6.5: Energy Storage and Release

Electron-transfer reactions play key roles in a great many biological processes, including collagen synthesis, steroid metabolism, the immune response, drug activation, neurotransmitter metabolism, nitrogen fixation, respiration, and photosynthesis. The latter two processes are of fundamental significance-they provide most of the energy that is required for the maintenance of life. From the point of view of global bioenergetics, aerobic respiration and photosynthesis are complementary processes (Figure 6.10). The oxygen that is evolved by photosynthetic organisms is consumed by aerobic microbes and animals. Similarly, the end products of aerobic respiratory metabolism (CO_2 and H_2O) are the major nutritional requirements of photosynthetic organisms. The global C, H, and O cycles are thus largely due to aerobic respiration and photosynthesis.

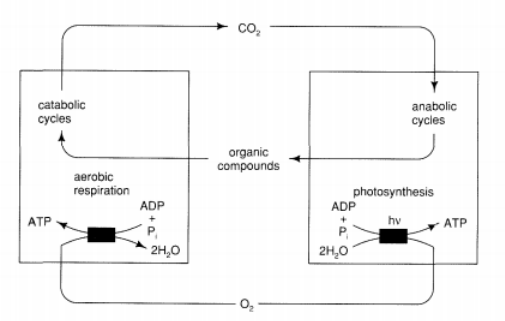

Figure 6.10 - Aerobic respiration and photosynthesis.

The extraction of energy from organic compounds, carried out by several catabolic pathways (e.g., the citric-acid cycle), involves the oxidation of these compounds to CO_2 and H_2O with the concomitant production of water-soluble reductants (NADH and succinate). These reductants donate electrons to components of the mitochondrial electron-transfer chain, resulting in the reduction of oxygen to water:

$$\frac{1}{2}O_2 + NADH + H^+ \rightarrow H_2O + NAD^+ \tag{6.5}$$

In aerobic organisms, the terminal oxidant is, of course, oxygen. However, some species of bacteria respire anaerobically and are able to use inorganic oxyanions (nitrate or sulfate) as terminal oxidants. The translocation of protons across the inner mitochondrial membrane accompanies the electron transfers that ultimately lead to the reduction of O_2; these protons, in turn, activate ATP synthase, which catalyzes the phosphorylation of ADP to ATP (a process known as oxidative phosphorylation). Because the hydrolysis of ATP is very exoergonic (i.e., $\Delta G < 0$), the newly synthesized ATP is used as a molecular energy source to drive thermodynamically unfavorable reactions to completion.

The rediscovery of cytochromes by Keilin[25] in 1925 led him to propose that the reduction of O_2 is linked to the oxidation of reduced substrates by a series of redox reactions, carried out by cellular components collectively referred to as the respiratory electron-transport chain. Progress toward a molecular understanding of these redox reactions has been painfully slow. Most of the components are multisubunit proteins that reside in the inner mitochondrial membrane (Figure 6.11). These proteins (Complexes I-IV) are quite difficult to purify with retention of *in vivo* properties, and they do not crystallize well.

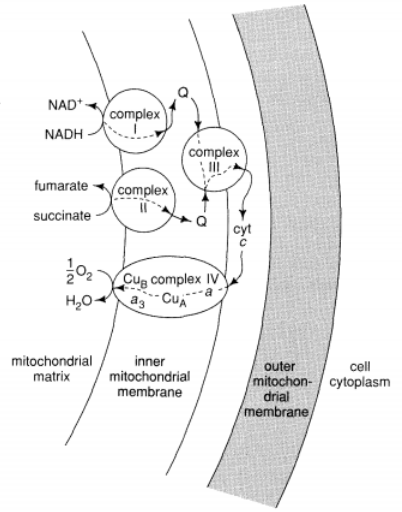

Figure 6.11 - Redox components in mitochondria.

The components[26-28] of the respiratory chain contain a variety of redox cofactors. Complex I (NADH-Q reductase; > 600 kDa) contains five iron-sulfur clusters and FMN. Complex II (succinate-Q reductase; 150 kDa) contains several iron-sulfur clusters, FAD (flavin adenine dinucleotide), and cytochrome b_{568}. Complex III (ubiquinol-cytochrome C reductase; 250 kDa) contains a [2Fe-2S] iron-sulfur center and cytochromes b_{562}, b_{566}, and c_1. Complex IV (cytochrome C oxidase; 200 kDa) contains at least two copper ions and cytochromes a and a_3; Q denotes coenzyme Q, which may be bound to hydrophobic subunits of Complexes I, II, and/or III *in vivo*. Cytochrome c (cyt c in Figure 6.11) is a water-soluble protein (12.4 kDa) that is only peripherally associated with the inner mitochondrial membrane; it has been so thoroughly studied that it is generally regarded as the prime example of an electron transferase.

More than 20 redox centers are involved in the electron-transport chain. Figure 6.12 depicts a simplified view of the flow of electrons from NADH to O_2 via this series of electron carriers. Electron flow through Complexes I, III, and IV is associated with the release of relatively large amounts of energy, which is coupled to proton translocation by these complexes (and therefore ATP production). The redox potentials of the electron carriers thus appear to playa role in determining the pathway of electron flow through the electron-transport chain.

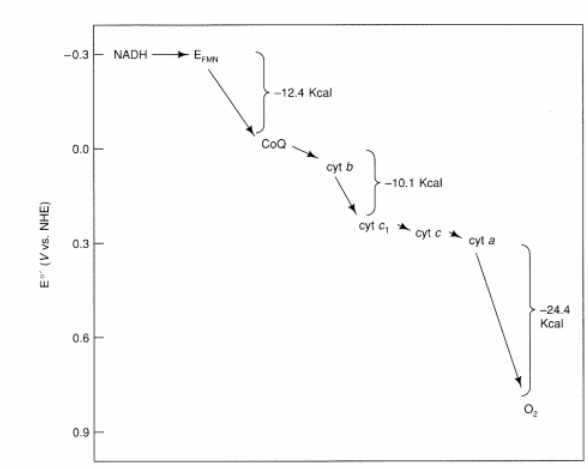

Figure 6.12 - Electron flow from NADH to O_2 in the mitochondrial electron-transport chain.

Approximately 50 percent of the surface area of the inner mitochondrial membrane is lipid bilayer that is unoccupied by membrane proteins and through which these proteins, in principle, are free to diffuse laterally. Kinetic (laser photobleaching and fluorescence recovery) and ultrastructural (freeze-fracture electron microscopy) studies[29,30] indicate that Complexes I-IV diffuse independently and laterally over the inner membrane, whereas cytochrome c diffuses in three dimensions (i.e., through the intramembrane space). Respiratory electron transport has been shown to be a diffusion-coupled kinetic process.[29,30] The term "electron-transport chain" is

thus somewhat misleading, because it implies a degree of structural order that does not exist beyond the level of a given protein complex.

In view of these observations, why are all of the electron transfers associated with mitochondrial respiration required? For example, why is cytochrome c needed to shuttle electrons in Figures 6.11 and 6.12 when the cofactor reduction potentials of Complex III are more negative than those of Complex IV? Evidently, factors other than $\Delta G°$ are of importance—these will be discussed in Sections III and IV.

Photosynthesis could be viewed as the most fundamental bioenergetic process. Biological reactions are driven by an energy flux, with sunlight serving as the energy source. Photosynthesis[31-36] is the process by which radiant solar energy is converted into chemical energy in the form of ATP and NADPH, which are then used in a series of enzymatic reactions to convert CO_2 into organic compounds. The photosynthetic algae that appeared on Earth two million years ago released oxygen into the atmosphere and changed the environment from a reducing to an oxidizing one, setting the stage for the appearance of aerobically respiring organisms.

Photosynthesis is initiated by the capture of solar energy, usually referred to as "light harvesting." A large number of organic pigments, including chlorophylls, carotenoids, phycoerythrin, and phycocyanin (in green plants and algae) are clustered together in pigment-protein complexes called photosystems. These pigments collectively absorb most of the sunlight reaching the Earth—their absorption spectra are displayed in Figure 6.13.

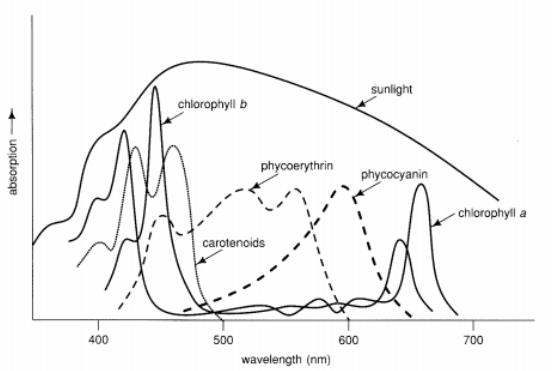

Figure 6.13 - Absorption spectra of the photosystem pigments.

Light is transformed into chemical energy in pigment-protein complexes called reaction centers. The concentration of reaction centers within a photosynthetic cell is too small to offer a suitable absorption cross section for sunlight. Hence, hundreds of these lightharvesting pigments function as molecular antennas; an x-ray structure[35] of one subunit of a bacteriochlorophyll-protein complex is displayed in Figure 6.14.

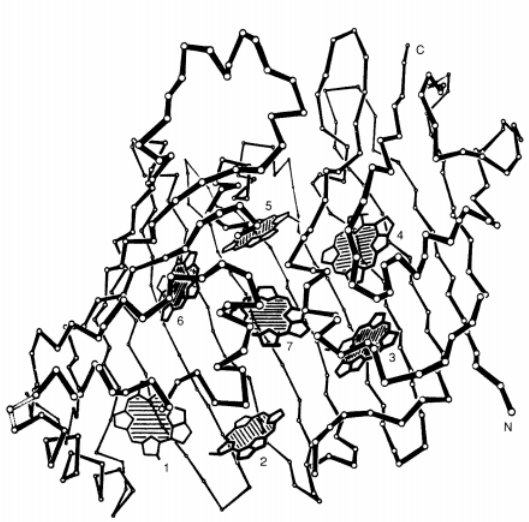

Figure 6.14 - Structure of a subunit of a bacteriochlorophyll-protein complex. Reproduced with permission from Reference 35.

Absorption of a photon by an antenna pigment promotes the pigment into an electronically excited state, which can return to the ground state by a variety of relaxation processes, including fluorescence or resonance transfer of excitation energy to a nearby pigment at picosecond rates. As much as 100 ps may elapse between the photon absorption and the arrival of the light energy at a reaction center. During this time, the energy may "migrate" in a random-walk fashion among hundreds of pigments.

The energy of the excited state is converted into electrochemical potential energy at the reaction center, which contains a primary electron donor P that transfers an electron to a nearby acceptor Al within the same protein (and P becomes oxidized to P^+):

$$P A_1 A_2 A_3 \cdots \xrightarrow{h\nu} P^* A_1 A_2 A_3 \cdots \rightarrow P^+ A_1^- A_2 A_3 \cdots \tag{6.5}$$

This *charge separation* is of paramount importance. The key problem is maintaining the charge separation, which involves minimization of the energy-wasting back reaction. Reaction centers contain an ordered array of secondary electron acceptors (A_1, A_2, A3•••) that optimize the $\Delta G°$ that occurs at each step:

$$P^+ A_1^- A_2 A_3 \cdots \rightarrow P^+ A_1 A_2^- A_3 \cdots \rightarrow P^+ A_1 A_2 A_3^- \cdots \tag{6.6}$$

Thus, the back reaction is circumvented by optimizing forward electron transfers that rapidly remove electrons from A_1^-. As the acceptors are separated by greater and greater distances from P^+, the probability of the back electron transfer to P^+ decreases. Put another way, the overlap of P^+ and each acceptor orbital decreases in the order $P^+/A_1^- > P^+/A_2^- > P^+/A_3^-$.

Photosynthetic bacteria contain only one type of reaction center (l00 kDa). The solution of the x-ray structure (at 2.9 Å resolution) of the *Rps. viridis* reaction center was reported[36] in 1984, providing conclusive proof that electrons can "tunnel" over 10-20 Å distances through protein interiors. The reaction-center protein contains many cofactors (Figure 6.15): two bacteriochlorophylls (BChl) in close proximity (the so-called "special pair"), two further bacteriochlorophylls that are spectroscopically identical, two bacteriopheophytins (BPh), two quinones (Q_A and Q_B), and one iron center. (Q_B was lost during isolation of the *Rps. viridis* reaction center and thus does not appear in Figure 6.15.) The reaction center contains an approximate two-fold rotation axis. Despite this strikingly high symmetry in the reaction center, one pathway of electron flow predominates, as the cartoon in Figure 6.16 indicates.

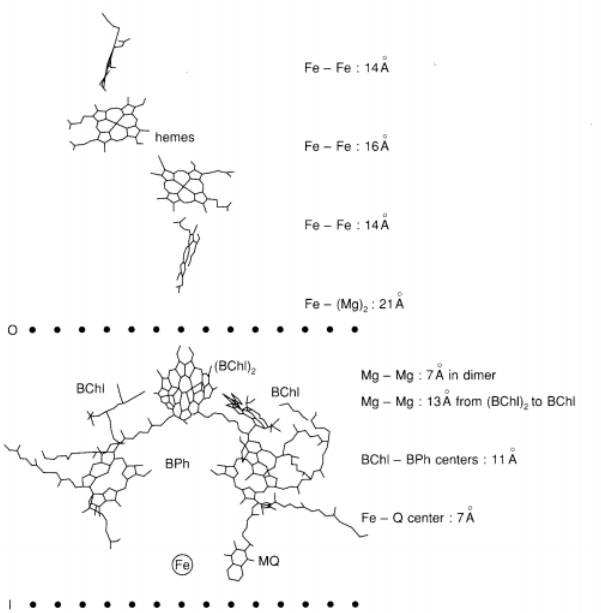

Figure 6.15 - Structure of the Rps. viridis photosynthetic reaction-center cofactors. The black dots delineate the outward (O)- and inward (I)-facing portions of the membrane. Adapted from Reference 36.

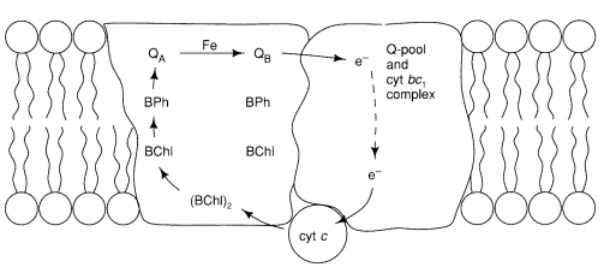

Figure 6.16 - Electron flow in the bacterial photosynthetic reaction center.