps, is synthesized to sequester unincorporated iron (10, 31). This has the effect of substantially suppressing the rate of DNA damage, especially in collaboration with the induction of Fur and Yaa proteins (71, 111, 119). The MntH manganese importer is induced, apparently to enable Mn—which does not react with H_2O_2—to displace Fe as the catalytic cofactor of mononuclear enzymes (3, 50, 103). Induction of the Suf iron-sulfur assembly system enables the repair of damaged iron-sulfur clusters. Although the cell normally uses an Isc system for this purpose, Isc does not work well when iron levels decline, a situation that ensues from the

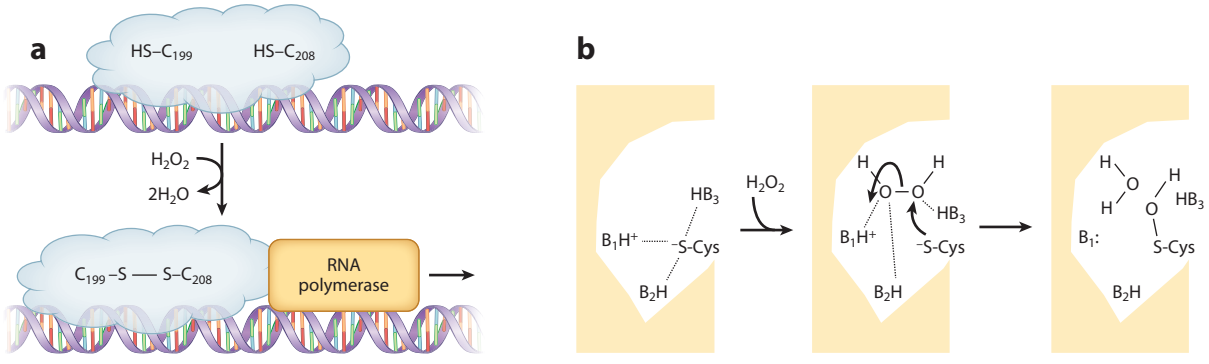

Figure 1

Activation of OxyR. (*a*) Oxidation of a sensory cysteine by H_2O_2 perturbs global conformation. In *E. coli* the disulfide-bonded form of the protein stabilizes the transcription complex. (*b*) The acute H_2O_2 sensitivity of OxyR may depend on a shift in hydrogen bonds from the sensory cysteine thiolate to the incoming H_2O_2. This idea derives from models of peroxiredoxin behavior (18, 106).

Table 1 The *Escherichia coli* OxyR regulon[a]

Gene	Activity	Impact
katG	Catalase	Scavenge H_2O_2
ahpCF	NADH peroxidase	
dps	Mini-ferritin	Minimize free iron
fur	Iron-import repressor	
yaaA	Unknown biochemistry	
mntH	Manganese importer	Activates mononuclear enzymes
sufA-E	Iron-sulfur assembly	Activates Fe/S enzymes
hemF	Coproporphyrinogen III oxidase	Sustain heme synthesis
hemH	Ferrochelatase	
gor	Glutathione reductase	Maintain thiol
trxC	Thioredoxin	
grxA	Glutaredoxin	
dsbG	Protein sulfenate reductase	

[a]Several genes of unknown function are not included. The PerR regulon of *Bacillus subtilis* includes catalase, mini-ferritin (MrgA), Fur, and heme biosynthesis genes (7). In that bacterium, Mn importers and Suf proteins are constitutively synthesized, whereas disulfide-reducing systems are controlled by an independent regulator, Spx, that does not respond to H_2O_2.

action of Dps (47, 85). Similarly, the iron-dependent enzymes in the heme biosynthetic pathway are replaced (HemF) or induced (HemH) to sustain this process as intracellular iron becomes scarce (77).

Three regulon members typically reduce disulfide bonds: glutathione reductase, glutaredoxin 1, and thioredoxin 2. The glutaredoxin assists in deactivating OxyR once H_2O_2 has dissipated (4), but the roles of the others are less obvious. Deletion of these genes does not cause obvious sensitivity to H_2O_2, which inspires other ideas about their purpose in this regulon (see below).

How Does OxyR Sense H_2O_2?

Recent studies have analyzed both the specificity and the sensitivity of OxyR. *E. coli* OxyR features a hyperreactive thiol (Cys199) that is quickly oxidized by H_2O_2 to a sulfenic acid (−SOH). The adducted residue moves from the hydrophobic cleft in which it is normally buried, and it swings into the proximity of Cys208, with which it condenses to form a disulfide bond (11). This shift locks the domain into a conformation that activates the protein as a transcription factor. The protein functions as a dimer, and it displays some cooperativity that may avoid activation when H_2O_2 is scant (61). The fact that OxyR uses cysteine oxidation to detect H_2O_2 enables it to work like a rheostat, given that disulfide formation is among the few amino acid oxidations that are reversible.

The surprising aspect of this chemistry is that cysteine per se reacts very sluggishly with H_2O_2 (2 M^{-1} s^{-1}) (116). With such a rate constant, the half-time for oxidation by 0.5 μM H_2O_2—an intracellular concentration that suffices to block *E. coli* growth (103)—is approximately one week. In sharp contrast, the sensing cysteine residue on OxyR exhibits a rate constant of 10^5 M^{-1} s^{-1} and detects micromolar H_2O_2 within seconds (4). What is the source of this kinetic improvement?

The hyperreactive behavior of OxyR C199 has not been deconstructed in detail, but it resembles that of the catalytic cysteine residues on peroxiredoxins, the ubiquitous peroxidases (including *E. coli* AhpC) that degrade H_2O_2 through cycles of cysteine oxidation and reduction

(18, 106). As with OxyR, their reaction involves a nucleophilic attack of a cysteine thiolate on H_2O_2 (**Figure 1*a***), with cleavage of the dioxygen bond. A nearby cationic residue sets the stage by ensuring deprotonation of the cysteine (83)—but this should provide only an order-of-magnitude improvement in reactivity. A plausible explanation for the remaining stimulation is depicted in **Figure 1*b***. The peroxidactic cysteine residue of peroxiredoxins participates in hydrogen-bond networks that shield its charge within a largely hydrophobic cleft (91). Entry of H_2O_2 may shift these bonds toward the H_2O_2 itself, freeing the thiolate. Several aspects of this are conducive to catalysis. Release of the cysteine residue generates a nucleophile whose potency is enhanced in a low-dielectric environment. The hydrogen bonds that shift toward H_2O_2 likely polarize its dioxygen bond, making it more conducive to attack, and one of these residues probably protonates the hydroxide leaving group to pull the reaction forward. The cysteine residue of OxyR is invariably flanked by arginine and histidine residues that may play the same role.

A hydrogen-bond network configured to fit H_2O_2 should also enhance the rate constant by disfavoring water in the active site, given that H_2O is too small a molecule to bridge a hydrogen-bond network tailored for H_2O_2 (93). The upshot is that thiol-based peroxidases achieve a level of reactivity (10^7 M^{-1} s^{-1}) that is appropriate for their physiological roles, and similar features may allow OxyR to do so as well. A new structure of the *Pseudomonas aeruginosa* OxyR protein suggests that H_2O_2 binding might engage a hydrogen-bond network that ultimately links the deprotonation of cysteine with the protonation of the product hydroxide group (48).

In *E. coli* OxyR quickly responds to the submicromolar levels of H_2O_2 that threaten to disrupt metabolism. The facultative bacterium *Vibrio vulnificus* takes it a step further, employing two OxyR proteins that are calibrated to sense distinct levels of H_2O_2: The more sensitive OxyR is activated by the low levels of H_2O_2 that are endogenously generated upon aeration, whereas the less sensitive one responds to more-severe influxes of H_2O_2 from the external environment (53).

Has OxyR Evolved to Respond to Stresses Other Than H_2O_2?

Because OxyR responds to adduction of its sensory thiol, it can also be activated by electrophiles (118). Experimenters have commonly used the synthetic chemical diamide as a proxy for such compounds. An interesting question is whether OxyR has an authentic role in sensing stressors other than H_2O_2. Seth et al. (99) reported that OxyR becomes nitrosylated during anaerobic nitrate reduction, presumably by nitric oxide that escapes from the system. Indeed, an *oxyR* mutant exhibited poorer growth in this circumstance, suggesting that some elements of the regulon help the cell to tolerate this stress.

Many bacteria contain transcription factors other than OxyR that appear to be dedicated to responding to electrophiles, whose attack on thiols can indirectly generate disulfide bonds. Both the Spx system of *Bacillus subtilis* and the SigR/RsrA system of *Streptomyces coelicolor* induce a mixture of glutaredoxins, thioredoxins, chaperones, and proteases, which collectively avert protein aggregation from the denaturing effects of disulfide formation and thiol adduction (49, 52, 86, 96, 121). These bacteria additionally contain OxyR or PerR systems that respond to H_2O_2—but in these bacteria, the latter systems do not include disulfide-reducing genes that are part of the *E. coli* OxyR regulon. Thus, it seems possible that *E. coli* is exceptional in multitasking OxyR with responding to both stresses: H_2O_2, which primarily threatens iron enzymes, and electrophiles, which react with cysteine residues much more avidly than does H_2O_2.

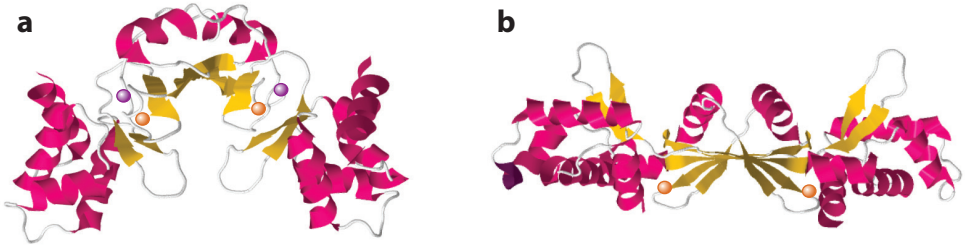

Figure 2

Structure of PerR in its (*a*) holoenzyme and (*b*) demetallated forms. Structures were derived from the Protein Data Bank (3F8N and 2FE3; **http://www.rcsb.org/pdb**) (45, 107). Orange spheres represent Zn, and purple spheres represent Mn.

PerR of *Bacillus subtilis*: An Alternative to OxyR

Gram-positive bacteria often employ a different sensor of H_2O_2: PerR. This dimeric protein belongs to the Fur family of metal-binding transcription factors, which have evolved in their various forms to sense iron (Fur proper), manganese (Mur), zinc (Zur), nickel (Nur), or H_2O_2 (PerR) (19, 38). Each subunit contains a structural site that irreversibly binds zinc, plus at least one regulatory site that reversibly binds the activating metal (**Figure 2*a***). Only the metal-bound forms of the proteins are competent to bind DNA, and they generally work as repressors. In *B. subtilis* PerR, the regulatory metal is ferrous iron, and it is affixed in a distorted square pyramid with four coplanar ligands and a single axial ligand (45). When H_2O_2 levels rise, H_2O_2 accesses the sixth coordination site and oxidizes the iron atom. The ferryl or hydroxyl radical that is thereby formed oxidizes the histidine ligands of the iron atom to 2-oxohistidine (64). The modified residues can no longer bind the metal, and the resultant apoprotein undergoes a global conformational change that eradicates its ability to bind DNA (**Figure 2*b***). Because holo-PerR acts as a repressor, the effect is to derepress regulon members whenever H_2O_2 levels rise.

Strikingly, *B. subtilis* PerR reacts with H_2O_2 at essentially the same rate constant as does *E. coli* OxyR (10^5 M^{-1} s^{-1}) (63). Other mononuclear iron proteins, such as *E. coli* peptide deformylase, have similar inactivation rate constants (2), indicating that this value is largely dictated by the fundamental rate of Fenton reactions. However, *B. subtilis* also employs a true Fur protein whose iron cofactor seems to be resistant to oxidation by H_2O_2. Inspection of this discrepancy elicited evidence that in the Fur protein a coordinating glutamate residue probably acts as a bidentate ligand to the iron atom, thereby occluding its sixth coordination site (89). The analogous aspartate ligand of PerR does not. Given that H_2O_2 must directly contact iron in order to oxidize it (28), H_2O_2 oxidizes PerR but not Fur.

The PerR regulon of *B. subtilis* includes many of the same genes that OxyR controls in other bacteria: genes encoding a catalase, a Dps homolog (MrgA), and Fur (7) (**Table 1**). Yet PerR offers several intriguing departures from the OxyR mechanism of H_2O_2 sensing. First, given that there is no known mechanism of oxo-histidine reduction, the reaction is presumably irreversible, perhaps making the system more of an on/off switch. This arrangement is a rarity: With few exceptions (80), bacterial regulators are reversible, and their activities are set by the dynamic equilibrium between activating and deactivating events. It is not clear whether the irreversibility of PerR deactivation provides any advantage.

Second, like other mononuclear proteins, PerR can bind manganese in place of iron, both in vitro and in vivo (9). Given that Mn is not oxidized by H_2O_2, the upshot is that PerR does not respond to H_2O_2 whenever the cytoplasm is rich in manganese. As manganese helps to defend

cells against H_2O_2, this arrangement suggests that a primary goal of the PerR and OxyR responses may be to raise the Mn/Fe ratio (117); if that ratio is already high, no induction is needed.

Finally, PerR does not control the synthesis of disulfide-reducing redoxins, as their synthesis is governed by the Spx response regulator (121), which is not responsive to H_2O_2. PerR appears to be focused exclusively on the detection and prevention of Fenton chemistry.

SUPEROXIDE STRESS: RETHINKING SoxR

The Threat of Redox-Cycling Compounds

Because O_2^- is a charged molecule, it cannot penetrate bacteria from outside (57, 74); therefore, any cytoplasmic O_2^- stress is due to O_2^- that is generated internally. Endogenous superoxide (O_2^-) that is formed by enzyme autoxidation is kept very scarce ($\sim 10^{-10}$ M) by the action of superoxide dismutases (SODs) (41). *E. coli* mutants that lack cytoplasmic SODs exhibit growth defects (8) that have been traced back to deficiencies in the same enzymes that H_2O_2 inactivates: [4Fe-4S] dehydratases and mononuclear iron enzymes (20, 24, 35, 60, 103). These mutants are unable to synthesize branched-chain or aromatic amino acids, and they cannot catabolize TCA-cycle substrates.

The same phenotypes arise even in wild-type *E. coli* if it encounters viologens (e.g., paraquat), quinones, or phenazines (37). These cyclic organic chemicals can penetrate cells, where they directly abstract electrons from redox enzymes and transfer them to oxygen. Toxic doses of O_2^- are thereby produced. Indeed, quinones and phenazines are released by various bacteria and plants, often to poison microflora (43, 87, 109). In 1977 it was discovered that *E. coli* responds to this stress by inducing higher titers of SOD (36). This adaptation is mediated by two proteins dubbed SoxR and SoxS (superoxide) (32, 108). SoxR is a [2Fe-2S]-containing transcription factor that senses the stress; it then induces transcription of *soxS*. SoxS is a second transcription factor that then activates scores of defensive genes scattered around the chromosome (6). Several of these encode proteins that help suppress the toxicity of O_2^-: superoxide dismutase; aconitase A and fumarase C, which are superoxide-resistant isozymes of vulnerable [4Fe-4S] enzymes (68, 110); and YggX, which facilitates cluster-repair processes (30, 34, 90). Like OxyR, the SoxRS system is not activated during normal aerobic growth and does not control the basal level of defensive systems; instead, it becomes active only when forcing conditions emerge.

The Sensing Mechanism of SoxR

SoxR is a homodimer that binds one $[2Fe\text{-}2S]^+$ cluster per monomer (**Figure 3**); a second domain includes a DNA-binding HTH motif (114). Electron paramagnetic resonance studies demonstrate that when cells are exposed to redox-cycling compounds, the clusters are quickly oxidized to the $[2Fe\text{-}2S]^{2+}$ state (15, 26). Upon removal of the oxidant the clusters revert within minutes to their reduced forms. The *E. coli soxS* gene features unusually long 20-bp spacing between its −10 and −35 promoter motifs, and the current model is that cluster oxidation contorts the SoxR dimer and thereby alters the conformation of the bound DNA, moving the promoter motifs to the same side of the helix so that RNA polymerase can productively bind. Physical studies have confirmed that changes in SoxR redox state distort bound DNA (23). Genetic studies indicate that the SoxR reduction to the deactivated form is catalyzed by Rsx/Rse proteins (56) that likely employ NADPH as a source of electrons (55). Thus these data collectively suggest that transcription of *soxS* depends on the dynamic equilibrium between oxidation and reduction of the SoxR iron-sulfur clusters.

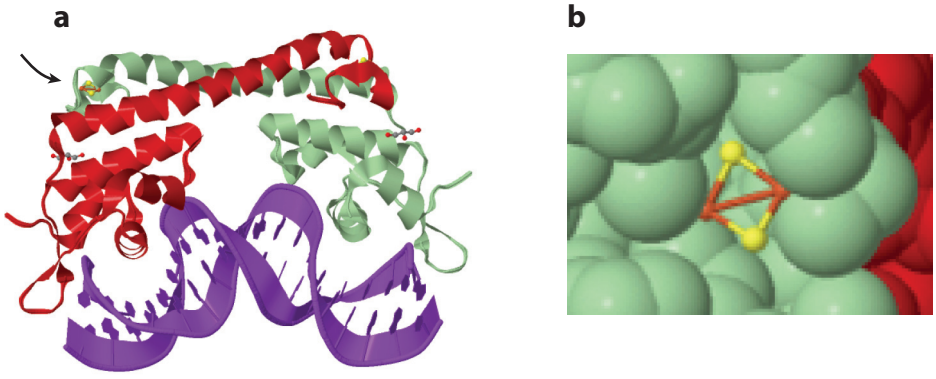

Figure 3

SoxR in association with DNA. (*a*) Oxidation of the cluster (*arrow*) allows the protein to twist the DNA, improving the −10 to −35 spacing. (*b*) The cluster is exposed on the protein surface so that it is accessible to diverse oxidants. The structure was derived from the Protein Data Bank (2ZHG; **http://www.rcsb.org/pdb**) (114).

When SoxR returns to its reduced form, the transcription of the full regulon quickly ceases owing to the rapid proteolysis of extant SoxS protein (33).

What Is the Inducer That Oxidizes SoxR?

The apparent parallel with H_2O_2 detection by OxyR suggested the obvious possibility that SoxR is activated when O_2^- directly oxidizes its clusters. The clusters are located near the protein surface and appear accessible to a small molecule like O_2^- (**Figure 3**) (114). Unfortunately, two efforts to directly detect cluster oxidation by O_2^- in vitro led to contradictory results (21, 34), suggesting that one of these complicated experiments was compromised in some way. The in vivo data, however, are clearer and somewhat surprising. In SOD^- mutants—which suffer debilitating levels of O_2^-—the *soxS* gene is induced only 2- to 4-fold (25, 34). This activation is consistent with the idea that O_2^- can oxidize the clusters, but it is far less than the ~40-fold induction that occurs when wild-type cells are treated with redox-cycling compounds. Similarly modest effects were observed for members of the regulon (34, 67, 69). This induction is too minor to be effectual: The SoxRS regulon provided no protection to SOD^- mutants unless *soxS* was forcibly induced from a heterologous promoter (34). Thus, high O_2^- concentrations are not sufficient to adequately activate SoxR.

Further, overproduction of SOD does not diminish the ability of redox agents to activate SoxR in wild-type cells (25, 29, 34, 68, 76), leading workers to conclude that these drugs oxidize SoxR independently of O_2^-. Indeed, exposure to redox compounds can activate SoxR even in anoxic cells, from which O_2^- is absolutely absent (14, 34, 58).

An alternative explanation for SoxR oxidation by redox agents is that they deplete NADPH pools and thereby disrupt the ability of Rse/Rsx to keep SoxR in its reduced form. Indeed, some genes within the *E. coli* SoxR regulon provide mechanisms to restore NADPH pools (59, 81). However, measurements indicate that the shift in NADPH redox status [to −0.314 V (58)] is not enough to explain the near-total oxidation of SoxR [$Eo' = -0.293$ V (54)], and mutations that impair cellular NADPH formation do not activate SoxR (34). Thus, although NADPH depletion might augment the inducing effects of redox agents, by itself it is probably not sufficient to turn on the system.

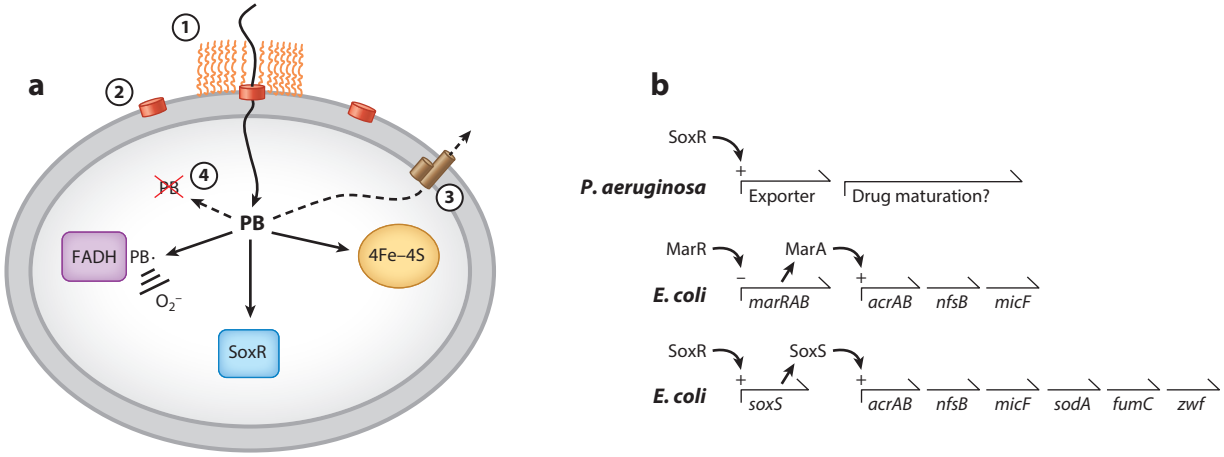

Figure 4

Toxic actions of a redox-cycling agent and the defensive tactics that minimize its accumulation. (*a*) Redox-cycling compounds such as plumbagin (PB) catalyze electron transfer from flavoproteins to oxygen, directly damage [4Fe-4S]-dependent dehydratases, and activate SoxR by oxidation. Components of the SoxR system exclude the agent by ① altering the lipopolysaccharide coat (via *waaYZ*) (62), ② inhibiting porin synthesis (*micF*) (12), ③ exporting the compound (*tolC*, *acrAB*) (75), and ④ modifying it (*nfsA*, *ygfZ*) (66, 70, 95). (*b*) Relationship of *E. coli* SoxRS to nonenteric SoxR and *E. coli* MarA. In nonenteric bacteria SoxR detects endogenous redox-active compounds like pyocyanin and induces exporters that excrete it. The SoxRS regulon could plausibly have been created by lateral transfer of *soxR* and duplication of *marA*, as SoxS and MarA exhibit 50% identity. The SoxRS regulon consists of MarA-controlled genes plus several genes that specifically defend against oxidizing compounds. Only representative genes are shown; for a full list, see EcoCyc (**http://ecocyc.org**).

Therefore, it seems likely that the redox compounds themselves directly oxidize the clusters of SoxR, just as they do the metal and flavin moieties of typical redox enzymes. This reaction has been demonstrated in vitro, with rate constants that accommodate the rates of SoxR oxidation that are observed in vivo (34). The position of the iron atom near the SoxR protein surface ensures that it can be oxidized by diverse univalent oxidants without regard for their structures.

The constituency of the regulon supports the idea that the SoxRS response specifically arose to defend bacteria against redox-cycling compounds. A major element of the SoxRS response in *E. coli* is the induction of proteins that collectively block the entry and accumulation of redox-active compounds within the cell: lipopolysaccharide-modifying enzymes that diminish the ability of such compounds to penetrate the envelope, broad-specificity export systems that actively pump them out of the cytoplasm, and derivatizing enzymes that deactivate them through covalent modification (1, 62, 66, 70, 75, 95) (**Figure 4*a***). Interestingly, SoxR is an important defense against redox-active agents even under anoxic conditions, indicating that these agents can exert O_2^--independent mechanisms of toxicity. Their primary action under these conditions is unclear, but such compounds can form adducts to proteins by acting as Michael acceptors, they can deactivate [4Fe-4S] dehydratases by directly oxidizing the clusters, and they can deplete cellular NADPH pools by oxidizing NADPH-reducible enzymes and then dumping the electrons into the anaerobic respiratory chain (34).

Finally, it is worth noting that although H_2O_2 is an ineffective activator of SoxR (34, 120), nitric oxide (NO) can activate it by forming dinitrosyl iron complexes with the atoms of the iron-sulfur cluster (22, 84). The significance of this effect is uncertain, because dedicated NO scavenging systems are controlled by independent regulatory proteins (NsrR and NorR) that are much more responsive to NO. In one study (92), NO doses that activated the aerobic and anaerobic NO

scavenging systems by 30- and 180-fold only induced *soxS* 5-fold. Furthermore, this slight SoxS induction had no apparent effect on SoxS-controlled genes, leading the authors to suggest that this degree of SoxS induction was inadequate for activation of the full regulon. That outcome dovetails with the failure of 3-fold SoxS induction to provide any protection to SOD^- cells (34).

A More Expansive View: SoxR Beyond *E. Coli*

Studies of SoxR and SoxS in other bacteria have prompted a substantial reappraisal of their biological role. Dietrich et al. (13) found homologs of SoxR among α-, β-, γ-, and δ-proteobacteria and among actinobacteria, but not elsewhere. Strikingly, only enterics employ SoxS; in all the other bacteria, SoxR directly controls expression of all regulon members. Just as striking is the fact that in these nonenterics the SoxR regulon is quite different from that of *E. coli*: It typically comprises only a handful of genes of unclear function (82), and these generally do not include *sodA* or other genes that might confer resistance to O_2^- (13, 14, 51, 82, 101). The implication is that the physiological purpose of SoxR in nonenterics is quite different from its purpose in *E. coli*.

Evidence regarding this purpose has come from studies of *P. aeruginosa* and *Streptomyces coelicolor*. Both bacteria synthesize and excrete redox-cycling compounds: pyocyanin for *P. aeruginosa* and actinorhodin for *S. coelicolor*. Different roles have been suggested for these compounds, including toxifying competitors, signaling, assisting electron transfer to distant respiratory substrates, and solubilizing iron (14, 112, 113). The pumps that excrete these compounds, and perhaps the enzymes that complete their synthesis, are induced in both bacteria when SoxR is oxidized by either the redox agent itself or a precursor to it (13, 101) (**Figure 4*b***). The SoxR protein can also respond to exogenous synthetic redox compounds such as paraquat, but this is presumably adventitious, as SoxR activation does not enhance resistance to such compounds (88). The implication is that these bacteria use SoxR to control the export of endogenous redox agents into the environment.

Whether or not it is to the advantage of the producing organism, these agents can penetrate and toxify other microbes. It is possible that the latter laterally acquired SoxR as a sensor that could perceive these substances and induce defenses. In *E. coli* the arriving *soxR* gene may have piggybacked onto a preexisting system that responds to other toxic chemicals (**Figure 4*b***). SoxS is a paralog of the MarA and Rob transcription factors, which respond to and provide protection against exogenous nonredox agents (salicylate and bile salts, for example). The three proteins are similar enough (~50% identity) that they recognize common regulatory sites, so their regulons overlap extensively (78). The simple model, then, is that *E. coli* acquired SoxR and, after duplication of MarA or Rob, fashioned SoxS to connect SoxR to a preexisting regulon that serves to exclude, modify, or export hazardous compounds. SoxR provided the means to activate this system in response to redox-active compounds. Notably, the reduction potential of *E. coli* SoxR is lower than those of *P. aeruginosa* and *S. coelicolor* SoxR proteins, and accordingly it responds to a larger range of redox agents (100, 102). Extant antioxidant defenses, such as *sodA* and *fumC*, may then have been added to the regulon. The latter genes are significantly more responsive to SoxS than to MarA (78).

A FINAL WORD ABOUT OTHER ANTIOXIDANT REGULONS

Workers have identified distinct bacterial transcription factors that are activated by singlet oxygen [e.g., RpoE/ChrR (65)], organic hydroperoxides [OhrR (17)], disulfide stress [Spx (121) and SigR/RsrA (52)], or hypochlorous acid [HypT (16)]. Unfortunately, space restrictions preclude a discussion of these systems here. Implicit in this diversity is the fact that "oxidative stress" is an

overbroad term that subsumes a panoply of oxidants that (*a*) vary in their chemical properties, (*b*) attack very different targets, and (*c*) are sensed and defended against in completely different ways. For example, none of those systems is activated by physiological doses of H_2O_2 or O_2^-, and they provide very little crossover resistance against them.

PROSPECTS

Because life evolved in an anoxic world, the iron-intensive biochemistry that was inherited by contemporary organisms exhibits some incompatibility with the presence of oxygen. This vulnerability is problematic for bacteria that experience fluctuations in local oxygen tension, and it is exploited by competitors that would stymie their growth. A key goal of current work on these regulons is to identify the natural circumstances in which they are activated. As this review emphasizes, important clues can be deduced from the biochemistry of the sensor proteins themselves and from the membership of the regulons that they control. The emergence of studies in nonenteric bacteria promises to provide clarity by offering new perspectives on these familiar regulators.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Work in the author's lab is supported by grants GM49640 and GM101012 from the National Institutes of Health.

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Annual Review of Microbiology

Volume 69, 2015

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