Regulation of Bacterial Responses to Oxidative Stress

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I. Introduction

Oxidative stress occurs when organisms encounter elevated levels of reactive oxygen species such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH\cdot$). The reactive oxygen species are produced at low rates during normal aerobic respiration in both prokaryotes and eukaryotes. For example, the intracellular superoxide anion concentrations for aerobically growing *Escherichia coli* cells have been measured to be 10^{-10} *M,* while the concentrations of hydrogen peroxide are maintained at around 10^{-7} *M* (27,35). A variety of environmental conditions, however, can lead to increased levels of these reactive oxygen species. Shifts between aerobic and anaerobic environments and exposure to radiation, metals, and xenobiotic drugs capable of reacting with oxygen species can all result in elevated superoxide, hydrogen peroxide, and hydroxyl radical concentrations. Another source of oxidants is the reactive species generated by phagocytes in a defense against microorganisms.

Reactive oxygen species can cause DNA mutations, enzyme inactivation, and membrane damage, but all bacteria, even strict anaerobes, appear to have mechanisms to detoxify these deleterious oxidants. Superoxide dismutases which convert superoxide anion to hydrogen peroxide and catalase/peroxidases which eliminate hydrogen peroxide are central to the defense against oxidative stress and are very con-

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served. Studies have shown that an alkyl hydroperoxide reductase which can convert peroxides such as lineolic hydroperoxide to their corresponding alcohols is also a ubiquitous defense activity (11). In addition, enzymes and DNA binding proteins that repair or protect against oxidative DNA damage are critical and appear to be conserved.

The expression of many of the defense activities is induced by changes in the levels of the hydrogen peroxide or superoxide, suggesting that many cells have mechanisms to sense reactive oxygen species. In this chapter, we review the properties of transcriptional regulators that are important for the induction of antioxidant defense genes in bacteria. The regulators have been best characterized in *E. coli,* but the studies of the oxidative stress responses in other bacterial species are pointing to some interesting similarities and differences between bacteria. Here we compare the oxidative stress responses of *E. coli, Salmonella, Haemophilus, Mycobacterium,* and *Bacillus;* discuss interesting connections between oxidative stress and pathogenesis and drug resistance in these organisms; and propose directions for future studies.

II. Regulators of *Escherichia coli* Responses to Oxidative Stress

Like many genetic responses, the defenses against oxidative stress have been best studied in *E. coli* (see refs. 17 and 22 for comprehensive reviews). *Escherichia coli* cells have two catalase/peroxidase activities, denoted hydroperoxidase I (HPI, encoded by *katG*) and hydroperoxidase II (encoded by *katE*), as well as three superoxide dismutase activities, manganese superoxide dismutase (encoded by *sodA*), iron superoxide dismutase (encoded by *sodB*), and copper-zinc superoxide dismutase (7). The *E. coli* alkyl hydroperoxide reductase activity is composed of two subunits: a 22-kDa subunit (encoded by *ahpC*) and a 52-kDa subunit (encoded by *ahpF*). DNA binding or repair activities that appear to be critical for protection against oxidative damage include a nonspecific DNA binding protein (encoded by *dps*), exonuclease III (*xthA*), endonuclease IV (encoded by *nfo*), and the RecA recombinase.

A. OxyR

Escherichia coli cells show an adaptive response to hydrogen peroxide and approximately 30 proteins are induced when treated with low concentrations of hydrogen peroxide (17,22). The expression of nine of the hydrogen peroxide-inducible proteins is controlled by OxyR. Several of the proteins whose expression is activated by OxyR have been identified and include HPI catalase, the alkyl hydroperoxide reductase, the

Dps protein, and glutathione reductase (encoded by *gorA*). All of these activities have understandable roles in protecting the cell against oxidative damage. OxyR also activates the expression of a small untranslated regulatory RNA denoted OxyS, but the role of this RNA in the defense against oxidative stress is not yet understood (S. Altuvia, D. Weinstein, A. Zhang, L. Postow, and G. Storz, unpublished). OxyR has also been shown to be a repressor and negatively autoregulates its own expression, so a constant level is maintained in the cell. In addition, two studies have shown that OxyR represses the expression of the Mu phage *mom* gene and the *E. coli flu* gene (30), neither of which has an understandable role in the oxidative stress response. An interesting direction for further research will be to elucidate all of the roles of OxyR within the cell, as well as to characterize the other proteins induced by hydrogen peroxide.

The tetrameric OxyR protein is a member of the LysR family of transcriptional activators and has been characterized extensively (17,22,41,42). The protein appears to exist in two forms, reduced and oxidized, but only the oxidized form is able to activate transcription. Direct oxidation of OxyR is therefore the likely mechanism whereby the cells sense hydrogen peroxide and induce the OxyR regulon. The redox-active center in OxyR has been proposed to be a single cysteine, and the next challenge will be to determine the nature of the oxidative reaction that activates OxyR.

OxyR has been shown to bind to promoters by contacting four major grooves on one face of the DNA, and a putative consensus of four repeats of ATAGnt has been defined for OxyR (63). Interestingly, the two forms of OxyR appear to have different binding specificities. The reduced form is able to bind the *oxyR* and *mom* promoters, but not the *katG* and *ahpC* promoters, and contacts ATAG nucleotide repeats in two pairs of adjacent major grooves separated by one helical turn. In contrast, oxidized OxyR has been found to bind all OxyR-regulated promoters that have been tested and binds in four adjacent major grooves. The differences in binding may allow OxyR to carry out different functions under different conditions. Therefore OxyR can repress the *oxyR* and *mom* promoters during normal growth and activate *katG* and *ahpC* in response to oxidative stress. OxyR activates transcription by increasing the binding of RNA polymerase to the promoters and has been shown to require specific surfaces on the C-terminal domain of the α subunit of RNA polymerase (α CTD) to activate transcription (61,62).

B. SoxRS

Escherichia coli cells also adapt to increased levels of superoxide, and key regulators of this response are the SoxR and SoxS proteins (17,22). The genes activated by SoxS include *sodA, nfo, micF, zwf, acnA,*

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fumC, *fpr*, and *acrAB*, in addition to a few other genes identified by *lacZ* fusions or by two-dimensional polyacrylamide gel electrophoresis (PAGE) (Table 1). The corresponding gene products help to eliminate superoxide (*sodA*), repair damaged DNA (*nfo*), reduce outer membrane permeability (*micF* mRNA interferes with the translation of *ompF*, which encodes an outer membrane porin), increase the reducing power of the cell (*zwf*), and encode superoxide-resistant isozymes of fumarase (*fumC*) and aconitase (*acnA*). Unexpectedly, the *soxRS* regulon also confers multiple antibiotic resistance as well as resistance to certain organic solvents and heavy metals, but the genes responsible for the latter defenses are not known.

Regulation of the *soxRS* regulon occurs by a two-stage process. SoxR is first converted to an active form which enhances *soxS* transcription (17,22). The increased level of SoxS, in turn, activates expression of the regulon. Curiously, the genes encoding the two regulators overlap each other, with the *soxR* promoter embedded in the *soxS* structural gene and transcribed in the opposite direction. The constitutively expressed SoxR protein resembles MerR, a regulator of mercury resistance. Like MerR, SoxR is a dimer and has four C-terminal cysteines that are critical for activity. SoxR can be isolated as Fe-free or Fe-containing forms, and both forms can bind the *soxS* promoter, but only the Fe form with two [2Fe:2S] centers per dimer is able to activate transcription *in vitro* (31,32,64). The mechanism of SoxR activation and the nature of the signaling molecule are still under debate. Possibly SoxR exists as an apoprotein, and the full [2Fe:2S] clusters in SoxR are assembled with the iron released from superoxide-sensitive enzymes in the cell (31). Alternatively, the SoxR protein is normally in a reduced [2Fe:2S]$^{+}$ state and is activated by oxidation to a [2Fe:2S]$^{2+}$ state (64). This oxidation may occur through direct exposure to superoxide anion, although Liochev *et al.* argue that SoxR responds to changes in reduced nicotinamide-adenine dinucleotide phosphate (NADPH) or to reduced flavodoxin or ferredoxin levels (46). They observe that when *zwf*$^{-}$ mutants, which have less ability to generate NADPH, are treated with low concentrations of paraquat, the expression of *fumC* and *sodA* is elevated, yet overexpression of manganese superoxide dismutase does not diminish these levels. However SoxR is activated, the regulator appears to distort the *soxS* promoter and thereby increases *soxS* transcription (31).

The SoxS protein activates the promoters of the *soxRS* regulon by mechanisms which involve binding near or at the −35 hexamer. SoxS and a MalE–SoxS fusion protein have been purified and shown to bind to several SoxS-regulated promoters (23,44). The core sequence of a

TABLE 1

GENES RESPONDING TO OVEREXPRESSION OR INDUCTION OF SOXS, MARA, OR ROB[a]

Gene	Map position	Required for:		Increased protein/mRNA expression (increased *lacZ* fusion expression[b]) constitutive expression of:			Treatment with PQ, SoxS	Transcriptional activation *in vitro*		
		Antibiotic resistance	$O_2^{\cdot-}$ resistance	SoxS	MarA	Rob		SoxS	MarA	Rob
acnA	28′			+[c]			+/o (+)			
acrAB	11′	Yes		(+)	(+)	(+)				
fpr	88′			+++			+++	+++	+	+
fumC	36′			+++ (+++)	+	+	+++ (+++)	+++	+	++
inaA	48′	No	No	(+)	(+++)	(+++)	(++)			
micF	48′	Partially	Partially	+ + (++)			+++	+++	++	+++
nfo	47′		Partially	++ (++)	+		+++ (+++)	++	+	+
ompF	21′	Yes		− (−)	− (−)		− (−)			
pqi	22′	No	No	(++)			(++)			
S6 A<C[d]				+	+		+			
sodA	88′			+ (+)	+ (+)	(+)	+ (++)	+	+/o	+
soi 17/19	45′–61′		Yes	+ (+)	+ (+/o)		+ (+++)			
soi 28	45′–61′		Yes	+ (+++)	o (+)		+ (+++)			
zwf	41′		Partially	+ (+)	+ (+)	+	++ (+)	++	+	+

[a] From refs. 2, 3, 14–17, 22, 23, 26, 28, 36–39, 45, 46, 48, and 56.

[b] Values in parentheses are for *lacZ* fusions which are transcriptional except for the *ompF* translational fusion.

[c] +++, 10-fold or greater increase; ++, 5- to 9-fold increase; +, 2- to 4-fold increase; +/o, 1- to 2-fold increase, o, no change, −, >3-fold decrease.

[d] This refers to the increased glutamination of the small ribosomal subunit protein S6, as seen by two-dimensional PAGE.

proposed SoxS box is AnnGCAY. For some promoters such as *zwf* and *fpr,* the sequences bound by SoxS do not overlap the −35 promoter sequence, and *in vitro* transcription experiments have shown that activation of these promoters requires the α CTD of RNA polymerase, indicating direct contact between SoxS and RNA polymerase (36). In contrast, at other promoters such as *micF* and *fumC,* SoxS binds at sites overlapping the −35 hexamer, and the α CTD of RNA polymerase is not required for activation. Thus, SoxS, like the *E. coli* cyclic adenosine monophosphate (cAMP) receptor protein (CRP), is an "ambidextrous" transcriptional activator and activates RNA polymerase differently at different promoters. SoxS also binds to its own promoter, where it appears to downregulate its own transcription (54).

C. MarA, Rob

A surprise has been the finding that the *soxRS* regulon overlaps the regulon controlled by MarA, a regulator identified as part of an operon conferring multiple antibiotic resistance, and the regulon controlled by Rob, a protein detected originally by its ability to bind DNA near the bacterial origin of replication (3,17) (see Table I). Transcription of the *marRAB* operon is repressed by MarR, which binds to two sites between the −35 transcriptional signal and the initial MarR methionine (49). The operon is induced by a variety of phenolic compounds, including salicylate and 2,4-dinitrophenol, and apparently by certain antibiotics, such as tetracycline. Salicylate, but not chloramphenicol or tetracycline, has been shown to bind MarR and thereby reduce the binding of MarR to the promoter (49). Derepression of the operon then results in synthesis of MarA, a transcriptional activator with about 40% sequence identity to SoxS and 50% identity to Rob. What regulates Rob and why it has a high constitutive level in the cell (5000 molecules) is not known (60).

SoxS, MarA, and Rob resemble each other is a number of ways. They share the DNA binding motif characteristic of the AraC subfamily of transcriptional activators; are ambidextrous activators of many, if not all, of the same promoters *in vitro* and *in vivo;* bind to these promoters at very similar, in some cases identical, sequences; bend the DNA; and bind as monomers (36–38). Each of these transcriptional activators is functional in the absence of the others. Nevertheless, the degree to which the *mar, soxRS,* and *rob* regulons differ from each other has not been systematically studied. It may be that SoxS activates functions involved in superoxide defense more than MarA or Rob does (Table I), but the basis for the differences in expression is not known. Furthermore, it has not been clearly established which of the functions are required for antibiotic or superoxide resistance, and none of the genes have been evaluated with regard to the heavy metal- and organic solvent-resistance phenotypes seen for strains that overexpress SoxS or Rob (52).

D. RpoS

One additional regulator that cannot be excluded from discussions of the *E. coli* response to oxidative stress is the *rpoS*-encoded σ^s, subunit of RNA polymerase (see ref. 47 for a comprehensive review). This sigma (σ) factor is important for the expression of a large group of genes that are induced when cells encounter a number of different stresses, including starvation, osmotic stress, and acid stress, as well as on entry into stationary phase. Starved and stationary phase cells are intrinsically resistant to a variety of stress conditions, including high levels of hydrogen peroxide, and RpoS has been shown to regulate the expression of the antioxidant defense activities encoded by *katG, katE, dps, xthA,* and *gorA* (1,6,47). The *katG, dps,* and *gorA* genes are activated by OxyR, suggesting that *E. coli* cells have two regulons that can protect against exosure to hydrogen peroxide: the OxyR regulon during exponential growth and the σ^s regulon in stationary phase. It seems likely that some of the SoxS/MarA/Rob-regulated genes are also regulated by σ^s, and one SoxS target, *acrAB,* has been shown to be induced in stationary phase (48).

The regulation of σ^s levels occurs at multiple levels, and much remains to be learned (47). The transcription, translation, and stability of σ^s are all modulated in response to different signals, including the starvation signal ppGpp, a cell density signal homoserine lactone, cAMP, and uridine diphosphate (UDP)-glucose (9,34,43,47). The regulators that transmit the different stress signals are now being identified. They include CRP, which regulates transcription, and the general regulator H-NS, which affects both the translation and stability of σ^s (5,65). Studies have also shown that the stability of σ^s is controlled by the ClpXP proteases and a response regulator protein alternatively denoted Hns/RssB/SprE (50,55,58). Among the interesting questions to be addressed in the future are how the different responses are integrated and whether oxidative stress has a direct impact on σ^s levels.

III. Oxidative Stress Responses in *Salmonella, Haemophilus, Mycobacterium,* and *Bacillus*

Antioxidant defense activities have been characterized in a number of different bacterial species, and it will be interesting to learn more

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about how the expression of the corresponding genes is regulated in the different organisms. In this section, we review the oxidative responses in prokaryotes in which regulators have been identified (see Table II).

A. *Salmonella typhimurium*

All studies of the responses to oxidative stress in *S. typhimurium* suggest that they are very similar to the responses in *E. coli*. *Salmonella typhimurium* has homologs of the peroxidases and superoxide dismutases encoded by *katG, katE, ahpCF, sodA,* and *sodB,* and mutational studies and sequence analysis have shown that the OxyR, SoxRS, MarA, and RpoS regulators are also present in *S. typhimurium* (17,22,47, 60a; E. A. Martins and B. Demple, personal communication).

The *S. typhimurium* responses to oxidative stress are interesting in that several connections to virulence have been reported. Strains carrying either an *oxyR* constitutive mutation or an *oxyR* deletion mutation are less virulent than the corresponding wild-type parent *in vivo,* suggesting that the OxyR-regulated response plays a role in virulence (24). The role of RpoS in *Salmonella* virulence is even more clearly established. Like *E. coli rpoS*$^-$ mutants, the *S. typhimurium* mutant strains show increased sensitivity to nutrient limitation, acid stress, and DNA-damaging agents, as well as oxidative stress (21). The *rpoS*$^-$ mutants also show significantly reduced virulence in mice. The oral lethal dose for the mutant strain is 1000-fold greater than for the wild-type parent. The role of RpoS in virulence is probably complex. Most likely, many of the chromosomally encoded RpoS-regulated genes which help the cells survive against general stress help the bacteria survive in the host environment. However, RpoS also modulates the expression of the *spvRABCD* genes carried on virulence plasmids (21,53). Studies of several different *lacZ* fusion constructs in combination with mutant backgrounds suggest that RpoS controls the level of SpvR, a LysR family-type transcriptional regulator, which, in turn, activates expression of the *spvABCD* genes (40).

TABLE II
REGULATORS OF RESPONSE TO OXIDATIVE STRESS IN PROKARYOTES[a]

Regulator	Map position of *E. coli*	Homologs found in
OxyR	89.6′	*Escherichia coli* *Salmonella typhimurium* *Haemophilus influenzae* *Mycobacterium*
SoxR/SoxS	92.2′	*Escherichia coli* *Salmonella typhimurium*
MarA	34′	*Escherichia coli* *Salmonella typhimurium*
RpoS	59′	*Escherichia coli* *Salmonella typhimurium* *Haemophilus influenzae* *Klebsiella pneumoniae* *Shigella flexneri* *Pseudomonas aeruginosa*

[a] From refs. 17, 18, 22, 25, 47, 59, and 60a; E. A. Martins and B. Demple, personal communication.

B. *Haemophilus influenzae*

A scan of the completed sequence of the entire genome of *H. influenzae* suggests that *H. influenzae* encodes homologs of *katE* (denoted *hktE*) and *sodA*. (25). Surprisingly, the *hktE*-encoded KatE homolog has been shown to be regulated like *E. coli katG*. The HktE catalase levels are higher in exponential cells than in stationary phase cells, and the *hktE* message and protein are induced by treatment with hydrogen peroxide or ascorbic acid, which generates hydrogen peroxide in the presence of oxygen (8).

The regulator of *hktE* induction has not been reported, and SoxS, SoxR, MarA, Rob, and RpoS homologs are not apparent from the genomic sequence. However, homology searches do indicate the presence of a gene encoding a protein that is more than 70% identical to *E. coli* OxyR. Interestingly, this gene was identified as *tbpR* in a screen for multicopy clones that lead to expression of a transferrin binding activity in *E. coli* (48a). The reason for the effect on transferrin binding activity is not known, but *H. influenzae* strains carrying mutations in *tbpR/oxyR* do show increased sensitivity to hydrogen peroxide. Therefore, TbpR/OxyR appears to play a role in regulating the response of *H. influenzae* to oxidative stress, and it is interesting that, as noted by Bishai *et al.*, the *hktE* promoter has some similarity to the OxyR consensus sequence (8).

C. *Mycobacterium*

It has been long been known that *Mycobacterium* strains have hydroperoxidase I that is similar to the *E. coli katG*-encoded peroxide. This activity has been the focus of several studies since strains carrying mutations that inactivate *katG* are resistant to isonicotinic acid hydrazide (isoniazid, INH), one of the main antimycobacterial drugs (for a review, see ref. 66). For wild-type *M. smegmatis,* the minimum inhibi-

tory concentration of INH is 32 μg/ml compared to 512 μg/ml for *katG*⁻ mutant strains. *Escherichia coli* cells are constitutively more resistant to INH, and *katG* mutants do not show increased resistance. Therefore, an observation that *E. coli oxyR*⁻ mutants or *katG*⁻ *ahpCF*⁻ double mutants are more sensitive to INH seemed almost contradictory. Further studies, however, showed that the *oxyR*⁻ mutants as well as the *katG*⁻ *ahpCF*⁻ double mutants had increased endogenous hydrogen peroxide levels, and hydrogen peroxide alone could potentiate the effects of INH (57). These and other results are consistent with the view that INH is a prodrug that can be activated by peroxides or by mycobacterial hydroperoxidase I and subsequently inactivate other targets within the cell. In *Mycobacterium,* the primary target of the activated INH appears to be mycolic acid biosynthesis (4), while in *E. coli,* DNA is likely to be a target since INH treatment of susceptible cells results in filamentation and mutagenesis (J. L. Rosner, R. G. Martin, and P. M. R. Achary, unpublished data). The different effects of the *E. coli* and *Mycobacterium katG*⁻ mutations can be explained by the finding that *M. tuberculosis* hydroperoxidase I is more effective at INH-dependent generation of radicals than is *E. coli* hydroperoxidase I (33).

The regulation of the *katG*-encoded hydroperoxidase and other antioxidant enzymes in *Mycobacterium* is not well understood. However, two studies have provided some interesting insights (18,59). Sequence homology searches showed that *M. avium* and *M. leprae* encode proteins with strong similarity to the AhpC protein of *S. typhimurium* and *E. coli* (11). A gene encoded directly upstream of both the *M. avium* and *M. leprae ahpC*-like genes showed homology to the LysR family of transcriptional regulators (18,59). The close proximity to *ahpC* led to the suggestion that this LysR family member encodes the *Mycobacterium* OxyR, but this remains to be proven rigorously. The putative homologs show only 33% identity to *E. coli* OxyR, in contrast to the 70% identity seen for *H. influenzae;* however, the amino acids surrounding the critical cysteine in *E. coli* OxyR are conserved. Intriguingly, whereas the putative *oxyR* genes from *M. avium* and *M. leprae* appear to be intact, the genes from *M. tuberculosis* and other members of the *M. tuberculosis* complex, such as *M. bovis* BCG, *M. africanum,* and *M. microti,* contain numerous deletions and frameshifts and are probably nonfunctional.

Studies of the oxidative stress responses of various myocobacteria show that the different strains have significantly different responses to hydrogen peroxide (59). Only the saphrophytic strain *M. smegmatis* showed adaptation analogous to the OxyR-regulated response. *Mycobacterium smegmatis* bacilli pretreated with 50 μ*M* hydrogen peroxide became resistant to 5 m*M* hydrogen peroxide and showed the induction of several proteins, as seen on two-dimensional gels. *Mycobacterium avium* and *M. bovis* BCG strains were constitutively more resistant to 10 m*M* hydrogen peroxide but did not show any adaptation. In *M. avium,* only three proteins were induced by hydrogen peroxide, and only the expression of the *katG*-encoded hydroperoxidase I expression was induced in *M. bovis.* These findings have led to the hypothesis that pathogenic mycobacteria may continuously encounter reactive oxygen species in their host environments and therefore constitutively express antioxidant defense activities, eliminating the need for a functional OxyR protein.

D. *Bacillus subtilis*

Several proteins that are counterparts to *E. coli* antioxidant defense activities have been identified in *B. subtilis.* These include a vegetative catalase (encoded by *katA*), a catalase present in spores (encoded by *katE*), and proteins with similarity to the two alkyl hydroperoxide reductase subunits, as well as a 16-kDa protein (encoded by *mrgA*) that is similar to Dps (12,20). The presence of an adaptive response to hydrogen peroxide has also been known in *B. subtilis* for many years. Pretreatment of exponentially growing *B. subtilis* cells with 50 μ*M* hydrogen peroxide results in protection against killing by 10 m*M* hydrogen peroxide and leads to the induction of eight proteins, as detected on one-dimensional gels (51).

The regulators of this response are now being identified by mutational studies. One mutant was isolated by screening for resistance to hydrogen peroxide (29). While the wild-type parent lysed in 100 m*M* hydrogen peroxide, the mutant strain grew with a doubling time of 85 min in minimal medium containing 150 m*M* hydrogen peroxide. The mutant was also more resistant to organic peroxides and synthesized a number of proteins at a much higher rate than the wild type. The constitutively expressed proteins included the KatA catalase, the two subunits of alkyl hydroperoxide reductase, MrgA/Dps, flagellin, and all the proteins which were induced by hydrogen peroxide in the wild-type strain (29). In a complementary screen, Chen *et al.* isolated mutants with increased *mrgA* expression in the presence of Mn(II) (13). Trans-acting mutants identified in this screen were also resistant to hydrogen peroxide and expressed increased levels of KatA, AhpCF, and MrgA/Dps, indicating that the mutations isolated in both screens affected the same locus. The outcome of these screens also suggested that the Mn(II)-dependent repression and hydrogen peroxide-dependent induction of these genes are mediated through the same pathway. Chen *et*

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al. noticed that the *katA* and *mrgA* promoters both have inverted repeats of the sequence TTAtAAt. Because point mutations in this region of the *mrgA* promoter lead to derepression of *mrgA,* the redox-sensitive regulator of *katA, ahpCF,* and *mrgA* is likely to be a repressor. Therefore, in contrast to OxyR in *E. coli,* the presence of hydrogen peroxide in *B. subtilis* may be sensed by a peroxide-sensitive repressor.

Like *E. coli,* stationary phase and starved *B. subtilis* cells are much more resistant to hydrogen peroxide than exponentially growing cells, and some of the proteins induced by protective concentrations of hydrogen peroxide are, also induced on entry into the late log phase (19). Because the starvation and sporulation responses are well characterized in *B. subtilis,* the response to hydrogen peroxide was examined in sporulation (*spoO*) mutants. Five *spoO* mutants (*spoOB, E, F, H, J*) were indistinguishable from wild-type cells; however, strains with *spoOA* mutations showed altered resistance to hydrogen peroxide. The stationary phase induction of a *katA–lacZ* fusion was also shown to be dependent on *spoOA* (10). The SpoOA DNA binding protein controls the expression of the negative regulator encoded by *abrB,* and the *SpoOA* mutant phenotypes can be suppressed by mutations affecting this downstream regulator. These studies suggest that the expression of the *B. subtilis katA* gene may be similar to the expression of the *E. coli katG* gene, with induction in both exponential phase and stationary phase, but the corresponding regulators are likely to be different. Studies have also shown that *B. subtilis* cells have a second catalase with homology to the *E. coli katE*-encoded hydroperoxidase. The expression of this *katE* gene is regulated by the *sigB*-encoded σ^B, which shows similarity to the *rpoS*-encoded σ^S (20).

IV. Concluding Remarks

Although much remains to be learned about the bacterial responses to oxidative stress, some general themes have emerged. From the studies of *E. coli,* it appears that bacterial cells perceive superoxide differently from hydrogen peroxide. However, while the responses to hydrogen peroxide and superoxide are distinct, the two regulons may both overlap with the general stress response that is induced by starvation or entry into stationary phase. It is also noteworthy that mutations affecting antioxidant defense genes or their corresponding regulators alter antibiotic resistance in both *E. coli* and *Mycobacterium.* In addition, a comparison of *E. coli* and *B. subtilis* suggests that the oxidative stress regulators may not necessarily be conserved, and bacteria may use different mechanisms for sensing the same oxidant.

Several interesting questions for future studies are raised by our current understanding of the bacterial responses to oxidative stress: What are the chemical reactions that lead to SoxR and OxyR activation? Why do diverse treatments such as salicylate and superoxide trigger the same set of diverse responses, superoxide resistance and multiple antibiotic resistance? What are the roles of the still unidentified proteins that are induced by the different oxidative stress conditions, and how do they protect the cell? The answers to these questions should help elucidate the mechanisms that are used to sense and defend against oxidative stress in all organisms.

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