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Oxidative Stress Responses in *Escherichia coli* and *Salmonella typhimurium*

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INTRODUCTION AND SCOPE OF REVIEW

Oxidative stress can be functionally defined as an excess of prooxidants in the cell. Active oxygen molecules have been shown to cause damage to DNA, RNA, protein, and lipids. Active oxygen species are produced as an inescapable by-product of normal aerobic metabolism, and their production is further enhanced by exposure to certain environments or by dietary or disease conditions. Oxygen toxicity results when the degree of oxidative stress exceeds the capacity of the cell defense systems. Oxidative stress is

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strongly implicated in a number of diseases such as rheumatoid arthritis, inflammatory bowel disorders, and atherosclerosis (102, 104). It is also emerging as one of the most important causative agents of mutagenesis, tumorigenesis, and aging (1, 3–5, 40, 75). Virtually all aerobic organisms have evolved complex defense and repair mechanisms to mitigate the damaging effects of active oxygen (160, 161).

Because of the powerful genetic techniques available for manipulations in bacteria, tremendous progress has been made toward understanding the genetic and physiological responses to oxidative stress in bacteria. Many aerobic bacteria tested to date appear to encode multigene responses to oxidative stress (for example, see reference 25). In this article, we will summarize what has been learned to date about cellular responses to oxidative stress, primarily in *Escherichia coli* and *Salmonella typhimurium*. We will briefly describe the reactivities of active species of oxygen, detail the genetics and molecular biology of oxidative stress responses, and discuss the physiological role of the responses in oxidative stress. More recently, responses analogous to those first characterized in bacteria have been found in mammalian cells (e.g., 55a, 131, 185a). We will conclude by briefly reviewing the similarities and differences between oxidative stress responses in bacteria and eukaryotic organisms.

This review is intended to be comprehensive and self-contained so that most readers will obtain a current picture of the field without frequent consultation with references. Readers familiar with the background information might skip the beginning section.

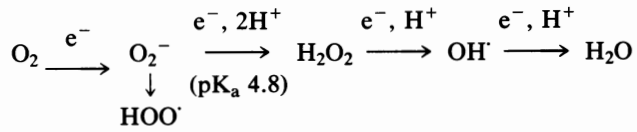
Portions of the material covered in this article have been reviewed elsewhere in greater detail. The reader is referred to the following reviews for further coverage of specific topics: the chemistry and biochemistry of oxygen radicals (34, 36, 95, 112, 205); the sources of active oxygen species (3, 74, 188); oxygen radicals and tissue damage, disease, aging, and cancer (75, 98, 104, 198, 207); oxygen radicals and DNA damage and repair (7, 56, 66, 119, 147, 197, 219, 233); lipid peroxidation (61, 79, 80, 103, 129); bacterial responses to other forms of stress (67, 87, 96, 159, 176, 177); and superoxide dismutases (77, 210, 222).

OXIDATIVE STRESS RESPONSES

Active Oxygen Species

Molecular oxygen (O_2) has an even number of electrons. However, it has two unpaired electrons in its molecular orbitals, one in the π^*Y antibonding orbital and one in the π^*Z antibonding orbital; the σ^*Z orbital is empty. These electrons have the same spin quantum number (parallel spins). Molecular oxygen cannot easily oxidize another molecule by accepting a pair of electrons since typically a pair of electrons in an atomic or molecular orbital would have antiparallel spins. As a consequence of its being spin restricted, molecular oxygen is unreactive with most compounds except radicals. Thus oxidation by molecular oxygen is limited to acceptance of electrons. For thermodynamic reasons, molecular oxygen is a poor acceptor of one electron [$E_0(O_2/O_2^-) = -0.33$ V] (see reference 36 and references therein). The spin restriction of molecular oxygen can be overcome by interaction with another paramagnetic center. Transition metals such as Fe or Cu are good catalysts for the one-electron reduction of O_2 (sources of active oxygen species are discussed below).

Its reactivity increases upon acceptance of one, two, or three electrons to form, respectively, a superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^\cdot$), or when it undergoes a spin flip to become singlet oxygen ($^1\Sigma_g$ O_2) (76, 112). The overall four-electron reduction of molecular oxygen to water is shown below.



Under more acidic conditions, O_2^- can be protonated to form $HOO^\cdot$ (hydroperoxyl radical) (19).

Reactivities. O_2^- will oxidize thiols, ascorbate, tocopherol, and catecholamines (78, 112, 156). Proteins containing $(Fe\text{-}S)_4$ clusters are highly sensitive to attack by O_2^- (80). Probably the most important reactions of O_2^- are its spontaneous dismutation to $H_2O_2 + O_2$ and its ability to reduce transition metals and metal complexes. Spontaneous dismutation of O_2^- in aqueous neutral pH environments produces H_2O_2 (78). As mentioned above, when O_2^- is protonated, the hydroperoxyl radical ($HOO^\cdot$) is produced ($O_2^- + H^+ \rightarrow HOO^\cdot$). The $HOO^\cdot$ radical is much more reactive than O_2^- because the negative charge has been neutralized. The pK_a of this species is 4.8. Thus, at physiological pH, the ratio of O_2^- to its protonated form would be about 100:1. When the pH of the cell (or mitochondria) decreases, such as when oxidative membrane damage has occurred (as will be discussed in a later section), the ratio of $HOO^\cdot$ to O_2^- increases.

O_2^- also can act as a reducing agent for transition metals. In vivo the relevant transition metals are Fe^{3+} and Cu^{2+}, although other transition metals such as titanium can be reduced (34). The general reaction is as follows:

$$O_2^- + M^{(n+1)+} \rightarrow O_2 + M^{n+}$$

The reduction of cupric and ferric ions by O_2^- can occur even when the metals exist in complexed form. For example, cytochrome *c* (Fe^{3+}) can be reduced by cytochrome *c* (Fe^{2+}) by O_2^- (8, 160).

The reactions of H_2O_2 with organic molecules remain unclear, partly because it reacts quickly with contaminating metals to form more reactive species which obscure its own role in oxidation reactions. It can act as a weak oxidizing agent and will attack thiol groups of proteins or reduced glutathione. It can also react directly with some keto acids (104, 237). Most significantly, H_2O_2 will react with reduced iron or copper ions to generate hydroxyl radicals ($OH^\cdot$) in the Fenton reaction (see reference 36 and references therein). Since O_2^- will reduce both Fe^{3+} and Cu^{2+} and since its dismutation produces H_2O_2, it is likely that when the intracellular concentration of O_2^- increases, the concentrations of H_2O_2 and $OH^\cdot$ will also rise.

Reactions of hydroxyl radicals are numerous because $OH^\cdot$ is so reactive that within the cell it will react with most biomolecules at diffusion-limited rates (206). The reactivity of $OH^\cdot$ is due to its very high standard electrode potential, +2.3 V (the standard potential of O_2 is ca. +0.8 V). It will oxidize almost anything but ozone. Because of the reactivity, the average diffusion distance of an $OH^\cdot$ radical is only a few nanometers (206), and thus its effects on any given biomolecule will depend largely upon the location of its formation.

Another form of highly reactive oxygen is ozone (O_3). O_3 is a ubiquitous air pollutant that is formed during photoreactions involving NO_2 and hydrocarbons. Ozone decom-

poses in pure water to form OH· and HOO· radicals with a bimolecular rate constant of 50 to 70 M^{-1} s^{-1} (209). Besides its decomposition to OH· and HOO·, ozone reacts rapidly with dienes, amines, and thiols. Mechanisms of ozone reactions with biomolecules include formation of ozonides (cyclic peroxides), or H_2O_2 and aldehydes, or both, through reactions with dienes such as polyunsaturated fatty acids. Ozone may also directly oxidize sulfhydryl (or thiol) groups of amino acids in low-molecular-weight compounds or proteins (166).

Almost all reactions involving singlet oxygen with biomolecules are additions of the $^1O_2$ ($^1\Sigma_g$ O_2) to conjugated bonds. Typically, peroxides are the initial products. $^1O_2$ can add to olefins to produce allylic hydroperoxides and a shift in the double bond, or it can add to diene systems to produce endoperoxides (76). Reactions of $^1O_2$ with unsaturated fatty acids or histidine are good examples of these types of reactions. $^1O_2$ can also undergo (2 + 2) cycloadditions to dienes to produce dioxetanes, which decompose to yield carbonyl compounds (137). The second-order rate constants for the reactions of singlet oxygen with histidine and tryptophan are 1×10^8 and 3×10^7 M^{-1} s^{-1}, respectively (205). Finally, $^1O_2$ will react with α-tocopherol to produce a stable tocopherol radical called chromoxal. Many of the sensitizers which produce $^1O_2$ are embedded within the membrane, and it is possible that $^1O_2$ is capable of initiating lipid peroxidation (129).

In addition to the above active oxygen species, there is growing evidence that iron-oxygen complexes may play a key role in several types of oxidative mechanisms. A superoxide anion will form a complex with Fe^{3+} to yield a "perferryl" radical, $[2Fe^{2+}O_2]^{\cdot}$. This species is not thermodynamically capable of undergoing oxidative reactions with most biomolecules, but will undergo a series of reactions to produce the ferryl radical, $[2Fe^{2+}O]^{\cdot}$ (10, 11, 27, 28). This species is electron rich, has radical characteristics, and is not spin restricted in its reactions. Consequently, it is proposed as one of the major initiating species of lipid peroxidation and possibly DNA damage as well (10, 119). It is worth noting that formation of the perferryl radical will be enhanced under conditions of increased O_2^-.

Sources. Active oxygen species are produced by a variety of compounds and enzymes. The four-electron reduction of O_2 to H_2O is done sequentially and is catalyzed enzymatically by several membrane-associated respiratory-chain enzymes. Recently, Imlay and Fridovich (116) have shown that autoxidation of NADH dehydrogenase, succinate dehydrogenase, and D-lactate dehydrogenase are major sources of O_2^- in *E. coli*. One cytosolic enzyme thought to be capable of generating significant amounts of O_2^- is glutathione reductase, which uses NADH as an electron source (116, 158). Cytochrome P-450s have been shown to be important sources of active oxygen species in mammalian tissues. In certain mammalian tissues, specifically the liver, P-450s can represent up to 4% of the total cell protein (68a). Whereas bacteria contain several P-450s, the role of these cytochromes in production of oxy-radicals is not known.

Nonenzymatic production of O_2^- occurs by autoxidation of several cellular components including ubiquinols, catechols, thiols, and flavins. Electrophilic quinone compounds, both natural cellular constituents (such as ubiquinone) and exogenous sources (such as plumbagin and menadione) (Fig. 1), are easily reduced to semiquinones, which in turn readily reduce O_2 to O_2^-, regenerating the oxidized quinone. The oxidized quinone can undergo this cycle numerous times and is thus referred to as a redox-cycling agent. Paraquat (methyl

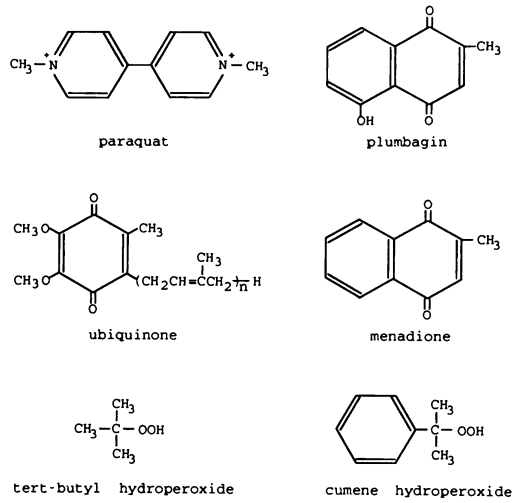


FIG. 1. Commonly used superoxide radical generators and organic peroxides.

viologen), a dipyridyl, is also a very effective redox-cycling agent (Fig. 1). As indicated above, reduced transition metals, both free and in complexed form, can donate a single electron to molecular O_2 to generate O_2^-. This reaction is readily reversible and depends upon the concentrations of reduced and oxidized metal and of O_2 and O_2^-.

Sources of H_2O_2 include spontaneous and superoxide dismutase (SOD)-catalyzed dismutation of O_2^-, as well as several oxidases such as D-amino acid oxidase. There is also abundant evidence that H_2O_2 a photoproduct of near-UV irradiation (67).

Sources of OH· are numerous. Tryptophan residues generate OH· upon absorption of a photon of 365 nm. Radiolysis of water produces OH· as well as a myriad of other active oxygen species (112). Another significant source of OH· is the reaction of H_2O_2 with reduced iron in the Fenton reaction:

$$H_2O_2 + Fe^{2+} + H^+ \rightarrow OH^{\cdot} + H_2O + Fe^{3+}$$

Since O_2^- will reduce transition metals such as iron and since O_2^- is dismutated to form H_2O_2, it is highly likely that when the steady-state concentration of O_2^- rises, the steady-state concentrations of H_2O_2 and OH· will also rise.

Singlet oxygen may be generated in vivo by the action of certain enzymes, decomposition of superoxide, decomposition of peroxidized glutathione (GSOO·), and photosensitization reactions with endogenous sensitizers such as riboflavin and bile pigments (76, 205). A combination of the photosensitizer methylene blue and visible-light radiation is also known to generate singlet oxygen in vitro (68).

Oxidative Stress and Cellular Responses

As discussed above, active species of oxygen naturally occur in aerobic cells, arising from a variety of intracellular and extracellular sources. It is not surprising, therefore, that cells maintain a strong defense against the threat. For example, aerobically growing *E. coli* cells are equipped with two superoxide dismutases (SODs), Mn-containing SOD (MnSOD, encoded by *sodA*) and Fe-containing SOD (FeSOD, encoded by *sodB*). SODs dismutate O_2^- to H_2O_2. Catalases (HPI catalase encoded by *katG* and HPII encoded

by *katE*) disproportionate H_2O_2 into H_2O and O_2. Alkylhydroperoxide reductase (Ahp; encoded by *ahpC* and *ahpF*) is thought to provide additional defense by reducing various organic hydroperoxides.

Under certain circumstances, the concentration of active oxygen rises to a level that overwhelms the basal level of the scavenging capacity of the cell, giving rise to an oxidative stress condition. Artificially, oxidative stress can be brought about by addition of H_2O_2 or superoxide radical generators such as paraquat and plumbagin (Fig. 1) or by raising the partial pressure of oxygen by bubbling pure oxygen through a culture. Mutational inactivation of genes encoding catalases and superoxide dismutases is also an effective means of imposing oxidative stress onto aerobically growing cells. For example, in *E. coli sodA sodB* double-mutant cells completely lacking SOD activity (37), the concentration of endogenous O_2^- may be elevated 2.5×10^3-fold over that in aerobically growing wild-type cells (116).

Bacteria respond to a variety of stresses and undergo derepression of a set of globally regulated genes (87). At least 13 different multigene systems (stimulons) are known to be induced in the response to a variety of stress stimuli (176). When oxidatively stressed, bacteria respond by invoking one or both of two known stimulons.

Peroxide stress response. In response to an increased flux of H_2O_2 and other organic peroxides such as *tert*-butyl hydroperoxide and cumene hydroperoxide (Fig. 1), the cellular concentrations of at least 30 proteins become elevated over the basal levels. We define this response as the peroxide-mediated stress response, although the actual stimulus for the induction is not known. The peroxide stimulon includes eight proteins in *E. coli* and at least nine proteins in *S. typhimurium* that are positively regulated by the locus *oxyR*. The genes encoding these proteins constitute the OxyR regulon (see below for details). Thus, both *E. coli* and *S. typhimurium* deleted for the *oxyR* gene (*oxyR*Δ) fail to induce these proteins. In OxyR(Con) mutants, the levels of these proteins are constitutively elevated.

Concomitantly with the derepression of the peroxide stimulon, bacteria acquire resistance to peroxide stress. For example, cells pretreated with a low dose of H_2O_2 display enhanced resistance to subsequent challenge doses of H_2O_2 (57). The increased survival derives, at least in part, from an induced DNA repair capacity. Thus, λ phage damaged by exposure to H_2O_2 have a higher plating efficiency on pretreated cells than on naive cells (57). Since λ phage inject naked DNA into the host cells, increased plating efficiency indicates an increased repair capacity for oxidatively damaged DNA. As the OxyR response does not appear to include a DNA repair pathway (70d), this response must be part of the broader peroxide stimulon.

Superoxide stress response. When stressed under elevated levels of the superoxide radical anion, O_2^-, bacteria respond by invoking an entirely different stimulon. Treatment of cells with paraquat, for example, results in induction of more than 30 proteins (91, 234). These superoxide stimulon proteins are for the most part different from those belonging to the peroxide stress stimulon (see below for details). At least six of the superoxide stimulon proteins are known to be regulated by the products of two regulatory genes, *soxR* and *soxS* (92, 226, 241). Mutations inactivating either of the two genes render cells noninducible for the proteins. Thus, SoxR and SoxS proteins positively control the expression of the SoxRS regulon genes.

As in the peroxide stress response, the superoxide stress response enhances survival of the stressed cells. Cells pretreated with a nonlethal dose of plumbagin, for example, display enhanced survival upon an exposure to a challenge dose (71). An increased capacity of pretreated cells for reactivating λ phage damaged by treatment with O_2^--generating agents indicates that the superoxide stress response includes an increased DNA repair capacity (71). This is supported by the finding that the level of endonuclease IV, a DNA repair enzyme belonging to the SoxRS regulon, is dramatically elevated in cells treated with O_2^--generating agents (41).

The two oxidative stress responses are distinct. A large volume of evidence has accumulated which indicates that the peroxide and superoxide stress responses are distinct. For example, cells pretreated with H_2O_2 and cells preinduced with O_2^- generators do not develop cross-resistance; i.e., H_2O_2-treated cells show no enhanced survival when exposed to plumbagin, and vice versa (71). Similarly, H_2O_2-pretreated cells reactivate H_2O_2-damaged phage but not O_2^--damaged phage, whereas O_2^--pretreated cells reactivate O_2^--damaged phage but not H_2O_2-damaged phage. Proteins induced with H_2O_2 are, for the most part, different from those induced under O_2^--generating conditions (234). Furthermore, the OxyR and SoxRS regulons are controlled by two distinct control elements. Most of the OxyR regulon genes are not inducible with O_2^- generators and vice versa. Detailed analyses of the two responses and their regulation are described below.

OXIDATIVE STRESS-INDUCIBLE PROTEINS

Several approaches have been taken to examine proteins and genes induced under oxidative stress conditions. Two-dimensional gel electrophoresis analysis of cell extract from ^{35}S-labeled cells is an effective means of probing induction of proteins (179). Since stress proteins are induced at different times after the onset of stress (44, 228), the timing of protein labeling is a crucial factor in determining the kinds of proteins induced. Also important is the means by which stress is brought about. For example, chronic O_2^- stress brought about by the lack of SOD in aerobically growing SOD$^-$ cells (i.e., *sodA sodB* double mutants) induces proteins that are not seen in wild-type cells stressed by addition of paraquat (234). Treatment with different redox-cycling agents (e.g., paraquat, plumbagin, and menadione), as well as with different concentrations of these agents, results in different patterns of induced proteins (91, 234). These variables would account at least in part for the considerable discrepancy found among reports in the literature.

The second approach is to monitor enzyme activities during the stress (44, 91, 107). Several enzymes have been identified as being induced under various oxidative stress conditions. These are included in Table 1. The third approach is to screen random operon fusions to a reporter gene (e.g., *lacZ*) for inducibility by oxidative stress (132). Since the fusion event often leads to inactivation of the gene, inspection of a two-dimensional electrophoresis gel for disappearance of a protein spot allows one to identify a putative protein which the gene encodes (234). The *soi*::*lacZ* fusions have been cloned (70d) and partially characterized (70a).

A number of proteins that are induced in *S. typhimurium* and *E. coli* under various oxidative stress conditions have been detected. Table 1 lists some of the oxidatively inducible proteins for which cellular activities or encoding genes, or both, are known. The examination of the patterns of induced proteins permits the following generalization. (i) The proteins that are induced by O_2^- stress are, for the most part,

TABLE 1. Stress proteins and genes inducible by oxidative stress

Protein	Species	Polypeptide	Gene	Inducible[a] by stress (reference):				
				H_2O_2	Paraquat	Menadione	Heat	Nalidixic acid
HPI catalase	*S. typhimurium*	D69/D71	*katG*	+ (44, 171)	?	?	− (171)	− (171)
	E. coli	D78/D78.1	*katG*	+ (91, 228, 234)	− (234)	+ (91)	− (228)	+ (228)
Ahp	*S. typhimurium*	F52a	*ahpF*	+ (44, 171, 212)	?	?	+ (44, 171, 212)	?
		C22	*ahpC*	+ (44, 171, 212)	?	?	− (44, 171, 212)	?
	E. coli	F50.6	*ahpF*	+ (91, 234)	+ (91, 234)	+ (91)	− (228)	− (228)
		B20.9	*ahpC*	+ (91, 234)	+ (91, 234)	+ (91)	− (228)	− (228)
Glutathione reductase	*S. typhimurium*	?[a]	?	+ (44)	?	?	?	?
	E. coli	F45.6	*gor*	?	?	?	?	?
MnSOD	*S. typhimurium*	?	?	+[b] (44)	?	?	?	?
	E. coli	I21.3	*sodA*	− (221), + (228)	+ (91, 108, 221)	+ (91, 108, 221)	?	+ (109)
Endo IV	*E. coli*	F30	*nfo*	?	+ (41, 91, 109, 226, 234)	+ (41, 91, 109, 226)	?	?
Glucose-6-P dehydrogenase	*E. coli*	F48.8	*zwf*	?	+ (91, 128, 226)	+ (91, 128, 226)	?	?
NADPH-dehydrogenase	*E. coli*	?	*ndh*	?	+ (70c)	?	?	?
Soi-28	*E. coli*	F120	*soi-28*	− (132, 234)	+ (132, 226, 234)	?	?	?
Soi-17/19	*E. coli*	H47	*soi-17/19*	− (132, 234)	+ (132, 226, 234)	?	?	?
GroES	*S. typhimurium*	?	?	− (171)	?	?	+ (177)	?
	E. coli	C15.4	*groES*	+ (228), − (91)	+ (91)	+ (91)	+ (228)	+ (228)
GroEL	*S. typhimurium*	C56	?	− (171)	?	?	+ (177)	?
	E. coli	C56.5	*groEL*	− (228), +[c]	+ (234)	?	+ (228)	+ (228)
DnaK	*S. typhimurium*	C69	?	+ (171)	?	?	+ (177)	?
	E. coli	B66.0	*dnaK*	+ (228)	− (234)	?	+ (228)	+ (228)
RecA	*E. coli*	C39.3	*recA*	+ (91, 228)	− (91)	− (91)	− (228)	+ (233)

[a] Symbols: +, inducible; −, not inducible; ?, not known.
[b] Constitutively elevated in *oxyR1* mutants.
[c] L. Shapiro, cited in reference 171.

different from those induced by peroxide stress. (ii) Each stress condition induces a set of proteins including those that are apparently unique to that stress. (iii) Some proteins that are induced by oxidative stress can also be induced by other types of stress, such as heat shock, starvation, and SOS. These points are elaborated in the sections that follow. (iv) The regulation of certain oxidative stress-inducible genes appears to be different in *S. typhimurium* and in *E. coli*. For example, a moderate degree of induction of MnSOD in *S. typhimurium* with H_2O_2 was reported (44), whereas no significant induction was detected in *E. coli* (221). The *ahpF* gene encoding the large subunit of Ahp (Table 1) is induced by heat shock in an OxyR-dependent fashion in *S. typhimurium*, but the cloned *E. coli ahpF* gene cannot be induced by heat in *E. coli* or when introduced into *S. typhimurium* (212). This suggests that the *S. typhimurium* gene has a *cis*-acting heat shock element that is not present in the *E. coli* gene. Curiously, the induction of the *ahpF* gene by heat shock does not depend on the heat shock sigma factor, σ^{32} (212).

Peroxide Stress Proteins

The two-dimensional gel analysis of *S. typhimurium* proteins labeled during the 60-min period following treatment with a low dose of H_2O_2 indicated that the rate of synthesis of 30 proteins is elevated over that seen in untreated cells (44, 171). Of those 30 proteins, 12 (early proteins) are maximally synthesized during the first 30 min whereas 18 (late proteins) continue to be synthesized at an elevated rate in the second 30 min. Nine of the 12 early proteins are constitutively expressed at an elevated level in *S. typhimurium oxyR1* mutants but are not at all induced with H_2O_2 in *oxyRΔ2* strains in which the *oxyR* gene is deleted (44). The *oxyRΔ2* mutation does not affect the inducibility of the remaining 21 proteins. Thus, only 9 of 30 peroxide stress proteins are regulated by the *oxyR* locus. Two of the OxyR-regulated proteins are heat-shock proteins. Four of the remaining seven proteins have been identified; two are electromorphs of the HPI catalase (228), and two are subunits of Ahp (Table 1). As expected, it was found that the activities of these enzymes are constitutively elevated significantly in *oxyR1* mutants (44). In addition, MnSOD and glutathione reductase activities are moderately elevated in the constitutive mutants. It is not known whether any of the remaining unidentified proteins are responsible for these two enzyme activities. Thus, in *S. typhimurium*, 8 to 11 observed proteins are under *oxyR* control. Of course, additional OxyR-regulated polypeptides may be hidden or unobservable on two-dimensional gels.

A similar two-dimensional protein analysis of *E. coli* cells treated with H_2O_2 revealed that more than 30 proteins are synthesized at an elevated rate after induction, 9 of which are constitutively expressed at an elevated level in *oxyR2* mutants (228). Five of the OxyR-regulated proteins are identified: two proteins of HPI catalase, two proteins of *ahp*, and MnSOD (228) (Table 1). However, a study with *sodA-lacZ* operon fusions failed to detect an elevated level of transcription of the *sodA* gene after treatment with H_2O_2 (221). Consistent with this observation, induction of MnSOD was shown to be not dependent on $OxyR^+$ (29). MnSOD is not likely to be induced by peroxide stress in *E. coli*. Thus, in *E. coli*, eight proteins are under *oxyR* control.

O_2^- Stress Proteins

The first enzyme that was identified as being induced by O_2^--generating conditions is MnSOD (93, 107). The activity of endonuclease IV (Endo IV), normally a minor apurinic/apyrimidinic (AP)-endonuclease, was found to be induced dramatically in *E. coli* after treatment with a sublethal dose of paraquat and moderately after treatment with plumbagin and menadione (41). Glucose-6-phosphate dehydrogenase (glucose-6-P dehydrogenase) (91) and NAD(P)H-dehydrogenase (diaphorase) were also found to be induced with paraquat or menadione (Table 1).

A more systematic survey for proteins inducible by O_2^--mediated oxidative stress was undertaken by use of two-dimensional protein gel analysis (234). An increase in O_2^- flux induces MnSOD, which in turn stimulates production of H_2O_2. The elevated level of H_2O_2 is expected to induce peroxide stress-inducible proteins. In this particular study, *sodA sodB* double mutants completely lacking SOD activity were used to minimize the expected secondary induction. Because of their lack of dismutase activity, use of the SOD^- cells also permitted the use of paraquat or plumbagin at approximately 100- to 1,000-fold lower concentrations than those normally used with SOD^+ cells. This was expected to minimize any effect that might arise from the action of the agents other than generation of O_2^- (234). Proteins elevated under these conditions were compared with the proteins induced in SOD^+ cells stressed with O_2^- generators. On the whole, about 30 proteins were found to be induced under O_2^- stress conditions. Of these ca. 30 Soi proteins (for superoxide inducible), 6 have been identified: 2 proteins associated with HPI catalase, 2 proteins of Ahp, heat shock protein GroEL, and endonuclease IV (Table 1). MnSOD, a basic protein, was not resolved on these gels. Except for the two proteins associated with HPI catalase and the two proteins of Ahp, no other H_2O_2-inducible proteins are elevated under O_2^- stress conditions (234). In a similar study, Greenberg and Demple (91) found that O_2^--generating agents, paraquat and menadione, induce in wild-type strains at least 33 proteins that are not seen with H_2O_2 (91). These include GroES and two other heat shock proteins, MnSOD, endonuclease IV, and glucose-6-P dehydrogenase (Table 1).

Screening random operon fusions for inducibility to paraquat treatment, Kogoma et al. (132) found three *soi::lacZ* fusions that are inducible specifically with O_2^--generating agents. One fusion, *soi-28::lacZ*, lacked the Soi protein F120, and the other two, *soi-17::lacZ* and *soi-19::lacZ*, did not synthesize the Soi protein H47 with or without O_2^- stress (234). The *soi-28* and *soi-17/soi-19* genes have been tentatively assigned to the genes encoding F120 and H47, respectively (Table 1). The functions of these Soi proteins in oxidative stress have not been determined. Since the *soi::lacZ* fusion strains are somewhat more sensitive to paraquat than is soi^+ (132), and since the absence of these Soi proteins in the fusion strains results in an increased expression of other Soi proteins (234), they are possibly protective proteins involved in scavenging active oxygen species.

A recessive mutation in the locus, *mvrA*, that renders cells sensitive specifically to paraquat (methyl viologen) has been isolated (172). The wild-type gene has been cloned, and the nucleotide sequence has been determined. The molecular weight and pI estimated for the amino acid sequence are similar to those of the Soi protein H30 (234). However, inducibility of MvrA protein with paraquat has not been demonstrated.

There are conflicting reports with regard to the inducibility of OxyR-regulated proteins with O_2^- generators. Hassan and Fridovich (108) detected significant induction of catalase activity with menadione and plumbagin but not with paraquat, whereas Kao and Hassan (128) reported induction of catalase with paraquat. Greenberg and Demple (91) observed the induction with paraquat or menadione of all of the proteins that are under OxyR control. The induction of OxyR proteins including HPI and Ahp proteins with paraquat, plumbagin, or menadione is clearly $oxyR^+$ dependent (91, 234). This suggests that the induction is mediated by H_2O_2, a product of the dismutation of O_2^- which is stimulated by induced MnSOD as discussed above. In keeping with this suggestion, Walkup and Kogoma (234) found that depletion of SOD activity as in *sodA sodB* double mutants abolishes the induction of these proteins with paraquat or plumbagin, with the exception of AhpF, which is present at a markedly elevated level in SOD^- cells with or without paraquat treatment. Greenberg and Demple (91) also reported significant levels of induction of HPI catalase proteins in SOD^- cells treated with menadione. It appears, therefore, that a significant amount of H_2O_2 is generated from O_2^- in the complete absence of SOD activity in *sodA sodB* double mutants. However, the possibility cannot be ruled out that the redox-cycling agents commonly used may generate H_2O_2 directly. Alternatively, some of the OxyR regulon genes may possess a *cis*-acting control element that is sensitive to an O_2^--mediated inducing signal (see below).

In summary, more than 30 proteins are induced under O_2^- stress conditions in *E. coli*. These proteins are, for the most part, different from those induced by H_2O_2 stress. For at least six superoxide stress proteins, enzyme activities and/or encoding genes are known (Table 1). It should be noted that there are no comparative studies for *S. typhimurium*. Therefore, we know little about proteins inducible by O_2^- stress in this organism, with the exception of MnSOD (44).

Overlaps with Other Stress Responses

Heat shock response. One of the remarkable observations, repeated in a number of studies, is that oxidative stress induces some of the proteins that are also induced under other stress conditions such as heat shock and DNA damage (44, 91, 171, 228, 234). In particular, heat shock proteins GroES and GroEL have been shown to be induced by both peroxide- and superoxide-mediated oxidative stresses as well as heat shock, starvation, and SOS, at least in *E. coli* (Table 1). Another heat shock protein, DnaK, has been shown to be induced by treatment with H_2O_2, nalidixic acid, UV irradiation, and starvation. Elevated levels of the protein, however, could not be seen under O_2^- conditions (234). An SOS protein, RecA, can be induced by both types of oxidative stress but not by heat shock (Table 1). These observations clearly indicate that several stress responses overlap and that the extent of the overlap varies in different overlapping responses.

Mutants of *E. coli* deleted for the *rpoH* gene, which encodes the major heat shock sigma factor, σ^{32}, are extremely sensitive to thermal stress; they cannot grow at temperatures above 20°C (246). Heat shock genes are expressed at much reduced rates in these mutants. However, overproduction of GroEL and GroES proteins alleviates the effects of the *rpoH* deletion and allows growth at up to 40°C (136). Growth at temperatures above 40°C requires concomitant overexpression of the *dnaK* gene (136). These results strongly suggest that GroE and DnaK are key proteins that

are important in protection against thermal stress. Together with these observations, the fact that these proteins are induced in at least four overlapping responses (Table 1) points to an attractive notion that some of the heat shock proteins may serve as general antistress proteins. In support of this idea, it has been demonstrated that the *rpoH* deletion renders *E. coli* SOD^+ and SOD^- cells extremely sensitive to both peroxide and superoxide stress, although a mutation in the *groES*, *grpE*, or *dnaK* gene does not by itself increase the sensitivity appreciably (132a).

Privalle and Fridovich (186) observed that heat shock (for 1 h at 48°C) followed by a period of recovery at 37°C resulted in a moderate induction of MnSOD. Since the induction was seen only under aerobic conditions, they speculated that heat shock might generate O_2^- as a result of disruption of the electron transport system of the membrane by heat. In support of this proposal, a recent study has shown that certain membrane-binding drugs (anesthetics) which inhibit the membrane-bound respiratory activity also induce MnSOD only under aerobic conditions (245). Whether these cells acquired resistance to heat shock or oxidative stress was not examined.

Cells adapted to peroxide stress exhibit increased resistance to heat shock. Thus, pretreatment of *S. typhimurium* with H_2O_2, which induces 30 proteins including 5 heat shock proteins (e.g., DnaK) (171), leads to markedly increased resistance to thermal stress (44). An OxyR(Con) mutant (*oxyR1*) of *S. typhimurium* was shown to be markedly more resistant to killing by heat than the wild type was (44). Similarly, transcription of several heat shock genes has been found to be constitutively elevated in *E. coli* SOD^- cells chronically stressed by high levels of O_2^-. However, these cells are no more resistant to thermal stress than are SOD^+ cells, partly because they are defective in the induction of heat shock genes upon temperature shift-up (132a).

Carbon starvation response. Glucose-starved stationary-phase cells of *E. coli* develop enhanced resistance to several forms of stress such as heat shock, oxidation, osmotic shock, and starvation, and more than 30 proteins are induced in starved cells, including several oxidative stress and heat shock proteins (126, 159). Apparently, three of the heat shock proteins (DnaK, GroEL, and HtpG) are regulated by σ^{32}, which has been shown to increase upon starvation (125a). However, the oxidative stress proteins seen in starved cells are not induced by oxidative stress. Instead, the induction of these proteins is regulated as part of a multigene system (the KatF regulon) which is turned on upon entry to the stationary phase or to carbon starvation (see below).

SOS response. Genetic stress invokes the SOS response, which includes an inducible DNA damage repair response (233, 239). Brawn and Fridovich (30) reported that paraquat treatment causes induction of a *din* (damage-inducible) gene and imparts resistance to UV irradiation only in the presence of oxygen. They proposed that O_2^- induces the SOS response. However, induction of MnSOD with paraquat has since been shown to be independent of $RecA^+$ (105). Plumbagin at the concentrations that induce a DNA repair response in *E. coli* has failed to activate the *din* gene or to confer resistance to UV irradiation (71). The concentrations of paraquat necessary to confer protection against UV irradiation (0.5 to 10 mM) (30) are much higher than those needed to fully induce SoxRS regulon genes (e.g., 0.1 mM for *sodA* and 0.05 mM for *soi* genes) (105, 132, 221). It is possible, therefore, that paraquat at only high concentrations causes SOS-inducing damage (e.g., single-strand breaks) by elevating both O_2^- and H_2O_2 concentrations, leading to formation of $OH^{\cdot}$. The $RecA^+$ dependence of the induction of UV resistance with paraquat is yet to be demonstrated.

Treatment with low (91) but not high (101) concentrations of H_2O_2 induces *recA*, an SOS gene. Similarly, H_2O_2 is known to cause the induction of two SOS functions, λ phage induction from lysogens and cell filamentation (118). Cell filamentation during the SOS response is due to the induction of the *sfiA* (*sulA*) gene encoding a cell division inhibitor (82). It is puzzling, therefore, that the H_2O_2-induced filamentation is not dependent on $sfiA^+$ (118) and yet the *sfiA* gene is inducible with H_2O_2 (65, 84). It is likely that H_2O_2 causes both SOS-dependent and SOS-independent inhibition of cell division.

REGULATION OF CELLULAR RESPONSES TO OXIDATIVE STRESS

OxyR Regulon

Positive regulatory protein, OxyR. The expression of a limited number of the proteins inducible with H_2O_2 is regulated by the *oxyR* locus. Thus, *oxyR* controls only a subset of the 30 H_2O_2-inducible proteins. This was demonstrated by the isolation of an *S. typhimurium* mutant, *oxyR1*, in which the expression of 9 of the early 12 proteins is constitutively elevated (44). The *oxyR1* mutation was mapped by linkage to Tn*10* insertions in the nearby *argH* (at 89.5 min on the standard *E. coli* linkage map [12]). By Tn*10*-mediated deletion, *S. typhimurium* and *E. coli* mutants deleted for the *oxyR* locus were generated (44). The deletion of the gene abolished the inducibility of the nine proteins with H_2O_2. Thus, it was inferred that the *oxyR* gene product is a positive factor essential for activation of the OxyR regulon genes (44). The *E. coli oxyR* gene has been cloned, and the nucleotide sequence has been determined (45, 216a). The gene encodes a protein of 34 kDa which shares significant homology with a family of bacterial regulatory proteins known as the LysR family (111). The *oxyR* gene expression is negatively autoregulated, as is the expression of many Lys family regulatory genes (45, 216a).

The regulation by OxyR operates primarily at the transcriptional level. Thus, the *katG* transcript is elevated more than 50-fold in *S. typhimurium oxyR1* mutants (171), which is in good agreement with the 50-fold increase in catalase activity observed in the mutants (44). Induction of β-galactosidase with H_2O_2 in strains carrying a *katG::lacZ* operon fusion is completely blocked by introduction of an *oxyR* deletion mutation (218). Similar transcriptional activation of the *ahp* operon by OxyR has been demonstrated by the presence of elevated levels of *ahpC* transcript and AhpC protein in *oxyR* constitutive mutants (218). Footprinting analysis with purified OxyR protein has identified the regions upstream of the *katG* gene, *ahpCF* operon, and *oxyR* gene to which OxyR protein binds. The OxyR-binding sites extend into the -35-σ^{70} binding sites, suggesting that the OxyR protein interacts with RNA polymerase to activate transcription. Intriguingly, inspection of the sequences of these OxyR-binding sites has revealed only a few conserved nucleotides (218).

OxyR is known to regulate the expression of one other gene: the phage Mu *mom* gene which encodes a DNA modification function (26). OxyR (MomR) protein prevents transcription of the *mom* gene by binding to a 5′ region of the *mom* structural gene which contains three GATC sites (the

target sequence for Dam methylase [157]), only when these sites are unmethylated. Thus, the OxyR protein can discriminate between unmethylated and methylated states of the *mom* promoter region (26). Whether the Dam methylation-dependent repression of the *mom* gene in any way relates to oxidative stress is not known. *E. coli dam* mutants are hypersensitive to killing by H_2O_2 (242). Thus there may be other OxyR-regulated genes whose repression or induction depends upon correct methylation. It is interesting that there is significant homology between the OxyR-binding site sequences of the *mom* and *oxyR* genes (26), in contrast to the absence of a consensus among those of the *katG*, *ahpCF*, and *oxyR* genes as mentioned above. This suggests that the modes of interaction with DNA might be different depending on whether the protein functions as a repressor or an activator.

Direct activation of OxyR protein by oxidative stress. In response to the elevated flux of H_2O_2, OxyR protein activates transcription of the OxyR regulon genes. How is an oxidative stress signal transduced to OxyR protein? Upon treatment of cells with H_2O_2, the transcription of *oxyR'-'lacZ* does not change (216b), nor does the rate of OxyR protein synthesis increase (213). Thus, induction of the OxyR regulon does not involve an increase in the amount of OxyR expression. The possibility of direct activation of OxyR protein by oxidation was suggested by the initially surprising observation that OxyR protein purified from unstressed cells is capable of activating transcription of *ahpCF* and *katG* genes in vitro. It was subsequently demonstrated that OxyR prepared in the absence of oxygen is inactive as a transcriptional activator, but it can be readily converted to an active form by exposure to air (213). In fact, addition and removal of dithiothreitol, a reducing agent, permits conversion between the active and inactive forms of the protein. The conversion to the active form by removal of the reductant can be prevented by the addition of catalase. Therefore, it appears that as soon as it is removed from the reducing environment within the cell, OxyR protein is oxidized, perhaps by H_2O_2 that is present under the aerobic conditions. These results led to the conclusion that upon oxidative stress the increased flux of H_2O_2 converts OxyR protein to an oxidized form, which in turn activates transcription of the OxyR regulon genes (213). Thus, in this scenario, OxyR is both the sensor and the transducer of an oxidative stress signal which is H_2O_2 itself.

The mechanism by which the oxidized form of OxyR activates transcription has not been clearly elucidated. OxyR protein contains six cysteine residues (45). Although good candidates for the redox-active center, these cysteine residues are not likely to be involved in formation of inter- or intramolecular disulfide bonds by oxidation because the conversion of five of the six residues to serine does not affect the activation of the protein (213). A change in the cysteine residue at position 199 to serine inactivates the protein, however. Both the reduced and oxidized forms of OxyR bind to the regulatory regions of *ahpCF*, *katG*, and *oxyR*, but the footprints obtained with the two forms are clearly different, suggesting a distinct conformational change upon oxidation and reduction of the protein (213). OxyR mutant protein, which is inactivated by conversion of the essential cysteine residue to serine, even when prepared in the presence of oxygen, yields a footprinting pattern identical to that seen with the reduced form of wild-type protein. It has been suggested that the postulated conformational change alters the interaction of the protein with RNA polymerase, leading to activation of transcription (213). It should be pointed out, however, that the same difference in the footprinting patterns between the reduced and oxidized forms can be seen with the regulatory region of the OxyR regulon gene which is bound and repressed by both the reduced and oxidized forms of OxyR (213). Therefore, the suggested conformational alteration may be a necessary change, but not a sufficient event leading to transcriptional activation. The understanding of the precise mechanism of the transcriptional activation would require detailed structural and genetic studies of the two forms of the OxyR protein.

SoxRS Regulon

Isolation of Sox(Con) mutants. The demonstration that the induction of *soi::lacZ* fusions is completely independent of previously described regulatory loci, *oxyR*, *rpoH*, and *recA*, led to the prediction of the existence of a new control locus termed *soxR* (for superoxide radical response) (132). The *soxR* regulatory locus was defined by subsequent isolation of mutations [SoxR(Con)] that render the expression of several O_2^--inducible genes constitutive in *E. coli*. Two mutations, *soxR1* and *soxR2*, were isolated by searching for extragenic mutations that elevated the expression of an *nfo'-'lac* fusion (226). Additionally, two mutations, *soxR101* and *soxR105*, were found among the mutations that conferred elevated levels of resistance to redox-cycling agents (92). The mutations have been mapped at 92.2 min on the *E. coli* chromosome (92, 226). The genes which are expressed constitutively in these mutants are *nfo*, *zwf*, *sodA*, *soi-17*, *soi-19*, and *soi-28* (Table 1). Four other proteins including a ribosomal protein were also found elevated in *soxR*(Con) mutants by two-dimensional gel analysis (92). The SoxR(Con) mutants are not particularly more resistant to O_2^--generating agents than is the wild type, except for *soxR101* and *soxR105* mutants, which are isolated by virtue of increased resistance to menadione. Thus, the mutants are resistant to this agent and plumbagin, but not to paraquat (92).

The regulation by the *soxR* locus appears to occur primarily, if not exclusively, at the level of transcription because SoxR(Con) mutations affect the expression of *nfo'-'lacZ*, which utilizes the ribosome-binding site of *lacZ* (226). Large deletions in the *soxR* locus lead to noninducibility by paraquat. The inducibility can be restored by introduction of a plasmid carrying this region of the chromosome (92, 226). Thus, the *soxR* locus encodes a *trans*-acting positive factor(s) essential for transcriptional activation of *soxR* regulon genes.

The *soxR* region encodes two proteins. The actual SoxR regulation appears to be much more complex than one positive factor activating transcription of the regulon in the response to stress. By analyzing proteins coded for by the DNA sequences in this region, Tsaneva and Weiss (226) have detected two polypeptides, of 17 and 13 kDa, which they have assigned to SoxR and SoxS proteins, respectively. Both SoxR and SoxS are essential for the inducibility of the SoxR regulon genes (241). Deletions and insertions near the 3' end of the soxR gene lead to the constitutive phenotype, thus raising the possibility that the activation of the SoxR protein involves cleavage by a protease activity in analogy to the RecA-mediated cleavage of LexA protein in SOS induction (233). The two genes, *soxR* and *soxS*, are transcribed divergently (226). Furthermore, the *soxR* promoter lies within the *soxS* gene (241). The significance of this divergent and overlapping transcription has not been elucidated. The mechanism by which the two proteins activate transcription of the SoxRS regulon genes is not known. One possibility

that the activation involves an increase in the amount of these proteins has been suggested by the observation that the *soxS* gene is inducible by paraquat (241). Since SoxR is normally expressed at barely detectable levels (226), the expression of *soxR* may also be inducible by O_2^- stress.

The individual roles of the SoxR and SoxS proteins in the regulation of SoxRS-regulated genes has not been elucidated. SoxS is related to the AraC family of proteins (2a, 241) that function as positive transcriptional regulators. The SoxR protein was found to share regions of homology to the MerR protein (2a), which, when bound by mercury, positively regulates several genes involved in mercury detoxification (180). SoxR contains four cysteines clustered in a region near the carboxy terminus (241). This suggests the possibility of a bound metal whose redox state might determine the activity of the SoxR protein as an inducer (signal sensor) or transcriptional activator. It has recently been shown that SoxS alone can switch on the SoxRS regulon genes in the absence of SoxR or paraquat (2a).

Pleiotropic effects of *soxR*(Con) mutations. Greenberg et al. (92) have made an interesting observation that SoxR(Con) mutants are multiply resistant to several antibiotics including chloramphenicol, nalidixic acid, ampicillin, tetracycline, and bleomycin. This multiple drug resistance phenotype can be accounted for at least in part by additional effects of *soxR*(Con) mutations on gene expression. First, SoxR(Con) mutants have diminished amounts of protein S6A, accompanied by concomitant increases in protein S6C (92). The gene encoding this activity (*rimK*) has recently been cloned and sequenced (127). Addition of glutamyl residues to the carboxy terminus of the S6 protein, yielding S6C, affects the level of resistance to several antibiotics (189). Second, the levels of the OmpF porin are significantly decreased in SoxR(Con) mutants (92). This change appears to be mediated by the increased expression of the *micF* gene (55a) encoding the antisense RNA, which inhibits translation of *ompF* mRNA (169). A similar multiple drug resistance conferred by the *marA* mutations has been shown to be caused at least in part by the decreased levels of OmpF porin in these mutants (46). It appears, therefore, that *soxR*(Con) mutations enhance the expression of the *micF* gene which decreases the OmpF porin level and in turn lowers the membrane permeability to antibiotics.

Garvey et al. (81) observed a significant decrease in the level of OmpF porin upon SOS induction by treatment with nalidixic acid. Since nalidixic acid treatment is known to induce some oxidative stress proteins (228), Greenberg et al. (92) speculated that nalidixic acid might generate oxygen radicals in the treated cells. This attractive hypothesis is unlikely because the decrease in OmpF porin is observed not only after SOS induction in *recA*$^+$ cells treated with nalidixic acid, but also in *recA730* mutants which constitutively express SOS genes in the absence of nalidixic acid (81).

Other Factors Involved in Regulation of SoxRS Regulon Genes

There is evidence indicating that SoxRS regulon genes are regulated by additional factors. For example, SoxRS regulon genes are not maximally expressed in SoxR(Con) mutants; thus, the expression of several SoxRS regulon genes can be further elevated by treatment of SoxR(Con) mutants with paraquat (92, 226). This partial expression might account for the puzzling observation that *soxR*(Con) mutants are not particularly more resistant to many redox-cycling compounds than is the wild type, as mentioned above. A group of mutations [*soxQ*(Con)] have been isolated which elevate the expression of some SoxRS regulon genes at the transcriptional level like *soxR*(Con) but map at a locus distinct from the latter (88). *soxQ*$^+$ is not required for the induction by paraquat. Thus, SoxQ could be a factor responsible for additional control of some SoxRS genes.

Multilayered Regulation of SodA Expression

In addition to the SoxRS control, the expression of the *sodA* gene is under at least two and possibly three other global controls. Moody and Hassan (170) made a seminal observation that MnSOD can be anaerobically induced by the addition of iron chelators. The observation led them to propose the involvement of an iron-containing protein in the negative control of the SodA expression at the transcriptional level. Niederhoffer et al. (178) noted that the promoter region of the *sodA* sequence (216) contains a stretch of sequence that is homologous to the iron box, a 19-bp consensus sequence that is found in the regulatory region of genes involved in iron uptake (14). The Fur protein which requires Fe^{2+} as cofactor binds to the iron box and represses the expression. The involvement of Fur protein was subsequently demonstrated by modest levels of derepression of the *sodA-lacZ* operon fusion in aerobically and anaerobically growing *fur* mutants and by gel shift of DNA fragments containing the *sodA* promoter by Fur protein (178).

The genetic study by Tardat and Touati (217) confirmed the involvement of Fur and further uncovered another layer of control over SodA expression. They isolated and analyzed mutants that are capable of anaerobic expression of SodA. The analysis revealed that high levels of the anaerobic derepression require two mutations, one each in the *fur* and *arc* regulatory systems, and that single mutations affecting only one of the systems allow only partial derepression. The *arc* regulatory system negatively controls expression of a number of genes involved in aerobiosis (121–123). Thus, SodA expression is coupled to respiration by the Arc regulation and to iron availability by the Fur system. It has been speculated that the derepression of MnSOD by the unavailability of ferric iron is designed to compensate decreased activities of FeSOD in case of insufficient cellular iron concentrations for full FeSOD activity (217). Importantly, the derepressed level of MnSOD in *fur arc* double mutants can be further elevated by paraquat in the presence of oxygen, indicating that the positive regulation by SoxRS is independent of the Fur and Arc systems (217). Recent evidence suggests a role for Fnr in SodA expression as well (109a). Thus, the SodA expression is under at least three independent global regulatory systems. In addition, evidence indicates that overproduction of MnSOD represses transcription of the *sodA* gene; thus, the SodA expression is also autoregulated (221).

KatF (RpoS) Regulon

E. coli possesses two catalases, HPI and HPII, encoded by *katG* and *katE*, respectively (152, 154). Whereas KatG catalase expression is under OxyR control, KatE expression is not inducible with H_2O_2, but is regulated in a growth phase-dependent manner (201). The synthesis of KatE catalase requires the *katF* gene product as a positive regulatory factor (153). The nucleotide sequence of a cloned *katF* gene has suggested that KatF protein is a σ factor (173). KatF synthesis is turned on before or coincidently with KatE expression as cells enter the stationary phase (174). Thus, it

appears that a starvation signal triggers the synthesis of KatF, which activates RNA polymerase to turn on transcription of the *katE* gene. Moreover, the observation that *katF* mutants die off more rapidly than the wild type upon prolonged exposure to starvation conditions led to the suggestion that KatF positively controls other genes involved in the response to starvation stress (174).

Exonuclease III (Exo III) encoded by *xthA*, is an important repair enzyme in H_2O_2-mediated oxidative stress (58, 59). Thus, *xthA* mutants are very sensitive to exposure to near-UV and H_2O_2 (58, 194). Although KatF regulates KatE expression, inactivation of the *katF* gene but not *katE* results in sensitivity to near-UV (195). This observation led Sak et al. (193) to examine a possible role of KatF in XthA expression. They found that *katF* mutant cells contain as little Exo III activity as *xthA* mutant cells. The expression of *xthA* is controlled in a growth phase-dependent manner, as is *katE* expression. Thus, KatF appears also to regulate XthA expression. The speculation that the *xthA* and *katE* genes belong to the same operon (193) has not been critically examined.

Furthermore, *katF* (*appR*) is also known to regulate the expression of the *appA* gene (138, 225), which encodes an acid phosphatase (224), and the *bolA* gene (22, 139), a "morphogene" which controls the cell morphology change from the rod shape of growing cells to the spherical shape upon entry to the stationary phase (2). It is not likely that these gene products play a significant role in the defense against oxidative stress. Instead, the *katE* and *xthA* genes are regulated as part of a multigene system (the KatF or RpoS regulon) which is invoked when cells enter the stationary phase (138, 159). The induction of *katE* and *xthA* would account for the remarkable degree of tolerance against H_2O_2 which develops in glucose-starved stationary-phase cells (126, 159a) since *katF* is one of the carbon starvation-inducible genes (138). Glucose starvation also elicits resistance to heat shock (126). However, *katF* is not inducible by heat (138). Thus, it appears that stationary-phase cells can develop tolerance to several types of stress by a unique mechanism which is not mediated by regular stress response systems such as the OxyR and heat shock regulons.

AppppN, Heat Shock, and Oxidative Stress

Ames and coworkers found that unusual nucleotides, AppppN and ApppN (N = A, C, G, or U), accumulate in cells stressed by heat shock or by exposure to a wide variety of oxidizing agents including menadione and H_2O_2 (141). An enzyme (dinucleotide tetraphosphate hydrolase) which hydrolyzes the nucleotides has been identified, and the gene (*apaH*) encoding the enzyme has been cloned (164). It has been proposed that these nucleotides may be alarmones signaling the onset of the stress (21). At least three lines of evidence resulting from subsequent investigations appear to rule out this possibility. First, the time course of the accumulation of AppppN lags behind induction of heat shock proteins (228). Second, cells carrying an $apaH^+$ gene on a high-copy-number plasmid do not accumulate the nucleotides during heat shock or oxidative stress as a result of an elevated level of the hydrolase activity. However, the pattern of the proteins induced under stress conditions is not altered (184). Nor are ApaH overproducers particularly sensitive to H_2O_2 (184). Third, *apaH*(Null) mutants, whose basal level of AppppN is the same as that reached in heat shock-induced wild-type cells, do not show elevated levels of constitutive expression of heat shock proteins (69).

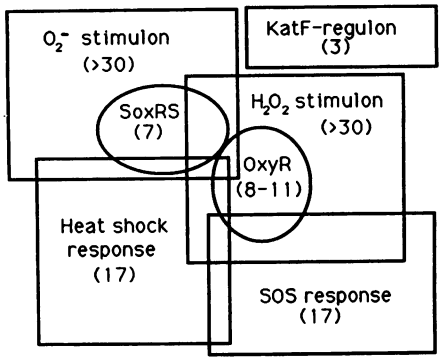


FIG. 2. Cellular responses to oxidative stress and possible overlaps with other stress responses. The number in parentheses indicates the number of proteins and genes known to be involved in the response. Recent studies (159, 159a) indicate that the KatF regulon may contain more than 30 proteins. See the text for details.

Although AppppN cannot be a signal for the heat shock or oxidative stress response, the following evidence (69, 126a) suggests that AppppN levels may modulate the heat shock response, perhaps by affecting DnaK functions. (i) *apaH* (Null) mutants are more sensitive to killing at 55°C than is the parental strain with or without preexposure to 43°C. (ii) *apaH*(Null) mutants show prolonged synthesis of some heat shock proteins including GroEL, DnaK, and E89 when heat-shocked cells are returned to low temperatures. (iii) *apaH*(Null) mutants exhibit an induction lag when lambda *c*I857(Ts) lysogens are shifted from 30 to 43°C. (iv) Although the level of phosphorylated DnaK increases upon heat shock in wild-type cells, the level in *apaH*(Null) mutants is already high at 30°C and does not increase after a heat shock. (v) Overproduction of DnaK decreases the heat sensitivity of an *apaH ksgA* double mutant and, as previously shown (142), DnaK overproduction suppresses the filamentation phenotype of the double mutant. Together with the observations that DnaK is involved in controlling the stability of σ^{32} (220) and that DnaK protein autophosphorylates (247), these results point to the possibility that AppppN levels affect DnaK functions, thereby modulating the heat shock response. How AppppN affects DnaK functions is not clear at present, although photocrosslinking experiments with N^8-azido-[^{32}P]AppppA show that several stress proteins, including DnaK, GroEL, E89, C45, and C40 are AppppA-binding proteins (126a).

Summary

Bacteria respond to oxidative stress by invoking two distinct stress responses, the peroxide stimulon and the superoxide stimulon (Fig. 2). The two stimulons each contain a set of more than 30 proteins which overlap slightly. The expression of a subset of genes in each stimulon is controlled by a unique regulatory element. These genes constitute the OxyR and SoxRS regulons (Fig. 2). No overlap has been detected between the two regulons, with the possible exception of the *sodA* gene in *S. typhimurium*. The signal for the OxyR regulon induction may be H_2O_2 itself, whereas the signal for the SoxRS regulon is not known. The nature of the proteins outside the regulons and the regulation of the genes encoding these proteins have not been elucidated.

H_2O_2 and O_2^- generators also induce some heat shock

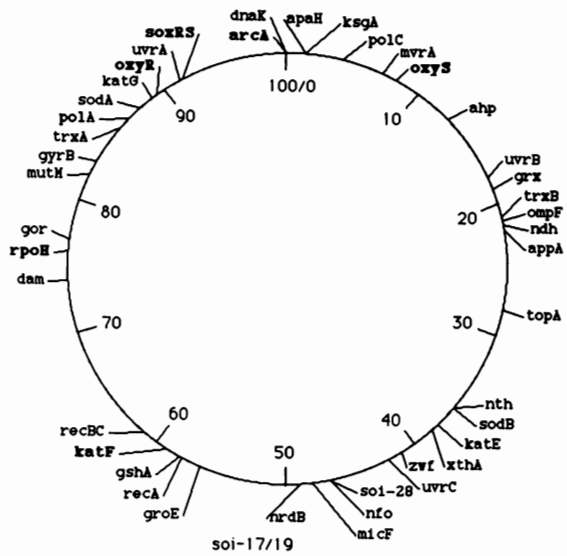


FIG. 3. Map positions of *E. coli* oxidative stress-related genes. The map positions of the genes listed in Table 2 are those of Bachmann (12). The genes in boldface letters are regulatory loci. See the text for details.

proteins, indicating definite overlaps between the heat shock response and the two oxidative stress responses (Fig. 2). Evidence indicates an overlap of the H_2O_2 stimulon with the SOS response. However, the overlap between the superoxide stimulon and SOS response is uncertain. At least three known genes including *xthA* and *katE* are regulated by a sigma factor, KatF (RpoS), whose synthesis is turned on during the stationary phase (Fig. 2). XthA and KatE are known to play important roles in the defense against oxidative stress. However, these KatF regulon genes are not induced by oxidative stress.

Figure 3 shows the locations of the genes that are involved in oxidative stress responses (as described above; Table 1), as well as those that are known to participate in the defense against oxidative stress (see below; Table 2). Although many additional genes are yet to be identified and characterized, the sheer number of genes identified thus far indicates the complexity of the defense systems. The map suggests some tendency toward clustering of the genes. This clustering and the pattern of the cluster locations may reflect the evolution of the *E. coli* chromosome (55, 190). The functional significance, if any, of the clustering is not apparent.

PHYSIOLOGICAL ROLE OF RESPONSES IN OXIDATIVE STRESS

Up to this point, we have discussed genes and proteins induced by oxidative stress only in the context of their regulation. It is equally important, if not, in some cases, equally difficult, to ascribe to them a physiological function vis-à-vis oxidative stress. At the onset, we admit to a teleological bias, namely, that all of the genes and proteins induced by oxidative stress serve a beneficial function. There are examples of proteins, however, whose expression during oxidative stress would appear more detrimental than beneficial, given what is known about their activities.

The defenses against deleterious effects of active oxygen can be logically divided into two broad classes, preventive and reparative. The former class serves to prevent the occurrence of oxidative damage by destroying the offending oxygen species or by limiting the length of certain reactions such as lipid peroxidation and one-electron redox- cycling of quinones. The latter class serves to repair damage caused by offending species that escaped elimination by the prophylactic defense system. Some enzymes, such as alkylhydroperoxide reductase, may serve both roles.

Prevention of Oxidative Damage

Cellular defenses against the damaging effects of oxidative stress involve both enzymatic and nonenzymatic components. The enzymatic components may directly scavenge active oxygen species or may act by producing the nonenzymatic antioxidants. The protective enzymes are, with rare exception, ubiquitous among aerobic organisms. There are four enzymes that provide the bulk of protection against deleterious reactions involving active oxygen in bacteria: SODs (encoded by *sodA* and *sodB*), catalases (*katE* and *katG*), glutathione synthetase (*gshAB*), and glutathione reductase (*gor*). Although some bacteria have NADH-dependent peroxidases specific for H_2O_2, *E. coli* and *S. typhimurium* do not; nor do they have glutathione peroxidase activities.

The reaction catalyzed by SOD is thought to occur in a two-step reaction as follows:

$$Enz_{oxidized} + O_2^- \longrightarrow Enz_{reduced} + O_2$$
$$Enz_{reduced} + O_2^- + 2H^+ \longrightarrow Enz_{oxidized} + H_2O_2$$

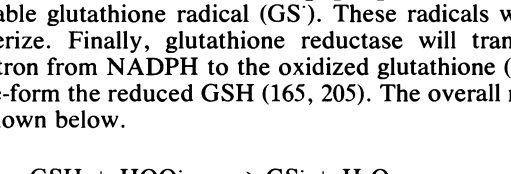
There are three types of SOD based upon the metal ligand(s) bound, CuZnSOD, FeSOD, and MnSOD. Each of the metals in the SOD isozymes is a transition metal that facilitates electron transfer. FeSOD is found primarily in prokaryotes. MnSOD is found in both prokaryotes and eukaryotes. CuZn SOD is generally not found in bacteria. One exception is the marine bacterium *Photobacter leiognathi* (210). The second-order rate constants are quite similar, at about 6×10^8 M^{-1} s^{-1}. The steady-state concentration of SOD in *E. coli* is approximately 10^{-5} M (77). The reaction between SODs and O_2^- is first order with respect to O_2^-. The steady-state concentration of O_2^- in a wild-type aerobically growing *E. coli* cell is about 10^{-9} to 10^{-10} M. In *sodA sodB* mutant cells lacking SOD activity, the calculated steady-state concentration of O_2^- is about 5×10^{-6} M (116). Thus the presence of SOD in the cell reduces the steady-state concentration of O_2^- by up to three orders of magnitude.

One of the products of O_2^- dismutation is H_2O_2, which is itself a reactive species. The function of SOD would not appear, therefore, to benefit the cell, unless (i) O_2^- is more toxic than H_2O_2, or (ii) H_2O_2 is disproportionated very rapidly, or (iii) SOD directs O_2^- to H_2O_2 versus other potentially toxic routes of clearance such as through the glutathione radical ($GS^{\cdot}$). Although it is difficult to draw firm conclusions about the relative toxicities of H_2O_2 and O_2^- in vivo, it is certainly true that cellular catalases destroy H_2O_2 with remarkable rapidity. The turnover number for typical catalase is about 10^9 molecules of H_2O_2 disproportionated per active site per second (at 1 M H_2O_2) (236). The electron source is from H_2O_2 itself, and so the reaction is a disproportionation and does not require an exogenous reducing source. Likewise, the reaction is exothermic and does not require ATP. Catalases therefore provide protection against H_2O_2 even in an energy-depleted cell. The two catalases in *E. coli* are found in different cellular locations. HPI and HPII are found in the periplasm and cytoplasm, respectively (110).

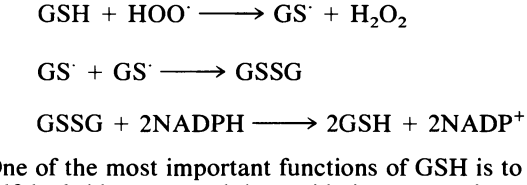
This differential localization suggests that sources of H_2O_2 may vary during starvation-dependent and starvation-independent oxidative stress.

Peroxidases are also capable of destroying H_2O_2. Peroxidases, unlike catalase, require NADH or NADPH as an electron source. Under conditions where reducing power is limited, the protective role of peroxidase is likely to be small.

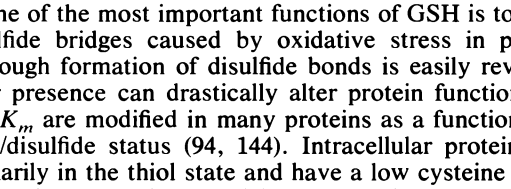
Glutathione (GSH) is an important antioxidant and is synthesized by glutathione synthetase. The high steady-state levels of glutathione in *E. coli* (and presumably in *S. typhimurium*) maintain a strong reducing environment in the cell (150). GSH will react with H_2O_2, O_2^-, or $HOO^\cdot$ to form a stable glutathione radical ($GS^\cdot$). These radicals will then dimerize. Finally, glutathione reductase will transfer an electron from NADPH to the oxidized glutathione (GSSG), to re-form the reduced GSH (165, 205). The overall reaction is shown below.



$$GSH + HOO^\cdot \longrightarrow GS^\cdot + H_2O_2$$



$$GS^\cdot + GS^\cdot \longrightarrow GSSG$$



$$GSSG + 2NADPH \longrightarrow 2GSH + 2NADP^+$$

One of the most important functions of GSH is to reduce disulfide bridges caused by oxidative stress in proteins. Although formation of disulfide bonds is easily reversible, their presence can drastically alter protein function. V_{max} and K_m are modified in many proteins as a function of the thiol/disulfide status (94, 144). Intracellular proteins exist primarily in the thiol state and have a low cysteine content (approximately 2%). Surprisingly, GSH is not essential for maintaining a relatively reduced state of most intracellular proteins, and mutants unable to synthesize GSH (*gshA*) show no increased sensitivity to H_2O_2 and only slight sensitivity to redox active compounds (55a, 89). Other low-molecular-weight thiols including thioredoxin and glutaredoxin may compensate for the lack of GSH (discussed below).

Another enzyme that may play an important protective function is Ahp. This enzyme will reduce many organic hydroperoxides in vitro, including cumene hydroperoxide and *t*-butyl hydroperoxide (124). Mutants lacking Ahp activity are sensitive to killing by these agents (212), and therefore these compounds may be substrates for this enzyme in vivo as well. In addition, a mutant overproducing *ahp* was found to suppress the H_2O_2 sensitivity of *E. coli* Δ*oxyR* cells (90), suggesting that Ahp may act on H_2O_2 directly.

These are the main enzymatic defenses against active oxygen species. It is important to note that peroxidases and the GSH system require a source of reducing power in order to function. In addition, at least in *E. coli*, the most important catalase, KatG, is inducible, as is MnSOD. As we shall see in a later section, reducing power and the ability to synthesize new gene products are limited under conditions of severe oxidative stress. The threshold level of oxidative toxicity may be at the concentration of active oxygen at which the cell can no longer induce or maintain an effective enzymatic defense.

In addition to the enzymatic defenses against active oxygen, most cells employ a wide variety of nonenzymatic organic antioxidants. The main nonenzymatic antioxidants in *E. coli* are GSH and thioredoxin (encoded by *trxA*) (155, 165). Ubiquinone and menaquinone may also serve as membrane-associated antioxidants.

Oxidative Damage and Repair

Oxygen radicals cause a great deal of damage to macromolecules in vitro and in vivo. The oxidative damage that leads to cell death, however, is not always clear. Treatment of cells with redox-active drugs causes both DNA and membrane damage, either of which can be lethal. Oxygen radicals also damage RNA and proteins in vivo. Although damage to RNA and proteins is not likely to be the cause of oxygen radical-induced death, such damage must, at the very least, waste cell energy and resources since the damaged molecule must be either repaired or degraded and replaced. In this section, we will present the major types of damage caused by active oxygen species in vivo. We will also discuss the repair mechanisms that ameliorate that damage.

DNA damage. Cell exposure to H_2O_2, O_2^--generating compounds, ionizing radiation, organic hydroperoxides, singlet oxygen, and ozone results in numerous types of DNA lesions. Both the base and the sugar moieties of DNA are attacked by active oxygen species. Attack on the base produces 8-hydroxyguanine, hydroxymethyl urea, urea, thymine glycol, thymine, and adenine ring-opened and ring-saturated products (for reviews, see references 7, 119, 147, and 197).

Hydroxyl radical attacks on the sugar moiety of DNA lead to sugar fragmentation and production of strand breaks with 3′-phosphate or 3′-phosphoglycolate termini (231). Thymine residues in DNA can be hydroxylated to produce 5-hydroxymethyluracil or oxidatively degraded to produce thymine glycol or a urea residue (31, 60, 133). Guanosine can also be oxidatively degraded in a photooxidative process (215). Addition reactions of $OH^\cdot$ with guanosine residues form 8-hydroxydeoxyguanosine.

In addition to the DNA damage caused directly by oxygen radicals, intermediate organic radicals that are formed during the propagation step of lipid peroxidation can react with DNA. For example, in the presence of autoxidizing methyl linoleate, either the oxidizing fatty acid transfers free radicals to purines (abstracts an H^+), resulting in decomposition of the purines, or the fatty acid radical adds to the purine to form a bulky adduct (200, 227). Incubation of DNA with linoleic acid hydroperoxide also causes site-specific cleavage of double-stranded DNA adjacent to guanidylate residues (200, 227).

Lipid peroxides formed during the initiation and propagation steps decompose into a number of nonradical, stable end products containing a wide range of functional oxygen groups. These include an array of aldehyde, epoxide, hydroxy, carboxy, and peroxy groups, as well as alkanes and alkenes (for reviews, see references 16 and 129). Many stable termination products, such as 4-hydroxyalkenals, epoxides, and other aldehydes have been shown to be directly reactive with DNA, either by alkylating bases (203) or by forming intrastrand and interstrand cross-links (214).

To what extent do these specific reactions occur, and what is their relative significance in vivo? Biochemical analyses of damaged DNA derived from treated cells, coupled with genetic experiments with known DNA repair-deficient mutants (Table 2), allow for an approximate answer to these questions.

Strand breaks and other lesions that block replication are likely to contribute more toward lethality than does base damage that does not hinder replication, although the latter may contribute significantly to mutagenesis. Thymine glycols and 8-hydroxydeoxyguanosine residues are two of the

TABLE 2. *E. coli* genes involved in defense against oxidative stress

Gene	Map position (min)	Regulon	Gene product or activity	Sensitivity to[a]:		References
				H_2O_2	O_2^-	
ahpCF	13	OxyR	Ahp	?	?	90, 212
apaH	1		Dinucleotide tetraphosphate hydrolase	+	+	69
appA	22	KatF	Acid phosphatase	?	?	138, 225
arcA	100		Resistance to methylene blue	?	?	217
dam	74		DNA adenine methylase	+	?	242
dnaK	0	HS	Heat shock protein	−	−	132a, 171
gor	77	OxyR	Glutathione reductase	?	?	44
groELS	57	HS	Heat shock proteins	−	−	132a
grx	19		Glutaredoxin	?	?	134
gshA	58		Glutathione synthetase	−	+	88a, 89
gyrB	83		DNA gyrase β subunit	?	+	233a
katE	38	KatF	Catalase HPII	+	−	151, 193
katF	59		σ factor (RpoS)	+	−	138, 153, 225
katG	89	OxyR	Catalase HPI	+	−	154
ksgA	1		16S rRNA methylase	+	+	126b
micF	48	SoxRS	Inhibitor of OmpF translation	?	?	55a, 92
mutM (*fpg*)	82		Formamidopyrimidine DNA glycosylase	−	?	23, 24, 50
mvrA	7		?	?	+	172
ndh	22	SoxRS (?)	Respiratory NADH dehydrogenase	+	?	118, 119
nfo	47	SoxRS	Endo IV	−[b]	−[b]	48
nrdB	49		Ribonucleoside diphosphate reductase	+	+	66a, 183
nth	36		Endo III	−	−	49
ompF	21		Porin	?	−	55a, 92
oxyR	89		OxyR regulon activator	+	+	44, 90
oxyS	8		General antioxidant/regulator	?	?	125
polA	87		DNA polymerase I	+	?	6
polC	4		DNA polymerase III	+	?	99
recA	58	SOS	Recombination/SOS regulator	+	−	39, 71, 118
recBC	61		Exonuclease V	+	?	117
rimK	?	SoxRS	S6 glutamic acid transferase	?	?	92, 127
rpoH	76		σ^{32} protein	+	+	132a
sodA	88	SoxRS	MnSOD	+	+	37
sodB	36		FeSOD	+/−	+	37
soi-17/19	45–61	SoxRS	?	−	+/−	132
soi-28	47	SoxRS	?	−	+	132
soxRS	92	SoxRS	Regulon activators	−	+	92, 226, 241
topA	28		DNA topoisomerase I	?	?	243
trxA	86		Thioredoxin	?	?	155
trxB	21		Thioredoxin reductase	?	?	155
uvrA	92	SOS	Excinuclease	−	?	50, 146, 196
uvrB	18	SOS	Excinuclease subunit	−	?	50, 146, 196
uvrC	42		Excinuclease subunit	−	?	50, 146, 196
xthA	38	KatF	Exo III	+	+/−	58, 193
zwf	41	SoxRS	Glucose-6-P dehydrogenase	?	?	91, 192

[a] Symbols: +, a mutation in the gene renders cells sensitive; −, not sensitive; ?, not known.
[b] Generally not sensitive, but sensitive to *tert*-butyl hydroperoxide and bleomycin.

most important predominant stable products of oxygen radical attack on DNA (60, 64). Thymine glycol has been shown to block replication in vitro (115). It will also spontaneously decompose to form formylpyruvylurea, urea, and N-substituted urea residues (31). Thymine glycol and its decomposition products are readily removed by an *N*-glycosylase activity associated with endonuclease III (Endo III) (49, 235). Mutants lacking Endo III activity have no detectable thymine glycol glycosylase activity. It is informative that Endo III deficient (*nth*) mutants show no increased sensitivity to killing by H_2O_2 or γ radiation, although *nth* mutations have a mutator effect (31, 117). These observations strongly suggest that thymine glycols are an important premutagenic lesion, but not necessarily a lethal lesion. Consistent with the finding that thymine residue damage is a significant component of general oxidative DNA damage, the bulk of the increased mutagenesis observed in *oxyR* deletion mutants was found to be A · T → G · C transitions (211). A · T base pairs have been shown to be very sensitive to transitions by many chemical oxidants (143).

H_2O_2 causes strand breaks in vivo in the presence of Fe^{2+} (117). Strand breaks also accumulate in H_2O_2-treated cells (6, 71, 100). Single-strand breaks induce the SOS response (148, 233), whereas thymine glycols apparently do not, since *nth* mutants, which lack thymine glycol glycosylase activity, do not show enhanced induction of the SOS response by H_2O_2 (84). In *E. coli*, killing by H_2O_2 is bimodal. The first mode (mode I) is maximal at 1 to 2 mM H_2O_2, is approximately zero order with respect to H_2O_2 concentration, and requires active metabolism. The second mode (mode II) appears to be first order with respect to H_2O_2 between 10 and 100 mM and does not require active metabolism (117). The SOS response is induced by H_2O_2 in proportion to the degree of killing at mode I concentrations (118). Further-

more, *recA* mutants are very sensitive to killing by H_2O_2 (39), and it is the RecA protein itself that is important in cell survival upon exposure to H_2O_2 (118). This suggests that it is the role of RecA in the recombinational repair pathway that is crucial for cell survival against peroxide stress. This conclusion is supported by the observation that *recB* mutants are hypersensitive to H_2O_2, and *sbcB* mutations which activate the RecF pathway of recombination suppress the hypersensitivity of *recBC* mutants to H_2O_2 (117).

The results cited above strongly suggest that the genotoxic damage induced by H_2O_2 is single-strand breaks. That *xthA* (Exo III) mutants are also hypersensitive to killing by H_2O_2 suggests that sugar fragments (blocking groups) must occur with high frequency at the 3′ end of the strand breaks. This has been confirmed in experiments showing that H_2O_2-nicked DNA cannot serve as primers for in vitro DNA synthesis unless incubated in the presence of Exo III (58, 59, 119).

Endo IV, the *nfo* gene product, like Exo III, also removes 3′-blocking groups (48, 59), and overexpression of Endo IV alleviates some of the H_2O_2 sensitivity in an *xthA* mutant (188a). There are differences between Exo III and Endo IV that are discussed below.

Excinuclease (A)BC (encoded by *uvrA*, *uvrB*, and *uvrC*), which plays a crucial role in repair of UV-damaged DNA, was considered to play only a minor role in repair of oxidative DNA damage because *uvrA*, *uvrB*, and *uvrC* mutants are not sensitive to ionizing radiation (114) or H_2O_2 (118). Saporito et al. (196) reported that a *uvrA nfo xthA* triple mutant cannot be constructed, although mutants with any combination of two mutations are viable. Similar results were reported by Goerlich et al. (84). These results suggest a functional redundancy of excinuclease (A)BC, Endo IV, and Exo III. In support of this suggestion, it has been demonstrated that excinuclease (A)BC is capable of removing thymine glycols and AP sites from oxidatively damaged DNA (146). Therefore, it is likely that excinuclease (A)BC also participates in repair of oxidatively damaged DNA. However, the results do not necessarily indicate overlapping specificities of the enzymes. An alternative explanation has been offered by Foster (76a), who showed that relieving the burden of AP sites by using an *ung* mutation allows construction of a *uvrA nfo xthA* triple mutant.

The involvement of excinuclease (A)BC in the repair of oxidative damage is further demonstrated by more recent experiments, which also indicate the participation of the Fpg protein, a DNA glycosylase which has the ability to excise the imidazole ring-opened form of purines. The photosensitizer methylene blue plus visible light generates singlet oxygen as the ultimate reactive species (68) and produces the damaged base 8-hydroxyguanine (202). It has been shown that pBR322 DNA treated with methylene blue plus light transforms *uvrA fpg* double mutants at a significantly lower frequency than it transforms the wild type, whereas the *uvrA* or *fpg* single mutation does not affect the transformation frequency (50). *fpg* mutants are defective in the formamidopyrimidine-DNA glycosylase activity (Fpg protein) (23, 24). The results indicate that both excinuclease (A)BC and Fpg protein are capable of excising damaged bases (perhaps 8-hydroxyguanine and other modified bases) and that these excision activities are a functional duplication for the damage repair.

Finally, H_2O_2 has been shown to stimulate ATP- and Mg^{2+}-independent DNA repair synthesis in vitro (100). As shown with *recA* and *xthA* mutants, *polA* mutants have also been found to be hypersensitive to killing by H_2O_2 (6). Taken together, these findings show that DNA repair synthesis after H_2O_2 damage requires DNA polymerase I activity. Furthermore, *polC*(Ts) mutations encoding thermolabile DNA polymerase III sensitize cells to H_2O_2 at 43°C and *polC*(Ts) mutants fail to repair single-strand breaks caused by H_2O_2 when incubated at the high temperature (99). Therefore, DNA polymerase III is also required for repair of DNA damage by H_2O_2. It has been proposed that at least two pathways are involved for repair of H_2O_2-induced DNA damage: one requires Exo III and DNA polymerases I and III, whereas the other utilizes DNA polymerase I (101).

Mutagenicity. Since oxygen radicals cause DNA damage in vitro and in vivo, and since DNA repair-deficient mutants are hypersensitive to oxidative stress, it is not surprising that oxidative stress leads to mutagenesis. It has been known since the mid-1950s that hyperbaric oxygen is mutagenic in *E. coli* (73). Even concentrations of oxygen physiological to humans have been reported to be mutagenic in *S. typhimurium* (35), although not so in *E. coli* (70).

The mutagenic potential and pathway for H_2O_2 has been fairly well established (38, 118). As with killing, H_2O_2 mutagenicity is biphasic. At low (mode I) concentrations, mutagenicity is strongly correlated with SOS induction as measured by induction of a lambda lysogen (118). At high (mode II) concentrations, the correlation is weaker. The SOS-dependent mutagenesis observed after UV irradiation depends largely upon expression of the *umuDC* operon (13, 15). Surprisingly, H_2O_2 mutagenesis was reported to be UmuDC independent in *E. coli* (118). And yet, *lexA*(Ind$^-$) mutants are even more sensitive to killing by H_2O_2 than are *recA* mutants, suggesting that other SOS functions are important for cell survival (118). Furthermore, as mentioned above, H_2O_2 induces cell filamentation in an *sfiA*$^+$-independent manner (118). The role of the SOS response in H_2O_2 killing and mutagenicity is therefore somewhat unusual.

Another approach to understanding the mutagenic potential and specificity of H_2O_2 was taken by Storz et al. (211), who used *oxyR*Δ mutants in an *S. typhimurium* His$^-$ → His$^+$ reversion assay. When *oxyR*Δ mutants are grown under normal oxygen tension, they show a His$^-$ → His$^+$ reversion frequency 11-fold higher than do isogenic wild-type cells. Interestingly, the mutagenesis in an *oxyR*Δ strain carrying *mucA*$^+$ and *mucB*$^+$ on a plasmid (pKM101) is 50-fold higher than in the wild-type with the same plasmid. These results suggest that the products of *mucA*$^+$ and *mucB*$^+$ enhance mutagenesis by converting premutagenic lesions to mutagenic lesions. Since *mucA* and *mucB* are plasmid-encoded analogs of the *E. coli umuDC* genes, this finding indicates either that the paths of H_2O_2 mutagenicity differ in *S. typhimurium* and *E. coli* or that there is an unresolved discrepancy in the results. The difference in results may simply reflect a difference in mutagenicity markers. The *oxyR* and pKM101 mutational analysis involved a His reversion assay (211), whereas Imlay and Linn (117, 118) used forward mutation markers.

As noted above, the most frequent type of mutation in the *oxyR*Δ strain is A · T → G · C transitions, as might be expected if oxidative damage to thymine residues is prevalent. Similar results were obtained with *E. coli* or *S. typhimurium* cells lacking catalase activity or Ahp activity (211).

The nature and pathway of O_2^- mutagenicity are less clear. Since O_2^- is not very reactive toward many biological substrates in vitro (18–20), there remains the important question of how dangerous an increase in the flux of O_2^- is in vivo and whether endogenously produced O_2^- poses a threat to genetic integrity. Some redox-active compounds

have been shown to be slightly mutagenic (43, 71, 170). Given the pleiotropic effect of many redox compounds, however, the results did not exclude the possibility of O_2^--independent mutagenicity of these compounds. In fact, O_2^- that is generated by the hypoxanthine-xanthine oxidase system has been shown to cause a significant increase in mutations only in a particular strain (TA104) of *S. typhimurium* which carries a *uvrB* mutation (54). The possible involvement of excinuclease (A)BC in oxidative DNA damage repair has been discussed above.

By using a different approach, both the mutation frequency and rate in SOD$^-$ cells (i.e., *sodA sodB* double mutants) have been determined in the absence of redox-active compounds (70), when the steady-state concentration of O_2^- is ca. 10^3 times higher than in wild-type cells (116). The mutation rate was determined by a fluctuation test measuring the rate of rifampin-sensitive to rifampin-resistant mutagenesis during aerobic growth of SOD$^-$ cells. The target of the antibiotic rifampin is the β subunit of RNA polymerase, and resistant mutants possess an altered subunit as a result of mutations in the *rpoB* gene. Such mutations must be mostly, if not exclusively, base substitutions (83). The mutation rate toward rifampin resistance is thus a sensitive assay for this class of mutational events. The *sodA sodB* double mutant shows a 40-fold increase in the rate of aerobic spontaneous mutations toward rifampin resistance, and the *sodA* single mutant shows a 9-fold increase compared with the wild type; the *sodB* single mutant do not show an increase in the mutation rate (70, 223).

The increased mutagenesis observed in SOD-less cells is oxygen dependent. In anaerobic cultures, the frequency of Thy$^+$ → Thy$^-$ mutations is essentially identical for the *sodA sodB* double mutant and the wild-type strain. When SOD-less cells are subjected to bubbling of pure oxygen through the culture or are exposed to plumbagin, they display a very high mutation frequency. These results indicate that the increase in spontaneous mutagenesis in cells lacking SOD activity depends on the presence of oxygen and that exposure to increased levels of O_2^- greatly enhances mutagenesis.

In keeping with the finding that O_2^--generating agents do not induce the SOS response (71), the presence of a *recA*(Def) mutation does not alter the oxygen-dependent mutagenesis enhancement in *sodA sodB* mutants (70). Furthermore, by using *dinD*::*lacZ* and *sfiA*::*lacZ* fusions (*dinD* and *sfiA* genes are repressed by LexA), it has been shown that SOD-less cells do not display induction of the SOS response. Thus, unlike H_2O_2 mutagenesis, O_2^- mutagenesis appears to be completely independent of the SOS response.

Exo III, encoded by the *xthA* gene, has several catalytic activities that are involved in the repair of certain types of DNA damage. The enzyme possesses 3′ exonuclease and phosphatase activities, RNase H, and apurinic endonucleolytic activities, as well as an endonucleolytic activity that recognizes bases containing urea residues (133, 168, 238). *xthA* mutants lack 90% of the normal Exo III activities (238). When mutation frequencies in wild-type, *sodA sodB*, and *sodA sodB xthA* strains were measured, it was found that the enhanced mutation frequency observed in the *sodA sodB* culture is largely dependent on the presence of a functional *xthA* gene (70, 113). The observation suggests that cells lacking SOD suffer from increased levels of premutagenic DNA damage and that the action of Exo III on these DNA lesions greatly enhances their mutagenicity.

Little is known about the mutagenic specificity of O_2^--inflicted DNA damage. Evidence shows that it cannot be identical to that induced by H_2O_2. First, it is SOS independent. Second, whereas SOD-less cells show higher plating efficiency of O_2^--damaged λ damage than wild-type cells do, they show no difference in plating efficiency of H_2O_2-damaged phage (71a). Finally, O_2^- induces synthesis of Endo IV, whereas H_2O_2 does not. It is probable that Endo IV and Exo III do not have identical substrate specificities since overproduction of Endo IV in an *xthA* mutant does not complement the H_2O_2 sensitivity defect of the mutant (48). Also, *nfo* mutants are sensitive to bleomycin and cumene hydroperoxide, whereas *xthA* mutants are not sensitive to bleomycin (48).

A · T base pairs are the major targets for substitutions in *E. coli* mutants lacking SOD activity (131a). Furthermore, both Endo III and MutY adenine glycosylase (specific for A-G mismatches) may be $(Fe\text{-}S)_4$-containing proteins (167). Several $(Fe\text{-}S)_4$-containing proteins have been shown to be remarkably sensitive to inactivation by O_2^- (80). It is therefore possible that the elevated mutagenesis in SOD-less cells comes from inactivation of one or both of these repair enzymes. *mutY* mutants show a high frequency of transversions (9), whereas H_2O_2 mutagenesis largely generates transitions. If determination of the mutagenic specificity in SOD-less cells turns up an elevated frequency of transversions, this might suggest a role of MutY in O_2^--dependent mutagenesis.

Since elevated concentrations of oxygen are mutagenic in *E. coli*, it was of interest to determine the role, if any, of the SOS response in that mutagenesis. The SOS response includes error-prone repair functions that create mutations at the site of DNA lesions (targeted mutagenesis), as well as functions that mutate undamaged DNA ("untargeted" mutagenesis) (239). It is possible, however, that all DNA contains cryptic lesions, revealed only when fixed as mutations in an SOS-induced cell. Active oxygen species are a potential source of such lesions. To determine whether untargeted SOS mutagenesis is oxygen dependent, the frequency of His$^-$ → His$^+$ reversions in a *recA441*(Tif) strain was compared when cells were grown aerobically or anaerobically. The *recA441*(Tif) mutation allows activation of RecA protein at 42°C without DNA damage, leading to an increase in the spontaneous mutation rate. The results revealed no reduction in Tif-mediated untargeted mutagenesis in the absence of oxygen. It was concluded that untargeted SOS mutagenesis is not responding to oxygen-dependent lesions (70).

Although ozone and singlet oxygen have both been shown to cause DNA damage in vivo and in vitro, their roles in oxidative mutagenesis remain controversial (51, 63, 68, 145, 199, 230).

DNA damage and transcription. As discussed above, thymine glycol and possibly other damaged bases, as well as AP sites, block replication by DNA polymerase. It has recently been shown that thymine glycols also interfere with transcription by causing RNA polymerase to pause or stop completely at or near the lesion (35a). Although other forms of oxidative DNA damage have not been tested, it is probable that single-strand breaks, AP sites, 8-hydroxydeoxyguanosine, and many other forms of oxidatively damaged DNA either block RNA polymerase or cause misreading. Both of these effects would result in production of truncated or abnormal proteins, a result that, if severe enough, would itself induce the heat shock response (85). The chaperone proteins, DnaK and GroEL, show increased synthesis after oxidative stress, suggesting the possibility that they serve to handle the increased level of misfolded proteins (discussed

in detail below). Toxicity induced by severe oxidative DNA damage may result directly from disruption of transcription as well as from mutagenesis.

Membrane damage. Oxidative damage to membranes can arise through either lipid or membrane protein damage. Oxidative stress has been shown to cause peroxidation of lipids both in vitro and in vivo. Of the active oxygen species formed during oxidative stress, $HOO^{\cdot}$, $OH^{\cdot}$, singlet oxygen, ozone, and perferryl radicals can all initiate lipid peroxidation in vitro and in vivo. Lipid peroxidation entails three steps: initiation, propagation, and termination. It is likely that a lipid peroxidation chain reaction begins after hydrogen abstraction from an unsaturated fatty acid to form a lipid radical. The lipid radical ($L^{\cdot}$) thus formed reacts with molecular oxygen to form a lipid peroxy radical ($ROO^{\cdot}$). The reaction is perpetuated when the lipid peroxy radical attacks another unsaturated fatty acid and abstracts a hydrogen atom to form a fatty acid hydroperoxide (ROOH) and perpetuate the initial reaction. The hydroperoxides thus formed will break down thermally or in the presence of O_2^- or reduced transition metals to form lipid peroxy radicals ($LOO^{\cdot}$) or lipid alkoxy radicals ($LO^{\cdot}$), both of which can initiate new rounds of peroxidation. It is also possible that NADH will donate an electron to the hydroperoxide to generate another lipid radical and water (129).

Lipid alkoxy radicals can undergo cleavage of C—C bonds to form unsaturated fatty acid aldehydes and alkyl radicals. The peroxidation of lipids thus generates products which are shorter than the initial fatty acid. In addition to producing fatty acyl chains that are shorter than the parent chain, the end products of lipid peroxidation include alkanes, ketones, epoxides, and aldehydes (16, 129, 163). When fatty acid chains become shortened or gain charges, their ability to rotate within the membrane is altered and the membrane becomes more fluid (162, 163). An increase in membrane fluidity results directly in a loss of structural integrity. Structural integrity is required for transport of most nutrients, F_1/F_0 ATPase activity, motility, and prevention of osmotic imbalance (62, 130, 140). Because membrane permeabilization will destroy the proton gradient across the cell membrane, the internal pH will drop, causing the $O_2^- \rightleftharpoons HOO^{\cdot}$ equilibrium to shift to the right, which in turn is likely to result in further oxidative damage (72).

A second hazard posed by lipid peroxidation is that peroxidation intermediates and end products are mutagenic. As stated above, lipid peroxidation products include an array of aldehyde, epoxide, hydroxy, carboxy, and peroxy groups, as well as alkanes and alkenes. Many stable termination products, such as 4-hydroxyalkenals, epoxides, and other aldehydes have been shown to be directly reactive with DNA, either by alkylating bases (203) or by forming intrastrand and interstrand cross-links as discussed above (214). Lipid oxidation products listed above also react with and inactivate proteins (42).

E. coli and *S. typhimurium* are defended against lipid peroxidation in both simple and complex ways. The rate of fatty acid peroxidation is directly proportional to the number of unsaturated C=C bonds (129), and bacteria have generally saturated or monounsaturated fatty acids in their membranes. It has been shown that *E. coli* fatty acid auxotrophs grown on oleic acid (monounsaturated fatty acid) were more sensitive to killing by oxidative stress than were those grown on saturated fatty acids (106); therefore, that there is a cost associated with maintaining even monounsaturated fatty acids in the membrane. Additionally, certain stress conditions such as exposure to ethanol have been shown to cause an increase in the level of unsaturated fatty acids into the membranes (17). Taken together, these data suggest that bacteria are not altogether immune to oxidative lipid damage.

In *E. coli*, there may be an inducible membrane repair response. It has been shown that H_2O_2 disrupts membrane functions at nonlethal doses and that cells which are pretreated with a low concentration of H_2O_2 acquire the ability to rapidly recover from the loss of membrane functions (72). The peroxide stress response is required for this inducible recovery of membrane function. Furthermore, *katG* (under control of *oxyR*) plays a necessary, although not sufficient, role in this response (72). It is possible that the OxyR-regulated Ahp (*ahpC ahpF*) plays an important role in inducible membrane repair by reducing fatty acid hydroperoxides. In these experiments, membrane functions were monitored by measuring ΔpH-dependent and ΔpH-independent transport. The concentrations of H_2O_2 sufficient to induce rapid recovery of transport inhibition has no effect upon transport activities. This would imply that the signal to induce transport recovery does not initiate with damage to the membrane itself.

Other evidence for the existence of an inducible membrane response comes from experiments showing that ionizing radiation induces a membrane repair response as measured by sensitivity of naive and preexposed cells to hypotonic medium (244). Cells treated with chloramphenicol prior to the inducing treatment failed to develop resistance to osmotic stress, suggesting that novel gene products are required for induced resistance.

Protein damage. Interactions between oxygen radicals and proteins leads to conversion of proline and arginine residues into carbonyl derivatives. Oxidative attack of histidinyl and prolyl residues converts them into asperginyl and glutamyl derivatives, respectively (208). Methionine and cysteinyl residues are oxidized to form methionine sulfoxide derivatives and disulfide derivatives, respectively. These alterations generally inactivate enzymes and can lead to their targeted degradation. Several *E. coli* enzymes have been shown to be quite sensitive to oxidative damage, including dihydroxy acid dehydratase, glutamine synthetase, ribosomal protein L12, quinolate synthetase, aconitase, and 6-phosphogluconate dehydratase (32, 80, 135, 144). Dihydroxy acid dehydratase, quinolate synthetase, aconitase, and 6-phosphogluconate dehydratase have $(Fe\text{-}S)_4$ clusters, and it is likely that these are the sites of O_2^- attack (80). It would appear to be generally true that metal-binding sites in proteins are especially sensitive to attack by active oxygen species. It will be of value to determine whether Endo III and MutY are inactivated by O_2^-, since they may contain $(Fe\text{-}S)_4$ clusters.

Repair of protein damage appears to be limited to reduction of disulfides and methionine sulfoxides. Reduction of both of these is facilitated by thioredoxin and thioredoxin reductase in an NADPH-dependent pathway (86, 155). Little is known about the regulation of inducibility of protein repair enzymes.

In *E. coli*, there appear to be specific proteinases that selectively degrade oxidized proteins (52). This degradation pathway is ATP independent and is distinct from the Lon protease associated with the heat shock response (53). A protease has been purified from *E. coli* that selectively degrades oxidized glutamine synthetase (191). Whether this specific degradation serves simply to expedite recycling of amino acids, or whether it has some other physiological function (for example, to prevent accumulation of damaged

proteins that can cause induction of the heat shock response or accumulation of glutamate owing to inactivation of a high proportion of the total glutamine synthetase) is not currently known. Regulation of these proteases is also poorly understood, although OyxR(Con) mutants show a two-fold increase in protease activity, suggesting a possible role for OxyR in their induction (53).

Role of the Peroxide Stress Response

We know the biochemical functions of only a minority of OxyR- and non-OxyR-regulated genes that are induced in the peroxide stress response. The OxyR-regulated genes whose functions are known are *katG* (HPI catalase), *gor* (glutathione reductase) and *ahpC-ahpF* (alkylhydroperoxide reductase). The role of HPI catalase is to reduce the concentration of H_2O_2. The reduction of GSSG by glutathione reductase would be useful for maintaining the reducing environment of the cell and for directly reacting with and destroying H_2O_2. Surprisingly, however, mutant cells completely devoid of GSH (*gshAB* mutants) show no increased sensitivity to H_2O_2 (89), raising the question of the role of glutathione reductase during exposure to peroxide stress. Ahp activity is likely to play two important roles during exposure to peroxides. By reducing the level of the offending peroxide, it diminishes the threat of damage. Also, by reducing the level of peroxidized cellular components, it repairs the damage that is not prevented from occurring in the first place.

Another activity that may be at least partially regulated by OxyR is that of ribonucleoside diphosphate reductase (66a), which converts all ribonucleoside diphosphates to 2′-deoxyribonucleoside diphosphates, precursors for DNA synthesis. Ribonucleoside diphosphate reductase can use either glutaredoxin or thioredoxin as a hydrogen source. GSH is required for synthesis of glutaredoxin. As discussed above, one of the OxyR-regulated genes, *gor*, encodes glutathione reductase. One possible role of enhanced levels of glutathione reductase may be to maintain sufficient levels of GSH so that glutaredoxin levels do not fall dramatically during oxidative stress. H_2O_2 has been shown to inhibit incorporation of radiolabeled thymine into the DNA, and pretreated cells show a more rapid recovery from inhibition of incorporation after a challenge dose than naive cells do (56). Since H_2O_2 treatment also disrupts uptake of thymine (72), it is not clear whether H_2O_2 also directly inhibits DNA synthesis. If it does, however, and recovery of DNA synthesis after H_2O_2 treatment requires OxyR functions, the candidate functions would be ribonucleoside diphosphate reductase and glutathione reductase.

The heat shock proteins DnaK and GroEL are inducible with H_2O_2 (171). GroEL is induced by O_2^- (234). DnaK makes up approximately 1% of the total cellular protein at 37°C and approximately 4% after a temperature shift (182). It possesses several activities in vitro: (i) it is essential for in vitro replication of λ phage, (ii) it has a 5′ nucleotidase activity that is inhibited by AppppA, and (iii) it autophosphorylates (247) in a temperature-dependent manner (159b). *dnaK* mutants also do not phosphorylate glutaminyl- and threonyl-tRNA synthetases (232). Finally, DnaK plays an important role as a chaperone protein (see reference 96 and references therein). Since DnaK is induced by H_2O_2, the presumption is that it must play a role during peroxide stress. Which, if any, of the above functions is important in the response of the cell to peroxide stress is not known. It is interesting that AppppA is strongly induced by H_2O_2, starvation, and heat shock conditions that all induce DnaK. AppppA has recently been shown to bind to DnaK and increase the level of DnaK autophosphorylation in vitro (126a).

GroEL functions as a chaperone that maintains prefolded proteins in that state and facilitates their export through the inner membrane. Increased production of GroEL during oxidative stress may be required to handle an increased number of misfolded proteins resulting directly from damaged nascent polypeptides or indirectly from mistranscribed or mistranslated genes. Alternatively, increased levels of GroEL may be required to compensate for damaged membrane export apparatus.

Finally, H_2O_2 and O_2^- exposure induces two distinct NADH-dependent diaphorase activities as determined by activity bands on nondenaturing protein gels (70c). The role of diaphorase (NADH dehydrogenase) activities during oxidative stress is not clear. One possible function is to reduce the concentration of intracellular NADH in order to prevent the reduction of Fe^{3+} to Fe^{2+} by NADH, as reported previously (97). Consistent with the idea that high NADH concentrations might pose a threat to cells during exposure to H_2O_2, *ndh* mutants (which accumulate NADH) are hypersensitive to killing by H_2O_2 (119). A second function might be to reduce the level of cellular respiration and thereby reduce the number of electrons flowing through the electron transport chain (a source of O_2^-). A third possible function of NADH-diaphorase activity is to transfer electrons to ubiquinone-8, which can then be oxidized in a coupled manner to generate ΔpH and Δψ (see reference 185 and references therein), both of which are lost during oxidative stress (72).

Role of the Superoxide Stress Response

As discussed above, the SoxRS-regulated genes that encode known functions are *sodA* (SOD), *nfo* (Endo IV), *micF* (antisense regulator of *ompF*), *zwf* (glucose-6-P dehydrogenase), and *rimK* (an enzyme that adds nonencoded glutamic acid residues to the carboxy terminus of the ribosomal protein S6) (92, 127). A SoxRS-regulated function whose gene has not been characterized is an NADH diaphorase (70c).

The function of SOD is to reduce the level of O_2^-. Endo IV repairs oxidatively damaged DNA by removing 3′-blocking groups such as phosphoglycolates. As discussed above, there must be some substrate specificities not shared by Endo IV and Exo III. The function of the *micF* antisense RNA in down-regulating *ompF* has been discussed above. *soxR*(Con) mutants show increased resistance to several antibiotics whose uptake may be OmpF dependent, and it has been hypothesized that the natural function of OmpF down-regulation by SoxR is to exclude naturally occurring antibiotics (92). Another possible function of OmpF down-regulation might be to limit the uptake of naturally occurring redox-active compounds that promote O_2^- production. However, *ompF* mutants are not more resistant to menadione than is the wild type (55a).

The role of glucose-6-P dehydrogenase is to produce NADPH. NADPH production would be useful under conditions of oxidative stress since it is required as the electron source for both thioredoxin reductase and glutathione reductase. Also, protein repair catalyzed by methionine sulfoxide reductase requires NADPH. Furthermore, NADPH does not reduce Fe^{3+}, whereas NADH does (146a). Thus NADPH production would provide the cell with reducing

equivalents without facilitating OH· production via the Fenton reaction.

The role(s) for the NADH-dependent diaphorase activity induced by O_2^- stress is likely to be the same as that of the H_2O_2-induced diaphorase, namely, to reduce the level of NADH, to decrease the rate of respiration, or to detoxify redox-active quinones to the hydroquinone state by a two-electron reduction. In *E. coli*, one of the NADH dehydrogenases is the product of the *ndh* gene (185). It is not known whether either of the oxidative stress-inducible diaphorases is the product of the *ndh* gene, although upstream sequence homology between the *ndh* gene and several SoxRS-regulated genes suggests that this gene may be regulated, at least in part, by SoxRS (82a). The functions of *soi-17/19* and *soi-28* are not yet known, although these genes must play an important role since they sensitize cells to O_2^- stress (132). The function of *mvrA* is presently unknown.

Possible Role of the Stringent Response

Another response that may play a role in oxidative stress is the stringent response. When uncharged tRNAs enter the A site of a ribosome during translation, the ribosome stalls and a ribosomal protein, the RelA protein, produces a stringent factor, 3′,5′-tetraphosphoguanosine (ppGpp). The ppGpp then binds to RNA polymerase and alters the transcription from *rrn* operons (149). The net effect is that during amino acid starvation, the cell decreases or suspends production of rRNA and ribosomal protein. This suspension serves at least one apparent purpose: it saves the cell the costly expenditure of precursors and energy required to synthesize ribosomes when those ribosomes would end up being unemployed owing to the lack of amino acids.

Interestingly, ppGpp is also produced during heat shock and during exposure to H_2O_2 and paraquat (33, 228). Certain quinones and quinonelike drugs that are capable of redox cycling have been shown to inhibit leucyl-tRNA synthetases in vitro, suggesting that in vivo, leucyl-tRNAs would be unloaded in the presence of these compounds, triggering the stringent response (181). In addition, increased levels of ppGpp have been detected in *E. coli* after exposure to hyperbaric oxygen, and it has been proposed that this accounts for the inhibition of protein synthesis observed in these cells (204). In *E. coli*, an enzyme required for branched-chain amino acid biosynthesis (dihydroxy-acid dehydratase) is highly sensitive to inactivation by O_2^- (80), and SOD-less mutants are auxotrophs for these amino acids (37). It is therefore likely that O_2^--mediated stress causes induction of the stringent response by inactivating dihydroxy-acid dehydratase. All of the above inducing stresses are likely to disrupt normal translation (229). Some oxidative stress disrupts translational machinery, the stringent response may serve to limit production of unnecessary stable RNA. It would make sense for the cell to slow general protein synthesis under conditions where the membrane and DNA are under oxidative attack. Indeed, there is preliminary evidence that in *relA* mutants exposed to plumbagin, DNA synthesis decreases while the optical density of the culture increases (70d).

Other Genes Involved in Protection against Oxidative Stress

In addition to the genes and proteins discussed above, several genes are constitutively expressed, and there are genes whose regulation is SoxRS and OxyR independent and whose functions have been demonstrated to be necessary during oxidative stress, based on the fact that mutations in these genes sensitize the cell to oxidative stress. These include, but are not limited to, *apaH* (AppppN hydrolase) (126a) and *ksgA* (16S rRNA methylase) (70b). Mutations in the *xthA* (Exo III), *dam* (DNA adenine methylase), *gyr* (DNA gyrase), and *topA* (topoisomerase I) genes also play a role in protection against the oxidative damage. There are also mutations in genes whose functions are unknown, but nevertheless render the cell more sensitive to oxidative stress. These include *soi-17/19*, *soi-28*, *mvrA*, and several *oxyS* genes (125, 132, 172). In addition, there are several other genes whose functions would strongly suggest that they play an important role in protection against oxidative damage, but whose sensitivity to oxidative stress has not been determined, e.g., *trxA* (thioredoxin). These genes are included in Table 2.

CONCLUSIONS

Most of the proteins induced by either peroxide or O_2^- stress remain unidentified. Consequently we know relatively little about the physiological functions or pathways used during oxidative stress. The few functions that have been identified, however, suggest that many, if not most, of the metabolic pathways in the cell are altered in some way by oxidative stress and the responses of the cell to it. For example, oxidative membrane damage leads to a plethora of subsequent problems for the cell; loss of ΔpH will change the intracellular pH, causing further production of HOO·, will decrease ATP synthesis, will increase permeability to toxic compounds, will prevent chemotaxis, etc. Each one of these events requires further metabolic adjustments by the cell. For example, the loss of energy charge is likely to affect macromolecular synthesis, which, in turn, might induce the stringent response, and oxidative DNA or protein damage is likely to generate its own sequelae of events.

One approach to unraveling the complex responses of the cell to oxidative stress may therefore be to determine which types of damage or disruption inevitably follow one another. For example, certain types of DNA damage may generally lead to production of truncated or abnormal proteins. Because of the general cooccurrence of these events, the cell may have evolved a regulon that coinduces a response to deal with abnormal proteins, whether or not abnormal proteins have actually been produced. An example of this might be in the SoxRS response, in which the cell coregulates induction of antioxidants such as MnSOD and a DNA repair enzyme, Endo IV. It is unlikely that at the time of initial activation of the SoxRS regulatory elements, severe DNA damage has already occurred. Rather, through evolutionary experience, the cell may "know" that whenever it needs extra MnSOD, it is likely to need extra Endo IV. In terms of assigning genes or functions to stimulons, coinduction of genes or functions rather than sequential inductions would be suggestive of, although not proof of, coregulated events. Final assignment of genes to stimulons can, however, be made only when a necessary interaction between the regulators (SoxR, SoxS, OxyR, etc.) and the genes they regulate (*nfo*, *soi-28* promoters, etc.) has been demonstrated.

Comparison and Extrapolation to Eukaryotes

There is a great deal of similarity in the overall strategy of defense against oxidative stress between bacteria and eukaryotic cells. On the other hand, there appear to be some

crucial tactical differences. Nearly all aerobic cells, prokaryotic and eukaryotic, possess SODs, catalases, and peroxidases to destroy active oxygen species. Indeed, SOD expressed from mammalian *sod* genes fully protects SOD^- bacteria from O_2^- (175). Repair functions also overlap extensively. For example, some bacterial and eukaryotic DNA repair enzymes specific for oxidative damage share extensive homology (55a, 185a).

The fundamental differences between bacteria and eukaryotes appear in the regulation and complexity of the responses to oxidative stress. The inducers of the oxidative stress responses in bacteria appear to be either the oxidant itself or interaction of the oxidant with a cell component, as in the case of OxyR. On the other hand, oxygen radicals do not, in general, induce limited production of antioxidant enzymes in mammalian cells. Rather, cytokines such as tumor necrosis factor, interleukin-1, or bacterial lipopolysaccharides induce SOD synthesis and multigene responses (157a, 240). Evolutionally this makes sense, given that most mammalian cells exist in an environment where the oxygen concentration is constant (ambient 4% in blood serum). An exception is upon sequestration and activation of neutrophils to produce active oxygen species. It is economical that the same signals that sequester and activate neutrophiles should also induce antioxidants in bystander cells. Indeed, although paraquat has no inductive effects on MnSOD and phase II enzymes in mammalian cells, tumor necrosis factor is a very potent inducer (240).

Sources of active oxygen species of evolutionary importance (that fluctuate sufficiently to warrant inducible responses) include near-UV radiation, ionizing radiation, and possible ingestion of redox-active or radical generating phytoalexins (i.e., psoralen in celery). Mammalian cells induce a few proteins in specific response to NUV stress, most notably heme-oxygenase and glutathione transferase. Since cells of internal organs that are never exposed to near-UV show strong inducibility of these proteins by near-UV (131), this suggests either that their regulation pathway is ancient, or that the actual natural inducer is not near-UV, but rather a subsequent reactant, possibly H_2O_2. The regulation pathway of H_2O_2-inducible genes in mammalian cells is unknown. It is possible, however, that simple activation of a DNA-binding protein results in gene expression, as appears to be the case in bacterial OxyR-regulated genes.

Although the regulatory pathway(s) appears to be enormously complex and involves several factors, recent work shows that O_2^- is a strong tumor promoter that works by activation and induction of growth competence-related gene products; c-*fos*, c-*jun*, c-*myc*, and β-actin (40a, 47). These gene products are transcription factors that regulate a battery of genes. Other factors involved in the regulation of eukaryotic antioxidant gene expression include an induction of calmodulin kinase by increase in Ca^{2+} concentrations, which in turn activates S6 phosphorylation. The elevation of Ca^{2+} concentration levels, which could occur when membranes are damaged by O_2^- radicals, also leads to phosphokinase C activation. Phosphokinase C activation in turn phosphorylates and activates topoisomerase I (184a). Phospholipid hydroperoxides, produced by membrane oxidation, will also activate phospholipid hydroperoxide reductase, glutathione peroxidase, and phospholipase II.

Finally, DNA strand breaks induced by oxidative stress will induce poly(ADP-ribose) polymerase. Several proteins, including Jun, Fos, TopoI, and a DNA polymerase α subunit, are poly-ADP-ribosylated. Poly-ADP-ribosylation is crucial for activation of Fos. The relationship between an increase in the concentration of calcium and DNA damage is unclear. Other factors involved in increased expression of the c-*fos* gene upon oxidative stress include the serum-responsive factor, serum-responsive element, and cyclic AMP-responsive element (40a).

In summary, the complexity in bacterial responses appears to be in the sheer number of proteins induced by oxidative stress, well over 60. Conversely, the relative number of proteins induced in mammalian cells is small, but the regulatory pathways are highly complex.

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REFERENCES

1. **Adelman, R., R. L. Saul, and B. N. Ames.** 1989. Oxidative damage to DNA: relation to species metabolic rate and life span. Proc. Natl. Acad. Sci. USA **85:**2706–2708.
2. **Aldea, M., T. Garrido, C. Hernandez-Chico, M. Vicente, and S. R. Kushner.** 1989. Induction of a growth-phase-dependent promoter triggers transcription of *bolA*, an *Escherichia coli* morphogene. EMBO J. **8:**3923–3931.

2a. **Amabile-Cuevas, C. F., and B. Demple.** Nucleic Acids Res., in press.

3. **Ames, B. N.** 1983. Dietary carcinogens and anti-carcinogens. Science **221:**1256–1264.
4. **Ames, B. N.** 1985. Oxidative damage as related to cancer and aging, p. 11–26. *In* L. Back and K. Nathan (ed.), Genetic toxicology of environmental chemicals. Alan R. Liss, Inc., New York.
5. **Amstad, P., and P. Cerutti.** 1990. Genetic modulation of the cellular antioxidant defense capacity. Environ. Health Perspect. **88:**77–82.
6. **Ananthaswamy, H. N., and A. Eisenstark.** 1977. Repair of hydrogen peroxide-induced single-strand breaks in *Escherichia coli* deoxyribonucleic acid. J. Bacteriol. **130:**187–191.
7. **Aruoma, O. I., and B. Halliwell.** 1991. DNA damage and free radicals. Chem. Br. **2:**149–152.
8. **Aruoma, O. I., B. Halliwell, E. Gajewski, and M. Dizdaroglu.** 1989. Damage to bases in DNA induced by hydrogen peroxide and ferric ion chelates. J. Biol. Chem. **264:**20509–20512.
9. **Au, K. G., M. Cabera, J. H. Miller, and P. Modrich.** 1988. *Escherichia coli mutY* gene product is required for specific AG → CG mismatch correction. Proc. Natl. Acad. Sci. USA **85:**9163–9167.
10. **Aust, S. D.** 1988. Sources of iron for lipid peroxidation in biological systems, p. 27–33. *In* B. Halliwell (ed.), Oxygen radicals and tissue injury. The Upjohn Co., Bethesda, Md.
11. **Aust, S. D., L. E. Morehouse, and C. E. Thomas.** 1985. The role of metals in oxygen radical reactions. Free Radic. Biol. Med. **1:**3–26.
12. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54:**130–197.
13. **Bagg, A., C. J. Kenyon, and G. C. Walker.** 1981. Inducibility of a gene product required for UV and chemical mutagenesis in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78:**5749–5753.
14. **Bagg, A., and J. B. Neilands.** 1987. Molecular mechanism of regulation of siderphore-mediated iron assimilation. Microbiol. Rev. **51:**509–518.
15. **Battista, J. R., T. Ohta, T. Nohmi, W. Sun, and G. C. Walker.** 1990. Dominant negative *umuD* mutations decreasing RecA-

mediated cleavage suggest roles for intact UmuD in modulation of SOS mutagenesis. Proc. Natl. Acad. Sci. USA **87**:7190–7194.

16. **Benedetti, A., M. Competi, R. Fulceri, and H. Esterbauer.** 1984. Cytotoxic aldehydes originating from the peroxidation of liver microsomal lipids. Biochim. Biophys. Acta **192**:172–181.
17. **Berger, B., C. E. Carty, and L. O. Ingram.** 1980. Alcohol-induced changes in the phospholipid molecular species of *Escherichia coli*. J. Bacteriol. **142**:1040–1044.
18. **Bielski, B. H. J.** 1978. Reevaluation of the spectral and kinetic properties of HO_2 and O_2^- free radicals. Photochem. Photobiol. **28**:645–649.
19. **Bielski, B. H. J.** 1985. Reactivity of HO_2/O_2^- radicals in aqueous solution. Phys. Chem. Ref. Data **14**:1041–1045.
20. **Bielski, B. H. J., and G. G. Shiue.** 1978. Reaction rates of superoxide radicals with the essential amino acids, p. 43–56. *In* D. W. Fitzsimons (ed.), Oxygen free radicals and tissue damage. Elsevier Science Publishing, Inc., New York.
21. **Bochner, B. R., P. C. Lee, S. W. Wilson, C. W. Cutler, and B. N. Ames.** 1984. AppppA and related adenylylated nucleotides are synthesized as a consequence of oxidation stress. Cell **37**:225–232.
22. **Bohannon, D. E., N. Connell, J. Keener, A. Tormo, M. Espinosa-Urgel, M. M. Zambrano, and R. Kolter.** 1991. Stationary-phase-inducible "gearbox" promoters: differential effects of *katF* mutations and role of σ^{70}. J. Bacteriol. **173**:4482–4492.
23. **Boiteaux, S., and O. Huisman.** 1989. Isolation of a formamidopyrimidine-DNA glycosylase (*fpg*) mutant of *Escherichia coli* K12. Mol. Gen. Genet. **215**:300–305.
24. **Boiteaux, S., T. R. O'Connor, F. Lederer, A. Gouyette, and J. Laval.** 1990. Homogeneous *Escherichia coli* FPG protein: a DNA glycosylase which excises imidazole ring-open purines and nicks DNA at apurinic sites. J. Biol. Chem. **265**:3916–3922.
25. **Bol, D. K., and R. E. Yasbin.** 1990. Characterization of an inducible oxidative stress system in *Bacillus subtilis*. J. Bacteriol. **172**:3503–3506.
26. **Bolker, M., and R. Kahmann.** 1989. The *Escherichia coli* regulatory protein OxyR discriminates between methylated and unmethylated states of the phage Mu *mom* promoter. EMBO J. **8**:2403–2410.
27. **Borg, D. C., and K. M. Schaich.** 1986. Pro-oxidant action of desferrioxamine: Fenton-like production of hydroxyl radicals by reduced ferrioxamine. J. Free Radic. Biol. Med. **2**:237–243.
28. **Borg, D. C., and K. M. Schaich.** 1988. Iron and iron-derived radicals, p. 9–19. *In* B. Halliwell (ed.), Oxygen radicals and tissue injury. The Upjohn Co., Bethesda, Md.
29. **Bowen, S. W., and H. M. Hassan.** 1988. Induction of the manganese-containing superoxide dismutase in *Escherichia coli* is independent of the oxidative stress (*oxyR*-controlled) regulon. J. Biol. Chem. **263**:14808–14811.
30. **Brawn, K., and I. Fridovich.** 1985. Increased superoxide radical production evokes inducible DNA repair in *Escherichia coli*. J. Biol. Chem. **260**:922–925.
31. **Breimer, L. H., and T. Lindahl.** 1984. Excision of oxidized thymine from DNA. J. Biol. Chem. **259**:5543–5548.
32. **Brot, N., and H. Weissbach.** 1983. Biochemistry and physiological role of methionine sulfoxide residues in proteins. Arch. Biochem. Biophys. **223**:271–281.
33. **Brown, O. R., and R. L. Seither.** 1983. Oxygen and redox-active drugs: shared toxicity sites. Fund. Appl. Toxicol. **3**:209–214.
34. **Brunori, M., and G. Rotilio.** 1984. Biochemistry of oxygen radical species. Methods Enzymol. **105**:22–35.
35. **Bruyninckx, W. J., H. S. Mason, and S. A. Morse.** 1978. Are physiological oxygen concentrations mutagenic? Nature (London) **247**:606–607.

35a. **Byrd, S., D. Reines, and P. W. Doetsch.** 1990. Effects of oxidative DNA damage on transcription by RNA polymerases. Free Radic. Biol. Med. **9**(Suppl. 1):47.

36. **Cadenas, E.** 1989. Biochemistry of oxygen toxicity. Annu. Rev. Biochem. **58**:79–110.
37. **Carlioz, A., and D. Touati.** 1986. Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide dismutase necessary for aerobic life? EMBO J. **5**:623–630.
38. **Carlsson, J., E. H. Berglin, R. Claesson, M.-B. K. Edlund, and S. Persson.** 1988. Catalase inhibition by sulfide and hydrogen peroxide-induced mutagenicity in *Salmonella typhimurium* strain TA102. Mutat. Res. **202**:59–64.
39. **Carlsson, J., and V. S. Carpenter.** 1980. The *recA*$^+$ gene product is more important than catalase and superoxide dismutase in protecting *Escherichia coli* against hydrogen peroxide toxicity. J. Bacteriol. **142**:319–321.
40. **Cerutti, P.** 1985. Prooxidant states and tumor promotion. Science **227**:375–381.

40a. **Cerutti, P., A. Peskin, G. Shaw, and P. Amsted.** 1990. Free Radic. Biol. Med. **9**(suppl. 1):167.

41. **Chan, E., and B. Weiss.** 1987. Endonuclease IV of *Escherichia coli* is induced by paraquat. Proc. Natl. Acad. Sci. USA **84**:3189–3193.
42. **Chio, K. S., and A. L. Tappel.** 1969. Inactivation of ribonuclease and other enzymes by peroxidizing lipids and malondialdehyde. Biochemistry **806**:2827–2832.
43. **Chises, P. L., D. E. Levin, M. T. Smith, L. Ernster, and B. N. Ames.** 1984. Mutagenicity of quinones: pathways of metabolic activation and detoxification. Proc. Natl. Acad. Sci. USA **81**:1696–1700.
44. **Christman, M. F., R. W. Morgan, F. S. Jacobson, and B. N. Ames.** 1985. Positive control of a regulon for defenses against oxidative stress and some heat shock proteins in *Salmonella typhimurium*. Cell **41**:753–762.
45. **Christman, M. F., G. Storz, and B. N. Ames.** 1989. OxyR, a positive regulator of hydrogen peroxide-inducible genes in *Escherichia coli* and *Salmonella typhimurium*, is homologous to a family of bacterial regulatory protein. Proc. Natl. Acad. Sci. USA **86**:3484–3488.
46. **Cohen, S. P., L. M. McMurry, and S. B. Levy.** 1988. *marA* locus causes decreased expression of OmpF porin in multiple-antibiotic-resistant (Mar) mutants of *Escherichia coli*. J. Bacteriol. **170**:5416–5422.
47. **Crawford, D., I. Zbinden, P. Amstad, and P. Cerutti.** 1988. Oxidant stress induces proto-oncogenes c-fos and c-myc in mouse epidermal cells. Oncogene **3**:27–32.
48. **Cunningham, R. P., S. M. Saporito, S. G. Spitzer, and B. Weiss.** 1986. Endonuclease IV (*nfo*) mutant of *Escherichia coli*. J. Bacteriol. **168**:1120–1127.
49. **Cunningham, R. P., and B. Weiss.** 1985. Endonuclease III (*nth*) mutants of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82**:474–478.
50. **Czeczot, H., B. Tudek, B. Lambert, J. Laval, and S. Boiteaux.** 1991. *Escherichia coli* Fpg protein and UvrABC endonuclease repair DNA damage induced by methylene blue plus visible light in vivo and in vitro. J. Bacteriol. **173**:3419–3424.
51. **Dahl, T. A., W. R. Midden, and P. E. Hartman.** 1988. Pure exogenous singlet oxygen: nonmutagenicity in bacteria. Mutat. Res. **201**:127–136.
52. **Davies, K. J. A., and S. W. Lin.** 1988. Degradation of oxidatively denatured proteins in *Escherichia coli*. Free Radic. Biol. Med. **5**:215–223.
53. **Davies, K. J. A., and S. W. Lin.** 1988. Oxidatively denatured proteins are degraded by an ATP-independent proteolytic pathway in *Escherichia coli*. Free Radic. Biol. Med. **5**:225–236.
54. **De Flora, S., C. Bennicelli, P. Zanacchi, F. D'Agostini, and A. Camoirano.** 1989. Mutagenicity of active oxygen species in bacteria and its enzymatic and chemical inhibition. Mutat. Res. **214**:153–158.
55. **de Massy, B., O. Fayet, and T. Kogoma.** 1984. Multiple origin usage for DNA replication of *sdrA* (*rnh*) mutants of *Escherichia coli* K12: initiation in the absence of *oriC*. J. Mol. Biol. **178**:227–236.

55a. **Demple, B.** Personal communication.

56. **Demple, B., Y. Daikh, J. Greenberg, and A. Johnson.** 1985. Alkylation and oxidative damages to DNA: constitutive and inducible repair systems, p. 205–218. *In* D. M. Schenkel, P. E. Hartman, T. Kada, and A. Hollaender (ed.), Antimutagenesis and anticarcinogenesis: mechanisms. Plenum Publishing

Corp., New York.
57. **Demple, B., and J. Halbrook.** 1983. Inducible repair of oxidative DNA damage in *Escherichia coli*. Nature (London) **304:** 446–448.
58. **Demple, B., J. Halbrook, and S. Linn.** 1983. *Escherichia coli xth* mutants are hypersensitive to hydrogen peroxide. J. Bacteriol. **153:**1079–1082.
59. **Demple, B., A. Johnson, and D. Fung.** 1986. Exonuclease III and endonuclease IV remove 3′ blocks from DNA synthesis primers in H_2O_2-damaged *Escherichia coli*. Proc. Natl. Acad. Sci. USA **83:**7731–7735.
60. **Demple, B., and S. Linn.** 1982. 5,6-Saturated thymine lesions in DNA: production by UV light and hydrogen peroxide. Nucleic Acids Res. **10:**3781–3789.
61. **Dianzani, M. U.** 1982. Biochemical effects of saturated and unsaturated aldehydes, p. 129–158. *In* D. McBrian and T. F. Slater (ed.), Free radicals, lipid peroxidation and cancer. Academic Press, Inc. (London), Ltd., London.
62. **Dills, S. S., A. Apperson, M. R. Schmidt, and M. H. Saier.** 1990. Carbohydrate transport in bacteria. Microbiol. Rev. **44:**385–418.
63. **Di Mascio, P., H. Wefers, H.-P. Do-Thi, M. V. M. Lafleur, and H. Sies.** 1989. Singlet molecular oxygen causes loss of biological activity in plasmid and bacteriophage DNA and induces single-strand breaks. Biochem. Biophys. Acta **1007:**151–157.
64. **Dizdaroglu, M.** 1985. Formation of an 8-hydroxyguanine moiety in deoxyribonucleic acid on gamma-irradiation in aqueous solution. Biochemistry **24:**4476–4481.
65. **Eder, E., A. Favre, C. Stichtmann, and C. Deininger.** 1989. Induction of *sfiA* SOS function by peroxides using three different *Escherichia coli*. strains. Toxicol. Lett. **48:**225–234.
66. **Eisenstadt, E.** 1987. Analysis of mutagenesis, p. 1016–1033. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
66a. **Eisenstark, A.** Personal communication.
67. **Eisenstark, A.** 1989. Bacterial genes involved in response to near-ultraviolet radiation. Adv. Genet. **26:**99–147.
68. **Epe, B., J. Hegler, and D. Wild.** 1989. Singlet oxygen as an ultimately reactive species in *Salmonella typhimurium* DNA damage induced by methylene blue/visible light. Carcinogenesis **10:**2019–2024.
68a. **Estabrook, R., and J. A. Peterson.** 1990. Cytochrome P-450 and oxidative stress. Free Radic. Biol. Med. **9**(suppl. 1):161.
69. **Farr, S. B., D. N. Arnosti, M. J. Chamberlin, and B. N. Ames.** 1989. An *apaH* mutation causes AppppA to accumulate and affects motility and metabolite repression in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **86:**5010–5014.
70. **Farr, S. B., R. D'Ari, and D. Touati.** 1986. Oxygen-dependent mutagenesis in *Escherichia coli* lacking superoxide dismutase. Proc. Natl. Acad. Sci. USA **83:**8268–8272.
70a. **Farr, S. B., and X. Gidrol.** 1991. Cold Spring Harbor Meet. Stress Proteins Heat Shock Response, abstr. 50.
70b. **Farr, S. B., and D. Johnstone.** 1991. 16S rRNA methylation and induction of the heat shock response in *E. coli*. J. Cell. Biochem. Suppl. **15D:**178.
70c. **Farr, S. B., and J. Kitzler.** Unpublished results.
70d. **Farr, S. B., and T. Kogoma.** Unpublished results.
71. **Farr, S. B., D. O. Natvig, and T. Kogoma.** 1985. Toxicity and mutagenicity of plumbagin and the induction of a possible new DNA repair pathway in *Escherichia coli*. J. Bacteriol. **164:** 1309–1316.
71a. **Farr, S. B., and D. Touati.** Unpublished results.
72. **Farr, S. B., D. Touati, and T. Kogoma.** 1988. Effects of oxygen stress on membrane functions in *Escherichia coli*. J. Bacteriol. **170:**1837–1842.
73. **Fenn, W. O., G. Gerschman, D. L. Gilbert, D. E. Terwilliger, and F. V. Cothran.** 1957. Mutagenic effects of high oxygen tensions on *Escherichia coli*. Proc. Natl. Acad. Sci. USA **43:**1027–1032.
74. **Fisher, A. B.** 1988. Intracellular production of oxygen derived free radicals, p. 34–42. *In* B. Halliwell (ed.), Oxygen radicals and tissue injury. The Upjohn Co., Bethesda, Md.
75. **Floyd, R. A.** 1990. Role of oxygen free radicals in carcinogenesis and brain ischemia. FASEB J. **4:**2587–2597.
76. **Foote, C. S.** 1982. Light, oxygen and toxicity, p. 21–44. *In* A. Autor (ed.), Pathology of oxygen. Academic Press, Inc., New York.
76a. **Foster, P. L.** 1990. *Escherichia coli* strains with multiple DNA repair defects are hyperinduced for the SOS response. J. Bacteriol. **172:**4719–4720.
77. **Fridovich, I.** 1983. Superoxide dismutase: regularities and irregularities. Harvey Lect. **79:**51–75.
78. **Fridovich, I.** 1989. Superoxide dismutases. J. Biol. Chem. **264:**7761–7764.
79. **Gardner, H. W.** 1989. Oxygen radical chemistry of polyunsaturated fatty acids. Free Radic. Biol. Med. **7:**65–86.
80. **Gardner, P., and I. Fridovich.** 1991. Superoxide sensitivity of the *Escherichia coli* 6-phosphogluconate dehydratase. J. Biol. Chem. **266:**1478–1483.
81. **Garvey, N., A. C. St. John, and E. M. Witkin.** 1985. Evidence for RecA protein association with the cell membrane and for changes in the levels of major outer membrane proteins in SOS-induced *Escherichia coli* cells. J. Bacteriol. **163:**870–876.
82. **George, J., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *Escherichia coli*. Mutations in *sfiA* and *sfiB* restore division in *tif* and *lon* strains and permit the expression of mutator properties of *tif*. Mol. Gen. Genet. **140:**309–332.
82a. **Gidrol, X., and S. B. Farr.** Unpublished observations.
83. **Glass, R. E., S. T. Jones, and A. Ishihama.** 1986. Genetic studies on the beta-subunit of *Escherichia coli* RNA polymerase. Mol. Gen. Genet. **203:**265–268.
84. **Goerlich, O., P. Quillardet, and M. Hofnung.** 1989. Induction of the SOS response by hydrogen peroxide in various *Escherichia coli* mutants with altered protection against oxidative DNA damage. J. Bacteriol. **171:**6141–6147.
85. **Goff, S. A., and A. L. Goldberg.** 1985. Production of abnormal proteins in *Escherichia coli* stimulates transcription of *lon* and other heat shock genes. Cell **41:**587–595.
86. **Gonzales-Porque, P., A. Baldesten, and P. Reichard.** 1970. The involvement of the thioredoxin system in the reduction of methionine sulfoxide and sulfate. J. Biol. Chem. **254:**2371–2374.
87. **Gottesman, S.** 1984. Bacterial regulation: global regulatory networks. Annu. Rev. Genet. **18:**415–441.
88. **Greenberg, J. T., J. H. Chou, P. Monach, and B. Demple.** 1991. Activation of oxidative stress genes by mutations at the *soxQ/cfxB/marA* locus of *Escherichia coli*. J. Bacteriol. **173:** 4433–4439.
88a. **Greenberg, J. T., and B. Demple.** Personal communication.
89. **Greenberg, J. T., and B. Demple.** 1986. Glutathione in *Escherichia coli* is dispensable for resistance to H_2O_2 and gamma radiation. J. Bacteriol. **168:**1026–1029.
90. **Greenberg, J. T., and B. Demple.** 1988. Overproduction of peroxide-scavenging enzymes in *Escherichia coli* suppresses spontaneous mutagenesis and sensitivity to redox-cycling agents in *oxyR* mutants. EMBO J. **7:**2611–2617.
91. **Greenberg, J. T., and B. Demple.** 1989. A global response induced in *Escherichia coli* by redox-cycling agents overlaps with that induced by peroxide stress. J. Bacteriol. **171:**3933–3939.
92. **Greenberg, J. T., P. Monach, J. H. Chou, P. D. Josephy, and B. Demple.** 1990. Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**6181–6185.
93. **Gregory, E. M., and I. Fridovich.** 1973. Induction of superoxide dismutase by molecular oxygen. J. Bacteriol. **114:**543–548.
94. **Grenier, F. C., E. B. Waygood, and M. H. Saier, Jr.** 1985. Bacterial phosphotransferase system: regulation of the glucose and mannose enzymes II by sulfhydryl oxidation. Biochemistry **24:**4872–4876.
95. **Grisham, M., and J. McCord.** 1986. Chemistry and cytotoxicity of reactive oxygen intermediates, p. 1–18. *In* A. Taylor, S. Matalon, and P. Ward (ed.), Physiology of oxygen radicals.

American Physiolical Society, Bethesda, Md.

96. **Gross, C. A., D. B. Straus, J. W. Erickson, and T. Yura.** 1990. The function and regulation of heat shock proteins in *Escherichia coli*, p. 167–189. *In* R. I. Morimoto, A. Tissieres, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
97. **Gutman, M., R. Margalit, and A. Schejter.** 1968. A charge-transfer intermediate in the mechanism of reduced diphosphopyridine nucleotide oxidation by ferric ions. Biochemistry **7**:2778–2785.
98. **Gutteridge, J. M., and B. Halliwell.** 1990. Reoxygenation injury and antioxidant protection: a tale of two paradoxes. Arch. Biochem. Biophys. **283**:223–226.
99. **Hagensee, M. E., S. K. Bryan, and R. E. Moses.** 1987. DNA polymerase III requirement for repair of DNA damage caused by methyl methanesulfonate and hydrogen peroxide. J. Bacteriol. **169**:4608–4613.
100. **Hagensee, M. E., and R. E. Moses.** 1986. Repair response of *Escherichia coli* to hydrogen peroxide DNA damage. J. Bacteriol. **168**:1059–1065.
101. **Hagensee, M. E., and R. E. Moses.** 1989. Multiple pathways for repair of hydrogen peroxide-induced DNA damage in *Escherichia coli*. J. Bacteriol. **171**:991–995.
102. **Halliwell, B.** 1987. Oxidants and human disease: some new concepts. FASEB J. **1**:358.
103. **Halliwell, B., and J. M. C. Gutteridge.** 1984. Lipid peroxidation, oxygen radicals, transition metals and disease. Biochem. J. **219**:1–14.
104. **Halliwell, B., and J. M. C. Gutteridge.** 1990. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol. **186**:1–85.
105. **Hancock, L. C., and H. M. Hassan.** 1985. Regulation of the manganese-containing superoxide dismutase is independent of the inducible DNA repair system in *Escherichia coli*. J. Biol. Chem. **260**:12954–12956.
106. **Harley, J. B., G. M. Santangelo, H. Rasmussen, and H. Goldfine.** 1979. Dependence of *Escherichia coli* hyperbaric oxygen toxicity on the lipid acyl chain composition. J. Bacteriol. **134**:808–820.
107. **Hassan, H. M., and I. Fridovich.** 1977. Regulation of the synthesis of superoxide dismutase in *Escherichia coli*: induction by methyl viologen. J. Biol. Chem. **252**:7667–7672.
108. **Hassan, H. M., and I. Fridovich.** 1979. Intracellular production of superoxide radical and of hydrogen peroxide by redox active compounds. Arch. Biochem. Biophys. **196**:385–395.
109. **Hassan, H. M., and C. S. Moody.** 1984. Induction of the manganese-containing superoxide dismutase of *Escherichia coli* by nalidixic acid and iron chelators. FEMS Microbiol. Lett. **25**:233–236.

109a. **Hassan, H. M., and H. Sun.** 1991. Proc. 75th FASEB Annu. Meet., abstr. 2585.

110. **Heimberger, A., and A. Eisenstark.** 1988. Compartmentalization of catalases in *Escherichia coli*. Biochem. Biophys. Res. Commun. **154**:392–397.
111. **Henikoff, S., G. W. Haughn, J. M. Calvo, and J. C. Wallace.** 1988. A large family of bacterial activator proteins. Proc. Natl. Acad. Sci. USA **85**:6602–6606.
112. **Hill, O., and H. Allen.** 1978. The chemistry of dioxygen and its reduction products, p. 5–12. *In* D. W. Fitzsimons (ed.), Oxygen free radicals and tissue damage. Elsevier Science Publishing, Inc., New York.
113. **Hoerter, J., A. Eisenstark, and D. Touati.** 1989. Mutations by near-ultraviolet radiation in *Escherichia coli* strains lacking superoxide dismutase. Mutat. Res. **215**:161–165.
114. **Howard-Flanders, P., and R. P. Boyce.** 1966. DNA repair and genetic recombination: studies on mutants of *Escherichia coli* defective in these processes. Radiat. Res. Suppl. **6**:156–184.
115. **Ide, H., Y. W. Kow, and S. S. Wallace.** 1985. Thymine glycols and urea residues in M13 DNA constitute replicative blocks in vitro. Nucleic Acids Res. **13**:8035–8042.
116. **Imlay, J. A., and I. Fridovich.** 1991. DNA damage by hydrogen peroxide through the Fenton reaction in vivo and in vitro. Science **240**:640–642.
117. **Imlay, J. A., and S. Linn.** 1986. Bimodal pattern of killing of DNA-repair-defective or anoxically grown *Escherichia coli* by hydrogen peroxide. J. Bacteriol. **166**:797–799.
118. **Imlay, J. A., and S. Linn.** 1987. Mutagenesis and stress responses induced in *Escherichia coli* by hydrogen peroxide. J. Bacteriol. **169**:2967–2976.
119. **Imlay, J. A., and S. Linn.** 1988. DNA damage and oxygen radical toxicity. Science **240**:1302–1309.
120. **Inouye, S.** 1984. Site-specific cleavage of double strand DNA by hydroperoxide of linoleic acid. FEBS Lett. **172**:231–234.
121. **Iuchi, S., D. C. Cameron, and E. C. C. Lin.** 1989. A second global regulator gene (*arcB*) mediating repression of enzymes in aerobic pathways of *Escherichia coli*. J. Bacteriol. **171**:868–873.
122. **Iuchi, S., and E. C. C. Lin.** 1988. *arcA* (*dye*), a global regulatory gene in *Escherichia coli* mediating repression of enzymes in aerobic pathways. Proc. Natl. Acad. Sci. USA **85**:1888–1892.
123. **Iuchi, S., Z. Matsuda, T. Fujiwara, and E. C. C. Lin.** 1990. The *arcB* gene of *Escherichia coli* encodes a sensor-regulator protein for anaerobic repression of the *arc* modulon. Mol. Microbiol. **4**:715–728.
124. **Jacobson, F. S., R. W. Morgan, M. F. Christman, and B. N. Ames.** 1989. An alkyl hydroperoxide reductase from *Salmonella typhimurium* involved in the defense of DNA against oxidative damage. J. Biol. Chem. **264**:1488–1496.
125. **Jamison, C. S., and H. I. Adler.** 1987. Mutations in *Escherichia coli* that effect sensitivity to oxygen. J. Bacteriol. **169**:5087–5094.

125a. **Jenkins, D. E., E. A. Auger, and A. Matin.** 1991. Role of RpoH, a heat shock regulator protein, in *Escherichia coli* carbon starvation protein synthesis and survival. J. Bacteriol. **173**:1992–1996.

126. **Jenkins, D. E., J. E. Schultz, and A. Matin.** 1988. Starvation-induced cross protection against heat or H_2O_2 challenge in *Escherichia coli*. J. Bacteriol. **170**:3910–3914.

126a. **Johnstone, D., and S. B. Farr.** Submitted for publication.

126b. **Johnstone, D., and S. B. Farr.** Unpublished data.

127. **Kang, W.-K., T. Icho, S. Isono, M. Kitakawa, and K. Isono.** 1989. Characterization of the gene *rimK* responsible for the addition of glutamic acid residues to the C-terminus of ribosomal protein S6 in *Escherichia coli* K12. Mol. Gen. Genet. **217**:281–288.
128. **Kao, S. M., and H. M. Hassan.** 1985. Biochemical characterization of a paraquat-tolerant mutant of *Escherichia coli*. J. Biol. Chem. **260**:10478–10481.
129. **Kappus, H.** 1985. Lipid peroxdation: mechanisms, analysis, enzymology and biological relevence, p. 273–310. *In* H. Sies (ed.), Oxidative stress. Academic Press, Inc., New York.
130. **Kashket, E. R.** 1985. The proton motive force in bacteria: a critical assessment of methods. Annu. Rev. Microbiol. **39**:219–242.
131. **Keyse, S. M., and R. M. Tyrrell.** 1989. Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide and sodium arsenite. Proc. Natl. Acad. Sci. USA **86**:99–103.

131a. **Knowles and Eisenstark.** Unpublished results.

132. **Kogoma, T., S. B. Farr, K. M. Joyce, and D. O. Natvig.** 1988. Isolation of gene fusions (*soi*::*lacZ*) inducible by oxidative stress in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **85**:4799–4803.

132a. **Kogoma, T., and T. Yura.** Submitted for publication.

133. **Kow, Y. W., and S. S. Wallace.** 1985. Exonuclease III recognizes urea residues in oxidized DNA. Proc. Natl. Acad. Sci. USA **82**:8354–8358.
134. **Kren, B., D. Parsell, and J. A. Fuchs.** 1988. Isolation and characterization of an *Escherichia coli* K-12 mutant deficient in glutaredoxin. J. Bacteriol. **170**:308–315.
135. **Kuo, C. F., T. Mashino, and I. Fridovich.** 1987. Alpha,beta-dihydroxy-isovalerate dehydratase: a superoxide sensitive enzyme. J. Biol. Chem. **262**:4724–4727.
136. **Kusukawa, N., and T. Yura.** 1988. Heat shock protein GroE of

Escherichia coli: key protective roles against thermal stress. Genes Dev. **2**:874–882.

137. **Kwon, B. M., and C. S. Foote.** 1988. Chemistry of singlet oxygen. Hydroperoxide intermediates in the photoxygenation of ascorbic acid. J. Am. Chem. Soc. **110**:6582.
138. **Lange, R., and R. Hengge-Aronis.** 1991. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. Mol. Microbiol. **5**:49–59.
139. **Lange, R., and R. Hengge-Aronis.** 1991. Growth phase-regulated expression of *bolA* and morphology of stationary-phase *Escherichia coli* cells are controlled by the novel sigma factor σ^s (*rpoS*). J. Bacteriol. **173**:4474–4481.
140. **Larsen, S. H., J. Adler, J. J. Gargus, and R. W. Hogg.** 1974. Chemomechanical coupling without ATP: the sourse of energy for motility and chemotaxis in bacteria. Proc. Natl. Acad. Sci. USA **71**:1239–1243.
141. **Lee, P. C., B. R. Bochner, and B. N. Ames.** 1983. AppppA, heat shock stress and cell oxidation. Proc. Natl. Acad. Sci. USA **80**:7596–7600.
142. **Leveque, F., S. Blanchin-Roland, G. Fayat, P. Plateau, and S. Blanquet.** 1990. Design and characterization of *Escherichia coli* mutants devoid of Ap4A-hydrolase activity. J. Mol. Biol. **212**:319–329.
143. **Levin, D. E., M. Holstein, M. F. Christman, E. A. Schwiers, and B. N. Ames.** 1982. A new *Salmonella* tester strain (TA102) with AT base pairs at the site of mutation detects oxidative mutagens. Proc. Natl. Acad. Sci. USA **79**:7445–7449.
144. **Levine, R. L., C. N. Oliver, R. M. Fulks, and E. R. Stadtman.** 1981. Turnover of bacterial glutamine synthetase: oxidative inactivation precedes proteolysis. Proc. Natl. Acad. Sci. USA **78**:2120–2124.
145. **L'Herault, P., and Y. E. Chung.** 1984. Mutagenicity of ozone in different repair-deficient strains of *Escherichia coli*. Mol. Gen. Genet. **197**:472–477.
146. **Lin, J. J., and A. Sancar.** 1989. A new mechanism for repairing oxidative damage to DNA: (A)BC excinuclease removes AP sites and thymine glycols from DNA. Biochemistry **28**:7979–7984.

146a. **Linn, S.** Personal communication.

147. **Linn, S.** DNA damage and stress responses due to oxygen radicals. *In* A. D. Bloom, L. Spatz, and N. W. Paul (ed.), Mechanisms and consequences of oxidative damage, in press. Environmental Health Institute, Pittsfield, Mass.
148. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29**:11–22.
149. **Little, P.** 1983. ppGpp acts at the level of RNA polymerase. J. Bacteriol. **154**:787–792.
150. **Loewen, P. C.** 1979. Levels of glutathione in *Escherichia coli*. Can. J. Biochem. **57**:107–111.
151. **Loewen, P. C.** 1984. Isolation of catalase-deficient *Escherichia coli* mutants and genetic mapping of *katE*, a locus that affects catalase activity. J. Bacteriol. **157**:622–626.
152. **Loewen, P. C., J. Switala, and B. L. Triggs-Raine.** 1985. Catalases HPI and HPII in *Escherichia coli* are induced independently. Arch. Biochem. Biophys. **243**:144–149.
153. **Loewen, P. C., and B. L. Triggs.** 1984. Genetic mapping of *katF*, a locus that with *katE* affects the synthesis of a second catalase species in *Escherichia coli*. J. Bacteriol. **160**:668–675.
154. **Loewen, P. C., B. L. Triggs, C. S. George, and B. E. Hrabarchuk.** 1985. Genetic mapping of *katG*, a locus that affects synthesis of the bifunctional catalase-peroxidase hydroperoxidase I in *Escherichia coli*. J. Bacteriol. **162**:661–667.
155. **Lunn, C. A., and V. P. Pigiet.** 1987. The effects of thioredoxin on the radiosensitivity of bacteria. Int. J. Radiat. Biol. **51**:29–38.
156. **Machino, T., and I. Fridovich.** 1987. Superoxide radical initiates the autoxidation of dihydroxy acetone. Arch. Biochem. Biophys. **254**:547–554.
157. **Marinus, M. G.** 1987. DNA methylation in *Escherichia coli*. Annu. Rev. Genet. **21**:113–131.

157a. **Marklund, S. L.** 1990. Free Radic. Biol. Med. **9**(suppl. 1):127.

158. **Massey, V., S. Strickland, S. G. Mayhew, L. G. Howell, P. C. Engel, R. G. Mathews, M. Shuman, and P. A. Sullivan.** 1969. O_2^- generation from flavins by photolysis. Biochem. Biophys. Res. Commun. **36**:891–897.
159. **Matin, A.** 1991. The molecular basis of carbon-starvation-induced general resistance in *Escherichia coli*. Mol. Microbiol. **5**:3–10.

159a. **McCann, M. P., J. P. Kidwell, and A. Matin.** 1991. The putative σ factor KatF has a central role in development of starvation-mediated resistance in *Escherichia coli*. J. Bacteriol. **173**:4188–4194.

159b. **McCarty, J., and G. Walker.** 1991. Cold Spring Harbor Meet. Stress Proteins Heat Shock Response, abstr. 116.

160. **McCord, J. M., and I. Fridovich.** 1988. Superoxide dismutase: the first twenty years (1968–1988). Free Radic. Biol. Med. **5**:363–369.
161. **McCord, J., R. B. Keele, and I. Fridovich.** 1971. An enzyme based theory of obligate anaerobiosis. Proc. Natl. Acad. Sci. USA **68**:1024–1027.
162. **McElhaney, R.** 1985. The effects of membrane lipids on permeability and transport in prokaryotes, p. 75–91. *In* G. Benga (ed.), Structure and properties of cell membranes. CRC Press, Inc., Boca Raton, Fla.
163. **Mead, J.** 1976. Free radical mechanisms of lipid damage and consequences for cellular membranes, p. 51–68. *In* W. A. Pryor (ed.), Free radicals in biology. Academic Press, Inc., New York.
164. **Mechulam, Y., M. Fromant, P. Mellot, P. Plateau, S. Blanchin-Roland, G. Fayat, and S. Blanquet.** 1985. Molecular cloning of the *Escherichia coli* gene for diadenosine 5′,5″,P^1,P^4-tetraphosphate pyrophosphohydrolase. J. Bacteriol. **164**:63–69.
165. **Meister, A., and M. E. Anderson.** 1983. Glutathione. Annu. Rev. Biochem. **52**:711–760.
166. **Menzel, D. B.** 1984. Ozone: an overview of its toxicity in man and animals. J. Toxicol. Environ. Health **13**:183–204.
167. **Michaels, M. L., L. Pham, Y. Nghiem, C. Cruz, and J. Miller.** 1990. MutY, an adenine glycosylase active on GA mispairs, has homology to endonuclease III. Nucleic Acids Res. **18**:3843–3845.
168. **Milcarek, C., and B. Weiss.** 1972. Mutants of *Escherichia coli* with altered deoxyribonucleases. J. Mol. Biol. **68**:303–318.
169. **Mizuno, T., M. Y. Chou, and M. Inouye.** 1984. A unique mechanism regulating gene expression: translational inhibition by a complementary RNA transcript (micRNA). Proc. Natl. Acad. Sci. USA **81**:1966–1970.
170. **Moody, C. S., and H. M. Hassan.** 1982. Mutagenicity of oxygen free radicals. Proc. Natl. Acad. Sci. USA **79**:2855–2859.
171. **Morgan, R. W., M. F. Christman, F. S. Jacobson, G. Storz, and B. N. Ames.** 1986. Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. Proc. Natl. Acad. Sci. USA **83**:8059–8063.
172. **Morimyo, M.** 1988. Isolation and characterization of methyl viologen-sensitive mutants of *Escherichia coli* K-12. J. Bacteriol. **170**:2136–2142.
173. **Mulvey, M. R., and P. C. Loewen.** 1989. Nucleotide sequence of *katF* of *Escherichia coli* suggests KatF protein is a novel σ transcription factor. Nucleic Acids Res. **17**:9979–9991.
174. **Mulvey, M. R., J. Switala, A. Borys, and P. C. Loewen.** 1990. Regulation of transcription of *katE* and *katF* in *Escherichia coli*. J. Bacteriol. **172**:6713–6720.
175. **Natvig, D. O., K. Imlay, D. Touati, and R. A. Hallewell.** 1987. Human CuZn-superoxide dismutase complements superoxide dismutase-deficient *Escherichia coli* mutants. J. Biol. Chem. **262**:14697–14701.
176. **Neidhardt, F. C.** 1987. Multigene systems and regulons, p. 1313–1317. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
177. **Neidhardt, F. C., and R. A. VanBogelen.** 1987. Heat shock response, p. 1334–1345. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbi-

ology, Washington, D.C.

178. **Niederhoffer, E. C., C. M. Naranjo, K. L. Bradley, and J. A. Fee.** 1990. Control of *Escherichia coli* superoxide dismutase (*sodA* and *sodB*) genes by the ferric uptake regulation (*fur*) locus. J. Bacteriol. **172:**1930–1938.
179. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250:**4007–4021.
180. **O'Halloran, T. V., B. Frantz, M. K. Shin, D. M. Ralston, and J. G. Wright.** 1989. The MerR heavy metal receptor mediates positive activation in a topologically novel transcription complex. Cell **56:**119–129.
181. **Olgilvie, A., A. L. Landgrin, K. Weibauer, and K. Kersten.** 1975. Quinone-induced stringent control. Biochim. Biophys. Acta **395:**136–145.
182. **Pedersen, S., P. L. Bloch, S. Reeh, and F. C. Neidhardt.** 1978. Patterns of protein synthesis in *Escherichia coli*: a catalogue of the amount of 140 individual proteins at different growth rates. Cell **14:**179–190.
183. **Peters, J.** 1977. In vivo photoinactivation of *Escherichia coli* ribonucleotide reductase by near ultraviolet light. Nature (London) **267:**256–258.
184. **Plateau, P., M. Fromant, and S. Blanquet.** 1987. Heat shock and hydrogen peroxide responses of *Escherichia coli* are not changed by dinucleotide tetraphosphate hydrolase overproduction. J. Bacteriol. **169:**3817–3820.
184a. **Pommier, Y., D. Kerrigan, K. D. Hartman, and R. I. Glazer.** 1990. Phosphorylation of mammalian DNA topoisomerase I and activation by protein kinase C. J. Biol. Chem. **265:**9418–9422.
185. **Poole, R. K., and W. J. Ingledew.** 1987. Pathways of electrons of oxygen, p. 170–200. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
185a. **Popoff, S. C., A. I. Spira, A. W. Johnson, and B. Demple.** 1990. Yeast structural gene *Escherichia coli* (*APN1*) for the major apurinic endonuclease: homology to *Escherichia coli* endonuclease IV. Proc. Natl. Acad. Sci. USA **87:**4193–4197.
186. **Privalle, C. T., and I. Fridovich.** 1987. Induction of superoxide dismutase in *Escherichia coli* by heat shock. Proc. Natl. Acad. Sci. USA **84:**2723–2726.
187. **Proctor, P. H., and E. Reynolds.** 1984. Free radicals and disease in man. Physiol. Chem. Phys. NMR **16:**175–194.
188. **Pryor, W.** 1986. Oxy-radicals and related species. Annu. Rev. Physiol. **48:**657–667.
188a. **Ramotar, D., S. C. Popoff, and B. Demple.** 1991. Complementation of DNA repair-deficient *Escherichia coli* by the yeast Apn1 apurinic/apyrimidinic endonuclease gene. Mol. Microbiol. **5:**149–155.
189. **Reeh, S., and S. Pederson.** 1979. Post-translational modification of *Escherichia coli* ribosomal protein S6. Mol. Gen. Genet. **173:**183–187.
190. **Riley, M., and S. Krawiec.** 1987. Genome organization, p. 967–981. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
191. **Roseman, J. E., and R. L. Levine.** 1987. Purification of protease from *Escherichia coli* with specificity for oxidized glutamine synthetase. J. Biol. Chem. **262:**2101–2110.
192. **Rowley, D. L., and R. E. Wolf, Jr.** 1991. Molecular characterization of the *Escherichia coli* K-12 *zwf* gene encoding glucose 6-phosphate dehydrogenase. J. Bacteriol. **173:**968–977.
193. **Sak, B. D., A. Eisenstark, and D. Touati.** 1989. Exonuclease III and the catalase hydroperoxidase II in *Escherichia coli* are both regulated by the *katF* gene product. Proc. Natl. Acad. Sci. USA **86:**3271–3275.
194. **Sammartano, L. J., and R. W. Tuveson.** 1986. Control of sensitivity to inactivation by H_2O_2 and broad-spectrum near-UV radiation by the *Escherichia coli katF* locus. J. Bacteriol. **168:**13–21.
195. **Sammartano, L. J., R. W. Tuveson, and R. Davenport.** 1983. *Escherichia coli xthA* mutants are sensitive to inactivation by broad-spectrum near-UV (300- to 400-nm) radiation. J. Bacteriol. **156:**904–906.
196. **Saporito, S. M., M. Gedenk, and R. P. Cunningham.** 1989. Role of exonuclease III and endonuclease IV in repair of pyrimidine dimers initiated by bacteriophage T4 pyrimidine dimer-DNA glycosylase. J. Bacteriol. **171:**2542–2546.
197. **Saul, R. L., and B. N. Ames.** 1986. Background levels of DNA damage in the population, p. 529–535. *In* M. Simic and L. Grossman (ed.), Mechanisms of DNA damage and repair. Plenum Publishing Corp., New York.
198. **Saul, R. L., P. Gee, and B. N. Ames.** 1987. Free radicals, DNA damage and aging, p. 113–129. *In* H. R. Warner, R. N. Butler, R. L. Sprott, and E. L. Schneider (ed.), Modern biological theories of aging. Raven Press, New York.
199. **Sawadaishi, K., K. Miura, E. Ohtsuka, T. Ueda, K. Ishizaki, and N. Shinriki.** 1985. Ozonolysis of supercoiled pBR322 DNA resulting in strand scission to open circular DNA. Nucleic Acids Res. **13:**7183–7194.
200. **Schaich, K. M., and D. C. Borg.** 1984. Radiomimetic effects of peroxidizing lipids on nucleic acids and their bases, p. 603–606. *In* W. Bors, M. Saran, and D. Tait (ed.), Oxygen radicals in chemistry and biology. Walter de Gruyter, Berlin.
201. **Schellhorn, H. E., and H. M. Hassan.** 1988. Response of hydroperoxidase and superoxide dismutase deficient mutants of *Escherichia coli* K-12 to oxidative stress. Can. J. Microbiol. **34:**1171–1176.
202. **Schneider, J. E., S. Price, J. M. C. Gutteridge, and R. A. Floyd.** 1990. Methylene blue plus light mediates 8-hydroxy-2′-deoxyguanosine formation in DNA preferentially over strand breakage. Nucleic Acids Res. **18:**631–635.
203. **Segerback, D.** 1983. Alkylation of DNA and hemoglobin in the mouse following exposure to ethene and ethene oxide. Chem. Biol. Interact. **45:**135–151.
204. **Seither, R. L., and O. R. Brown.** 1982. Induction of stringency by hyperoxia in *Escherichia coli*. Cell. Mol. Biol. **23:**285–291.
205. **Sies, H.** 1986. Biochemistry of oxidative stress. Angew. Chem. Int. Ed. Engl. **25:**1058–1071.
206. **Singh, A., and H. Singh.** 1982. Time scale and nature of radiation biological damage. Prog. Biophys. Mol. Biol. **39:**69–107.
207. **Slater, T. F.** 1984. Free radical mechanisms in tissue injury. Biochem. J. **222:**1–15.
208. **Stadtman, E. R.** 1986. Oxidation of proteins by mixed function oxidation systems: implications in protein turnover, aging and neutrophil function. Trends Biochem. Sci. **11:**11–12.
209. **Staehelin, J., and J. Hoigne.** 1982. Decomposition of ozone in water: rate of initiation by hydroxide ions and hydrogen peroxide. Environ. Sci. Technol. **16:**676–681.
210. **Steinman, H. M.** 1982. Superoxide dismutases: protein chemistry and structure function relationships, p. 11–68. *In* L. W. Oberley (ed.), Superoxide dismutase. CRC Press, Inc., Boca Raton, Fla.
211. **Storz, G., M. Christman, H. Sies, and B. N. Ames.** 1987. Spontaneous mutagenesis and oxidative damage to DNA in *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA **84:**8917–8921.
212. **Storz, G., F. S. Jacobson, L. A. Tartaglia, R. W. Morgan, L. A. Silveira, and B. N. Ames.** 1989. An alkyl hydroperoxide reductase induced by oxidative stress in *Salmonella typhimurium* and *Escherichia coli*: genetic characterization and cloning of *ahp*. J. Bacteriol. **171:**2049–2055.
213. **Storz, G., L. A. Tartaglia, and B. N. Ames.** 1990. Transcriptional regulator of oxidative stress-inducible genes: direct activation of oxidation. Science **248:**189–194.
214. **Summerfeild, F. W., and A. L. Tappel.** 1983. Determination by fluorescence quenching of the environment of DNA crosslinks made by malondialdehyde. Biochim. Biophys. Acta **740:**185–189.
215. **Sussenbach, J. S., and W. Berends.** 1964. Photodynamic degradation of guanine. Biochem. Biophys. Res. Commun. **16:**263–226.

216. **Takeda, Y., and H. Avila.** 1986. Structure and gene expression of the *Escherichia coli* Mn-superoxide dismutase gene. Nucleic Acids Res. **14:**4577–4589.

216a. **Tao, K., K. Makino, S. Yonei, A. Nakata, and H. Shinagawa.** 1989. Molecular cloning and nucleotide sequencing of *oxyR*, the positive regulator gene of a regulon for an adaptive response to oxidative stress in *Escherichia coli*: homologies between OxyR protein and a family of bacterial activator proteins. Mol. Gen. Genet. **218:**371–376.

216b. **Tao, K., K. Makino, S. Yonei, A. Nakata, and H. Shinagawa.** 1991. Purification and characterization of the *Escherichia coli* OxyR protein, the positive regulator for a hydrogen peroxide-inducible regulon. J. Biochem. **109:**262–266.

217. **Tardat, B., and D. Touati.** 1991. Two global regulators repress the anaerobic expression of MnSOD in *Escherichia coli*: *fur* (ferric iron uptake) and *arc* (aerobic respiration control). Mol. Microbiol. **5:**455–465.

218. **Tartaglia, L. A., G. Storz, and B. N. Ames.** 1989. Identification and molecular analysis of OxyR-regulated promoters important for the bacterial adaptation to oxidative stress. J. Mol. Biol. **210:**709–719.

219. **Teebor, G. W., R. J. Boorstein, and J. Cadet.** 1988. The reparability of oxidative free radical mediated damage to DNA: a review. Int. J. Radiat. Biol. **54:**131.

220. **Tilly, K., N. McKittrick, M. Zylicz, and C. Georgopoulos.** 1983. The DnaK protein modulates the heat-shock response of *Escherichia coli*. Cell **34:**641–646.

221. **Touati, D.** 1988. Transcriptional and posttranscriptional regulation of manganese superoxide dismutase biosynthesis in *Escherichia coli*, studied with operon and protein fusions. J. Bacteriol. **170:**2511–2520.

222. **Touati, D.** 1988. Molecular genetics of superoxide dismutases. Free Radic. Biol. Med. **5:**393–402.

223. **Touati, D., and S. B. Farr.** 1990. Elevated mutagenesis in bacterial mutants lacking superoxide dismutase. Methods Enzymol. **186:**646–651.

224. **Touati, E., and A. Danchin.** 1987. The structure of the promoter and amino terminal region of the pH 2.5 acid phosphatase structural gene *appA* of *Escherichia coli*: a negative control of transcription mediated by cAMP. Biochimie **69:**215–221.

225. **Touati, E., E. Dassa, L. Dassa, P. L. Boquet, and D. Touati.** 1991. Are *appR* and *katF* the same *Escherichia coli* gene encoding a new sigma transcription initiation factor? Res. Microbiol. **142:**29–36.

226. **Tsaneva, I. R., and B. Weiss.** 1990. *soxR*, a locus governing a superoxide response regulon in *Escherichia coli* K-12. J. Bacteriol. **172:**4197–4205.

227. **Vaca, C. E., J. Wilhelm, and M. Harms-Ringdahl.** 1988. Interaction of lipid peroxidation products with DNA: a review. Mutat. Res. **195:**137–149.

228. **VanBogelen, R. A., P. M. Kelley, and F. C. Neidhardt.** 1987. Differential induction of heat shock, SOS, and oxidative stress regulons and accumulation of nucleotides in *Escherichia coli*. J. Bacteriol. **169:**26–32.

229. **VanBogelen, R. A., and F. C. Neidhardt.** 1990. Ribosomes as sensors of heat and cold shock in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**5589–5593.

230. **Van Hemmen, J. J., and W. J. A. Melving.** 1975. Inactivation of biologically active DNA by gamma-ray-induced superoxide radicals and their dismutation products singlet molecular oxygen and hydrogen peroxide. Biochim. Biophys. Acta **402:**133–141.

231. **Von Sontag, C.** 1979. Radiation chemistry of carbohydrates and of the sugar moiety in DNA, p. 85–98. *In* H. E. Edward, S. Navaratnam, B. J. Parsons, and G. O. Phillips (ed.), Radiation biology and chemistry. Elsevier Biomedical Press, Amsterdam.

232. **Wada, M., K. Sekine, and H. Itikawa.** 1986. Participation of the *dnaK* and *dnaJ* gene products in phosphorylation of glutaminyl-tRNA synthetase and threonyl-tRNA synthetase of *Escherichia coli* K-12. J. Bacteriol. **168:**213–220.

233. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48:**60–93.

233a. **Walkup, L. K. B., and T. Kogoma.** Unpublished results.

234. **Walkup, L. K. B., and T. Kogoma.** 1989. *Escherichia coli* proteins inducible by oxidative stress mediated by the superoxide radical. J. Bacteriol. **171:**1476–1484.

235. **Wallace, S. S.** 1988. AP endonucleases and DNA glycosylases that recognize oxidative DNA damage. Environ. Mol. Mutagen. **12:**431–477.

236. **Wang, J. H.** 1955. On the detailed mechanism of a new type of catalase-like action. J. Am. Chem. Soc. **77:**4715–4719.

237. **Wefers, H., and H. Sies.** 1983. Oxidation of glutathione by the superoxide radical to the disulfide and the sulfonate yielding singlet oxygen. Eur. J. Biochem. **137:**29–36.

238. **White, B. J., S. J. Hochhauser, N. M. Cintron, and B. Weiss.** 1976. Genetic mapping of *xthA*, the structural gene for exonuclease III in *Escherichia coli* K-12. J. Bacteriol. **126:**1082–1088.

239. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli* B/r. Mol. Gen. Genet. **142:**87–103.

240. **Wong, G. H. W., and D. V. Goeddel.** 1988. Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. Science **242:**941–944.

241. **Wu, J., and B. Weiss.** 1991. Two divergently transcribed genes, *soxR* and *soxS*, control a superoxide response regulon in *Escherichia coli*. J. Bacteriol. **173:**2864–2871.

242. **Yallaly, P., and A. Eisenstark.** 1990. Influence of DNA adenine methylase on the sensitivity of *Escherichia coli* to near-UV radiation and hydrogen peroxide. Biochem. Biophys. Res. Commun. **169:**64–69.

243. **Yamamoto, N., and M. L. Droffner.** 1985. Mechanism determining aerobic and anaerobic growth in the facultative anaerobe *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA **82:**2077–2081.

244. **Yatvin, M. B., P. G. Wood, and S. M. Brown.** 1972. Repair of plasma membrane injury and DNA single strand breaks in γ-irradiated *Escherichia coli* B/r and Bs-1. Biochim. Biophys. Acta **287:**390–403.

245. **Zhang, Q.-M., and S. Yonei.** 1991. Induction of manganese-superoxide dismutase by membrane-binding drugs in *Escherichia coli*. J. Bacteriol. **173:**3488–3491.

246. **Zhou, Y.-N., N. Kusukawa, J. W. Erickson, C. A. Gross, and T. Yura.** 1988. Isolation and characterization of *Escherichia coli* mutants that lack the heat shock sigma factor sigma-32. J. Bacteriol. **170:**3640–3649.

247. **Zylicz, M., J. Lebowitz, R. McMacken, and C. Georgopoulos.** 1983. The DnaK protein of *Escherichia coli* possesses an ATPase and autophosphorylation activity and is essential in an in vitro DNA replication system. Proc. Natl. Acad. Sci. USA **80:**6431–6435.