The atmosphere of planet earth was anaerobic until the advent of water-splitting, O$_2$-evolving photosynthesis. The accumulation of O$_2$ changed the environment for, and therefore changed the selection pressures on, all living organisms. It also increased the mutation rate and therefore hastened subsequent evolution. Advantages could be gained by using the O$_2$ to increase the useful energy derivable from foodstuffs, to carry out novel metabolic transformations, to solubilize and detoxify numerous compounds and even to generate heat and light.

But there was a price to pay for these benefits and that was to provide defenses against the considerable toxicity of this paramagnetic gas. Those organisms that succeeded in developing the requisite defenses could reap the benefits, and they gave rise to the enormous variety of aerobic life forms that are now so evident on earth. Those that could not accommodate to the challenge of O$_2$ toxicity evolved into the sensitive microscopic anaerobes now restricted to those anaerobic niches that remain even on a thoroughly aerobic planet. So, why is O$_2$ toxic and what sorts of defenses have been evolved to blunt that toxicity?

**Paramagnetism and the univalent pathway**

Rotating electrical charges generate magnetic fields. This applies to electrical current in a coil of wire or to a single spinning electron. The pairing of electrons with opposite spin states neutralizes this effect. Most substances are not influenced by imposed magnetic fields because the electrons they contain are all in spin-opposed pairs. Such substances are diamagnetic. O$_2$ is unusual in being paramagnetic, and that implies unpaired electronic spins. Indeed, O$_2$ contains two unpaired electrons and they have the same spin state.

This electronic structure constitutes a barrier to the insertion of a pair of electrons. Thus, the electrons of the incoming spin-opposed pair would be trying to join the parallel-spinning unpaired electrons of O$_2$, and one of them would have the same spin state as its partner to be. This situation, schematized in reaction 1, is energetically very unfavorable; as stated by the Pauly exclusion principle:

\[
\uparrow\uparrow + \uparrow\downarrow \rightarrow \bigcirc + \uparrow\downarrow .
\]

There is a way around this barrier, and that way is to add the electrons to O$_2$ one at a time. This works because electronic spins can be inverted by interaction with nuclear spins. However, this is a slow process relative to the lifetime of collisional complexes and is not likely while the reacting partners are in contact. But when the electrons are added one at a time, during separate collisional events, there is time between collisions for the inversion of electronic spin. As a result, the facile route of O$_2$ reduction is by a series of univalent electron transfers.

**Intermediates on the univalent pathway**

The reduction of O$_2$ to H$_2$O requires four electrons. Hence, intermediates will be encountered on this univalent pathway and these are superoxide (O$_2^-\$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (HO$^\cdot\$). It is these intermediates that are responsible for the toxicity of O$_2$ and defenses against that toxicity must include minimizing their production to the maximum extent possible and eliminating those whose production cannot be avoided.

Most of the O$_2$ consumed by respiring cells is reduced by cytochrome c oxidase which, by virtue of two ferrihemes and two Cu(II) prosthetic groups, manages the four-electron
reduction of O\textsubscript{2} to 2H\textsubscript{2}O without releasing intermediates. But there are enzymes that reduce O\textsubscript{2} to H\textsubscript{2}O\textsubscript{2}, and there are both enzymic and spontaneous processes within cells that produce O\textsubscript{2}\textsuperscript{−}. It has been estimated that only approximately 0.1 % of the O\textsubscript{2} reduced by *Escherichia coli* is reduced to O\textsubscript{2}\textsuperscript{−} (Imlay and Fridovich, 1991). Nevertheless, so great is the rate of O\textsubscript{2} utilization by these cells that, were the O\textsubscript{2}\textsuperscript{−} stable, this would correspond to the production of approximately 5 \(\mu\)mol\( \cdot l^{-1}\) intracellular O\textsubscript{2}\textsuperscript{−} per second. Similarly, in mitochondria, a small fraction of total O\textsubscript{2} reduction gives rise to O\textsubscript{2}\textsuperscript{−} (Gardner andBoveris, 1990).

*Hydroxyl radical*

HO\textsuperscript{*} is an extraordinarily powerful oxidant, which attacks most organic compounds at diffusion-limited rates (Czapski, 1984). First encountered during studies of the effect of ionizing radiation on water, it can also be produced by the reduction of H\textsubscript{2}O\textsubscript{2} by metal cations such as Fe(II) or Cu(I). It is sobering to contemplate the ease with which HO\textsuperscript{*} can be produced. During enzymatic reactions, producing both O\textsubscript{2}\textsuperscript{−} and H\textsubscript{2}O\textsubscript{2}, this is a metal-catalyzed process, as follows:

\[
\begin{align*}
\text{O}^\text{2−} + \text{Me}(n) &\leftrightarrow \text{O}_2 + \text{Me}(n-1), \quad (2) \\
\text{Me}(n-1) + \text{H}_2\text{O}_2 &\rightarrow \text{Me}(n) + \text{OH}− + \text{HO}^\text{*}. \quad (3)
\end{align*}
\]

Reaction 3 above can be broken down into a series of steps as follows:

\[
\begin{align*}
\text{Fe(II)} + \text{HOOH} &\rightleftharpoons (\text{Fe-OOH})^+ + \text{H}^+ \quad (3a) \\
(\text{Fe-OOH})^+ &\rightleftharpoons (\text{FeO})^{2+} + \text{OH}− \quad (3b) \\
(\text{FeO})^{2+} + \text{H}^+ &\rightleftharpoons (\text{Fe-OH})^{3+} \quad (3c) \\
(\text{Fe-OH})^{3+} &\rightleftharpoons \text{Fe(III)} + \text{HO}^\text{*}. \quad (3d)
\end{align*}
\]

It is almost a matter of semantics whether the powerful oxidant produced by this process is (FeO)\textsuperscript{3+} or (FeOH)\textsuperscript{3+} or HO\textsuperscript{*}. The point is that the products of this process would be damaging to cells. This is particularly the case since Fe(III) would not exist in free solution, but rather would bind to polyanions such as nucleic acids or to phospholipid membranes. In that case, the HO\textsuperscript{*}, or (Fe-OH)\textsuperscript{3+} or (FeO)\textsuperscript{2+} would be produced adjacent to, and would selectively attack, those critically important targets.

Cells are rich in reductants, such as thiols and enediols, and these are able to reduce Fe(III) to Fe(II), thus obviating the need for reaction 2. None the less, O\textsubscript{2}\textsuperscript{−} does collaborate with H\textsubscript{2}O\textsubscript{2} in producing HO\textsuperscript{*} within cells and it does so by oxidizing the [4Fe–4S] clusters of dehydratases, such as aconitase, causing the release of Fe(II). In this way, O\textsubscript{2}\textsuperscript{−} increases the availability of iron for reactions 3a–3d. This mechanism had been proposed (Liochev and Fridovich, 1994) and was subsequently experimentally verified (Keyer *et al.* 1995; Keyer andImlay, 1996).

*Superoxide anion radical (O\textsubscript{2}\textsuperscript{−})*

O\textsubscript{2}\textsuperscript{−} is more selective in its reactivity than is HO\textsuperscript{*} and paradoxically is therefore potentially more damaging. Thus, HO\textsuperscript{*} will react with something, perhaps something expendable, within a very small radius of its site of generation, whereas O\textsubscript{2}\textsuperscript{−} can diffuse a considerable distance before it encounters a suitable, and possibly critical, target. O\textsubscript{2}\textsuperscript{−} is the conjugate base of the hydroperoxyl radical (HO\textsuperscript{2−}) whose pK\textsubscript{a} is approximately 4.8, and the following spontaneous dismutations can occur (Bielski, 1978):

\[
\begin{align*}
\text{HO}^2\text{−} + \text{HO}^2\text{−} &\rightleftharpoons \text{H}_2\text{O}_2 + \text{O}_2 \quad (k_{2}=8\times10^5\text{\,mol}^{-1}\text{\,l}\,\text{s}^{-1}), \quad (4) \\
\text{HO}^2\text{−} + \text{O}_2^- + \text{H}^+ &\rightleftharpoons \text{H}_2\text{O}_2 + \text{O}_2 \quad (k_{2}=1\times10^8\text{\,mol}^{-1}\text{\,l}\,\text{s}^{-1}), \quad (5) \\
\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ &\rightleftharpoons \text{H}_2\text{O}_2 + \text{O}_2 \quad (k_{2}<0.3\text{\,mol}^{-1}\text{\,l}\,\text{s}^{-1}). \quad (6)
\end{align*}
\]

O\textsubscript{2}\textsuperscript{−} is thus intrinsically unstable in protic solvents such as water but, because reaction 6 is so slow, the higher the pH the more stable it becomes. At neutral pH, it is stable enough to oxidize such compounds as polyphenols, thiols, ascorbate, catecholamines, leukoflavins, tetrahydropterins and sulfite. More importantly, it can rapidly inactivate aconitase and similar [4Fe–4S]-containing dehydratases.

*Chain reactions*

Reactions of radicals with non-radicals have a special feature and that is amplification of the consequences of the initiating event by a chain reaction. This happens because a reaction of a radical with a non-radical always begets another radical. Consider the oxidation of sulfite to sulfate, which can be initiated by O\textsubscript{2}\textsuperscript{−}. Thousands of sulfites can be oxidized per O\textsubscript{2}\textsuperscript{−} introduced and this occurs as follows:

\[
\begin{align*}
\text{SO}_3^{2−} + \text{O}_2^{−} + 2\text{H}^+ &\leftrightarrow \text{SO}_3^{−}\cdot + \text{H}_2\text{O}_2 \quad \text{initiation}, \quad (7) \\
\text{SO}_3^{−}\cdot + \text{O}_2 &\leftrightarrow \text{SO}_5^{−}\cdot \quad \text{chain} \quad (8) \\
\text{SO}_5^{−}\cdot + \text{SO}_3^{2−} &\leftrightarrow \text{SO}_3^{2−} + \text{SO}_3^{−}\cdot \quad \text{formation} \quad (9) \\
\text{SO}_3^{2−} + \text{H}_2\text{O} &\leftrightarrow \text{SO}_4^{2−} + \text{H}_2\text{O}_2 \quad \text{stable\,products} \quad (10)
\end{align*}
\]

The sulfur trioxy anion radical produced by reaction 7 and the sulfur pentoxy anion radical produced by reaction 8 are strong oxidants capable of oxidizing amines or alcohols, which act as antioxidants because the radicals produced by their oxidation are not reactive enough to propagate the chain oxidation of sulfite. A chain mechanism similarly occurs during the oxidation of the polyunsaturated lipids so abundant in our cell membranes, and this amplifies the damage caused by the initiating event. In this case, the important antioxidant is \(\alpha\)-tocopherol (vitamin E).

*Defenses against O\textsubscript{2}\textsuperscript{−}*

A common selection pressure applied to a varied biota is apt to call forth multiple adaptations. In this light, it is not surprising that we find multiple defenses against O\textsubscript{2}\textsuperscript{−}. These are the superoxide dismutases (SODs) which catalyze reaction 6 at diffusion-limited rates. These supremely
efficient catalysts are abundant in aerobic cells and they keep the steady-state level of \( \text{O}_2^- \) in the \( 10^{-10} \text{mol} \cdot \text{L}^{-1} \) range (Inlay and Fridovich, 1991). It is useful to point out that with [SOD] at approximately \( 10^{-5} \text{mol} \cdot \text{L}^{-1} \) and \([\text{O}_2^-]\) at \( 10^{-10} \text{mol} \cdot \text{L}^{-1} \), an \( \text{O}_2^- \) is \( 10^9 \) times more likely to encounter a molecule of SOD than it is to encounter another \( \text{O}_2^- \). Add to this the fact that the rate constant for the reaction of \( \text{O}_2^- \) with SOD is \( k_2 = 2 \times 10^9 \text{mol}^{-1} \text{s}^{-1} \), while that of its uncatalyzed reaction with another \( \text{O}_2^- \) at neutral pH is \( k = 2 \times 10^5 \text{mol}^{-1} \text{s}^{-1} \). Hence, the lifetime of an \( \text{O}_2^- \) would be shortened by a factor of \( 10^9 \) by the SOD. This would be the case if the spontaneous and the SOD-catalyzed dismutations were the only fates open to \( \text{O}_2^- \). However, there are targets that would be attacked by \( \text{O}_2^- \), were it not removed by SOD. It has recently been estimated that the SODs in \( E. \text{coli} \) provide approximately 95 % protection for all targets susceptible to \( \text{O}_2^- \) attack in that cell (Liochev and Fridovich, 1997). Returning to the multiplicity of SODs, we note that there are SODs that depend for their activity on active sites containing Cu and Zn, Mn, Fe and even Ni. There are SODs that are cytosolic, localized to specific subcellular organelles and also secreted from the cell. We will consider these in turn.

**CuZnSODs**

These enzymes have Cu and Zn at their active sites. The copper undergoes valence changes during the catalytic cycle while the Zn is thought to play a mainly structural role. CuZnSODs are found in the cytosols of eukaryotic cells, in the periplasms of gram-negative bacteria, in the plastids of plants and in the extracellular spaces of mammals. The cytosolic CuZnSOD is homodimeric, and its structure has been determined to high-resolution by X-ray crystallography (Tainer et al. 1982). The Cu(II) and the Zn(II) are ligated to a bridging imidazolate that plays a role in proton conduction, which might otherwise become rate-limiting. Thus, upon reduction of the Cu(II) by \( \text{O}_2^- \), the Cu–imidazolate bond is broken and the imidazolate becomes protonated. Then, during reoxidation of the Cu(I) by the next \( \text{O}_2^- \), the Cu-imidazolate bond is re-established while the proton converts the reduced Cu(I)–Im–Zn(II) into Cu(II)–Im–Zn(II) + H_2O_2. This can be schematically presented as follows:

\[
\begin{align*}
\text{Cu(II)} & \rightarrow \text{Cu(I)} + \text{Cu}^+ \\
\text{Cu(I)} + \text{Im–Zn(II)} + \text{H}^+ & \rightarrow \text{Cu(II)} + \text{H}^+ + \text{Im–Zn(II)} \\
\text{Cu(II)} + \text{Im–Zn(II)} + \text{O}_2^- & \rightarrow \text{Cu(II)} + \text{Im–Zn(II)} + \text{HO}_2^- \\
\text{HO}_2^- + \text{H}^+ & \leftrightarrow \text{H}_2\text{O}_2 \\
\end{align*}
\]

This mechanism was proposed on kinetic grounds (Hodgson and Fridovich, 1975) and recently verified through X-ray absorption fine structure (XAFS) and crystallography (Murphy et al. 1997).

How is it possible for an enzyme to react with its substrate at a rate of approximately \( 2 \times 10^9 \text{mol}^{-1} \text{s}^{-1} \), which is close to the diffusion limit? This question arises because the Cu site, at which reactions with \( \text{O}_2^- \) occur, represents less than 1 % of the surface of the enzyme. On the basis of random collisions, one would expect more than 99 % of the collisions between \( \text{O}_2^- \) and the SOD to be fruitless. Koppenol (1982) was the first to consider this problem, and he suggested electrostatic guidance as a possible answer. Subsequently, the superoxide dismutases were seen to utilize electrostatic facilitation on the basis of ionic strength effects (Benovic et al. 1983; Cudd and Fridovich, 1982). Site-specific modifications have been used to enhance electrostatic facilitation and thus to make a more active SOD (Getzoff et al. 1992).

An extracellular SOD (ECSOD), which is a homotetrameric glycoprotein with a high affinity for heparin sulfate, has been described (Tibell et al. 1993). It presumably functions to scavenge the \( \text{O}_2^- \) that is released from the surfaces of cells. The discovery of the signaling roles of NO, and the extremely rapid reaction of NO with \( \text{O}_2^- \), increases our appreciation of the importance of extracellular SOD. Knockout mice, which lack ECSOD, appear superficially normal under ordinary conditions, but they exhibit enhanced sensitivity towards hyperoxia (Carlsson et al. 1995). Parasitic nematodes have been found to make extracellular SOD (Tang et al. 1994; James et al. 1994). In the case of such parasites, the ECSOD may provide a defense against \( \text{O}_2^- \) produced by host leukocytes. Microorganisms have also been seen to produce extracellular SODs, and these may similarly act as pathogenicity factors. Thus, *Mycobacterium avium* make an extracellular MnSOD (Escuyer et al. 1996), as does *Nocardia asteroides* (Alcendor et al. 1995).

Mutations in the cytosolic CuZnSOD have been associated with the familial form of amyotrophic lateral sclerosis (FALS). To date, approximately 50 different amino acid replacements have been seen in the CuZnSOD of FALS patients, and the activities of these mutant enzymes have ranged from 0.1 to 100 % of normal. That range of activities, as well as the genetic dominance of FALS, suggested that a toxic gain of function, rather than a loss of SOD activity, was the problem. This was established to be the case when transgenic mice expressing FALS-associated mutant CuZnSODs were seen to develop paralysis (Dal Canto and Gurney, 1994). Transgenic mice expressing normal human CuZnSOD did not develop symptoms of paralysis.

**MnSODs**

These enzymes, which are as active as the CuZnSODs, but are unrelated as judged by sequence, may be dimeric or tetrameric. They contain one Mn(III) per subunit and their structures have been determined by X-ray crystallography (Wagner et al. 1993). The *E. coli* MnSOD is dimeric and it is not ordinarily produced when the cells are growing anaerobically. However, it is induced under aeriation and is further induced by compounds that can increase intracellular \( \text{O}_2^- \) production. Compounds such as viologens, quinones, pyocyanine and a host of synthetic dyes are in this category (Hassan and Fridovich, 1979). This induction of the *E. coli*
MnSOD is controlled by the soxRS regulon, which will be further discussed below.

There is a homotetrameric MnSOD in the matrix of mitochondria, which is closely related, in terms of sequence, to the prokaryotic enzyme. It is fascinating that this eukaryotic organelle should contain a SOD very similar to the prokaryotic enzyme and totally unrelated to the SOD in the surrounding cytosol. This is certainly suggestive of the endosymbiotic origin of these organelles. Knockout mice, which are unable to make this mitochondrial MnSOD, are severely affected and live only a few days after birth (Li et al. 1995). The deleterious consequences of knocking out the cytosolic CuZnSOD are notable but much less dramatic (Reaume et al. 1996; Kondo et al. 1997).

**FeSODs**

It behoves a facultative enteric organism such as *E. coli* to contain a constitutive SOD, in addition to the inducible MnSOD, so that it will not be devoid of defense against O$_2^-$ when faced with the abrupt transition from anaerobic to aerobic conditions. In *E. coli*, this standby defense is provided by a FeSOD which, although specific for the metal it contains, is related by sequence to the MnSOD. There are some SODs, such as those found in *Bacteroides fragilis* (Gregory and Dapper, 1983) and *Propionibacterium shermanii* (Meier et al. 1982), which can be active with either Fe or Mn at the active site. These are called cambialistic SODs. The organisms that contain these cambialistic SODs insert Fe under anaerobiosis and Mn under aerobiciosis. This is a sensible adaptation since environmental Fe(II) would autoxidize to the much less soluble Fe(III) and thus be less available in the presence of oxygen. Mn, in contrast, remains Mn(II), and soluble, under both conditions. This, of course, leaves open the question of why these organisms insert Fe when anaerobic.

*Lactobacillus plantarum*, which ordinarily lives in the Mn-rich environment provided by fermenting plants, shows a fascinating adaptation. It accumulates large amounts of Mn(II), under relatively high concentrations of H$_2$O$_2$ because their Km for H$_2$O$_2$ lies in the millimolar range. Hence the packaging of catalase into peroxisomes, along with many H$_2$O$_2$-producing enzymes. Most of these are ferriheme enzymes, and their action involves the divalent oxidation of the heme to an Fe(IV) cation radical by H$_2$O$_2$, followed by divalent reduction by H$_2$O, in the case of catalase, and by two successive univalent reductions by the organic substrate, in the case of the peroxidases (Dolphin et al. 1971).

**Defenses against H$_2$O$_2$**

The catalases, which dismutate 2H$_2$O$_2$ into O$_2$ + 2H$_2$O, and the peroxidases, which use diverse reductants to reduce H$_2$O$_2$ to 2H$_2$O, are the enzymes that deal with H$_2$O$_2$. Some of the peroxidases can also reduce alkyl hydroperoxides to the corresponding alcohols. As was the case with the SODs, the catalases and peroxidases constitute a diverse family of enzymes. Most of these are ferriheme enzymes, and their action involves the divalent oxidation of the heme to an Fe(IV) σ cation radical by H$_2$O$_2$, followed by divalent reduction by H$_2$O$_2$, in the case of catalase, and by two successive univalent reductions by the organic substrate, in the case of the peroxidases (Dolphin et al. 1971).

**Catalases**

Mammalian catalases are homotetrameric ferriheme-containing enzymes whose subunit mass is approximately 60 kDa. These enzymes are most efficient when dealing with relatively high concentrations of H$_2$O$_2$ because their Km for H$_2$O$_2$ lies in the millimolar range. Hence the packaging of catalase into peroxisomes, along with many H$_2$O$_2$-producing enzymes. Mammalian catalase can also act as a peroxidase towards a few small molecules such as methanol, ethanol, nitrite and formate. Thus, it can use H$_2$O$_2$ to oxidize these substrates, which are small enough to gain access to the heme iron. The structure, as determined by X-ray crystallography, indicates that the heme lies deeply buried in the protein and is thus accessible only to small substrates (Reid et al. 1981). Catalase contains tightly bound NADPH, which may function to prevent the accumulation of an inactive Fe(IV) form of the enzyme (Kirkman et al. 1987). Yet the structure shows no interaction between the NADPH and the heme (Fita and Rossman, 1985).

*E. coli* makes two catalases, which have been named hydroperoxidases (HP) I and II (Claiborne and Fridovich, 1979; Claiborne et al. 1979). HP-I is unusual in that it is active
as a catalase and as a peroxidase, which can utilize large reducing substrates such as diamsidine. HP-II, in contrast, is a catalase without this peroxidase activity. The significance of the peroxidase activity of HP-I is not known since it had no peroxidase activity towards any dialyzable component of \textit{E. coli} extract. HP-I is induced as a member of the oxy \textit{R} regulon in response to \textit{H}_{2}\textit{O}, while HP-II is induced as cells enter stationary phase by the sigma factor \textit{rpos} (Visick and Clarke, 1997). The structures of the genes coding for HP-I and HP-II of \textit{E. coli} have been elucidated (Loewen and Stauffer, 1990; von Ossowski \textit{et al.} 1991). HP-I and HP-II are differently localized in \textit{E. coli}, with HP-I appearing in the periplasm and associated with the cytoplasmic membrane, while HP-II is cytosolic (Heimberger and Eisenstark, 1988).

The importance of catalase is nicely illustrated by the behavior of \textit{L. plantarum}, which cannot synthesize heme. When heme is available in the medium, this organism makes a heme-containing catalase. When heme is not available, it makes a different catalase which contains manganese (Kono and Fridovich, 1983). This manganese catalase was called pseudocatalase because, unlike the more familiar heme-containing catalases, it was not inhibited by CN\textsuperscript{−} or \textit{N}_{3}\textsuperscript{−}. Such is the need for catalase that \textit{L. plantarum} contrives to supply itself with this activity whether or not heme is available.

**Peroxidases**

Enzymes that use a variety of electron donors to reduce \textit{H}_{2}\textit{O} to \textit{2H}_{2}\textit{O} are widespread. Thus, yeast contains a cytochrome \textit{c} peroxidase, plants contain ascorbate peroxidase as well as peroxidases acting on a variety of phenols and amines, and \textit{E. coli} makes an NADPH peroxidase which is called alkyl hydroperoxide reductase. The principal peroxidase in mammals is glutathione peroxidase (GSH Px). Of course, GSH is oxidized to the corresponding disulfide during its action, but glutathione reductase converts that back to GSH, using NADPH as the reductant. GSH Pxs are important not only for the elimination of \textit{H}_{2}\textit{O}, Its specificity encompasses alkyl hydroperoxides as well, and it reduces them to the corresponding alcohols. As was the case with the SODs and the catalase, there are several GSH Pxs, one of which is secreted and found in the extracellular space (Perry \textit{et al.}, 1992), and another acts specifically on phospholipid hydroperoxides (Ursini \textit{et al.} 1985). All have proved to be seleno-enzymes. GSH is present and abundant in most cells but is not found in trypanosomes which contain, in its place, diglutathionyl spermidine. This compound has been named trypanothione, and the organisms that contain it also produce a trypanothione peroxidase and a trypanothione reductase. The latter enzyme seems to be a pathogenicity factor in \textit{Leishmania} (Dumas \textit{et al.}, 1997).

**Purposeful \textit{O}^{2}\textsuperscript{−} production**

Phagocytic leukocytes, when stimulated, increase their rate of \textit{O}_{2} consumption 15- to 20-fold in what has been called the respiratory burst. This is due to the activation of a membrane-bound NADPH oxidase which reduces \textit{O}_{2} to \textit{O}_{2}\textsuperscript{−} (Curnutte \textit{et al.}, 1974). The respiratory burst is an important component of the armamentarium used by these leukocytes to kill invading microorganisms, and its genetic lack results in a hypersusceptibility to infection termed chronic granulomatous disease (Jendrossek \textit{et al.}, 1997). The \textit{O}^{2}\textsuperscript{−} produced during the respiratory burst is converted to \textit{H}_{2}\textit{O} by the dismutation reaction, and the \textit{H}_{2}\textit{O} is used to oxidize \textit{Cl}\textsuperscript{−} to hypochlorite under the catalytic influence of myeloperoxidase (Klebanoff, 1996). Hypochlorite is bacteriocidal and is, moreover, the precursor of \textit{N}-chlorotaurine, which is also an effective antibacterial compound.

**Defense in depth – the soxRS regulon**

Removal of \textit{O}^{2}\textsuperscript{−}, by the superoxide dismutases, and of \textit{H}_{2}\textit{O}, by the catalases and peroxidases, is important; but a well-rounded defense requires more. This is beautifully illustrated by the response of \textit{E. coli} to conditions that increase intracellular \textit{O}^{2}\textsuperscript{−} production. Under this stress, \textit{E. coli} activates the soxRS regulon, which is a family of approximately 12 coordinately regulated genes whose products provide the needed defenses. The SoxR protein is the sensor. It is a [2F–2S] protein that can exist in a reduced and an oxidized state. It is the oxidized state that transcriptionally activates the sox\textit{S} gene, and the SoxS protein in turn then activates all the other genes in the regulon (Gaudu and Weiss, 1996). What are the members of this regulon?

MnSOD is one and of course its role is to remove \textit{O}^{2}\textsuperscript{−}. Glucose-6-phosphate dehydrogenase is another and it serves to supply the NADPH needed by glutathione reductase and alkyl hydroperoxide reductase, among others. Fumarase C is part of this regulon and it serves as an \textit{O}^{2}\textsuperscript{−}-stable replacement for fumarases A and B, which are rapidly inactivated by \textit{O}^{2}\textsuperscript{−}. Aconitase A is controlled by SoxRS because the aconitases too are rapidly inactivated by \textit{O}^{2}\textsuperscript{−} (Grue and Guest, 1994), and more synthesis is needed to replace the activity lost to \textit{O}^{2}\textsuperscript{−}. Ferredoxin-flavodoxin reductase is a member of this regulon probably because reduced ferredoxin/flavodoxin function in the reductive reactivation of fumarases A and B, aconitases A and B and the other [4Fe–4S]-containing dehydratases that are inactivated by \textit{O}^{2}\textsuperscript{−}. Endonuclease IV is a member because it serves in the repair of oxidatively damaged DNA. This incomplete listing of the members of the SoxRS regulon gives some appreciation of what it takes to blunt the negative impacts of \textit{O}^{2}\textsuperscript{−}. A recent review provides more detail (Nunoshiba, 1996).

**Orchestrated defense against \textit{H}_{2}\textit{O}**

The stress imposed upon \textit{E. coli} by \textit{H}_{2}\textit{O}_{2} turns on a distinct group of genes under the control of oxy\textit{R}, which is transcriptionally active only in its oxidized state. The proteins whose production is positively regulated by Oxy\textit{R} include hydroperoxidase I, alkyl hydroperoxide reductase and glutathione reductase. There are at least half a dozen more that...
have yet to be identified. The OxyR regulon has been reviewed (Iuchi and Weiner, 1996). Although the proteins whose production is known to be regulated by OxyR make perfect sense in terms of defense against $\mathrm{H}_2\mathrm{O}_2$, it is surprising that the soxRS regulon is activated by $\mathrm{O}_2^-$ and the oxyR by $\mathrm{H}_2\mathrm{O}_2$. This is because one would think that a source of $\mathrm{O}_2^-$ would necessarily also be a source of $\mathrm{H}_2\mathrm{O}_2$ because of the dismutation reaction. One might therefore expect that a source of $\mathrm{O}_2^-$ should activate both soxRS and oxyR.

**Oxidative stress and pathology**

The multiplicity of the defenses that have arisen to deal with oxidative stress serves as an index of the gravity of this stress. Yet no defense can be perfect. This is specially the case in living cells, which must balance finite resources to meet diverse needs. In fact, we have just enough defense to allow reproductive success, which is what evolution requires. We are thus subject to low-level, but chronic, oxidative damage. It is therefore not surprising that oxidative damage contributes to ‘spontaneous’ mutation, to senescence and to numerous pathologies. We may anticipate great strides in moderating these types of damage, or at least in understanding their bases, now that the role of oxidative stress is coming to light.

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**References**


