The Two Faces of Oxygen
The byproducts of aerobic metabolism might promote aging—and normal cellular processes
Rabiya Tuma
(Published 3 October 2001)

Scientists have suspected for half a century that reactive oxygen species (ROS) are major instigators of aging. These byproducts of metabolism batter a wide variety of molecules within cells, and an organism’s ability to repair the damage declines with age. Now, some researchers say they’re wrapping up the case against ROS, at least for lower organisms. By countering this destruction with protective enzymes, researchers have extended the average lifetime of some invertebrates. But the verdict isn’t in yet, because recent studies have revealed that ROS also make key contributions to normal cell signaling.

Introduction
Every day, shiny new cars stream down the roadways. Their engines purr with the carefully timed explosions that convert chemical energy into mechanical force. But as time passes, each of these small, controlled bursts takes its toll on the engine, slowly etching away at the metal of the cylinders, pitting previously smooth surfaces, and causing leaks around the gaskets. Eventually the damage causes the engine to falter, first dropping its efficiency, then triggering its outright failure. Yet the internal bruising is accepted as the long-term cost for the short-term gain of moving the car down the road and is tolerated by even the best engineers.

Many living creatures make a similar compromise: The oxygen they need to survive damages their cells and over time perhaps causes their demise. Reactive oxygen species (ROS) can corrupt lipids, proteins, and nucleic acids. Although many of the marred molecules heal, some remain, and as an organism ages, the damage builds up.

In the mid-1950s, Denham Harman (1) proposed that this accumulated damage is not just a passive side effect of aging but rather a cause of it. Half a century later, researchers continue to grapple with the evidence for—and against—this theory.

Creating the Culprits
During metabolism, cells transform carbohydrates and fat into adenosine triphosphate (ATP), which they use to fuel essential biochemical reactions. The process begins in the cytoplasm, where the cell generates a small amount of ATP by converting sugars to pyruvate. It then continues in the mitochondria if oxygen is available. There, pyruvate from sugars and acetyl-CoA from fats are broken down into carbon dioxide, a waste product. The same reactions also produce hydrogen, which creates oodles of ATP—and ROS.

Four large enzyme machines compose the respiratory chain (also called the electron transport chain), which converts energy from the hydrogens in fats and sugars into ATP. These protein conglomerations—or complexes—lie within the inner membrane of the mitochondria; they use the energy released when electrons move from one molecule to another to pump hydrogen ions across the membrane. Another complex, sometimes called complex V or ATP synthase, taps the potential energy generated by this proton gradient to produce ATP.

In the respiratory chain, electrons pass from the strongest reducing agent to the strongest oxidizing agent (oxygen) in a series of steps. The complexes hand them off as if they were hot potatoes no one wanted to hold (Fig. 1). If all goes as planned, oxygen (O2) acquires four electrons and four hydrogen ions (H+) to form two molecules of water in the final step of the chain, in complex IV. If, however, oxygen encounters a molecule with an unpaired electron, it soaks it up. In this event, the product—an ROS—carries an unpaired electron (Fig. 2). Because electrons yearn to pair up, these intermediates readily react with other molecules.

\[
\begin{align*}
O_2 & \rightarrow 2e^- \rightarrow H_2O_2 \rightarrow H_2O + 2e^- \rightarrow OH^- + 2H^+ \rightarrow 2H_2O
\end{align*}
\]

Fig. 2: Single and looking. Oxygen reduction occurs one electron at a time, giving rise to unstable intermediates that can damage other cellular components.
The complexes that interact with ubiquinone, including complex I and complex III, generate the preponderance of ROS (2). In complex I, electrons move from NADH to ubiquinone, also called coenzyme Q. In a parallel step, coenzyme Q also harvests electrons from succinate in complex II. Regardless of how it’s generated, the reduced form of coenzyme Q tosses its electrons to complex III, which then hands them off to cytochrome c. Because coenzyme Q rides itself of its two electrons one at a time, it forms an unstable intermediate (+Q, called ubiquisemiquinone). Most of the time this intermediate hands off its remaining electron to complex III without drama, but if it comes into contact with oxygen instead, it inappropriately donates its single electron to oxygen—and thereby generates an ROS. To complete the chain, cytochrome c passes electrons to complex IV, where oxygen receives them to form water.

In addition to these metabolic sources of ROS, external stimuli and stresses such as ultraviolet light, ionizing radiation, and hyperthermia can also induce their production, although these sources generate smaller quantities.

The three most common ROS are superoxide anion (\(\text{O}_2^−\)), hydrogen peroxide (\(\text{H}_2\text{O}_2\)), and hydroxyl radical (\(\cdot\text{OH}\)). Scientists estimate that between 0.1% and 4% of the electrons transferred to oxygen in a cell result in the production of an ROS (3-7). At that rate, a single rat liver mitochondrion generates \(3 \times 10^7\) superoxide anions a day (8). In addition, superoxide can react with nitric oxide (NO), which is used in cell signaling and as a neurotransmitter. This reaction yields peroxynitrite (ONOO\(^−\)); like the other ROS, this molecule causes cellular mayhem by reacting promiscuously with whatever it encounters (lipids, proteins, DNA).

**Cellular Delinquents**

All tissues are vulnerable to oxidative damage, but because most ROS arise from metabolic processes, the more active tissues suffer the most. ROS crash around the cell, wrecking molecules in their path. They attack the polyunsaturated fatty acids of lipids, eventually decreasing their numbers enough to reduce membrane fluidity. The increased rigidity can curb the function of proteins and enzyme complexes in the membrane, including those in the respiratory chain.

Oxidation of proteins produces carbonyl groups by direct modification of the amino acid side chains or by peptide cleavage, and these carbonyl groups can alter a protein’s function. Complex I of the respiratory chain appears particularly susceptible to oxidation, says David Nicholls of the Buck Institute for Age Research in Novato, California. Because the complex already limits the rate of electron transport, damage to this molecular machine can reduce the amount of cellular ATP, potentially crippling the cell’s function.

ROS can also modify DNA bases or induce deletions in the DNA strands. During DNA replication and transcription, the cellular machinery can misread the modified bases, which propagates the error to the next generation of cells. Both nuclear and mitochondrial DNA accumulate oxidative damage, but mitochondrial DNA gets whacked harder. Less efficient repair mechanisms and proximity to the predominant sources of ROS in the cell might underlie this inequity (8). Regardless of the reason, once these organelles suffer a critical number of hits, they can’t function properly. And stressed mitochondria leak more ROS into the cytoplasm, which spreads the misery.

**Fighting Back**

To cope with this burden, evolution has provided a number of protective enzymes, including superoxide dismutases (SOD), catalase, and glutathione peroxidase. Higher organisms produce three types of SOD proteins, all of which rapidly convert superoxide anion into oxygen and hydrogen peroxide, a relatively stable ROS. The three species of SOD differ both in the type of metal found in their active sites and in their subcellular location. SOD2 (also called MnSOD) dwells in the mitochondria, where most of the free radicals are produced. SOD1 and SOD3 are CuZn proteins and inhabit the cytosol and extracellular space, respectively.

Catalase and glutathione peroxidase break down hydrogen peroxide—including that released by the SODs—into \(\text{O}_2\) and water. Although hydrogen peroxide is less reactive than superoxide, some researchers think it causes more damage, especially in neural tissue. Superoxide carries a negative charge, so it can’t traverse the mitochondrial membrane and remains stuck inside the organelle. But hydrogen peroxide can diffuse through membranes, it lives longer, and when it comes in contact with metal ions such as iron or copper, it gives rise to the most vexing ROS, hydroxyl radical. Together, these traits allow hydrogen peroxide to deliver trouble to a larger portion of the cell.

**Instigators or Bystanders?**

No one denies the correlation between oxidative damage and aging, nor the decline of organisms’ ability to eliminate the damage as they age. But is oxidative damage like rust on a car’s fender: an unfortunate but largely cosmetic consequence of aging? Or is it like the explosions of gas that slowly erode away at the engine, eventually causing the car’s—or organism’s—demise?

John Tower of the University of Southern California in Los Angeles points to four experiments for an answer. In three of them—including one from his lab—flies engineered to overproduce cytoplasmic SODs lived up to 48% longer than their normal counterparts (9-11). In the third, worms fed a novel antioxidant lived an average of 40% longer than those that did not receive the dietary supplement (12). These experiments are “a pretty direct demonstration, in my opinion, that oxidative stress is at least one cause of aging,” says Tower.

The evidence in mammals isn’t so convincing, though—at least not yet. In the most persuasive experiment, researchers documented an acceleration in the rate of oxidative damage and mitochondrial decline in mice that carry only one functional copy of SOD2 (13). This result supports the notion that ROS induces aging, they conclude.

Although Toren Finkel of the National Heart, Lung, and Blood Institute in Bethesda, Maryland, does not quibble with this particular result, he thinks such experiments can oversimplify the situation. ROS concentrations are controlled by an elaborate system in which SOD and catalase rely on one another, and manufacturing extra SOD might not lower ROS concentrations in a predictable way. “I am not so sure that a simple overexpression or knockout experiment is as revealing as you would hope it would be,” he says. His lab has overproduced SOD in tissue culture cells, with variable effects. “When we directly look at the amounts of oxidants released, it is not that clear that [the amount] relates that much to how much we’ve overexpressed SOD.”
Vulnerable Neurons

Of all tissues, the link between oxidative damage and aging in the brain is most firmly cemented. That organ seems to suffer from oxidative damage more than other tissues do, and lesions in neurons may provoke many diseases associated with aging, including neurodegenerative diseases such as Parkinson's (see Andersen Review), Alzheimer's (see “Detangling Alzheimer's Disease”), and CBGD (see Scarmeas case study); Huntington's; amyotrophic lateral sclerosis; and stroke. Researchers suggest that at least three major factors contribute to the hypersensitivity of neurons.

First, the brain produces a disproportionate amount of ROS because it is the most metabolically active tissue in the body. It consumes 20% of an organism’s oxygen but represents only 2% of body mass.

The brain also sustains excess ROS damage because it contains a lot of metals, cofactors required for converting hydrogen peroxide into hydroxyl radicals. Ashley Bush, an oxidation biologist at Massachusetts General Hospital in Boston, speculates that these high metal concentrations facilitate some sort of sophisticated oxygen chemistry crucial for the brain’s function. “But there is a price to pay,” says Bush. “It is sort of like having a high-octane engine: It gets you better performance, but there is greater risk of damage to the cylinders.” In addition to holding these metals, the brain also harbors a class of neurotransmitters that reduce them, the catecholamines. This reduction reaction prepares the metals to react with hydrogen peroxide.

Finally, not only do nerve cells get pummeled by ROS, but they also can’t tolerate the drops in energy stores that result when their mitochondria suffer abuse, says Nicholls. Neurons use most of their energy to maintain a large difference in ion concentrations between the cytoplasm and the extracellular space, which they need to transmit signals. But if energy stores dwindle—such as when complex I of the respiratory chain suffers oxidative damage—the mitochondria end up with excess ions, which compromise their function and induce more ROS production; the ROS in turn seep into the cytoplasm and cause more damage.

Substantial evidence suggests that the mitochondria of older animals display less complex I activity than do those of younger ones. “Whether [the drop in activity] is [a] cause or effect [of aging], you can’t tell at this stage,” says Nicholls. The resulting drop in production of usable energy makes neurons more susceptible to catastrophic damage from small, disruptive events as well as larger ones such as a stroke, Nicholls adds.

In some cases, the link between neurodegenerative diseases and oxidative damage has been established. For example, numerous lines of evidence implicate oxidative damage in Parkinson’s disease.

Patients regularly display a decrease in complex I function. Significantly, compounds that inhibit the activity of complex I in the class of neurons facilitate Parkinson’s disease induce similar symptoms in primates, and excess SOD blocks that effect (14-16). Also, a decrease in glutathione peroxidase activity signals the beginning of disease, notes Julie Andersen of the Buck Institute, although it is not yet clear whether this drop in enzyme activity causes the pathology or results from it.

Bush, who works primarily on Alzheimer’s disease, says that a number of neurodegenerative diseases share an underlying mechanism that involves proteins that can oxidize or reduce other cellular components, including oxygen. For example, the β amyloid protein in Alzheimer’s disease catalyses the production of hydrogen peroxide, according to studies from his lab (17). His group has even found that small-molecule metal chelators, which bind free metals, might fight the disease (18). Similarly, Bush points out, hydrogen peroxide levels are high in familial amyotrophic lateral sclerosis patients, and a point mutation in the SOD1 gene is associated with 25% of the familial cases (19). Extrapolating from these data, he suggests that hydrogen peroxide production triggers a number of neurodegenerative diseases. Because the aberrant protein responsible for each disease is different, as is its location, the symptoms are unique. “But the attack at the microscopic level in both [diseases] is caused by hydrogen peroxide,” says Bush (20).

A Necessary Evil

Ever since Harman proposed that free radicals, particularly ROS, perpetrate aging, scientists have considered them nothing more than a noxious byproduct of metabolism. But recently, several researchers have discovered a twist: Under some circumstances, ROS function as signaling molecules required for normal cellular communications (21-23).

Although the full role ROS play in signaling remains obscure, it is clear that they activate the stress response (24, 25), which causes mitochondria to release oxidants. This process may alert the rest of the cell to trouble. For example, stress may condition the cell to resist apoptosis, the mechanism by which cells commit suicide when they sustain irreparable amounts of damage or experience other catastrophic events. Paradoxically, oxidative damage can also induce apoptosis (reviewed in 24, 25) (Fig. 3). Given these conflicting responses, it’s not yet clear how ROS signals affect a cell’s life-and-death choices.

“My prejudice is—and this is, well, if not really heretical, certainly unproven—that maybe the mitochondria talk to the

**Fig. 3: Delaying death.** Caenorhabditis elegans that were fed a synthetic compound (EUK-134) that inactivates both superoxide anion and hydrogen peroxide lived longer than worms that were not given the dietary supplement. The effect on life-span depended on concentration of the antioxidant, with the worms that consumed larger amounts of the antioxidant living longer than those that ate less of it.
cell by the release of oxidants and maybe that is important in aging,” says Finkel. Unlike most scientists, who think oxidative damage occurs randomly, accumulates, and eventually overwhelms the system, Finkel speculates that aging might result from targeted hits in particular pathways, although he doesn’t know which ones are most important. “I would argue that metabolism leading to oxidant generation should not be just thought of as a random, damaging, New Jersey-type of cesspool accumulation in the cell,” he says.

From that angle, it makes sense that Finkel is wary of the blunt-instrument approach of overexpression studies and wide-scale pharmacological use of antioxidants. In addition to reducing the amount of ROS, extra SOD or synthetic antioxidants could foul up normal cellular pathways and cause as yet unpredictable defects. Several studies have already shown that excess ROS-scavenging proteins can inhibit particular signaling pathways (26, 27). However, Tower says that he didn’t see any gross defects in the long-lived flies that overexpress SOD. Similarly, Buck Institute researchers Simon Melov and Gordon Lithgow didn’t notice any gross malfunctions in the antioxidant-fed worms, although all of the researchers caution that they didn’t look hard for such effects.

Given the newfound role of ROS in signaling, many researchers agree that the cell must perform a balancing act: Too many ROS spur cellular damage, but too few decrease critical signaling capacity. Bush says the perfect example of this seesaw is the extracellular SOD story. Mice that lack SOD3 show a loss of short-term memory function, but so do those that overproduce the enzyme (28). Too much is just as bad as too little.

**Caloric Connections**

Early in the last century, researchers realized that the longevity of several species was inversely proportional to their energy expenditure and proposed the rate-of-living hypothesis (29). At the time, scientists speculated that an organism had a limited supply of some substance at birth; when depleted, the animal died. The more slowly the organism consumed the substance, the longer it would live. A more modern version of the hypothesis proposes that cells have a fixed quantity of energy that they can produce or expend during their lifetime; once they reach that magic amount, they expire. In this scenario, ROS would serve as a critical link between metabolic rate and longevity: The higher an animal’s energy expenditure per unit mass, the more ROS it will produce, and the shorter its life-span will be.

Critics of this hypothesis point out that the simplicity of the inverse relation between energy expenditure and life-span just doesn’t hold up. Numerous species—such as some long-lived birds—boast both long life-spans and high energy expenditure per unit mass (reviewed in 30). Also, when scientists experimentally increase a mammal’s metabolic rate, they don’t see a corresponding decrease in life-span. Nor do mutations that extend life-span always come with a concomitant decrease in metabolic rate.

For these reasons and others, Roger McCarver of the University of Texas Health Science Center in San Antonio says, “it is a wonderful idea. Unfortunately, it needs to be relinquished at this stage. The evidence against it [for warm-blooded creatures] is simply overwhelming.”

The rate-of-living hypothesis owes its persistence in part to the observation that significantly reducing an animal’s food intake increases its life-span—a fact that at least superficially seems to be consistent with the idea that metabolic activity is inversely proportional to life-span. This effect of calorie restriction holds true for a wide variety of organisms, including protozoans, Caenorhabditis elegans, Drosophila, rodents, dogs, and probably even monkeys. Rodents can be restricted to about 40% of the ad libitum, or unrestricted, food intake without harm as long as their micronutrient needs are met; under these conditions, the mean life-span extends by 40% to 50%.

Although no one debates the fact that calorie restriction can prolong an animal’s life, the mechanism is still contentious. Most studies do not support the idea that the increased longevity observed in calorically restricted animals is simply a matter of decreased metabolic rate. For example, mitochondria isolated from calorie-restricted and control animals show similar metabolic rates in vitro. “There is something more subtle going on,” says Brian Merry, a biologist at the University of Liverpool in the United Kingdom, who studies calorie restriction.

Calorie-restricted animals seem to have a higher tolerance for many different types of stress than control animals, for example. But Merry thinks the primary reason aging is delayed in these creatures is a change in the rate of ROS production per unit oxygen consumed (31, 32). Not only do the animals produce less ROS overall because they are eating less, but also a smaller percentage of the oxygen they consume is converted into ROS.

His group peered inside mitochondria to collect clues about why calorie restriction slows ROS production. He speculates that changes in the membrane lipid composition may explain, at least in part, why calorie-restricted animals experience less oxidative damage. The membranes of calorie-restricted rodents “tend to lose double bonds,” he says, and because ROS attack the double bonds of lipids, “this [change] makes them more resistant to ROS damage.” Because injured mitochondria leak more ROS, this protection might translate into less seepage in the future, thereby providing a double layer of insulation from ROS-induced damage—resistance and prevention.

The altered membranes of the calorie-restricted rodents resemble the membranes of some naturally long-lived animals, including some whose life history contradicts the rate-of-living hypothesis. Some birds, for example, have an unusually low number of double bonds in their membranes.

In an open-ended search for clues to the life-span extension benefits of calorie reduction, Richard Weindruch of the University of Wisconsin, Madison, is using complementary DNA microarrays to measure changes in expression levels of a large number of genes over time (33-35). Approximately 70% to 80% of the messenger RNA transcripts that change with age in the controls are stable in the food-restricted animals. The calorie-restricted rodents also show some gene expression pat-
ters that suggest a “youthful” physiology, such as large amounts of protein turnover machinery and small amounts of cellular repair proteins, compared with age-matched controls. These data suggest, says Weindruch, that “caloric restriction provides opportunities [for researchers] to intervene in the process of aging” by identifying individual proteins that might control it. Once the researchers pinpoint genes of interest, they can use genetic manipulations to test those genes’ roles in the aging process. By learning more about altered events in these animals, scientists might eventually find more palatable ways to mimic the effect for humans than steering clear of hot fudge sundaes, he suggests.

Future Directions

A number of researchers say they expect the area of drug design and therapeutics in the field of oxidation biology to flourish in the next several years, and the field has already begun to bloom. The antioxidant compounds that extend the life-span of C. elegans represent a new class of compounds. These drugs are SOD/catalase mimetics and work like enzymes, unlike traditional antioxidant dietary supplements, which react only once. In addition, these compounds, which possess both catalase and SOD activity in vitro, are smaller and can diffuse across membranes—something traditional dietary antioxidants such as vitamin C can’t do. They might even gain entrance to the mitochondria. Hence, a single drug molecule may inactivate numerous ROS molecules before they have a chance to exit the mitochondria.

Melov, who led the C. elegans work, and Bush both suggest that it might be possible to develop similar compounds into disease therapies. Such compounds, they predict, would generally protect all cells and might provide the positive side effect of slowing the aging process.

Not everyone, however, thinks that development of therapeutics will be so straightforward. “Tuck” C. Finch of the University of Southern California and others express concern that such antioxidant compounds could interfere with important processes, including the newfound signaling activities.

Finch and several other researchers say that comparative biology is going to become more and more important. For example, Finch thinks that looking at very long-lived species such as whales or comparing short- and long-lived bird species might enable scientists to establish why some organisms can cope with oxidative stress whereas others can’t.

Many researchers emphasize the need to fill the large gaps in their understanding of ROS and signaling. For the most part, the work in this area has involved testing one pathway at a time for ROS involvement. But this strategy requires scientists to identify candidate processes in advance. To circumvent this problem, several scientists propose using unbiased methods, such as microarrays of genes and proteins.

“If you think ROS act nonspecifically” to promote aging by randomly battering cells, “then you are home free,” says Finkel, because researchers generally understand this process. But if, on the other hand, ROS activate particular, programmed pathways to induce aging, then “the burden is on you to figure out what the targets are,” he says. “I think what the field needs are methodologies to understand the specific targets” of ROS.

Although the connections between oxygen and aging remain to be proven, it is obvious that a compromise must be struck: Like the car and its internal combustion engine, organisms use oxygen for metabolism and risk damage from its byproducts. Yet recent investigations paint a more complex picture for aerobic creatures than what occurs in the engine, where the wear and tear of repeated explosions is unproductive. What was seen previously as a hazardous but non-negotiable trade-off for energy now appears to be a precarious balancing act in which an organism needs enough ROS to maintain normal signaling, but too much harms essential components of the cell.

Rabiya Tuma writes about science from Manhattan. She refuses to hold her breath.

Glossary

**Amyotrophic lateral sclerosis** A degenerative disease that causes the progressive loss of motor neurons and results in muscle atrophy; also called ALS or Lou Gehrig’s disease. Less than 25% of ALS cases are associated with a dominant mutation in the SOD1 gene; the mutant protein seems to take on an abnormal function and perhaps generates reactive oxygen species.

**β-amyloid** A protein fragment lopped off of the β-amyloid precursor protein; aggregates of it are associated with Alzheimer’s disease.

**Catecholamine** A class of neurotransmitters, including dopamine, that are strong reducing agents.

**Catalase** Cytosolic protein responsible for converting hydrogen peroxide to water and oxygen.

**Complex I** The first enzyme complex in the electron transport chain; converts NADH to NAD while transferring electrons to ubiquinone.

**Complex II** The smallest complex of the electron transport chain and the only one that doesn’t transport protons across the membrane; transfers electrons from succinate to ubiquinone.

**Complex III** The major site of reactive oxygen species production in the respiratory chain. Also called ubiquinone-cytochrome c reductase.

**Complex IV** Executes the final electron transport step in the respiratory chain, in which oxygen accepts electrons.

**Complex V** Also referred to as adenosine triphosphate (ATP) synthase. This complex executes the last step in the respiratory chain; uses the potential energy to generate ATP from adenosine diphosphate (ADP) and inorganic phosphate.

**Cytochrome c** One of a group of closely related metalloproteins called cytochromes that contain a heme group in their reaction center. The iron in this heme center can cycle between a ferric (Fe³⁺) and a ferrous (Fe²⁺) state as it donates or accepts electrons from neighboring electron carriers in the respiratory chain. Acts as an electron acceptor and donor in the respiratory chain.

**Glutathione peroxidase** A mitochondrial enzyme that converts hydrogen peroxide into water. A decrease in the amount of glutathione peroxidase in the substantia nigra region of the brain is one of the first signs of Parkinson’s disease.

**Hydrogen peroxide** A relatively long-lived reactive oxygen species (ROS) produced as a byproduct when superoxide dismutase enzymes convert superoxide to oxygen; also called H₂O₂. This radical is unusually destructive, perhaps because it can diffuse through membranes; furthermore, if it reacts with a metal catalyst, it gives rise to hydroxyl radical—the most reactive of all the ROS.

**Hydroxyl radical** The most reactive of all the reactive oxygen species; also called •OH. Formed when transition metals such as iron and copper catalyze the breakdown of hydrogen peroxide into hydroxyl radical and hydroxide ion (OH⁻); this reaction is referred to as the Fenton reaction.

**Nitric oxide** A small molecule used both as a neurotransmitter and as a signal molecule; also called NO. Under some circumstances, it can react with superoxide to yield peroxynitrite.

**Peroxynitrite** A highly reactive oxidizing agent that forms when nitric oxide reacts with superoxide; also called ONOO⁻.

**Reactive oxygen species** Unstable chemical intermediates that carry unpaired electrons formed during the reduction of molecular oxygen (O₂); abbreviated ROS. The garden-variety ROS are superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (•OH).

**Respiratory chain** Also called the electron transport chain. A collection of mitochondrial protein complexes that constitutes the main site of adenosine triphosphate (ATP) and reactive oxygen species production in the cell. It is executed by three large complexes (I, III, and IV) and two small ones (II and V) that lie within the inner membrane of the mitochondria. passes electrons from strongly reducing molecules to strongly oxidizing ones in complexes I to IV and uses the energy produced by this process to generate a proton gradient across the inner membrane of the mitochondria; this poten-
tional energy is then used to synthesize ATP in complex V, also called ATP synthase.

**SOD1** A superoxide dismutase expressed in the cytoplasm that contains copper and zinc in its reaction center. One of three superoxide dismutase enzymes in higher animals.

**SOD2** Also referred to as MnSOD. A superoxide dismutase that is expressed in the extracellular space that contains copper and zinc. One of three superoxide dismutase enzymes in higher animals.

**Superoxide anion** A reactive oxygen species produced predominantly by complex III and, to a lesser extent, by complex I of the respiratory chain in the mitochondria; also called O$_2^-$.

**SOD2** A superoxide dismutase expressed in the extracellular space that contains copper and zinc. One of three superoxide dismutase enzymes in higher animals.

**Ubiquinone** Also known as coenzyme Q. A small, hydrophobic molecule that floats in the inner membrane of the mitochondria and functions as an electron carrier in the respiratory chain. Can accept either one or two electrons at a time, picking up an equal number of protons to maintain its uncharged state.

**Further Reading**


**References**


