Annu. Rev. Biochem. 1995. 64:97–112

SUPEROXIDE RADICAL AND SUPEROXIDE DISMUTASES

Irwin Fridovich
Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

KEY WORDS: hydroxyl radical, extracellular SOD, free radicals, regulation of superoxide disutase, glycation and O_2^- production, targets for O_2^-, nitric oxide

CONTENTS

ABSTRACT

O_2^- oxidizes the [4Fe-4S] clusters of dehydratases, such as aconitase, causing inactivation and release of Fe(II), which may then reduce H_2O_2 to $OH^- + OH•$. SODs inhibit such HO• production by scavenging O_2^-, but Cu, ZnSODs, by virtue of a nonspecific peroxidase activity, may peroxidize spin trapping agents and thus give the appearance of catalyzing OH• production from H_2O_2.

There is a glycosylated, tetrameric Cu, ZnSOD in the extracellular space that binds to acidic glycosamino-glycans. It minimizes the reaction of O_2^- with NO. *E. coli*, and other gram negative microorganisms, contain a periplasmic Cu, ZnSOD that may serve to protect against extracellular O_2^-. Mn(III) complexes of multidentate macrocyclic nitrogenous ligands catalyze the dismutation of O_2^- and are being explored as potential pharmaceutical agents.

SOD-null mutants have been prepared to reveal the biological effects of O_2^-. SodA, sodB *E. coli* exhibit dioxygen-dependent auxotrophies and en-

0066-4154/95/0701-0097$05.00

hanced mutagenesis, reflecting O_2^--sensitive biosynthetic pathways and DNA damage. Yeast, lacking either Cu, ZnSOD or MnSOD, are oxygen intolerant, and the double mutant was hypermutable and defective in sporulation and exhibited requirements for methionine and lysine. A Cu, ZnSOD-null *Drosophila* exhibited a shortened lifespan.

Introduction

Situations that involve opposing and well-balanced forces, or reactions, are often effectively invisible. Discovery of the true dynamic balance is further confounded when the relevant species are short lived. These statements are offered as a rationalization, or even as an apology, for how slowly we have gained an understanding of the basis of dioxygen toxicity and the nature of the counterbalancing defenses. The demonstrations that xanthine oxidase produced O_2^- (1) and that erythrocytes contained an enzyme that very efficiently and specifically catalyzed the conversion of O_2^- into $H_2O_2 + O_2$ (2) opened the door on the true state of affairs: that O_2^- is commonly produced within aerobic biological systems, and superoxide dismutases (SODs) provide an important defense against it.

Although late in getting started, we now seem to be making up for lost time. The past 25 years have witnessed a very impressive increase in our knowledge of the biology of O_2^- and of the SODs that remove it. The relevant literature has been reviewed at regular intervals (3–56). Here, we touch upon only portions of this broad field that appear to be of current interest or that promise either important insights or practical applications.

Targets

O_2^- can act as either a univalent oxidant or reductant. Investigators have exploited this dual reactivity in devising assays for the activity of SODs. Thus, in some assays, O_2^- reduces tetranitro methane (2), cytochrome *c* (2), or nitro blue tetrazolium (57), and in others it oxidizes epinephrine (2, 58), tiron (59), pyrogallol (60), or 6-hydroxydopamine (61). The ability of O_2^- to oxidize sulfite, and thus to initiate its free-radical chain oxidation, was one of the early clues to the production of O_2^- by xanthine oxidase (62).

Given the chemical diversity of biological systems, intracellular O_2^- will surely find targets it can either oxidize or reduce. For example, it can oxidize the family of dehydratases that contain [4Fe-4S] clusters at their active sites. This group of enzymes, which includes dihydroxy acid dehydratase (63), 6-phosphogluconate dehydratase (64), aconitase (65, 66), and fumarases A and B (67, 68), undergoes rapid oxidation by O_2^- with a resultant loss of Fe(II) from the cluster and concomitant inactivation (66).

Release of Fe(II) from the cluster sets the stage for the Fenton reaction in which Fe(II) reduces H_2O_2, thereby producing Fe(II)O or Fe(III) + HO•. This

reaction may be the basis for the in vivo cooperation between O_2^- and H_2O_2, which produces an oxidant capable of attacking virtually any cellular target, most notably DNA (69). Much evidence indicates that this deleterious cooperative interaction occurs between O_2^- and peroxides within cells. Thus, the *sodA sodB* strain of *Escherichia coli*, which lacks both the Mn- and Fe-containing SODs, was hypersensitive not only to O_2 and to agents that increase production of O_2^- but also to H_2O_2 (70). Delivery of SODs into hepatocytes by liposomal fusion increased resistance towards an alkyl hydroperoxide (71). In an opposite approach, diethyl dithiocarbamate was used to inactivate the Cu,ZnSOD in cultured aortic endothelial cells, and this inactivation increased sensitivity towards damage by H_2O_2 (72).

Although the outcome is the same, i.e. the production of HO• or Fe(II)O from a cooperative interaction of O_2^- and H_2O_2, the in vivo and in vitro variants of this interaction differ profoundly. In vitro, O_2^- acts as a reductant towards available Fe(III) and so generates Fe(II) that can reduce H_2O_2. In contrast, O_2^- produces Fe(II) in vivo by acting as an oxidant towards susceptible [4Fe–4S] clusters. The Fe(II) released from the oxidized clusters could bind to DNA and provide a site for production of powerful oxidants immediately adjacent to this critical target. Such a mechanism could account for the enhanced dioxygen-dependent mutagenesis exhibited by *sodA sodB E. coli* (73) and for the in vivo hydroxylation of the bases of DNA (74–78).

SOD Prevents Production of HO•

As expected for a mechanism in which HO• or Fe(II)O is generated from the metal-catalyzed interaction of O_2^- with H_2O_2, the in vitro process is inhibited by SOD, or catalase, or by chelating agents that restrict the redox cycling of the catalytic metal. Such inhibitions have been observed repeatedly (79–112). The report that Cu,ZnSOD, rather than inhibit HO• production from H_2O_2, actually catalyzes it (113) contradicted all of this earlier work and demanded some explanation. Yim et al (113) used high concentrations of H_2O_2 (30 mM) and of Cu,ZnSOD (1.25 μM). Sato et al (114) concluded that Cu(II), released from the Cu,ZnSOD as it was inactivated by the H_2O_2, was the actual catalyst of HO• production. Voest et al (115) concluded that an HO•-like entity is generated at the active site of Cu,ZnSOD in the presence of H_2O_2, but that free HO• is not produced.

Another explanation for the apparent catalysis of HO• formation by Cu,ZnSOD can be derived from much earlier work on the interaction of H_2O_2 with this enzyme (116, 117). These studies showed that H_2O_2 rapidly reduced the Cu(II) at the active site and then more slowly inactivated the reduced enzyme. This inactivation could be prevented by xanthine, urate, formate, and azide, but not by alcohols. Thus the reaction of Cu(I) with H_2O_2 at the active

site, apparently generated a potent oxidant [Cu(I)O, or Cu(II)–OH] that could attack an adjacent histidine residue and thus inactivate the enzyme or alternately could attack xanthine, urate, formate, or azide. These exogenous reductants would thus serve as sacrificial substrates and spare the essential histidines.

In accord with these observations, Cu,ZnSOD acts as a peroxidase towards these and other substrates (116, 117). This peroxidase activity can fully account for the results of Yim et al (113). Thus Cu,ZnSOD probably acted as a peroxidase towards DMPO, producing DMPO-OH, whichappeared to have been produced by reaction of $HO^{\bullet}$ with DMPO (Dimethyl pyolline-N-oxide. Had free $HO^{\bullet}$ actually been produced, it would have been able to convert ethanol to the hydroxyethyl radical, which is eminently trappable by DMPO. Yim et al did not observe such trapping (113), which indicates that HO• production was not being catalyzed by Cu,ZnSOD. The inability of ethanol to protect Cu,ZnSOD against inactivation by H_2O_2 (116, 117) indicates that the Cu,ZnSOD cannot catalyze the peroxidation of ethanol. Were it able to do so, Yim et al (113) would have been able to trap the hydroxyethyl radical and would have had further reason to believe, incorrectly, that free HO• was being produced through a catalytic interaction of Cu,ZnSOD with H_2O_2.

The SOD Family of Enzymes

The cyanobacteria started a gradual oxygenation of the biosphere that applied a common selection pressure to a varied anaerobic biota. At least two of the classes of SODs were among the adaptations called forth. One consists of SODs with Cu(II) plus Zn(II) at the active site, wheras the other comprises SODs with either Mn(III) or Fe(III) at the catalytic center. These enzymes have been reviewed (14, 48), and we need now only consider relatively recent developments.

Extracellular SODs (ECSODs)

O_2^- should not easily cross biological membranes, with the exception of those membranes that are richly endowed with anion channels, such as the erythrocyte stroma (118). O_2^- must consequently be detoxified in the compartment within which it is generated. This necessity explains the presence of distinct SODs in the cytosol and in the mitochondria of eukaryotic cells (119–127) and why complementation of a defect in the mitochondrial MnSOD in yeast was effective only when the leader sequence of the maize gene, which assured importation of the gene product into the mitochondrion, was present (128).

Against this background of compartmentation of SODs, the existence of extracellular SODs bespeaks the need for defense against the numerous extracellular sources of O_2^- For example, ultraviolet irradiation of water produces O_2^- (129) continually in surface waters (130). In the presence of photosensitizers and a wide variety of electron donors, irradiation with visible light will

suffice (57, 131–136). Autoxidations routinely produce O_2^-. Moreover the collapse of cavities introduced into aerated water by ultrasonication produces O_2^- (137–139), and cavities produced by turbulence presumably will also do so, albeit to a lesser degree. In living systems, the membrane-associated NADPH oxidase, which is so abundant in phagocytic leukocytes, releases O_2^- into the extracellular phase (140–146).

The mammalian extracellular SOD is a Cu,ZnSOD, but unlike its dimeric cytosolic counterpart, it is tetrameric and glycosylated (147,148). This enzyme exhibits affinity for heparin and other acidic glycosamino-glycans (149, 150) because of a C-terminal heparin-binding domain that is rich in basic amino acid residues (151, 152). This heparin affinity results in binding to the endothelium and to other cell types (153) such that the amount of mammalian extracellular SOD actually free in the blood plasma is low but can be elevated by injection of heparin (149). The amino acid sequence of the human ECSOD is known from the sequence of the cDNA (152), which indicated the presence of an 18-residue signal peptide, characteristic of secreted proteins. Residues 96–193 show strong sequence homology to the cytosolic Cu,ZnSODs, whereas residues 1–95 do not. The residues that compose the active site are conserved in all Cu,ZnSODs, including the ECSOD.

The nucleotide sequence coding for the heparin-binding domain has been fused onto the gene coding for the cytosolic Cu,ZnSOD and the resultant fusion gene expressed in *E. coli* (154). The artificial ECSOD so produced exerted a strong antiinflammatory effect. ECSOD also protects against reperfusion injury (155–158), enhances arterial relaxation (159), and thereby diminishes hypertension (160). ECSOD may prove to be a useful pharmaceutical, and the production of transgenic mice that secrete the human ECSOD in milk has clearly brought us closer to realizing a convenient source (161).

Extracellular SODs have been found in severalnonmammalian sources, such as phloem sap (162), *Nocardia asteroides* (163), *Schistosoma mansoni* (164), and *Onchocerca volvulus* (165). *N. asteroides* elicits the respiratory burst of neutrophils and of monocytes, yet resists killing by these phagocytes (166). Treatments of *N. asteroides* with an antibody to the extracellular SOD increased its susceptibility to killing by the leukocytes, but treatment with nonspecific immunoglobulin did not (167). In this organism, the surface SOD thus appears to be a pathogenicity factor.

Periplasmic SODs

Surveys of a variety of bacterial species had indicated the presence of FeSOD and/or MnSOD but not Cu,ZnSOD (168–170). Such data led to the view that the Cu,ZnSODs were characteristic of eukaryotes. Nevertheless, some studies reported finding Cu,ZnSOD in a few bacteria. The first such instance dealt

with *Photobacter leiognathi*, which was found to contain both FeSOD and Cu,ZnSOD (171). Because this microorganism ordinarily lives as a symbiont in the luminescent organ of the ponyfish, a gene transfer seemed a reasonable explanation for this instance of a bacterial Cu,ZnSOD (172, 173), but this notion was subsequently dismissed (174). Furthermore, Cu,ZnSOD was found in several bacterial species including *Caulobacter crescentis* (175), *Pseudomonas diminuta* and *Pseudomonas maltophila* (176), *Brucella abortus* (177), and in several species of *Hemophilus* (178). The *C. crescentis* (179, 180) and the *B. abortus* (181) Cu,ZnSODs are periplasmic.

The much-studied *E. coli* also expresses a Cu,ZnSOD, in addition to the MnSOD and FeSOD (182). Moreover, this *E. coli* Cu,ZnSOD is selectively released by osmotic shock and thus is periplasmic. It is induced during aerobic growth, yet the net activity remains much less than the total activity. This Cu,ZnSOD seems to be important for the aerobic growth of *E. coli* mutants that cannot make the FeSOD and MnSOD (*sodA sodB*). Thus, diethyl dithiocarbamate, which inactivates Cu,ZnSOD but not FeSOD or MnSOD, inhibits the aerobic growth of the *sodA sodB* strain on a rich medium but has no effect on anaerobic growth. A true SOD-null *E. coli* i.e. a *sodA sodB sodC* strain, would probably behave like a sensitive obligate anaerobe and is a goal for future studies. Another important aim is to find out why a SOD must be targeted to the periplasm. Are there sources of O_2^- within the periplasm or does the periplasmic SOD protect against extracellular sources of O_2^-?

SOD Mimics

Because of the role of O_2^- in free-radical chain oxidations, oxygen toxicity, inflammations, reperfusion injuries, and very likely also in senescence, low-molecularweight mimics of SOD activity should be very useful. The simplicity of the O_2^- dismutation reaction, and the catalytic abilities of certain transition-metal cations, encouraged the view that such mimics could be found. To date, many reports of SOD mimics have been published—too many to adequately review here. The Mn(III) and Fe(III) complexes of substituted porphines (183, 184) are of particular interest because they are quite active and very stable. Manganese complexes are special because Mn(II), should it be liberated from the complex, does not participate in Fenton chemistry, and because in certain lactobacilli (185–187) high intracellular concentrations of Mn(II) salts have replaced SOD. This substitution indicates that low-molecular-weight Mn complexes can provide functional replacements for SOD and are well tolerated within at least certain types of cells.

Recent studies of a porphine Mn(III) complex with N-methyl pyridyl groups on the methine bridge carbons [Mn(III)TMPyP] reveal that the porphine compound eliminates the growth inhibition imposed by aerobic paraquat on a SOD-competent *E. coli*, as well as the growth inhibition imposed by oxygen

on a sodA sodB strain (188). Indeed, the protective effects of this Mn(III) porphine exceeded expectations based on its ability to catalyze the dismutation of O_2^- as measured in vitro. This result is at least partially explained by the large difference between the rate constants for the first and second steps of the catalytic cycle (184) and by the observation that the complex remains reduced within *E. coli* (188).

Below, the rate constant for Reaction 1 is $\sim 2 \times 10^7\ M^{-1}\ s^{-1}$, while for Reaction 2 it is $\sim 4 \times 10^9\ M^{-1}\ s^{-1}$ (184).

$$\text{Por-Mn(III)} + O_2^- \leftrightarrow \text{Por-Mn(II)} + O_2 \qquad 1.$$

$$\text{Por-Mn(II)} + O_2^- + 2H^+ \leftrightarrow \text{Por-Mn(III)} + H_2O_2. \qquad 2.$$

When the dismutation is catalyzed in vitro, the slower of these steps, Reaction 1, is rate limiting. However, this step is irrelevant in vivo, because the Mn(III) porphyrin remains reduced at the expense of GSH and NADPH (188), and the rate constant of Reaction 2 limits O_2^- scavenging. This Mn(III) porphine thus protects against O_2^- by acting not as a superoxide dismutase but as an O_2^-:GSH/NADPH oxidoreductase. The concentration of this Mn(III) porphine within the cell also increases its protective effect. Thus when the complex is at 25 μM in the medium, it reaches 1 mM within *E. coli*. Mammalian cell lines are also protected against paraquat (B Day, in preparation) or against pyocyanine (PR Gardner, personal communication) by this Mn(III) porphine.

Another promising group of SOD mimics comprises the cyclic polyamine complexes of Mn(III) (190a). These compounds catalyze the dismutation of O_2^- at approximately 1% of the rate exhibited by SOD. Nevertheless, they could protect endothelial cells against damage by a flux of O_2^- produced by activated neutrophils or by the xanthine oxidase reaction (190b). Because catalase did not protect in this system, O_2^- is apparently the damaging species. The damage may result from protonation of the O_2^- in the acidic domain adjacent to the anionic cell membrane. Alternatively, the O_2^- may have been converted to $ONOO^-$ by reaction with the NO produced by endothelial cells.

Oxygen Radicals from Sugars

Small sugars, such as glycolaldehyde, glyceraldehyde, or dihydroxy acetone, autoxidize by a free radical pathway in which O_2^- serves as a chain propagator (191–193). Enolization precedes autoxidation, and small sugars, unable to block the carbonyl by cyclization, are consequently most readily autoxidized; in contrast, aldohexoses, which exist primarily as pyranoses, are relatively stable. Production of O_2^- and H_2O_2, during autoxidation probably explains the mutagenicity of small sugars (194), their abilities to inactivate the transsul-

furase called rhodanese (195), and their abilities to cause the peroxidation of polyunsaturated fatty acids (196).

The relative resistance of aldohexoses towards autoxidation is abrogated when they react with amino compounds and are converted to fructosyl amines (197). This is the situation in glycated proteins, which do autoxidize with production of O_2^- and H_2O_2 (198). Oxidative damage, subsequent to glycation, has been reported for LDL (199), collagen (200), the cytosolic Cu,ZnSOD (201), the extracellular Cu,ZnSOD (202), and serum albumin (203). The inhibition of the autoxidative degradation of such fructosylamines by SOD (197) bespeaks a role for O_2^- as a chain propagator.

SOD Mutants

An excellent way to explore the functions of an enzyme is through the phenotype of null mutants. Mutants of *E. coli* unable to produce MnSOD (*SodA*) or FeSOD (*SodB*) were first reported by Carlioz & Touati (70). This *sodA sodB* strain was indistinguishable from the parental strain under anaerobic conditions, but under aerobic conditions it exhibited dioxygen-dependent nutritional auxotrophies, hypersensitivity towards paraquat, and enhanced mutagenesis (73); these deficits were reversed by introduction of a plasmid carrying a SOD gene. Because the *sodA sodB* strain will not grow on aerobic minimal medium, owing to its multiple O_2-dependent auxotrophies, it lends itself to complementation studies. Thus *E. coli* that have reacquired a functional SOD gene can be easily selected from among many that have not by means of growth on aerobic minimal plates.

This technique has been exploited to show that any functional SOD gene will complement the *sodA sodB* strain of *E. coli*. Investigators have obtained this result with genes coding for plant FeSODs (204), human Cu,ZnSOD (204a), the *Legionella pneumophila* FeSOD (205), the *Listeria monocytogenes* MnSOD (206), the *Coxiella burnetii* FeSOD (207), and others (208, 209). This sort of complementation has been extended to other organisms. A yeast with a defect in its MnSOD was complemented by a MnSOD gene from maize (210); extra copies of a MnSOD gene protected tobacco against paraquat (211); and a gene coding for the bovine Cu,ZnSOD complemented a Cu,ZnSOD null mutant of *Drosophila melanogaster* (212).

Some of the specific deficits associated with mutations in SOD genes are instructive, but others remain to be explained. The O_2-dependent auxotrophy for branched-chain amino acids, exhibited by the *sodA sodB E. coli*, is explained by the O_2^- oxidative inactivation of [4Fe-4S]–containing dehydratases. The section on targets for O_2^- has already discussed this topic. In this instance the dihydroxy acid dehydratase, which catalyzes the penultimate step in the

biosynthesis of the branched-chain amino acids, is the O_2^--sensitive dehydratase. The 41-fold increase in spontaneous mutagenesis seen with *sodA sodB E. coli* (73) reflects attack on DNA, probably by HO• or by Fe(II)O (69). We have recently observed an additional O_2-dependent deficit in *sodA sod B E. coli* that is a rapid loss of viability in stationary phase (LT Benov & I Fridovich, unpublished results). A SOD-null mutant of *Porphyromona gingivalis* exhibited a similar O_2-dependent loss of viability (214). The causes of such viability losses are not known, but damage to cell membranes is a possibility, as shown by the protective effect of osmolytes on the *sodA sodB* strain (215) and by the role of osmolytes in facilitating loss of a SOD-1–bearing plasmid from an otherwise SOD-defective yeast (216).

Increases in spontaneous mutagenesis also occurs in *Salmonella typhimurium* with a deletion in *oxyR*. This defect was partially complemented by plasmids bearing genes coding for either catalase or SOD (217). This observation reflects the roles of both O_2^- and hydroperoxides in generating HO• or Fe(II)O (69).

Sensitivity towards O_2 was seen in both Cu,ZnSOD (218) and MnSOD defective yeast (219). Hypersensitivity toward paraquat and increased spontaneous mutagenesis were also characteristic of Cu,ZnSOD-null yeast (220), whereas a mutant defective in both the Cu,Zn- and the Mn-SODs was O_2-sensitive, hypermutable, auxotrophic for methionine and lysine, and defective in sporulation (221). In *D. melanogaster*, a Cu,ZnSOD null mutant exhibited hypersensitivity toward paraquat, male sterility, and a shortened lifespan (222).

To date, the only humans known to bear genetic defects in the coding regions of a SOD are those with the familial variant of amyotrophic lateral sclerosis (FALS), or Lou Gehrig's Disease. This autosomal dominant condition is associated with point mutations in the Cu,ZnSOD gene (223) and with decreased cytosolic SOD activity (224, 225). Inhibition of the cytosolic Cu,ZnSOD, either with diethyl dithiocarbamate or by expression of antisense message, causes apoptosis of spinal neurons (226). Yet, whether or not moderately decreased SOD activity causes the late-onset motor-neuron degeneration that results in the progressive and ultimately fatal disease is not clear. Transgenic mice expressing a human FALS Cu,ZnSOD gene exhibited a late onset and progressive paralysis (227). The fact that these symptoms occurred against a background of normal mouse Cu,ZnSOD activity suggests that a toxic property of the mutated Cu,ZnSOD, rather than a loss of its SOD activity, is the cause of the problem.

Cu,ZnSOD catalyzes two low-level, non-SOD activities, either of which might be increased by the mutations associated with FALS. One of these is a nonspecific peroxidase activity (117), and the other is the catalysis of the nitration of tyrosyl residues by peroxynitrite (228, 229).

NO and O_2^-

The endothelium-derived relaxing factor (EDRF), which regulates smooth muscle tone and thereby blood flow and blood pressure (230), is NO (231). Even before its identification as NO, EDRF was found to react with O_2^-. Thus, the half life of EDRF is extended by SOD but not by catalase (232, 233). Furthermore O_2^-, whether produced by the endothelium or by xanthine oxidase plus xanthine, acted like a contracting factor (234). This action can now be explained on the basis of the production of peroxynitrite from O_2^- + NO (235) at the diffusion-limited rate of $6.7 \times 10^9\ M^{-1}\ s^{-1}$ (236). Peroxynitrite is a strong oxidant and reacts with thiols (237), initiates lipid peroxidation (238), and kills *E. coli* (239) and *Trypanosoma cruzi* (240). In all likelihood the reaction of O_2^- with NO significantly modulates the biological activities of both substances.

Regulation in E. coli

Two of the SODs in *E. coli*, i.e. the cytosolic MnSOD and the periplasmic Cu,ZnSOD, are induced during aerobiosis, whereas the FeSOD is expressed both aerobically and anaerobically. Why should *E. coli* make a SOD under anaerobic conditions when O_2^- production cannot occur? One answer is that a facultative organism must maintain a standby defense to ward off the toxicity of O_2^- that must be faced following the transition from anaerobic to aerobic conditions. Such abrupt transitions must, of course, be a selection pressure for enteric organisms.

Experimental evidence supports this view. For example, *E. coli* defective in the *sodB* gene, which encodes the FeSOD, exhibited a 2-h growth lag when transferred from anaerobic to aerobic media, whereas the parental strain did not (241). Induction of the MnSOD, following exposure to aerobic conditions, finally ended the growth lag. The anaerobically grown cells evidently contained an enzyme capable of the univalent reduction of oxygen. The fumarate reductase, which allows anaerobic *E. coli* to use fumarate as an electron sink, reduced O_2 to O_2^- when supplied with NADH. If the anaerobic fumarate reductase was a major source of O_2^- in the cells, after the anaerobic-to-aerobic transition, a mutational defect in the fumarate reductase should eliminate the growth lag that attended this transition. It did (241).

The biosynthesis of MnSOD within *E. coli* is transcriptionally activated as a member of the *soxRS* (242, 243) and the *soxQ* (244) regulons and is also transcriptionally repressed by the products of the *fur* (245), *arcA* (246), and *fnr* (247) genes, as well as by the integration host factor (248). All of these regulatory elements have been explored and discussed (249). Iron plays a key role in the action of two of these transcriptional repressions, i.e. that of *fur* (250) and *fnr* (251) and is moreover a component of the SoxR protein that functions as the redox sensor of the *soxRS* regulon (252). Furthermore, iron

competes with manganese for insertion into the nascent MnSOD polypeptide and when inserted in place of manganese yields a catalytically inactive product (253, 254). Indeed, iron starvation, whether imposed by chelation or by depletion of the medium, has repeatedly been reported to increase MnSOD in *E. coli* even under anaerobic conditions (253, 255–259).

Is there some rationale that can be offered for this intimate involvement of iron in the regulation of the biosynthesis of MnSOD in *E. coli*?One possible scenario depends upon the great susceptibility of the [4Fe-4S]–containing dehydratases to oxidative inactivation by O_2^- (63–68). Reactivation of these enzymes, which include fumarases A and B, aconitase, dihydroxy acid dehydratase, and 6-phosphogluconate dehydratase, requires Fe(II). The levels of activity of these enzymes depend upon a balance between the rates of inactivation and reactivation. Hence, when [Fe(II)] is low, and reactivation is slow, only a low rate of inactivation, achievable by elevating [MnSOD] and thereby lowering O_2^-, can be tolerated. Therefore a low level of Fe(II) should lead to an increase in active MnSOD, and it does so through multiple effects. Transcriptional repression by Fur and by Fnr depends upon binding of iron to these regulatory proteins; low Fe(II) will lift these repressions. Oxidation of the SoxR protein activates transcription of *soxS*, and SoxS, in turn, activates transcription of the MnSOD gene. Because SoxR is an iron-sulfur protein, we may suppose that oxidation leads to iron loss and that the active form is iron depleted. Hence, low Fe(II) will lead to activation via the *soxRS* regulon. Finally, at the level of maturation of nascent MnSOD polypeptide, low Fe(II) will favor insertion of manganese and production of the active enzyme.

Epilogue

This review does not begin to do justice to the state of knowledge of the biology of oxygen radicals. As in all aspects of biology, beauty and perceived complexity increase with increased study. We may confidently expect that this will continue in the future. There will be more.

Literature Cited

1. McCord JM, Fridovich I. 1968. *J. Biol. Chem.* 243:5753–60
2. McCord JM, Fridovich I. 1969. *J. Biol. Chem.* 244:6049–55
3. Fridovich I. 1972. *Acct. Chem. Res.* 5:321–26
4. Halliwell B. 1974. *New Phytol.* 73: 1075–86
5. Fridovich I. 1974. *Adv. Enzymol.* 41: 35–97
6. Fridovich I. 1975. *Annu. Rev. Biochem.* 44:147–59
7. Fridovich I. 1978. *Photochem. Photobiol.* 28:733–41
8. Halliwell B. 1978. *Cell Biol. Int. Rep.* 2:113–28

9. Fridovich I. 1978. *Science* 201:875–80
10. Hassan HM, Fridovich I. 1979. *Rev. Infect. Dis.* 1:357–69
11. Hassan HM, Fridovich I. 1980. In *Enzymatic Basis of Detoxication*, ed. WB Jacoby, 1:311–32. New York: Academic
12. Fridovich I. 1981. In *Oxygen in Living Processes*, ed. DL Gilbert, pp. 250–72. New York: Springer
13. Elstner EF. 1982. *Annu. Rev. Plant Physiol.* 33:73–96
14. Steinman HM. 1982. In *Superoxide Dismutase*, ed. LW Oberley, pp. 11–68. Boca Raton, FL: CRC
15. Freeman BA, Crapo JD. 1982. *Lab. Invest.* 47:412–26
16. Michelson AM. 1982. *Agents Actions* 11:179–201 (Suppl.)
17. Parks DA, Bulkley GB, Granger DN. 1983. *Surgery* 94:415–22
18. Karnovsky ML, Badwey JA. 1983. *J. Clin. Chem. Clin. Biochem.* 21: 545–53
19. McCord JM. 1983. *Physiologist* 26:156–58
20. Halliwell B, Gutteridge JMC. 1984. *Biochem. J.* 219:1–14
21. Marklund SL. 1984. *Med. Biol.* 62:130–34
22. DiGuiseppi J, Fridovich I. 1984. *CRC Crit. Rev. Toxicol.* 12:315–42
23. Halliwell B, Gutteridge JMC. 1985. *Mol. Aspects Med.* 8:89–93
24. McCord JM. 1986. *Free Radic. Biol. Med.* 2:307–10
25. Halliwell B. 1987. *FASEB J.* 1:358–64
26. Weiss SJ. 1986. *Acta Physiol. Scand. Suppl.* 548:9–37
27. Bannister JV, Bannister WH, Rotilio G. 1987. *CRC Crit. Rev. Biochem.* 22:111–80
28. Fridovich I. 1986. *Adv. Enzymol.* 58:61–97
29. Michelson AM. 1987. *Life Chem. Rep.* 6:1–42
30. McCord JM. 1987. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 46:2402–6
31. Touati D. 1988. *Free Radic. Biol. Med.* 5:393–402
32. Cotgreave IA, Moldeus P, Orrenius S. 1988. *Annu. Rev. Pharmacol. Toxicol.* 28:189–212
33. Bannister WH. 1988. *Free Radic. Res. Commun.* 5:35–42
34. Koppenol WH. 1988. *Prog. Clin. Biol. Res.* 274:93–109
35. Hochstein P, Atallah AS. 1988. *Mutat. Res.* 202:363–75
36. Hassett DJ, Cohen MS. 1989. *FASEB J.* 3:2574–82
37. Hassan HM. 1989. *Adv. Genet.* 26:65–97
38. Joenje H. 1989. *Mutat. Res.* 219:193–208
39. Touati D. 1989. *Free Radic. Res. Commun.* 8:1–9
40. Floyd RA. 1990. *FASEB J.* 4:2587–97
41. Grace SC. 1990. *Life Sci.* 47:1875–86
42. Leff JA, Repine JE. 1990. *Blood Cells* 16:183–91
43. Storz G, Tartaglia LA, Farr SB, Ames BN. 1990. *Trends Genet.* 6:363–68
44. Fridovich I. 1991. *Curr. Top. Plant Physiol.* 6:1–5
45. Bielski BHJ, Cabelli DE. 1991. *Int. J. Radiat. Biol.* 59:291–319
46. Fridovich I. 1989. *J. Biol. Chem.* 264: 7761–64
47. Farr SB, Kogoma T. 1991. *Microbiol. Rev.* 55:561–85
48. Beyer W, Imlay J, Fridovich I. 1991. *Prog. Nucleic Acid Res. Mol. Biol.* 40: 221–53
49. Bowler C, van Montagu M, Inzé D. 1992. *Annu. Rev. Plant Physiol. Mol. Biol.* 43:83–116
50. Harris ED. 1992. *FASEB J.* 6:2675–83
51. Asada K. 1992. In *Molecular Biology of Free Radical Scavenging Systems*, ed. JG Scandalios, pp. 173–92. Cold Spring Harbor, NY: Cold Spring Harbor
52. Taniguchi M. 1992. *Adv. Clin. Chem.* 29:1–59
53. Omar BA, Flores SC, McCord JM. 1992. *Adv. Pharmacol.* 23:109–61
54. Halliwell B, Gutteridge JMC, Cross CE. 1992. *J. Lab. Clin. Med.* 119:598–620
55. Rice-Evans CA, Diplock AT. 1993. *Free Radic. Biol. Med.* 15:77–96
56. McCord JM. 1993. *Clin. Biochem.* 26: 351–58
57. Beauchamp C, Fridovich I. 1971. *Anal. Biochem.* 44:276–87
58. Misra HP, Fridovich I. 1972. *J. Biol. Chem.* 247:3170–75
59. Greenstock CL, Miller RW. 1975. *Biochim. Biophys. Acta* 396:11–16
60. Marklund S, Marklund G. 1974. *Eur. J. Biochem.* 47:469–74
61. Cohen G, Heikkila R. 1974. *J. Biol. Chem.* 249:2447–52
62. Fridovich I, Handler P. 1958. *J. Biol. Chem.* 233: 1578–80
63. Kuo CF, Mashino T, Fridovich I. 1987. *J. Biol. Chem.* 262:4724–27
64. Gardner PR, Fridovich I. 1991. *J. Biol. Chem.* 266:1478–83
65. Gardner PR, Fridovich I. 1991. *J. Biol. Chem.* 266:19328–33
66. Flint DH, Tuminello JF, Emptage MH. 1993. *J. Biol. Chem.* 268:22369–76
67. Liochev SI, Fridovich I. 1992. *Proc. Natl. Acad. Sci. USA* 89:5892–96
68. Flint DH, Emptage MH, Guest JR. 1992. *Biochemistry* 31:10331–37

69. Liochev SI, Fridovich I. 1994. *Free Radic. Biol. Med.* 16:29–33
70. Carlioz A, Touati D. 1986. *EMBO J.* 5:623–30
71. Nakae D, Yoshiji H, Amanuma T, Kinugasa T, Farber JL, Konishi Y. 1990. *Arch. Biochem. Biophys.* 279:315–19
72. Hiraishi H, Terano A, Razandi M, Sugimoto T, Harada T, Ivey KJ. 1992. *J. Biol. Chem.* 267:14812–17
73. Farr SB, D'Ari R, Touati D. 1986. *Proc. Natl. Acad. Sci. USA* 83:8268–72
74. Cathcart R, Schwiers E, Saul RL, Ames BN. 1984. *Proc. Natl. Acad. Sci. USA* 81:5633–37
75. Adelman R, Saul RL, Ames BN. 1988. *Proc. Natl. Acad. Sci. USA* 85:2706–08
76. Richter C, Park JW, Ames BN. 1988. *Proc. Natl. Acad. Sci. USA* 85:6465–67
77. Park EM, Shigenaga M, Degan P, Korn TS, Kitzler JW, et al. 1992. *Proc. Natl. Acad. Sci. USA* 89:3375–79
78 Wagner JR, Hu CC, Ames BN. 1992. *Proc. Natl. Acad. Sci. USA* 89:3380–84
79. Beauchamp C, Fridovich I. 1970. *J. Biol. Chem.* 245:4641–46
80. Goscin SA, Fridovich I. 1972. *Arch. Biochem. Biophys.* 153:778–83
81. Cohen G, Heikkila RE. 1974. *J. Biol. Chem.* 249:2447–52
82. Elstner EF, Konz JR. 1974. *FEBS Lett.* 45:18–21
83. Kellogg EW III, Fridovich I. 1975. *J. Biol. Chem.* 250:8812–17
84. Babior B, Curnutte JT, Kipnes RS. 1975. *J. Lab. Clin. Med.* 85:235–44
85. Lown JW, Begleiter A, Johnson D, Morgan AR. 1976. *Can. J. Biochem.* 54: 110–19
86. Cone R, Hasan SK, Lown JW, Morgan AR. 1976. *Can. J. Biochem.* 54:219–23
87. Hodgson EK, Fridovich I. 1976. *Arch. Biochem. Biophys.* 172:202–05
88. Van Hemmen JJ, Meuling WJA. 1977. *Arch. Biochem. Biophys.* 182:743–48
89. Heikkila RE, Cabbat FS. 1977. *Res. Commun. Chem. Pathol. Pharmacol.* 17:649–62
90. McCord JM, Day ED Jr. 1978. *FEBS Lett.* 86:139–42
91. Halliwell B. 1978. *FEBS Lett.* 92:321–26
92. Halliwell B. 1978. *FEBS Lett.* 96:238–42
93. Winterbourn CC. 1979. *Biochem. J.* 182:625–28
94. Gutteridge JMC, Richmond R, Halliwell B. 1979. *Biochem. J.* 184: 469–72
95. Klein SM, Cohen G, Cederbaum AI. 1980. *FEBS Lett.* 116:220–22
96. DiGuiseppi J, Fridovich I. 1980. *Arch. Biochem. Biophys.* 205:323–29
97. Sagone AL, Decker MA, Wells RM, DeMocko C. 1980. *Biochim. Biophys. Acta* 628:90–97
98. Trelstad PL, Lawley KR, Holmes LB. 1981. *Nature* 289:310–12
99. Fridovich SE, Porter NA. 1981. *J. Biol. Chem.* 256:260–65
100. Lown JW, Joshua AV, Lee JS. 1982. *Biochemistry* 21: 419–28
101. Legge RL, Thompson JE, Baker JE. 1982. *Plant Cell. Physiol.* 23:171–77
102. Rowley DA, Halliwell B. 1982. *FEBS Lett.* 138:33–36
103. Gutteridge JMC. 1984. *Biochem. Pharmacol.* 33:3059–62
104. Girotti AW, Thomas JP. 1984. *Biochim. Biophys. Acta* 118:474–80
105. Gutteridge JMC. 1984. *Biochem. J.* 224: 761–67
106. Graf E, Mahoney JR, Bryant RG, Eaton JW. 1984. *J. Biol. Chem.* 259:3620–24
107. Gutteridge JM. 1985. *FEBS Lett.* 185: 19–23
108. Gutteridge JMC, Bannister JV. 1986. *Biochem. J.* 234:225–28
109. Markey BA, Phan SH, Varanii J, Ryan US, Ward PA. 1990. *Free Radic. Biol. Med.* 9:307–14
110. Gutteridge JMC, Maidt L, Poyer L. 1990. *Biochem. J.* 269:169–74
111. Tukeshelashvili LK, McBride T, Spence K, Loeb LA. 1991. *J. Biol. Chem.* 266: 6401–6
112. Egan TJ, Barthakur SR, Aisen P. 1992. *J. Inorg. Biochem.* 48:241–49
113. Yim MB, Chock PB, Stadtman ER. 1990. *Proc. Natl. Acad. Sci. USA* 87: 5006–10
114. Sato K, Akaike T, Kohno M, Ando M, Maeda H. 1992. *J. Biol. Chem.* 267: 25371–77
115. Voest E, Van Faassen E, Marx JJM. 1993. *Free Radic. Biol. Med.* 15:589–95
116. Hodgson EK, Fridovich I. 1975. *Biochemistry* 14:5294–99
117. Hodgson EK, Fridovich I. 1975. *Biochemistry* 14:5299–5303
118. Lynch RE, Fridovich I. 1978. *J. Biol. Chem.* 253:4697–99
119. Weisiger RA, Fridovich I. 1973. *J. Biol. Chem.* 248:3582–92
120. Autor A. 1982. *J. Biol. Chem.* 257: 2713–18
121. Marres CA, Van Loon AR, Oudshoorn P, Van Steeg H, Grivell LA, Slater EC. 1985. *Eur. J. Biochem.* 147:153–61
122. White JA, Scandalios JG. 1987. *Biochim. Biophys. Acta* 926:16–25
123. Ho YS, Crapo JD. 1988. *FEBS Lett.* 229:377–82
124. White JA, Scandalios JG. 1989. *Proc. Natl. Acad. Sci. USA* 86:3534–38
125. Bowler C, Alliotte T, Van Den Bulcke M, Bauw G, Venderkerckhove J, et al.

1989. *Proc. Natl. Acad. Sci. USA* 86: 3237–41
126. Wispé JR, Clark JC, Burhans MS, Kropp KE, Korfhagen TR, Whitsett JA. 1989. *Biochim. Biophys. Acta* 994:30–36
127. Church SL, Grant W, Meese EU, Trent JM. 1992. *Genomics* 14:823–25
128. Scandalios JG. 1992. In *Molecular Biology of Free Radical Scavenging Systems*, ed. JG Scandalios, pp. 117–52. Cold Spring Harbor, NY: Cold Spring Harbor Lab.
129. McCord JM, Fridovich I. 1973. *Photochem. Photobiol.* 17:115–21
130. Jardin WF, Solda MI, Gimenez SMN. 1986. *Sci. Total Environ.* 58:47–54
131. Massey V, Strickland S, Mayhew SG, Howell LG, Engel PC, et al. 1969. *Biochim. Biophys. Acta* 36:891–97
132. Ballou D, Palmer G, Massey V. 1969. *Biochim. Biophys. Acta* 36:898–904
133. Martin JP, Logsdon N. 1987. *Arch. Biochem. Biophys.* 256:39–49
134. Martin J, Colina K, Logsdon N. 1987. *J. Bacteriol.* 169:2516–22
135. Ander S. 1967. *Strahlentherapie* 132:35
136. Cunningham ML, Krinsky N, Giovanazzi SM, Peak MJ. 1985. *J. Free Radic. Biol. Med.* 1:381–85
137. Lippitt B, McCord JM, Fridovich I. 1972. *J. Biol. Chem.* 247:4688–90
138. Makino K, Mossoba MM, Riesz P. 1982. *J. Am. Chem. Soc.* 104:35–37
139. Riesz P, Kondo T. 1992. *Free Radic. Biol. Med.* 13:247–70
140. Babior BM, Kipnes RS, Curnutte JT. 1973. *J. Clin. Invest.* 52:741–44
141. Curnutte JT, Whitten DM, Babior BM. 1974. *N. Engl. J. Med.* 290:593–97
142. Cheson BD, Curnutte JT, Babior BM. 1977. *Prog. Clin. Immunol.* 3:1–65
143. Babior BM. 1978. *N. Engl. J. Med.* 298:659–68
144. Babior BM. 1984. *Blood* 64:959–66
145. Haas A, Goebel W. 1992. *Free Radic. Res. Commun.* 16:137–57
146. Bellavite P. 1988. *Free Radic. Biol. Med.* 4:225–61
147. Marklund SL, Holme E, Hellmer L. 1982. *Clin. Chim. Acta* 126:41–51
148. Marklund SL. 1982. *Proc. Natl. Acad. Sci. USA* 79:7634–38
149. Karlsson K, Marklund SL. 1987. *Biochem. J.* 242:55–59
150. Karlsson K, Lindahl U, Marklund SL. 1988. *Biochem. J.* 256:29–33
151. Adachi T, Kodera T, Ohta H, Hayashi K, Hirano K. 1992. *Arch. Biochem. Biophys.* 297:155–61
152. Hjalmarsson K, Marklund SL, Engstrom A, Edlund T. 1987. *Proc. Natl. Acad. Sci. USA* 84:6340–44
153. Karlsson K, Marklund SL. 1989. *Lab. Invest.* 60:659–66
154. Inoue M, Watanabe N, Morino Y, Tanaka Y, Amachi T, Sasaki J. 1990. *FEBS Lett.* 269:89–92
155. Johansson MH, Deinum J, Marklund SL, Sjöquist PO. 1990. *Cardiovasc. Res.* 24:500–3
156. Sjöquist PO, Carlsson L, Jonason G, Marklund SL, Abrahamsson T. 1991. *J. Cardiovasc. Pharmacol.* 17:678–83
157. Wahlung G, Marklund SL, Sjöquist PO. 1992. *Free Radic. Res. Commun.* 17:41–47
158. Hatori N, Sjöquist PO, Marklund SL, Petrsson SK, Rydén L. 1992. *Free Radic. Biol. Med.* 13:137–42
159. Abrahamsson T, Brandt U, Marklund SL, Sjöquist PO. 1992. *Circ. Res.* 70: 264–71
160. Nakazono K, Watanabe N, Matsuno K, Sasaki J, Sato T, Inoue M. 1991. *Proc. Natl. Acad. Sci. USA* 88:10045–48
161. Hansson L, Edlund M, Edlund A, Johansson T, Marklund SL, et al. 1994 *J. Biol. Chem.* 269:5358–63
162. McEuen AR, Hill HAO. 1982. Planta 154:295–97
163. Beaman BL, Scates SM, Moring SE, Deem R, Misra HP. 1983. *J. Biol. Chem.* 258:91–96
164. Hong Z, LoVerde PT, Thakur A, Hammarskjöld ML, Rekosh D. 1993. *Exp. Parasitol.* 76:101–14
165. James ER, McLean DC Jr, Pealer F. 1994. *Infect. Immun.* 62:713–16
166. Filice GA, Beaman BL, Krick JA, Remington JS. 1980. *J. Infect. Dis.* 142: 432–38
167. Beaman BL, Black CM, Doughty F, Beaman L. 1985. *Infect. Immun.* 47: 135–41
168. Britton L, Malinowski DP, Fridovich I. 1978. *J. Bacteriol.* 134:229–36
169. Jurtshuck P, Lin JK, Moore ERB. 1984. *Appl. Environ. Microbiol.* 47: 1185–87
170. Baumann P. 1984. *Arch. Microbiol.* 138: 170–78
171. Puget K, Michelson AM. 1974. *Biochimie* 56:1255–67
172. Martin JP Jr, Fridovich I. 1981. *J. Biol. Chem.* 256:6080–89
173. Bannister JV, Parker MW. 1985. *Proc. Natl. Acad. Sci. USA* 82:149–52
174. Leunissen J, de Jong W. 1986. *J. Mol. Evol.* 23:250–58
175. Steinman HM. 1982. *J. Biol. Chem.* 257:10283–93
176. Steinman HM. 1985. *J. Bacteriol.* 162: 1255–60
177. Beck BL, Tabatai LB, Mayfield JE. 1990. *Biochemistry* 29:372–76

178. Kroll JS, Langford PR, Loynds BM. 1991. *J. Bacteriol.* 173:7449–57
179. Steinman HM. 1987. *J. Biol. Chem.* 262:1882–87
180. Steinman HM, Ely B. 1990. *J. Bacteriol.* 172:2901–10
181. Stabel TJ, Sha Z, Mayfield JE. 1994. *Vet. Microbiol.* 38:307–14
182. Benov LT, Fridovich I. 1994. *J. Biol. Chem.* 269:25310–14
183. Pasternack RF, Barth A, Pasternack JM, Johnson CS. 1981. *J. Inorg. Biochem.* 15:261–67
184. Faraggi M. 1984. In *Oxygen Radicals in Chemistry and Biology*, ed. W Bors, M Saran, D Tait, pp. 419–30. Berlin: de Gruyter
185. Archibald FS, Fridovich I. 1981. *J. Bacteriol.* 145:442–51
186. Archibald FS, Fridovich I. 1981. *J. Bacterid.* 146:928–36
187. Archibald FS, Fridovich I. 1982. *Arch. Biochem. Biophys.* 215:589–96
188. Faulkner KM, Liochev SI, Fridovich I. 1994. *J. Biol. Chem.* 269:23471–76
189. Deleted in proof
190. Deleted in proof
190a. Riley DP, Weiss RH. 1994. *J. Am. Chem. Soc.* 116:387–88
190b. Hardy MS, Flickinger AG, Riley D, Weiss RH, Ryan US. 1994. *J. Biol. Chem.* 269:18535–40
191. Robertson P, Fridovich SE, Misra HP, Fridovich I. 1981. *Arch. Biochem. Biophys.* 207:282–89
192. Mashino T, Fridovich I. 1987. *Arch. Biochem. Biophys.* 252:163–70
193. Mashino T, Fridovich I. 1987. *Arch. Biochem. Biophys.* 254:547–51
194. Garst J, Stapleton P, Johnston J. 1983. In *Oxy Radicals and Their Scavenger Systems: Proc. Int. Conf. Superoxide and Superoxide Dismutase, 1982*, ed. G Cohen, RA Greenwald, pp. 125–30. New York: Elsevier
195. Cannella C, Berni R. 1983. *FEBS Lett.* 162:180–84
196. Hicks M, Delbridge L, Yue DK, Reeve TS. 1988. *Biochem. Biophys. Res. Commun.* 151:649–55
197. Smith PR, Thornalley PJ. 1992. *Eur. J. Biochem.* 210:729–39
198. Sakurai T, Tsuchiya S. 1988. *FEBS Lett.* 236:406–10
199. Sakurai T, Kimura S, Nakano M, Kimura H. 1991. *Biochim. Biophys. Acta* 177:433–39
200. Bailey AJ, Sims TJ, Avery NC, Miles CA. 1993. *Biochem. J.* 296:489–96
201. Ookawara T, Kawamura N, Kitagawa Y, Taniguchi N. 1992. *J. Biol. Chem.* 267:18505–10
202. Adachi T, Ohta H, Hayashi K, Hirano K, Marklund SL. 1992. *Free Radic. Biol. Med.* 13:205–10
203. Hunt JV, Bottoms MA, Mitchinson MJ. 1993. *Biochem. J.* 291:529–35
204. Van Camp W, Bowler C, Villarroel R, Tsang EWT, Van Montagu M, Inzé D. 1990. *Proc. Natl. Acad. Sci. USA* 87: 9903–7
204a. Natvig DO, Imlay K, Touati D, Hallewell RA. 1987. *J. Biol. Chem.* 262: 14697–14701
205. Steinman HM. 1992. *Mol. Gen. Genet.* 232:427–30
206. Brehm K, Haas A, Goebel W, Kreft J. 1992. *Gene* 118:121–25
207. Heinzen RA, Frazier ME, Mallavia LP. 1992. *Infect. Immun.* 60:3814–23
208. Chambers SP, Brehm JK, Michael NP, Atkinson T, Minton NP. 1992. *FEMS Microbiol. Lett.* 70:277–84
209. Haas A, Goebel W. 1992. *Mol. Gen. Genet.* 231:313–22
210. Zhu D, Scandalios JG. 1992. *Genetics* 131:803–9
211. Bowler C, Slooten L, Vandenbranden S, de Rycke R, Butterman J, et al. 1991. *EMBO J.* 10:1723–32
212. Reveillaud I, Phillips J, Duyf B, Hilliker A, Kongpachith A, Flemin J. 1994. *Mol. Cell. Biol.* 14:1302–07
213. Deleted in proof
214. Nakayama K. 1994. *J. Bacteriol.* 176: 1939–43
215. Imlay JA, Fridovich I. 1992. *J. Bacteriol.* 174:953–61
216. Rech SB, Stateva LI, Oliver SG. 1992. *Curr. Genet.* 21:339–44
217. Storz G, Christman MF, Sies H, Ames BN. 1987. *Proc. Natl. Acad. Sci. USA* 84:8917–21
218. Bilinski T, Krawiec Z, Liczmanski A, Litwinska J. 1985. *Biochem. Biophys. Res. Commun.* 130:533–39
219. van Loon APGM, Pesold-Hurt B, Schatz G. 1986. *Proc. Natl. Acad. Sci. USA* 83:3820–24
220. Gralla EB, Valentine JS. 1991. *J. Bacteriol* 173:5918–20
221. Liu XF, Elashvili I, Gralla EB, Valentine JS, Lapinskas P, Culotta VC. 1992. *J. Biol. Chem.* 267:18298–18302
222. Phillips JD, Campbell SD, Michaud D, Charbonneau M, Hilliker AJ. 1989. *Proc. Natl. Acad. Sci. USA* 86:2761–65
223. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, et al. 1993. *Nature* 362:59–62
224. Deng HX, Hentati A, Tainer JA, Iqbal Z, Cayabyab A, et al. 1993. *Science* 261:1047–51
225. Bowling AC, Schulz JB, Brown RH Jr, Beal MF. 1993. *J. Neurochem.* 61:2322–25

226. Rothstein JO, Bristol LA, Hosler B, Brown RH Jr, Kunal RW. 1994. *Proc. Natl. Acad. Sci. USA* 91:4155–59
227. Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, et al. 1994. *Science* 264:1772–74
228. Ischiropoulos H, Zhu L, Chen J, Tsai M, Martin JC, et al. 1992. *Arch. Biochem. Biophys.* 298:431–37
229. Beckman JS, Ischiropoulos H, Zhu L, van der Woerd M, Smith C, et al. 1992. *Arch. Biochem. Biophys.* 298:438–45
230. Furchgott RF, Zawadski JV. 1980. *Nature* 288:373–76
231. Palmer RMJ, Ferrige AG, Moncada S. 1987. *Nature* 327:524–26
232. Gryglewski RJ, Palmer RM, Moncada S. 1986. *Nature* 320:454–56
233. Rubanyi GM, Vanhoutte PM. 1986. *Am. J. Physiol.* 250:H822–27
234. Katusic ZS, Vanhoutte PM. 1989. *Am. J. Physiol.* 257:H33–37
235. Bielski BHJ, Arudi RL. 1985. *Inorg. Chem.* 24:3502–4
236. Huie RE, Padmaja S. 1993. *Free Radic. Biol. Med.* 18:195–99
237. Radi R, Beckman JS, Bush KM, Freeman BA. 1991. *J. Biol. Chem.* 266: 4244–50
238. Radi R, Beckman JS, Bush KM, Freeman BA. 1991. *Arch. Biochem. Biophys.* 288:481–87
239. Zhu L, Gunn C, Beckman JS. 1992. *Arch. Biochem. Biophys.* 298:452–57
240. Denicola A, Rubbo H, Rodriguez D, Radi R. 1993. *Arch. Biochem. Biophys.* 304:279–86
241. Kargalioglu Y, Imlay JA. 1994. *Free Radic. Biol. Med.* 15:472 (Abstr.)
242. Greenberg JT, Monach P, Chou JH, Josephy PD, Demple B. 1990. *Proc. Natl. Acad. Sci. USA* 87:6181–85
243. Tsaneva IR, Weiss B. 1990. *J. Bacteriol.* 172:4197–4205
244. Greenberg JT, Chou JH, Monach PA, Demple B. 1991. *J. Bacteriol.* 173: 4433–39
245. Privalle CT, Fridovich I. 1993. *J. Biol. Chem.* 268:5178–81
246. Tardot B, Touati D. 1993. *Mol. Microbiol.* 9:53–63
247. Beaumont MD, Hassan HM. 1993. *J. Gen. Microbiol.* 139:2677–84
248. Friedman DI. 1988. *Cell* 55:545–54
249. Compan I, Touati D. 1993. *J. Bacteriol.* 175:1687–96
250. Bagg A, Neilands JB. 1987. *Microbiol. Rev.* 51:509–18
251. Sharrocks A, Green J, Guest J. 1991. *Proc. R. Soc. London Ser. B* 245:219–26
252. Hidalgo E, Demple B. 1993. *EMBO J.* 13:138–46
253. Privalle CT, Fridovich I. 1992. *J. Biol. Chem.* 267:9140–45
254. Beyer WF Jr, Fridovich I. 1991. *J. Biol. Chem.* 266:303–8
255. Hassan HM, Moody CS. 1984. *FEMS Microbiol. Lett.* 25:233–36
256. Moody CS, Hassan HM. 1984. *J. Biol. Chem.* 259:12821–25
257. Pugh SY, Fridovich I. 1985. *J. Bacteriol.* 162:196–202
258. Touati D. 1988. *J. Bacteriol.* 170:2511–20
259. Privalle CT, Kong SE, Fridovich I. 1993. *Proc. Natl. Acad. Sci. USA* 90:2310–14