$$4\,\text{Cyt}\,c_{red} + 8\,H^+_{matrix} + O_2 \longrightarrow 4\,\text{Cyt}\,c_{ox} + 2\,H_2O + 4\,H^+_{cytosol}$$

As discussed earlier, molecular oxygen is an ideal terminal electron acceptor, because its high affinity for electrons provides a large thermodynamic driving force. However, *danger lurks in the reduction of* O_2. The transfer of four electrons leads to safe products (two molecules of H_2O), but partial reduction generates hazardous compounds. In particular, *the transfer of a single electron to* O_2 *forms superoxide anion, whereas the transfer of two electrons yields peroxide.*

$$O_2 \xrightarrow{e^-} O_2^{\cdot-} \xrightarrow{e^-} O_2^{2-}$$

Superoxide anion — Peroxide

These compounds and, particularly, their reaction products can be quite harmful to a variety of cell components. The strategy for the safe reduction of O_2 is clear from the discussion of the reaction cycle: *the catalyst does not release partly reduced intermediates.* Cytochrome *c* oxidase meets this crucial criterion by holding O_2 tightly between Fe and Cu ions.

18.3.6. Toxic Derivatives of Molecular Oxygen Such as Superoxide Radical Are Scavenged by Protective Enzymes

Although cytochrome *c* oxidase and other proteins that reduce O_2 are remarkably successful in not releasing intermediates, small amounts of superoxide anion and hydrogen peroxide are unavoidably formed. Superoxide, hydrogen peroxide, and species that can be generated from them such as OH· are collectively referred to as *reactive oxygen species* or *ROS.*

What are the cellular defense strategies against oxidative damage by ROS? Chief among them is the enzyme *superoxide dismutase.* This enzyme scavenges superoxide radicals by catalyzing the conversion of two of these radicals into hydrogen peroxide and molecular oxygen.

$$2\,O_2^{\cdot-} + 2\,H^+ \xrightleftharpoons{\text{Superoxide dismutase}} O_2 + H_2O_2$$

Dismutation

A reaction in which a single reactant is converted into two different products.

Eukaryotes contain two forms of this enzyme, a manganese-containing version located in mitochondria and a copper-zinc-dependent cytosolic form. These enzymes perform the dismutation reaction by a similar mechanism (Figure 18.22). The oxidized form of the enzyme is reduced by superoxide to form oxygen. The reduced form of the enzyme, formed in this reaction, then reacts with a second superoxide ion to form peroxide, which takes up two protons along the reaction path to yield hydrogen peroxide.

The hydrogen peroxide formed by superoxide dismutase and by other processes is scavenged by *catalase,* a ubiquitous heme protein that catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen.

$$2\,H_2O_2 \xrightleftharpoons{\text{Catalase}} O_2 + 2\,H_2O$$

Superoxide dismutase and catalase are remarkably efficient, performing their reactions at or near the diffusion-limited rate (Section 8.4.2). Other cellular defenses against oxidative damage include the antioxidant vitamins, vitamins E and C. Because it is lipophilic, vitamin E is especially useful in protecting membranes from lipid peroxidation.

The importance of the cell's defense against ROS is demonstrated by the presence of superoxide dismutase in all aerobic organisms. *Escherichia coli* mutants lacking this enzyme are highly vulnerable to oxidative damage. Moreover, oxidative damage is believed to cause, at least in part, a growing number of diseases (Table 18.3).

18.3.7. The Conformation of Cytochrome *c* Has Remained Essentially Constant for More Than a Billion Years

Cytochrome c is present in all organisms having mitochondrial respiratory chains: plants, animals, and eukaryotic microorganisms. This electron carrier evolved more than 1.5 billion years ago, before the divergence of plants and animals. Its function has been conserved throughout this period, as evidenced by the fact that *the cytochrome* c *of any eukaryotic species reacts in vitro with the cytochrome* c *oxidase of any other species tested thus far.* Finally, some prokaryotic cytochromes, such as cytochrome *c2* from a photosynthetic bacterium and cytochrome *c* 550 from a denitrifying bacterium, closely resemble cytochrome *c* from tuna heart mitochondria (Figure 18.23). This evidence attests that the structural and functional characteristics of cytochrome *c* present an efficient evolutionary solution to electron transfer.

The resemblance among cytochrome *c* molecules extends to the level of amino acid sequence. Because of the molecule's relatively small size and ubiquity, the amino acid sequences of cytochrome *c* from more than 80 widely ranging eukaryotic species were determined by direct protein sequencing by Emil Smith, Emanuel Margoliash, and others. Comparison of these sequences revealed that *26 of 104 residues have been invariant for more than one and a half billion years of evolution.* A phylogenetic tree, constructed from the amino acid sequences of cytochrome *c*, reveals the evolutionary relationships between many animal species (Figure 18.24).