CHAPTER 1

Photosynthesis: The Light Reaction

CHAPTER OUTLINE

1.1 **Overview**

1.2 **The Hill Reaction**

1.3 **Photosynthetic Pigments**
- Chlorophyll *a*
- Accessory Pigments

1.4 **Origin of the Z-Scheme**
- Emerson Enhancement
- Photosystems I and II

1.5 **Light Reaction: Details**
- The Reaction Center of Purple Bacteria: A Model for PSII
- Structure of the Bacterial Reaction Center
- Light Energy Is Used To Reduce a Quinone

1.6 **Photosystem II**
- Electron Transfer
- Oxidation of Water

1.7 **Photosystem I**

1.8 **Cytochrome b_6f Complex**

1.9 **Photophosphorylation (ATP Synthesis)**

1.10 **Light-Harvesting Complexes**
- Light Energy
- Light Harvesting
- State Transitions

1.11 **Nonphotochemical Quenching**

1.12 **Photosynthesis Inhibitors: Herbicides**

1.1 Overview

Life on Earth runs on solar energy. The biological solar age started 3.5 to 3.0 billion years before the present when ancient microorganisms first evolved the ability to convert light energy from the sun into chemical bond energy of organic molecules. These ancient microorganisms lived in an anoxic world and used sunlight to produce adenosine triphosphate (ATP). Using this photochemically derived energy, they were thus able to take advantage of the various reduced compounds, such as H_2S, on the primitive Earth and subsequently reduce atmospheric CO_2 to sugars. About 0.5 billion years later, cyanobacteria developed a slightly different process, replacing reduced sulfur and organic compounds with H_2O as their reducing agent and releasing oxygen into the primitive atmosphere. The oxygen released from this oxygenic photosynthesis slowly built up in the Earth's atmosphere, reaching a partial pressure of 1 kPa approximately 1.5 billion years ago. At this time the oxygen concentration was sufficient for formation of ozone (O_3) in the stratosphere that screened out the more energetic solar radiation and made it possible for the first organisms to colonize the land. Today, oxygenic photosynthesis is the most common biochemical reaction on our planet, both in aquatic and terrestrial systems, and sustains almost all heterotrophic life forms. This complex series of reactions, often referred to as green plant photosynthesis, has been conserved throughout Earth's history and is the mechanism for energy transduction in cyanobacteria, algae, and nonvascular and vascular plants.

The overall chemical process of green plant photosynthesis was first described in the nineteenth century by the following equation:

$$6CO_2 + 6H_2O \xrightarrow{\text{Light}} C_6H_{12}O_6 + 6O_2$$

A large amount of energy is required to drive this reaction; the standard free energy change (ΔG^o) is calculated to be 2,880 kJ mol^{-1}. This energy is provided by visible light with wavelengths of 400 to 700 nm. The equation, as written, describes an oxidation-reduction (redox) reaction. Historically, the reaction was not recognized as a redox reaction because water does not readily release electrons and thus is not an effective reducing agent. However, in the 1930s Cornelis van Niel and his coworkers demonstrated that anaerobic photosynthetic bacteria perform the following reaction:

$$nCO_2 + nH_2A \xrightarrow{\text{Light}} (CH_2O)_n + nA$$

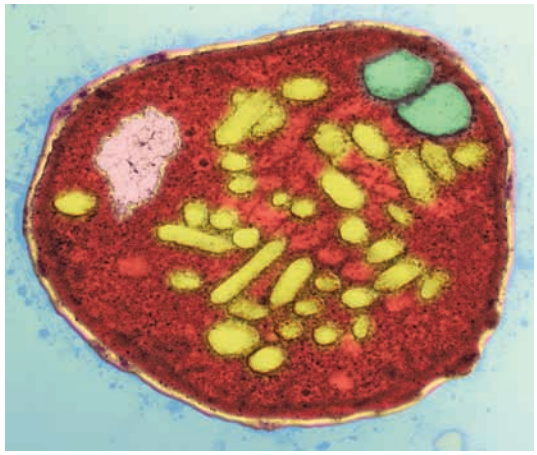


FIGURE 1.1 Light micrograph of a purple sulfur bacterium. This example of a Proteobacterium photosynthesizes only under anoxic conditions. Bacteriochlorophylls, other pigments, and proteins needed to harvest light energy and produce ATP are located in its cell membrane. Hydrogen sulfide (H_2S) is the reducing agent giving rise to the grains of elemental sulfur.

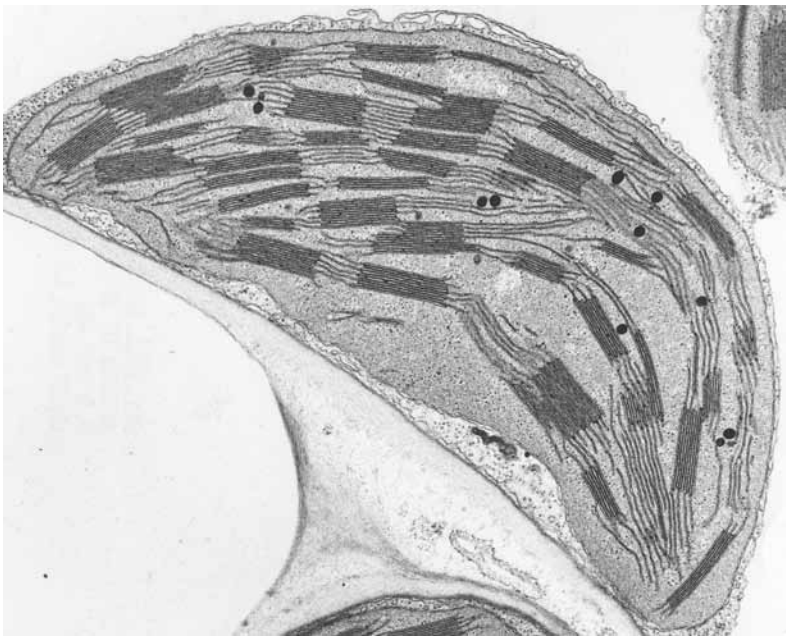
In anaerobic sulfur bacteria such as *Rhodospirillum rubrum*, H_2A is H_2S, which is a good reducing agent, and elemental sulfur is excreted to the outside of the cell (**FIGURE 1.1**) or oxidized to other compounds. By analogy, H_2A in green plant photosynthesis is H_2O and oxygen is released. Subsequent experiments by Ruben and Kamen in 1941 using ^{18}O-labeled water clearly showed that the oxygen generated in green plant photosynthesis is derived from the oxidation of water. Unlike anaerobic bacteria, plants absorb sufficient light energy to remove electrons from water. Subsequent research in the past 67 years has sought to elucidate the mechanisms behind this deceptively simple redox reaction.

Photosynthesis occurs in the chloroplasts of eukaryotic algae and plants and in the cytoplasm of cyanobacteria. The chloroplast is an organelle with two or more outer membranes and a set of internal membranes called thylakoids. The soluble portion of the organelle is called the stroma (**FIGURE 1.2**).

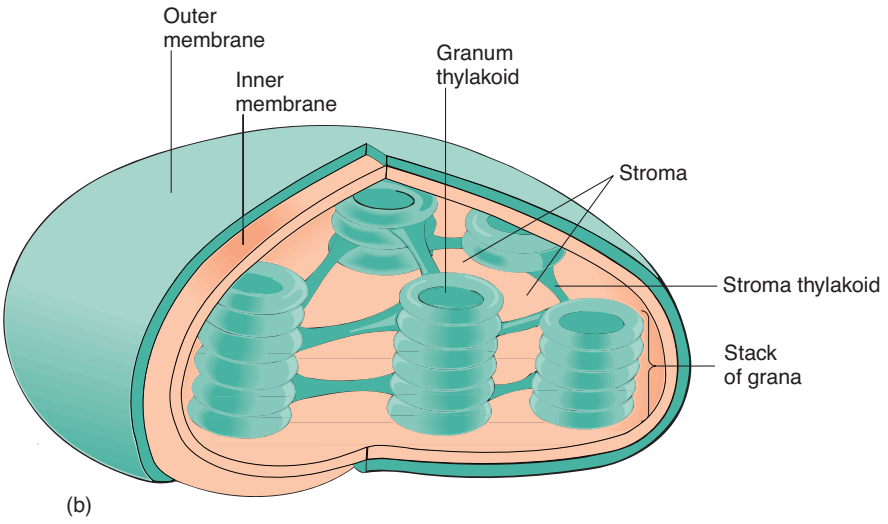
1.2 The Hill Reaction

Although the chloroplast is clearly the site of photosynthesis in plants, experiments with isolated chloroplasts in the early twentieth century were disappointing. When removed from the plant cell, these organelles were inactive. However, in 1937 Robin Hill showed that isolated chloroplasts evolve oxygen when supplied with an artificial electron acceptor such as ferricyanide:

$$4Fe^{3+} + 2H_2O \xrightarrow{\text{Light}} 4Fe^{2+} + 4H^+ + O_2$$



(a)



(b)

FIGURE 1.2 Electron micrograph (a) and diagram (b) of a chloroplast from maize. Chloroplasts in eukaryotic algae and plants have two or more outer membranes. In addition, the organelle has an extensive array of interior membranes called thylakoids. In angiosperms (flowering plants), these are arranged in stacks called grana (singular, granum). The thylakoid membranes are the site of the light reaction. These membranes are small sacs with an internal space or lumen. The soluble portion of the chloroplast is the stroma. It contains enzymes and cofactors needed for CO_2 fixation.

He concluded that the biological electron acceptor must be a water-soluble compound that is leached out of the organelle during the isolation procedure. In the most commonly used methods for chloroplast isolation, the outer membranes are damaged and soluble components of the stroma can be lost. The membranes, including thylakoid membranes, remain associated with each other and can be separated by centrifugation into a pellet. The components of the stroma are present in the supernatant fraction, usually in a highly dilute form. Isolated chloroplasts evolve O_2 (oxidize H_2O) but cannot produce carbohydrates (reduce CO_2). Gentle procedures for chloroplast isolation that preserved intact organelles capable of the complete photosynthetic process were not devised until the 1960s.

However, Hill's work was a major breakthrough in the study of photosynthesis. He clearly showed that photosynthesis is basically two oxidation-reduction reactions. The light reaction involves the oxidation of water, release of oxygen, and reduction of a soluble component. This occurs in thylakoid membranes. The second redox reaction, the reduction of carbon dioxide and oxidation of the soluble component, occurs in the water-soluble portion (i.e., the stroma) of the chloroplast. The Hill reaction can be written as follows:

$$2H_2O + 2A \xrightarrow{\text{Light}} 2AH_2 + O_2$$

where A is a redox dye such as dichloroindophenol, methyl viologen, or ferricyanide. Hill's

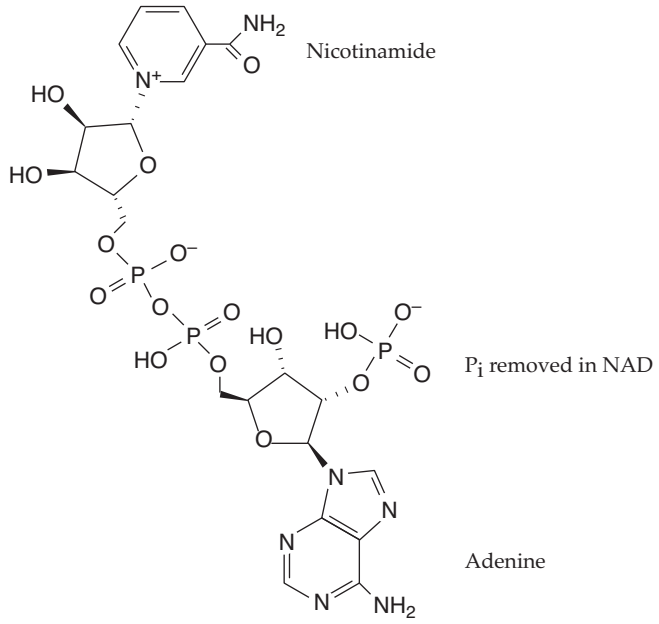


FIGURE 1.3 Nicotinamide adenine dinucleotide phosphate (NADP). (a) NADP consists of two nucleotides connected by a phosphodiester bond. A similar redox cofactor, NAD, lacks the second phosphate group on the adenine ribose. (b) The nicotinamide ring accepts two electrons and one proton (hydride, H^- ion) to produce the reduced cofactor, NADPH.

contribution made it possible to study the light reaction in isolated organelles either spectrophotometrically or polarographically by monitoring the evolution of oxygen. In the 1950s Daniel Arnon and coworkers demonstrated that the soluble cofactor, NADP (nicotinamide adenine dinucleotide phosphate; **FIGURE 1.3**), is the physiological electron acceptor. This research group also showed that isolated chloroplasts produce ATP when adenosine diphosphate (ADP) and PO_4^{3-} (inorganic phosphate, Pi) are added to the incubation mixture. The light reaction could now be written in its complete form:

$$2\text{NADP} + \text{Pi} + \text{ADP} + 2\text{H}_2\text{O} \xrightarrow{\text{Light}} 2\text{NADPH} + 2\text{H}^+ + \text{ATP} + \text{O}_2$$

1.3 Photosynthetic Pigments

Chlorophyll *a*

This basic energy conversion, light energy to chemical bond energy, occurs in the thylakoid membranes. Visible light supplies the energy for the oxidation of water and the transfer of electrons to NADP. Electron transfer also provides the energy for the synthesis of ATP. Light energy is captured by photosynthetic pigments. All organisms that perform oxygenic photosynthesis use a pigment called chlorophyll *a* (**FIGURE 1.4**). The basic structural element of all chlorophylls is a tetrapyrrole ring that is also found in related molecules containing heme groups such as cytochromes. In the chlorophyll ring the four nitrogens are coordinated to a Mg ion, as opposed

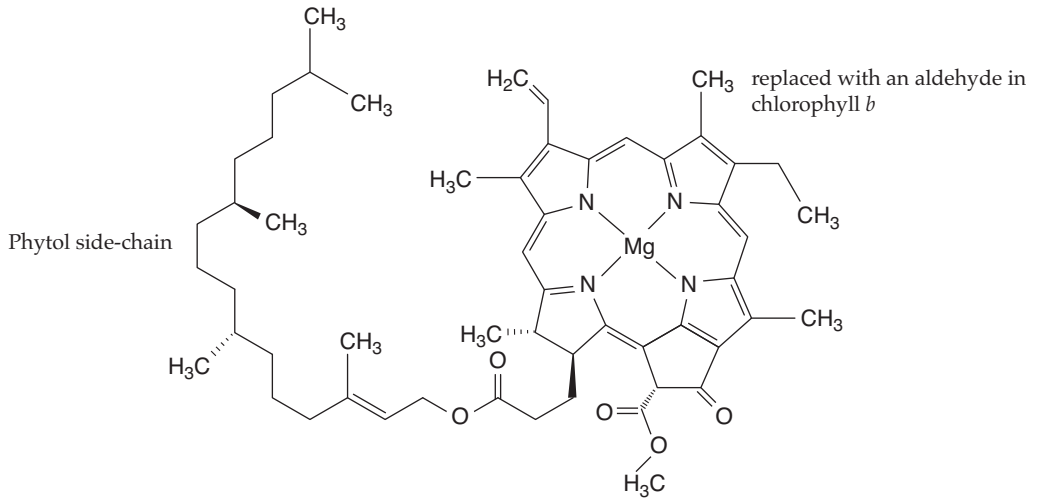


FIGURE 1.4 Structure of chlorophyll *a*. The tetrapyrrole ring is a conjugated system, making this portion of the molecule rigid and planar. The four nitrogens of the tetrapyrrole ring are conjugated to a Mg ion. Various substitutions can be made to the groups on the ring to produce other types of chlorophylls. For example, in chlorophyll *b* a methyl group is substituted with an aldehyde. The 20-carbon long phytol side chain makes the entire molecule very hydrophobic.

to an Fe in heme groups. The ring is a conjugated system (i.e., alternating double and single bonds), which means that it has a rigid, planar structure because of the overlap of the hybrid molecular orbitals. In addition, chlorophylls have a 20-carbon hydrocarbon side chain, called a phytol unit, making these molecules very hydrophobic. Chlorophylls are associated with integral membrane proteins and can be extracted using organic solvents such as acetone or ethanol.

In addition to chlorophyll *a*, most plants also contain other types of chlorophylls with different chemical groups on the side chains of the tetrapyrrole ring. Chlorophylls absorb visible light in the blue and red regions of the visible spectrum (**FIGURE 1.5**; **see Appendix 7 for discussion of spectrophotometry**). The absorption spectrum is influenced by the chemical environment (e.g., the solvent in which the molecules are dissolved). Figure 1.5 shows the spectrum of isolated chlorophyll *a* and associated carotenoid pigments dissolved in ethanol. The major absorption peak at approximately 430 nm is characteristic of all tetrapyrrole rings and is called the Soret band. The absorption bands in the red region of the spectrum (approximately 630 and 660 nm) are characteristic of chlorophyll *a* and are called the Q bands. Most photosynthetic organisms also synthesize some accessory pigments that absorb light in other regions of the visible spectrum. In chlorophyll *b*, a common accessory pigment in angiosperms, an aldehyde replaces a methyl group in the tetrapyrrole ring (Figure 1.4). This substitution shifts the Soret band toward the red end of the spectrum and the Q bands toward the blue end of the spectrum compared with chlorophyll *a*, thus providing a wider range of light absorption (**FIGURE 1.6**). "Green" light, at approximately 500 to 600 nm, is not absorbed but is

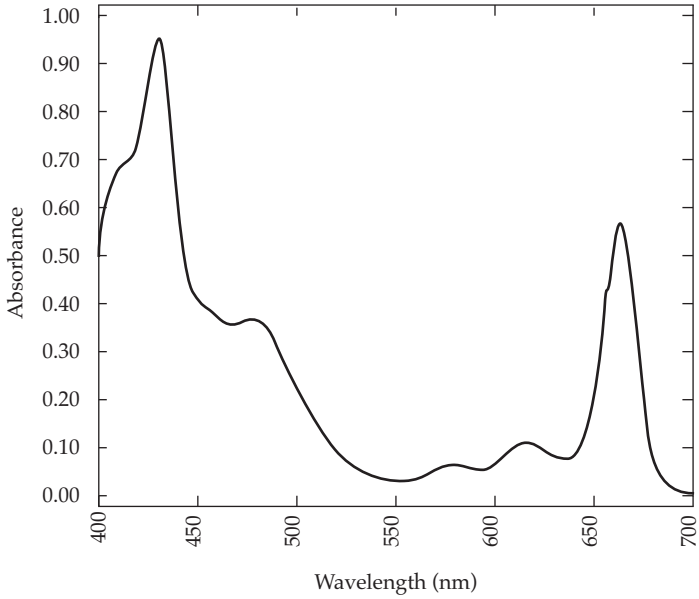


FIGURE 1.5 The visible absorption spectrum of photosynthetic pigments from a cyanobacterium, *Gloeocapsa alpicola*. The photosynthetic pigments were extracted from the cells with ethanol. The major peak at 430 nm is called the Soret band and is characteristic of most cyclic tetrapyrroles. The bands at 630 and 660 nm, Q bands, are characteristic of chlorophyll *a*. The shoulder at approximately 470 nm is due to absorption of light by accessory carotenoid pigments.

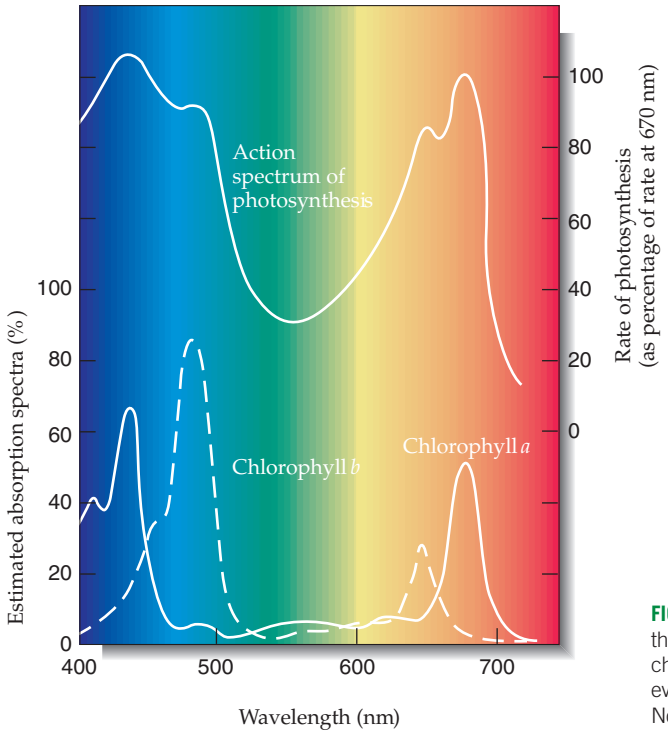
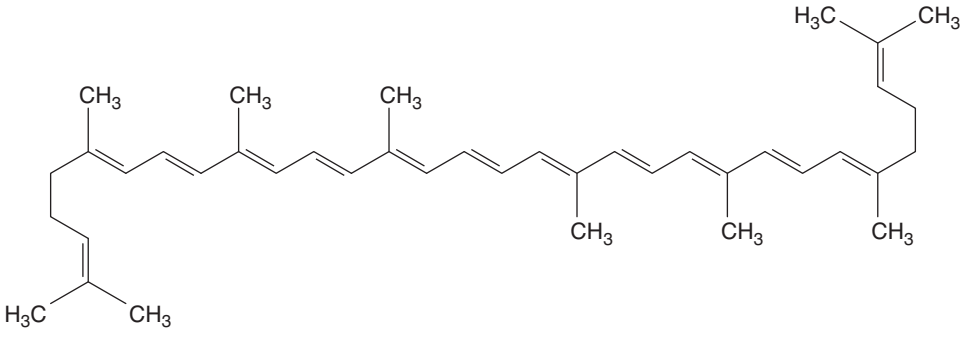


FIGURE 1.6 Action spectrum of the light reaction. In this diagram the action spectrum is superimposed above the absorption spectra of chlorophylls *a* and *b*. The rate of photosynthesis, measured as oxygen evolution, roughly parallels the absorption spectra of the chlorophylls. Note the abrupt "red drop" in the rate of photosynthesis above 680 nm.

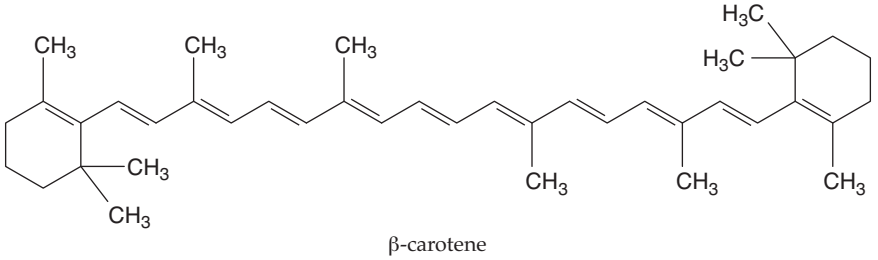
transmitted or reflected, and the solution appears green to human eyes.

Accessory Pigments

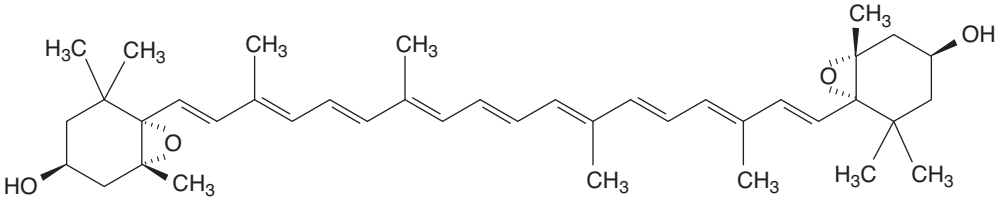
Other types of chlorophylls and pigments are found in photosynthetic organisms. Bacteriochlorophylls are characteristic of the purple and green photosynthetic bacteria. These absorb light at longer wavelengths (800–890 nm) than chlorophyll *a*. Chlorophylls *c* and *d* are accessory pigments in algae such as diatoms. In addition to various chlorophylls, most plants also contain accessory pigments called carotenoids. These are 40-carbon, conjugated hydrocarbons that absorb light in the violet and blue regions of the visible spectrum (**FIGURE 1.7**). They function in



Lycopene



β-carotene



Violaxanthin

FIGURE 1.7 Examples of some common carotenoids. These 40-carbon-long hydrocarbons are conjugated systems that absorb visible light in the blue region of the spectrum. They act as accessory pigments in the light reaction and also protect the proteins in the photosynthetic reaction centers from excessive light and free radical damage.

harvesting light energy and in protecting the photosynthetic apparatus from photo-oxidative damage by excess illumination. Phycobilins are linear tetrapyrroles that absorb light at various wavelengths, including the green region of the spectrum. These are water-soluble pigments found in cyanobacteria and some eukaryotic algae. Most photosynthetic pigments, however, are not soluble in water and are an integral part of a cellular membrane. Chromatophores in photosynthetic bacteria are invaginations of the cell membrane called chlorosomes. Thylakoid membranes are found in the cytoplasm of cyanobacteria. In eukaryotic algae and plants, these membranes are in the chloroplast and are not connected to the organelle's outer membranes. They can often have elaborate stacked arrangements, as seen in Figure 1.2.

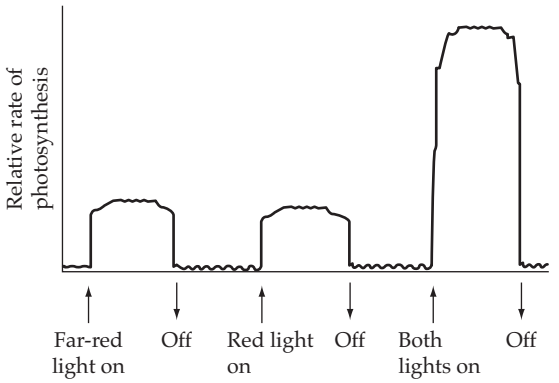


FIGURE 1.8 Emerson enhancement effect. Algal cultures are briefly exposed to far red light (700 nm) or/and red light (680 nm). The rate of photosynthesis with both types of light is more than the sum of activity with either single light source. These data suggest that the light reaction requires two RCs working in series for maximum efficiency. (*Adapted from:* Taiz, L. and Zeiger, E., *Plant Physiology*, 2nd ed. Sunderland, MA: Sinauer Associates, Inc., 1998. Pg. 165, Fig. 7.10.)

1.4 Origin of the Z-Scheme

Emerson Enhancement

Although Robin Hill's discovery of the oxygen-evolving capacity of isolated chloroplasts made it possible to study the light reaction in isolated organelles, unicellular algae were often a better experimental system to study the whole process of photosynthesis. The eukaryotic green alga, *Chlorella* sp., was commonly used. In the 1950s, R. Emerson and his colleagues measured photosynthetic efficiency in *Chlorella* cultures with very short light flashes. They concluded that the light reaction was much faster than CO_2 fixation. They also found that approximately 2,500 chlorophyll molecules were required to evolve one O_2 molecule. Most of these pigment molecules served to harvest light energy (i.e., they functioned as an antenna system). Sunlight is a diffuse source of energy that must be concentrated to be effective. Light energy is also easily dissipated before it can be used. Most of these 2,500 chlorophylls are associated with integral membrane proteins called light-harvesting complexes (LHCs). LHCs consist of several polypeptides that generally have alpha helices traversing the thylakoid membranes. The pigments are noncovalently attached to the membrane-embedded part of these proteins. The LHCs trap light energy and transfer it to a photosynthetic reaction center (RC) that includes only a small fraction of the total pigment molecules in the membrane. Emerson's group determined the action spectrum for photosynthesis by measuring the rate of oxygen evolution from *Chlorella* cultures at different wavelengths (Figure 1.7). Oxygen evolution parallels the absorption spectrum of chlorophyll and accessory pigments except for the abrupt decline in efficiency at the red end of the spectrum. Chlorophyll molecules that absorb light energy at wavelengths longer than 680 nm do not appear to be as efficient as those that absorb at shorter wavelengths. This anomaly was referred to as the "red drop." Using brief flashes of light at different wavelengths, these researchers also found that exposure to far-red light (greater than 680 nm), when supplemented with a brief flash of red light (less than 680 nm), yielded a rate greater than the sum of exposure to each wavelength alone. This phenomenon was called the Emerson enhancement effect (**FIGURE 1.8**).

Photosystems I and II

The data of the Emerson group were interpreted as a requirement for several LHCs to gather and transfer light energy to a reaction center (RC) and for two RCs, working in series, for maximum efficiency. This led to the proposed "Z-scheme" of photosynthesis that was first published in 1960 (**FIGURE 1.9**). Two RCs, photosystem I (PSI), and photosystem II (PSII), which constitute only 1% of the total pigment-protein complexes in thylakoid membranes, act together to transfer electrons from H_2O to NADP. PSI is a supramolecular complex of proteins and pigments with maximal absorption at 700 nm (P_{700}). PSI performs the light-induced reduction of a soluble 2Fe-2S protein, ferredoxin, which then transfers electrons to NADP via a flavoprotein, ferredoxin-NADP reductase (FNR).

$$\text{Light} \rightarrow P_{700} \rightarrow P_{700}^{+} + e^{-} \rightarrow \text{Acceptor-}e^{-} \rightarrow \text{Ferredoxin} \rightarrow \text{NADP} \rightarrow \text{NADPH}$$

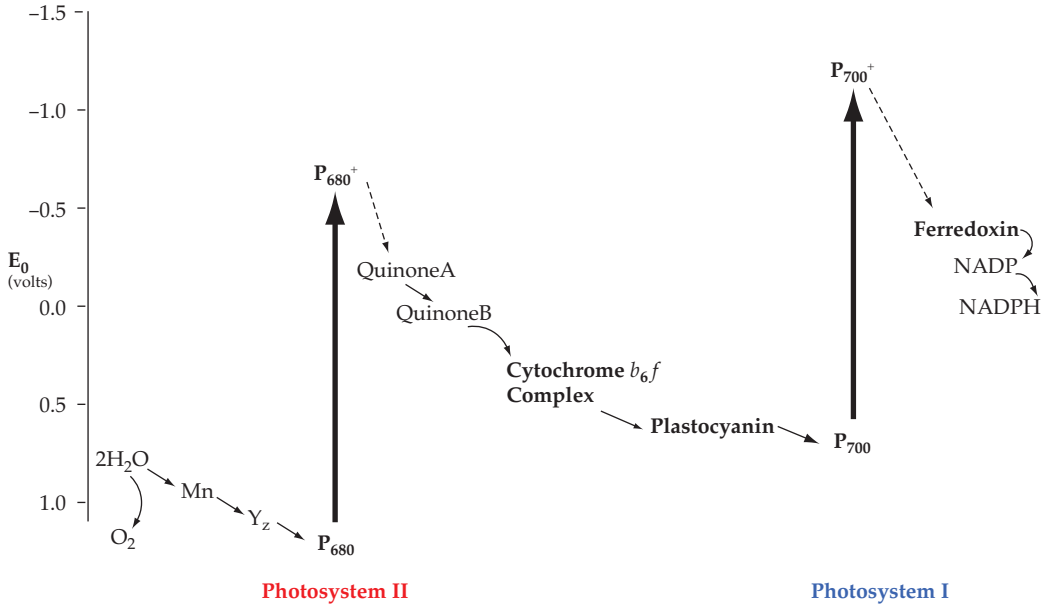


FIGURE 1.9 Z-scheme. After being published in the 1960s, this basic outline became the paradigm for further experimental work on the light reaction. PSI (P_{700}) absorbs far red light and transfers an electron to ferredoxin and ultimately to NADP. This electron is replaced in the RC by transfer of electrons from PSII via a cytochrome b_6f complex. The shorter wavelength of light absorbed by PSII (P_{680}) also provides sufficient energy to oxidize H_2O. PSII uses H_2O as the ultimate source of electrons.

P_{700}^+ is reduced by plastocyanin, a membrane-associated copper-containing redox protein located in the thylakoid space (lumen). PSII is a supramolecular complex of proteins and pigments with maximal absorption at 680 nm (P_{680}). It carries out the light-induced oxidation of H_2O and the reduction of a small molecule called a quinone.

$$\text{Light} \rightarrow P_{680} \rightarrow P_{680}^+ + e^- \rightarrow \text{Quinone B reduced}$$

After accepting two electrons and acquiring two protons, the reduced quinone leaves the PSII reaction center and diffuses within the lipid bilayer of the thylakoid membrane. It is reoxidized by a cytochrome b_6f complex that transfers electrons to plastocyanin. The PSII RC simultaneously catalyzes the oxidation of water:

$$2H_2O \rightarrow 4H^+ + O_2 + 4e^- \rightarrow P_{680}^+ \rightarrow P_{680}$$

The four electrons are channeled, one at a time, into the PSII reaction center to replace those transferred to quinone B. The oxygen escapes from the cell, but the H^+s remain in the thylakoid lumen, contributing to a proton gradient.

The two photosystems are connected by a cytochrome b_6f complex. Cytochromes are proteins that contain heme groups (i.e., tetrapyrrole rings coordinated to iron). The b_6f complex contains both *b*-type and *f*-type cytochromes in addition to a Rieske 2 Fe-2S protein consisting of Fe coordinated to sulfur groups in the protein. This complex oxidizes the quinones reduced in PSII and reduces plastocyanin to replace electrons transferred out of PSI (Figure 1.9). It also transfers protons from the stroma to the thylakoid lumen. The proton gradient in the thylakoid lumen drives the synthesis of ATP via a fourth membrane-bound component of the light reaction, the chloroplast F0F1 ATP synthase.

1.5 Light Reaction: Details

The Reaction Center of Purple Bacteria: A Model for PSII

The purple bacteria, members of the Proteobacteria phylum, photosynthesize under anaerobic conditions, using sulfide, thiosulfate, or hydrogen as their reducing agent. They have a single RC containing proteins and bacteriochlorophylls associated with the cell membrane. Purple bacteria use light energy to produce a transmembrane proton gradient that drives the synthesis of ATP. Mechanistically, their RCs have much in common with PSII, and these organisms have been used as a model system to study green plant photosynthesis. A major breakthrough in research on the mechanism of photosynthesis occurred in the 1980s when a group led by J. Deisenhofer, H. Michel, and R. Huber reported the x-ray crystal structure of the RC from *Rhodopseudomonas viridis* (renamed *Blastochloris viridis*). In addition to its direct

relevance to photosynthesis, their work also described procedures for the crystallization and structure determination of membrane-bound proteins using octyl glucoside detergents to isolate and maintain the proteins in a native state. Previously, integral membrane proteins were almost impossible to isolate and characterize using crystallographic techniques.

Structure of the Bacterial Reaction Center

The transmembrane core of the *R. viridis* RC consists of three proteins, designated H, M, and L. These letters refer to their relative mobility during sodium dodecyl sulfate-polyacrylamide gel electrophoresis, where H has the largest relative molecular weight, M is intermediate in size, and L is the smallest subunit. Subsequent amino acid analyses showed that in reality H is the smallest protein, L is intermediate in size, and M is the heaviest; this illustrates the anomalous behavior of membrane proteins in denaturing gels designed to separate water-soluble proteins. These RC preparations often contained a *c*-type cytochrome that was easily lost during purification. In addition to these four proteins, the RC also contained four bacteriochlorophylls, two bacteriopheophytins (chlorophyll molecules lacking the coordinated Mg), two quinones, an Fe^{2+}, and a carotenoid. The L and M proteins are anchored in the membrane. As shown in **FIGURE 1.10**, each has five membrane-spanning helices. All RC cofactors are associated with these two core proteins. The H polypeptide has one helix embedded in the membrane with the bulk of the protein in the cytoplasm. The cytochrome is positioned on the edge of the cell membrane on the periplasmic side.

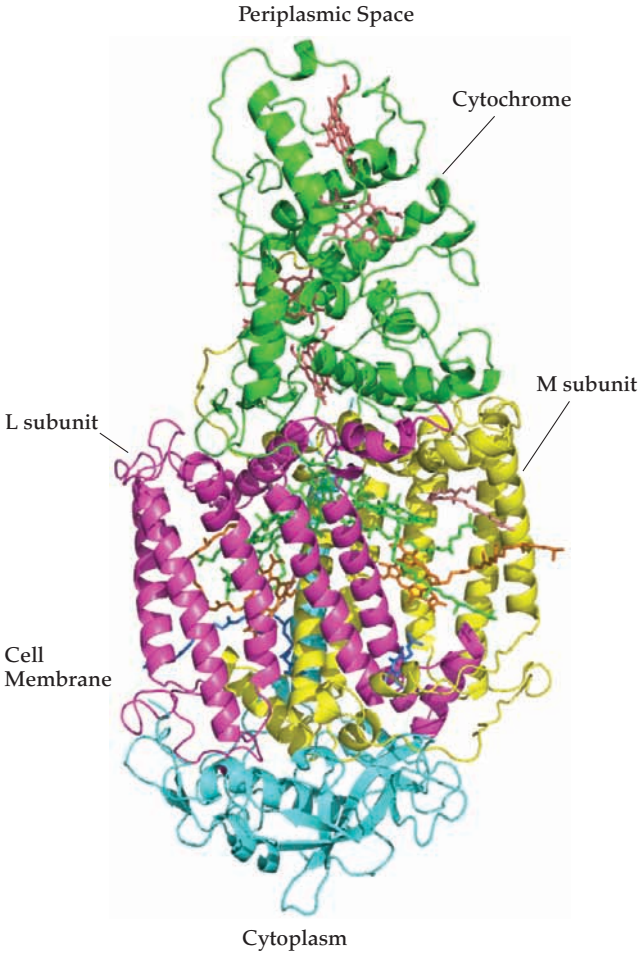
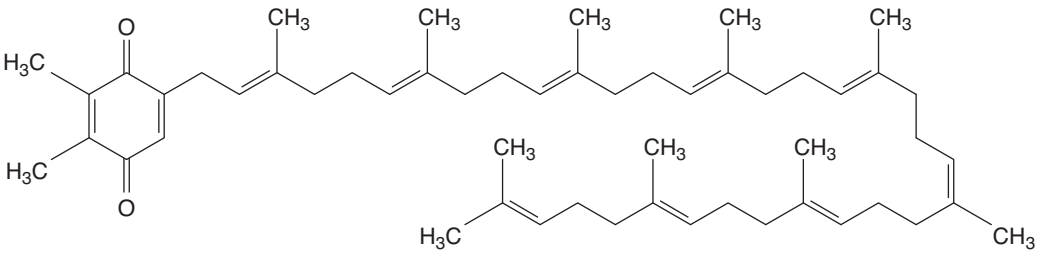
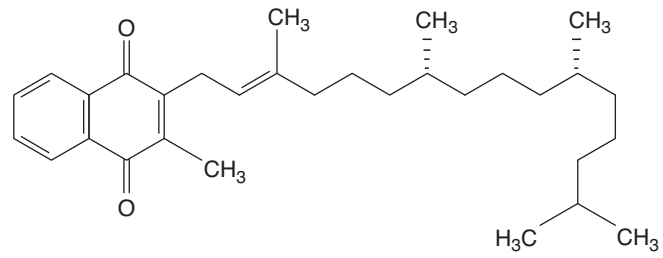


FIGURE 1.10 Reaction center of the Proteobacterium, *Rhodopseudomonas viridis*. The L subunit (magenta) and the M subunit (yellow) each contain five helices that span the cell membrane. The H subunit (blue) is located mainly in the cytoplasm with one helix embedded in the membrane. The cytochrome (green) sits atop the L-M dimer in the periplasm. Bacteriochlorophylls in L and M are shown in green, bacteriopheophytins in orange, and the quinones in deep blue. The heme groups in the cytochrome are shown in pink. (Structure from Protein Data Bank, 1prc. J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, *J. Mol. Biol.* 246 [1995]: 429–457.)

Light Energy Is Used To Reduce a Quinone

How does this simple RC work? Light energy is absorbed and transferred by the antenna pigments into the "special pair" of bacteriochlorophyll molecules in the RC (P_{870}). An excited electron is freed from this primary electron donor and captured by the primary acceptor, bacteriopheophytin, in the L subunit. This transfer takes approximately 3 picoseconds (ps). The M subunit, with its corresponding cofactors, is placed in a symmetrical position, but electron transfer does not seem to proceed through this pathway under normal physiological conditions. From the reduced bacteriopheophytin, the electron is transferred to quinone A in approximately 200 ps. Electron transfer to this quinone results in a one electron-reduced quinone (i.e., a semiquinone or quinone radical anion). Fully reduced quinones (quinols) carry two electrons and two protons (**FIGURE 1.11**). From quinone A the electron is transferred to quinone B in 100 µs. The Fe^{2+} positioned between quinones A and B does not directly participate in electron transfer but acts to stabilize the semiquinones (**FIGURE 1.12**). The transfer proceeds if this Fe is removed, but at a much slower rate. The cycle repeats so that quinone B receives two electrons and two protons. The protons are transferred from amino acid side chains in the RC proteins but ultimately come from water in the cytoplasm. Fully reduced quinone B no longer binds to the RC and diffuses into





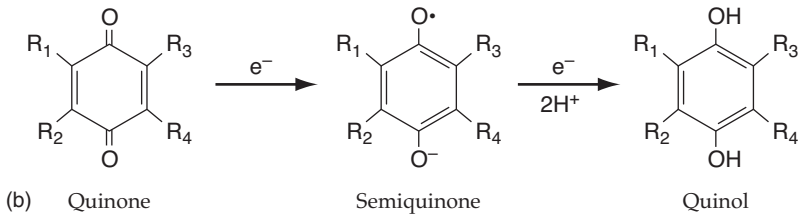


FIGURE 1.11 Structure and redox activity of quinones. Part a shows the structure of plastoquinone associated with PSII and phylloquinone in PSI. The long hydrocarbon side chains make these molecules very hydrophobic. Part b shows the redox activity of a quinone. A quinone can accept one electron and become a semistable radical anion. A fully reduced quinone (quinol) carries two electrons and two protons.

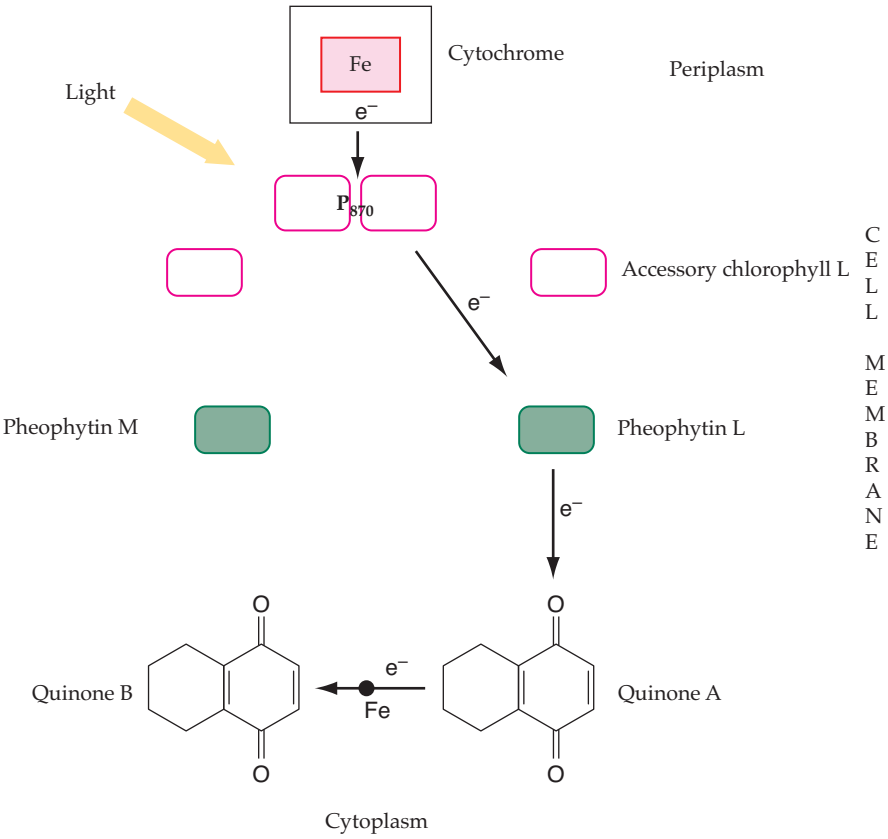


FIGURE 1.12 Electron transfer in the *R. viridis* reaction center. The special pair of bacteriochlorophylls (P_{870}) absorb light at far red wavelengths. An electron is rapidly transferred to the bacteriopheophytin in the L subunit and subsequently to quinone A and finally to quinone B. The electron lost by P_{870} is replaced by transfer from the cytochrome in the periplasm. Compare the diagram with the crystal structure of the RC shown in Figure 1.10. After two photo events and proton transfer from the cytoplasm, quinone B is fully reduced and diffuses out of the RC into the lipid bilayer. It is reoxidized by a cytochrome complex in the cell membrane.

the membrane. The rapid electron transfer in the RC ensures that the back reaction or radical production does not occur.

The electron lost from the oxidized special pair of bacteriochlorophylls in the P_{870}^{+} RC is replaced by an electron transferred from the cytochrome positioned in the periplasm. This cytochrome, in turn, is reduced by a cytochrome bc_1 complex located in the cell membrane in the vicinity of the RC. The cytochrome bc_1 complex oxidizes the reduced quinone B and then reduces cytochrome *c*. In the electron transfer process, it also transfers protons from the cytoplasm into the periplasmic space. The bacterial RC and associated cytochromes perform a cyclic electron transfer that serves to create a proton gradient across the cell membrane. The energy stored in this H^+ gradient can then be used to generate ATP.

1.6 Photosystem II

Electron Transfer

The purple bacterial RC serves as a good model for the plant PSII reaction center. Gene and protein sequencing data show significant similarities between the L and M proteins of the bacterial RC and the core proteins of PSII, confirming that a bacterial type II center was the evolutionary precursor of PSII RC in cyanobacteria and plants. PSII consists of approximately 25 proteins that can be isolated as a complex from thylakoid membranes. The proteins are called Psb proteins with the corresponding *psb* genes, but some of the proteins are still referred to by their original, pre-genomic designations. The RC core of PSII consists of the D1 (PsbA) and D2 (PsbD) proteins. Like the L and M proteins of the bacterial RC, the D1–D2 core consists mainly of five transmembrane helices. Cofactors associated with the D1–D2 core include six chlorophyll molecules, two pheophytin molecules, two quinones, an Fe^{2+}, and a four Mn-one Ca cluster that is unique to oxygenic photosynthesis (**FIGURE 1.13**).

The mechanism of action is similar to that in the bacterial RC. Light energy is funneled into a special pair of chlorophyll *a* molecules (P_{680}) located at the interface of the D1 and D2 proteins. Electron transfer proceeds in 3 to 4 ps to the pheophytin in the D1 protein. As in the bacterial center, electrons are presumably not funneled through the symmetric cofactors in the D2 protein. The electron is then transferred to a quinone A and, subsequently, to quinone B (plastoquinones). After two electron transfers and addition of two H^+, quinone B is fully reduced and the quinol diffuses into the thylakoid bilayer. It is replaced by an oxidized quinone and the cycle continues. The oxidized chlorophyll special pair is reduced by electron transfer from a transient tyrosine radical in the D1 protein referred to as Yz. This specific tyrosine residue is the electron transfer conduit from the water-oxidizing reaction, which is the ultimate source of electrons in green plant photosynthesis (**FIGURE 1.14**).

Oxidation of Water

The water-splitting/oxygen-evolving reaction takes place on the lumenal side of the D1 protein.

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2$$

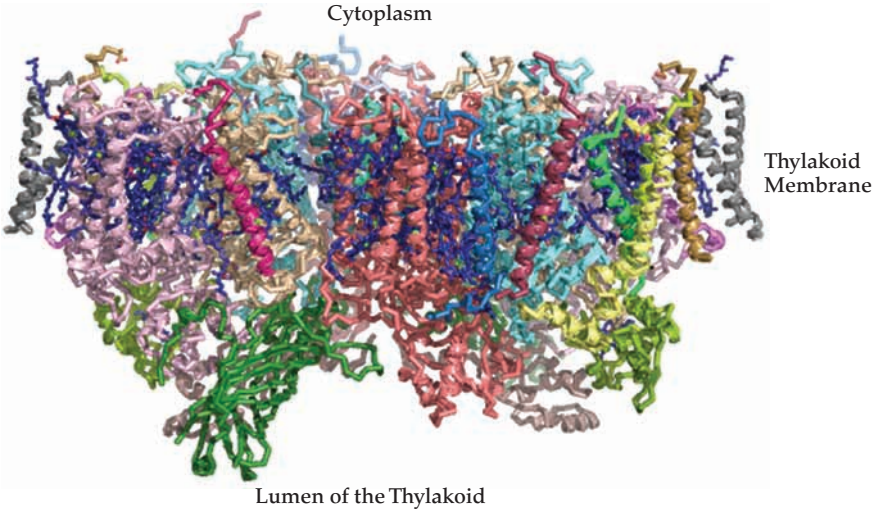


FIGURE 1.13 Structure of PSII from the cyanobacterium *Thermosynechococcus elongatus*. This view of PSII is parallel to the thylakoid lipid bilayer with the cytoplasmic side at the top and the thylakoid lumen at the bottom. The PSII complex crystallizes as a dimer with an axis in the center. Each monomer consists of 19 protein subunits. The D1 protein is in light blue and D2 in yellow. These two proteins, which correspond to the L and M subunits of the bacterial RC in Figure 1.10, consist of five transmembrane helices oriented in the membrane. In addition, each subunit contains 36 chlorophyll *a*, 7 carotenoids, 4 Mn, 1 Ca, 2 plastoquinones, 2 pheophytins, and 1 Fe. Some of the chlorophylls are depicted in red. The majority of the chlorophylls are found in light-harvesting proteins that flank the D1–D2 core. The PsbO protein that stabilizes the oxygen-evolving complex with its Mn–Ca cluster is located on the lumenal side and is shown in dark green. (Structure from Protein Data Bank, 1s51. K. N. Ferreira, T. M. Iverson, K. Maghlaoui, J. Barber, and S. Iwata, *Science* 303 [2004]: 1831–1838.)

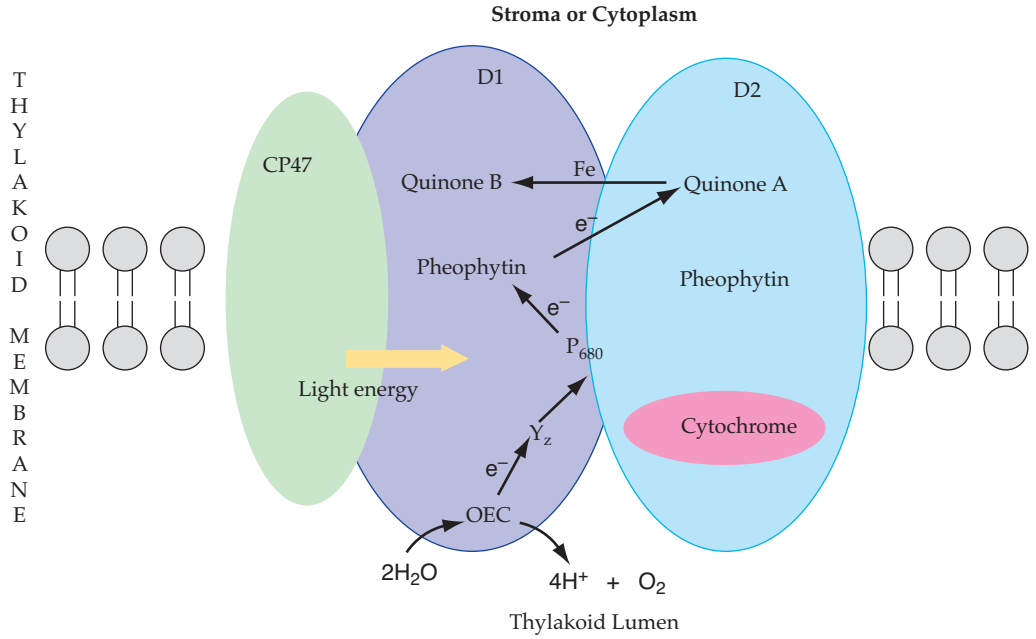


FIGURE 1.14 Electron transfer in PSII. The D1–D2 core proteins are flanked by light harvesting complexes. One of these antenna proteins, chlorophyll protein (CP) 47, is shown transferring light excitation energy into P_{680}, which consists of two chlorophyll *a* molecules centered between the D proteins. An electron is freed from P_{680} and captured by a pheophytin in the D1 protein. The electron is then transferred to quinone A and subsequently to quinone B. An Fe^{2+} in the D1–D2 core facilitates this transfer but does not undergo any redox changes. The electron lost by P_{680} is replaced by a tyrosine, Yz in the D1 protein. This amino acid residue channels electrons one at a time from the oxygen-evolving complex, which consists of 4 Mn and 1 Ca ion and uses light energy to split H_2O and channel electrons to Yz. The core complex of PSII also includes one or more cytochromes. These cytochromes may serve as alternate electron pathways to protect the core complex from radical damage and/or to provide for cyclic electron transport in PSII.

The function of the water-oxidizing complex is to transfer the four electrons, one at a time, through Yz to P_{680}^+ without releasing free radical intermediates. In 1967, P. Joliot and B. Kok set up an experimental system with isolated thylakoid membranes that they subjected to very brief, intense flashes of light. They found that oxygen evolution exhibited a periodicity of approximately one O_2 evolved per three to four flashes (**FIGURE 1.15**). Based on their experimental data, these researchers proposed the S state-cycle or "water-oxidizing clock." Five different states are possible, S_0 to S_4, corresponding to different oxidation states of the Mn ions associated with D1. Electrons are channeled one at a time to the tyrosine residue, Y_Z. O_2 is released only in the transition from S_4 to S_0 when the clock is "reset" (**FIGURE 1.16**). The oxygen-evolving complex has a cofactor containing four Mn, one Ca, and one Cl ions. A crystal structure of PSII from cyanobacteria was published in 2001, but the oxygen-evolving part of the D1 protein was not resolved until very recently. Although the oxygen-evolving complex is part of D_1, another protein, PsbO (also called MSP [manganese-stabilizing protein]), is required to maintain the stability of the complex. Because of the instability of the isolated complex, it is difficult to crystallize; in addition, the x-ray beam reduces all the Mn ions to the same redox state. Biophysical techniques have led to several models of the complex that

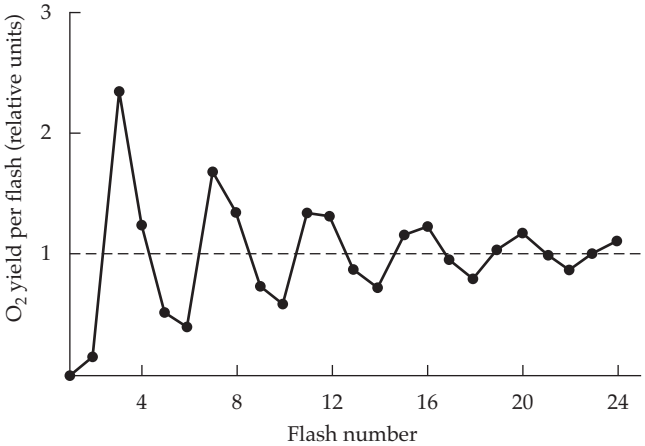


FIGURE 1.15 Joliot-Kok experiment. When removed from darkness and subjected to brief, intense flashes of light, isolated thylakoid membranes release O_2 in discrete bursts of one O_2 for every three to four flashes. This type of experiment led to the proposed "water clock" model that has served as a guide to further investigation of the water-splitting reaction in PSII. (*Adapted from*: Blankenship, R., *Molecular Mechanisms of Photosynthesis*, Wiley-Blackwell, 2002. Pg. 114, Fig. 6.10A.)

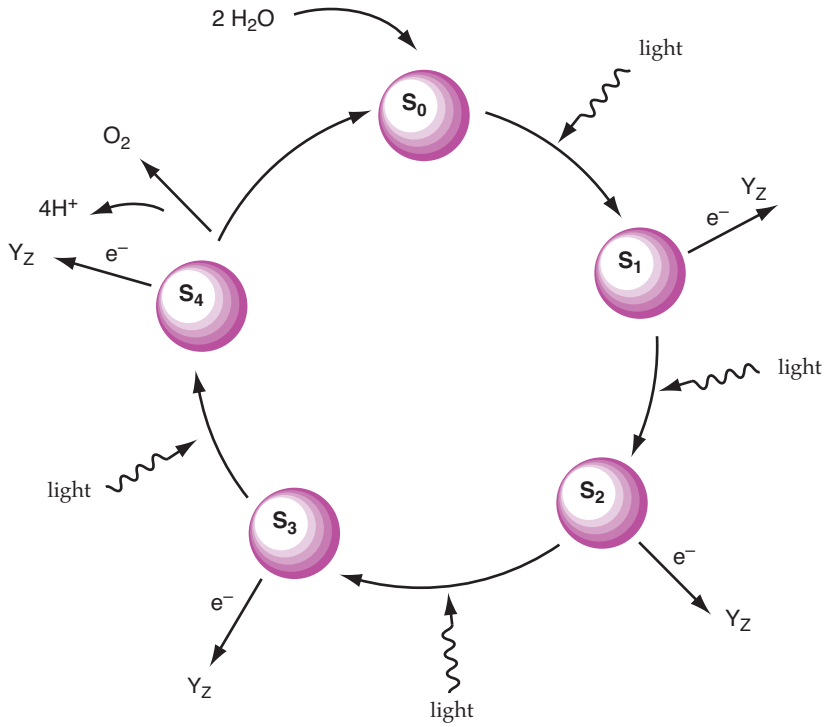


FIGURE 1.16 The "water clock" in PSII. Based on the Joliot-Kok experiment (Figure 1.15), five states of the 4Mn–1Ca ions in the oxygen-evolving complex were proposed. Two H_2O molecules are bound in a "resting" state, S_0. Light absorbed by P_{680} serves to change the redox state of the Mn ions, leading to S_1, and results in the transfer of one electron from the complex to a tyrosine residue, Yz. Subsequent light absorption by P_{680} continues the process through three additional states. O_2, in various oxidation states, remains bound until it is released as a stable molecule at S_4. Protons are also released from the complex at various states but remain in the thylakoid lumen.

involve binding and sequential breakdown of two water molecules coordinated with redox changes in the Mn ions. The precise details whereby $2H_2O$ molecules are oxidized without release of intermediate radicals are still unknown.

1.7 Photosystem I

The bacterial counterpart of PSI is the type of RC found in green sulfur bacteria. In these microorganisms the RC catalyzes cyclic electron transfer as in the purple bacteria or can in some cases reduce NAD. H_2S serves as the reducing agent and replaces the electrons transferred out of the RC. X-ray crystal structures of PSI have been solved for complexes isolated from cyanobacteria and angiosperms. The overall structure resembles that of PSII, suggesting the core protein complexes evolved from the same ancestral RC. The PSI RC in angiosperms consists of 12 core proteins, designated Psa proteins with *psa* corresponding genes. The core of the complex consists of two integral membrane proteins, PsaA and PsaB, six chlorophyll *a* molecules, two phylloquinones, and an 4Fe-4S cluster. The PsaA and PsaB proteins correspond to the D proteins in PSII. They are both helical, integral membrane proteins with a mass of approximately 84 kDa each. These proteins bind the RC pigments, and the primary events in electron transfer occur here.

Light energy is channeled into a pair of chlorophyll *a* molecules (P_{700}) where an electron is freed and rapidly transferred to a phylloquinone in PsaA protein and then to the Fe-S cluster located between the PsaA and B proteins (**FIGURE 1.17**). A membrane-associated protein, PsaC, on the stromal side of the thylakoid accepts the electron from the PsaA cluster. PsaC has a mass of approximately 9 kDa and contains two 4Fe-4S clusters. The PsaC protein binds and reduces the soluble electron carrier, ferredoxin (Fdx). As in other RCs, there are two parallel pathways of electron transfer to the iron-sulfur acceptors. Biophysical studies suggest that, unlike PSII, in PSI both branches are used. Ferredoxin is a soluble protein with a transient association with the PsaD protein on the stromal side of the thylakoid membrane. Fdx is a 2Fe-2S protein in most plants and carries one electron. It associates with the Psa proteins of the RC by ionic interactions. In reduced Fdx these interactions are weakened,

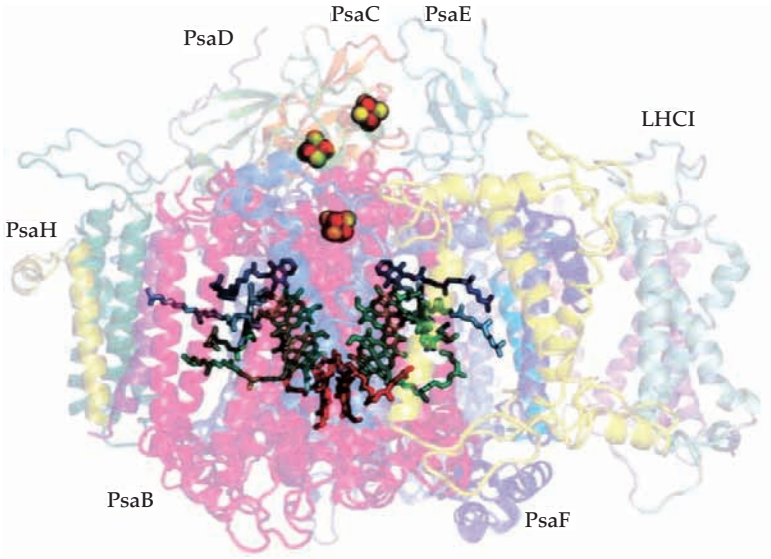


FIGURE 1.17 Structure of PSI from *Pisum sativum*. The crystal structure is shown in the background, viewed parallel to the thylakoid membrane. The stromal side is at the top and the lumenal side is at the bottom. PSI from pea is monomeric with 16 protein subunits and 127 cofactors, mostly chlorophyll a molecules associated with LHCs. Subunits PsaA and PsaB form the core of PSI. The cofactors that function in electron transfer are superimposed in the foreground. The special pair of chlorophyll molecules, P_{700}, are shown in red and the accessory pigments in green. The phylloquinones are colored blue and the iron (red)–sulfur (yellow) cluster is depicted by balls in the center of the core. Electron transfer from this cluster proceeds through two additional clusters located in the PsaC protein. The final electron acceptor, ferredoxin, binds on the stromal side to sites on the PsaC, D, and E proteins. On the lumenal side, the PsaF protein binds reduced plastocyanin that replaces the electron lost by P_{700}. (*Reproduced from*: Nelson, N. and Yocum, C.F., "Structure and Function of Photosystems I and II," *Annu. Rev. Plant Biol.* 57 [2006]: 521–565, Fig. 5.)

and Fdx diffuses away from PSI and reduces NADP on the stromal side of the thylakoid membrane.

$$2Fdx_{reduced} \rightarrow NADP + H^+ \rightarrow NADPH + 2Fdx_{oxidized}$$

The above reaction is catalyzed by a flavoprotein, ferredoxin-NADP reductase (FNR).

The electron lost from the PSI chlorophyll pair is replaced by transfer from plastocyanin. PsaF is a 19-kDa protein found on the lumenal side of the thylakoid membrane. It docks reduced plastocyanin, a Cu-containing redox protein, and facilitates reduction of the oxidized primary electron donor, P_{700}^+.

1.8 Cytochrome b_6f Complex

The integral membrane proteins that form an electron transfer pathway between PSII and PSI consist of a cytochrome-Fe complex. This complex in thylakoids resembles the cytochrome bc_1 complex in purple bacteria and a similar electron-transfer complex found in the mitochondrial inner membrane. The chloroplast complex consists of several proteins, including two cytochrome b_6 molecules, cytochrome *f*, and a Rieske 2Fe-2S protein. Its function is to oxidize quinols to quinones and reduce plastocyanin. In addition, it transports protons from the stroma into the thylakoid lumen.

$$2QH_2 + 2PC_{ox} + 2H^+_{stroma} + Q \rightarrow 2Q + 2PC_{red} + 4H^+_{lumen} + QH_2$$

The proton-pumping activity of this complex is referred to as a "Q cycle" and results from the properties of the redox components. Reduced quinones carry two electrons and two protons. Cytochromes, nonheme iron proteins, and plastocyanin each carry one electron and no protons. In step 1 of the Q cycle, a reduced quinone (QH_2) from PSII binds to the b_6f complex (**FIGURE 1.18**). One of its electrons is transferred via the Rieske iron-sulfur protein and cytochrome *f* to plastocyanin. The second electron from the bound quinone is transferred to the cytochrome *b* complex. The two protons carried by the reduced quinone are left in the thylakoid lumen as the oxidized quinone is released from the cytochrome complex.

In step 2 oxidized quinone binds to a second quinone binding site on the complex. The reduced cytochrome *b* transfers its electron to this oxidized quinone producing a semiquinone at the second binding site. Now the cytochrome complex is reset to its initial state and can bind another reduced quinone from PSII.

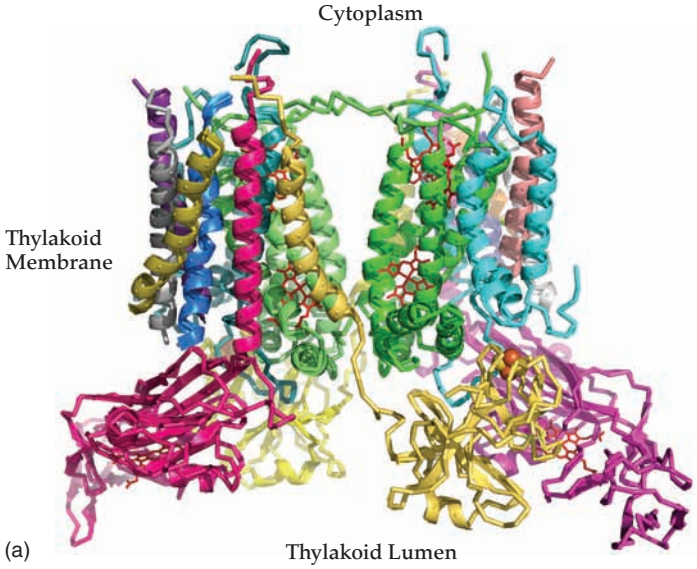
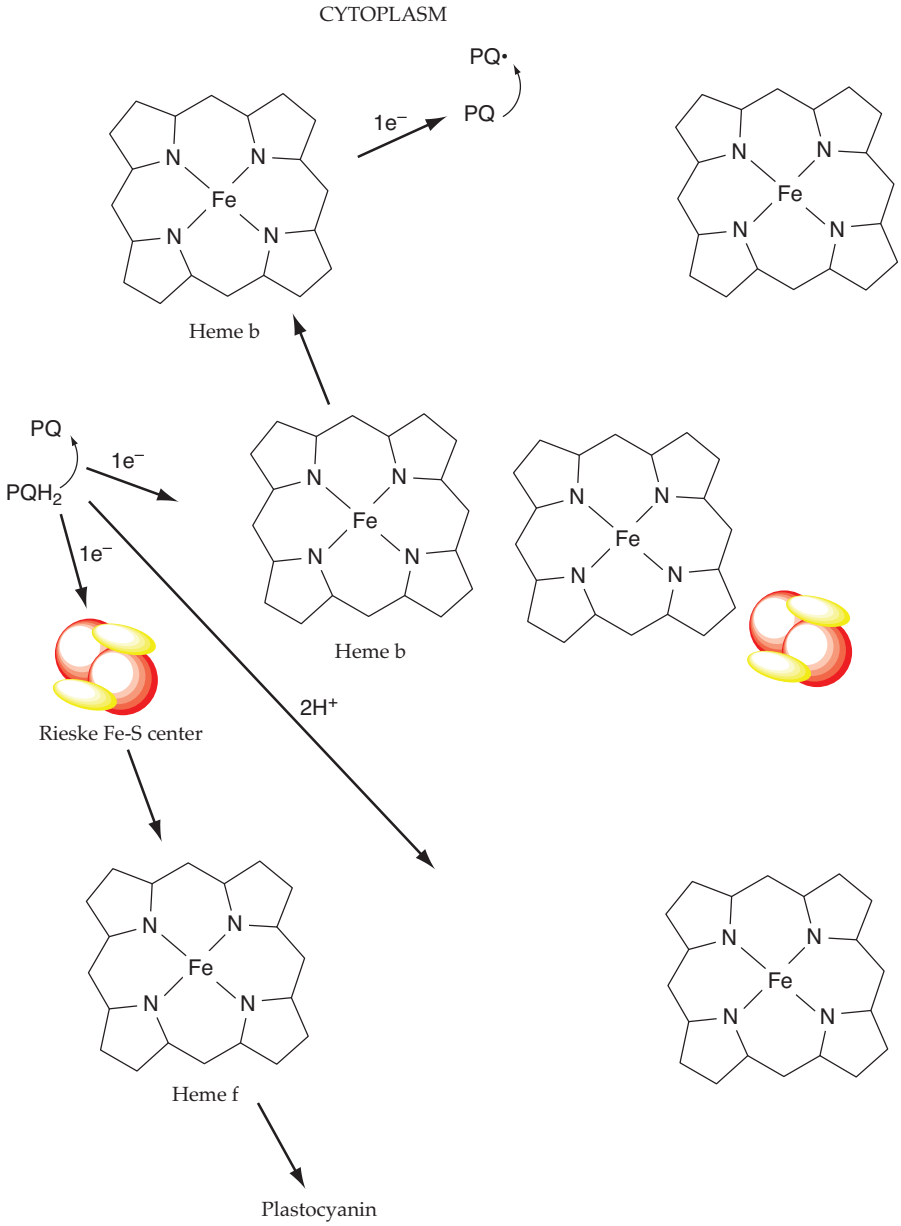


FIGURE 1.18 Structure of the cytochrome b_6f complex from the cyanobacterium, *Mastigocladus laminosus*. (a) The complex is shown parallel to the thylakoid membrane with the cytoplasmic side at the top and the lumenal side at the bottom. The cytochrome complex is a dimer with a twofold axis running perpendicular through the center of the complex. The monomer consists of four large and four small protein subunits and cofactors. The helical portion of the complex is embedded in the thylakoid membrane. Four heme groups are shown in red. A Rieske 2Fe-2S center is shown as red and yellow spheres. The cytochrome *f* protein is on the lumenal side of the complex and is shown as a magenta stick diagram. It provides a binding site for oxidized plastocyanin (not shown). (b) Q cycle in the cytochrome b_6f complex. Reduced plastoquinone binds at the left side of the figure to the Q_0 side. One electron is transferred to the Rieske 2Fe-2S cluster and then to the heme group in cytochrome *f*. Plastocyanin docks near this part of the complex on the lumenal side of the thylakoid membrane and accepts an electron from the cytochrome. The second electron from the reduced quinone is transferred to a heme group in cytochrome *b*. An oxidized quinone then binds at the Q_1 site on the right side of the figure. It accepts one electron from cytochrome *b* and remains bound to the complex. The cycle then repeats. Protons are transferred to the thylakoid lumen when the quinones are oxidized. (Structure from Protein Data Bank, 1vf5. G. Kurisu, H. Zhang, J. L. Smith, and W. A. Cramer, *Science* 302 [2003]: 1009–1014.)



During this second turnover, the semiquinone that is still bound to the complex is fully reduced, which requires two protons that are obtained from the stroma and transferred through the complex. The fully reduced quinol is released from its binding site and can be reoxidized by binding to the other site on the complex. The net result is that two protons are transferred from the stroma to the thylakoid lumen for every one electron that is transferred through the complex to plastocyanin. This enhances the production of an electrochemical potential (proton gradient) across the thylakoid membrane that can be used to drive the synthesis of ATP.

1.9 Photophosphorylation (ATP Synthesis)

The hydrolysis of ATP yields the energy needed to drive most cellular reactions.

$$ATP + H_2O \rightarrow ADP + Pi + H^+$$
$$\Delta G^o = -30 \text{ kJ mol}^{-1}$$

Under conditions in the chloroplast, $\Delta G \approx -52$ kJ mol^{-1}.

The energy needed to drive the reaction in the direction of ATP synthesis is obtained from the proton gradient that builds up in the thylakoid lumen during the light reaction. As a result of water oxidation and proton pumping in the Q cycle, the lumen becomes more acidic and the stroma, more alkaline.

Various methods have been used to demonstrate that the stroma becomes more alkaline by 1 to 1.5 pH units during the light reaction. A corresponding decrease in the lumen pH has been calculated so that a proton motive force can be described as

$$\Delta p = \Delta\Psi - 59\Delta\text{pH}$$

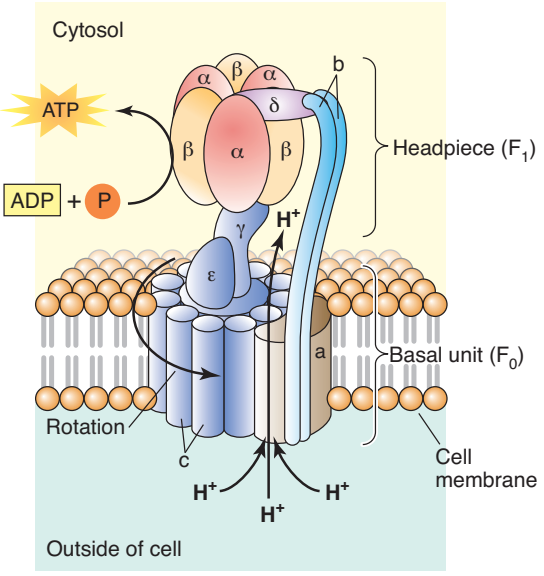
where p is the proton motive force and Ψ is the electrical potential. In chloroplasts, Mg^{2+} ions are released from the lumen in the light. This approximately balances the charge on the H^+ ions accumulated in the lumen, and, therefore, $\Delta\Psi$ is negligible. This is not true in mitochondria and in some photosynthetic bacteria.

The synthesis of ATP requires a membrane protein complex called the CF0CF1 ATP synthase (**FIGURE 1.19**). CF0 is a membrane-spanning protein complex that provides a "passage" for protons from the lumen to the stroma. CF1 is a protein complex that is connected to CF0 and protrudes into the stroma. It is the catalytic unit that, when isolated, has ATPase activity. The ATP synthase complexes from bacteria, mitochondria, and photosynthetic organisms are highly similar both at the primary and tertiary structural levels. Crystal structures of these enzyme complexes from *Escherichia coli* and bovine mitochondria are available at high resolution, and the spinach chloroplast enzyme is partially resolved.

CF1 is composed of five different polypeptides designated with Greek letters: α, β, γ, δ, and ε, in order of decreasing mass. The three α and three β subunits form the bulk of CF1 and have three binding sites for nucleotides at the α/β interfaces. The α and β subunits are arranged around a single γ protein that, together with the ε protein, form a connection to the CF0 complex.

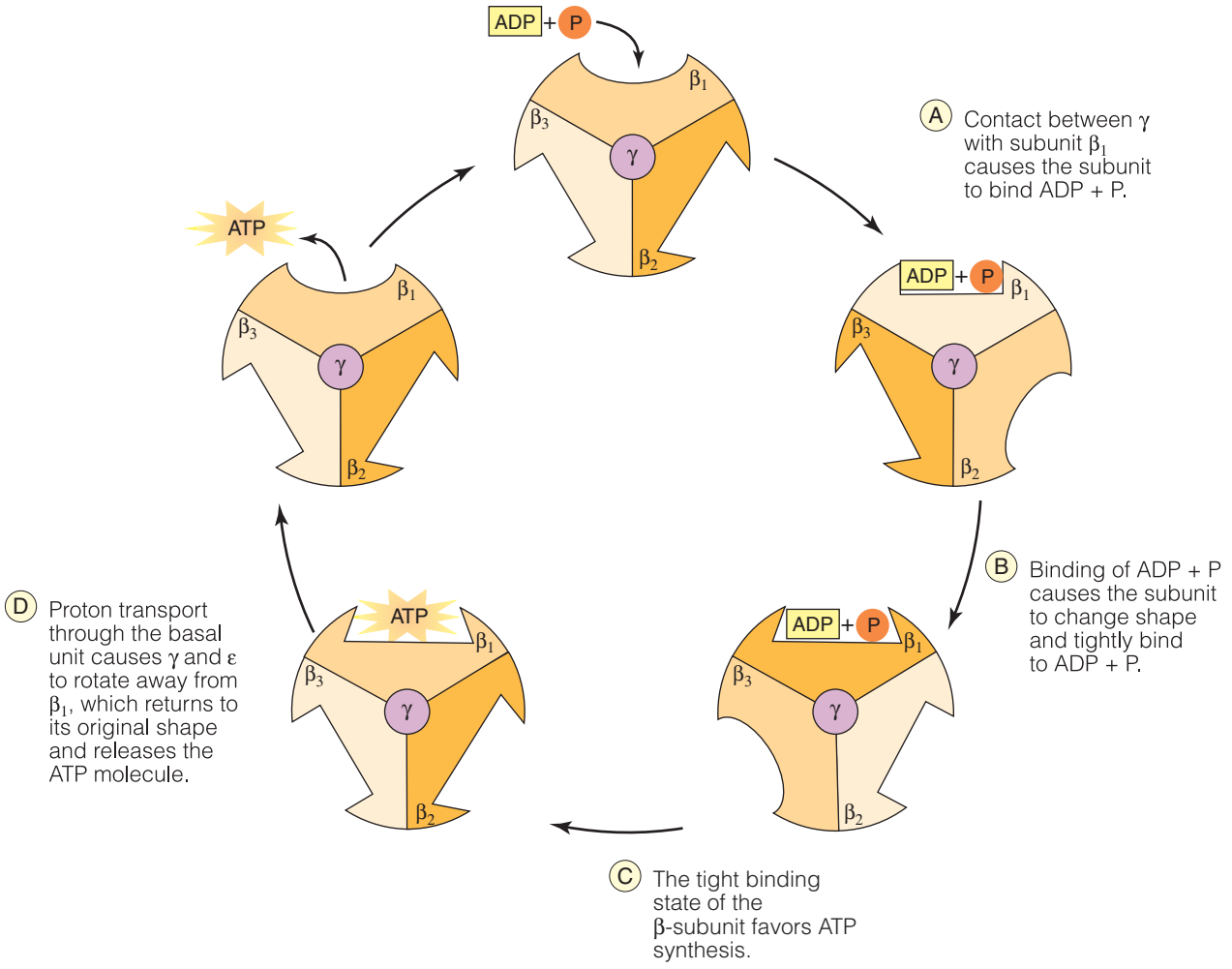
CF0 is embedded in the thylakoid membrane. It is composed of a single *a* subunit, a single *b* subunit, and a variable number of *c* subunits. The *c* subunits provide a passage for protons into the stroma through the otherwise impermeable thylakoid membrane. A specific aspartic acid residue (asp 61 in the *E. coli* enzyme) on *c* is protonated in the lumen and the uncharged protein then rotates into the hydrophobic bilayer. Deprotonation occurs at the *a/c* interface on the stromal side aided by an arginine residue in the *c* subunit (arg 210). The deprotonated aspartate must have an unusually high pK_a because it would be easier to move an uncharged residue through the bilayer back to a position facing the lumen. The physical rotation of the *c* subunits together with the connected γ/ε proteins provides the energy for a series of conformational changes in the three α/β subunit pairs. In the open conformation, an α/β unit binds ADP and Pi. In the subsequent closed conformation, ATP is produced in an anhydrous compartment where thermodynamics favors ATP synthesis.

A final, energy-requiring conformational change is required to return to the open conformation and release the newly synthesized ATP. In the above model the *b* and δ subunits form a stator that ensures that the *a* subunit and the α/β units do not rotate along with the γ/ε and *c* subunits and dissipate the kinetic energy generated by proton movement into the stroma. This elaborate ATP synthase complex converts the kinetic energy of proton movement into chemical bond energy of ATP.



(a)

FIGURE 1.19 Structure and function of ATP synthase. The model shown here is based on the crystal structure of the *E. coli* complex. Part a illustrates the basic architecture of the F_0 subunit composed of a, b, and several c polypeptides. This part of the complex is embedded in the cell membrane in the bacterium but would occupy a similar position in thylakoids of photosynthetic organisms. The γ peptide of F_1 protrudes into the cytoplasm or the chloroplast stroma, and is embedded in the center of the $\alpha_3\beta_3$ cluster of the complete F_0F_1 synthase. Protons in the periplasm or lumen bind to an aspartic acid residue in a C subunit that then rotates into the membrane. The proton is released on the cytoplasmic or stromal side of the membrane. This movement provides energy for conformational changes in the α/β subunits. Part b illustrates the nucleotide binding sites at the α/β interfaces. At A, ADP and inorganic phosphate bind to the loose (L) conformation. Proton transport in F_0 causes a conformation change to B where the binding site is closed or tight (T). The phosphodiester bond of ATP is formed in this state. Another conformation change, shown at D, is required to produce the open (O) state and release the ATP.



(b)

1.10 Light-Harvesting Complexes

Light Energy

The photosynthetic apparatus in the thylakoid membranes can be divided into two components, the electron transport chain described above and the antenna system, which consists of the proteins of the light-harvesting complexes (LHCs). The light energy that initiates electron transfer in PSI and PSII is rarely the result of direct excitation of the RCs from sunlight. Instead, solar energy is captured by the antenna system and funneled into the RCs. The light used in photosynthesis, 400 to 700 nm, has energy that is inversely proportional to the wavelength: $E = hc/\lambda$, where h is Planck's constant and c is the velocity of light in a vacuum (see Appendix 7 for definitions and values of constants). Light, however, can also be envisioned as massless particles called photons. The energy of a photon is a unit called a quantum. A quantum must be absorbed totally by the material with which it interacts. In the case of chlorophyll, this includes photons in the blue and red regions of the visible spectrum (Figure 1.7 and **FIGURE 1.20**). The light that is effective in photosynthesis is referred to as photosynthetically active radiation (PAR, usually expressed in µmol photons $m^{-2}s^{-1}$). Absorption of a quantum of energy boosts an electron in the chlorophyll molecule to a higher orbital. This excited state is unstable, and the electron will quickly return to the ground state. The energy is released as light (fluorescence) or heat or can be transferred to another molecule with the same quantum states. When calculating the energy absorbed by chlorophyll, light at 680 nm provides 175 kJ mol^{-1}, whereas light at 400 nm provides 296 kJ mol^{-1}.

Blue light might seem to be more efficient at promoting photosynthesis. However, as seen in Figure 1.20, the absorption of shorter wavelengths leads to a highly unstable second singlet state that quickly decays to the somewhat more stable energetic state similar to that produced by the absorption of red light. As experiments described previously have shown, red light is most effective in driving photosynthesis.

Light Harvesting

The major LHCs function to absorb most of the photosynthetically active radiation from light and transfer the excitation energy to the RCs. LHCs consist of several protein subunits, designated Lhca for those associated with LHCI and Lhcb for peptides associated with LHCII. These proteins bind various chlorophylls and carotenoids. LHCI is the complex associated with PSI, and LHCII is most often associated with PSII. The antenna peptides are sometimes referred to as cabs, chlorophyll *a/b* binding proteins, although in some organisms chlorophyll *b* is replaced by other types of chlorophylls. These proteins may also be referred to as CP proteins (i.e., chlorophyll-binding proteins) and have a number indicating their relative mobility on sodium dodecyl sulfate-polyacrylamide gels.

Because membrane proteins often have anomalous mobility on gels, the numbers may not reflect an accurate mass of the protein. The proteins of the LHCs are encoded by a family of nuclear genes referred to as *lhca* genes for proteins associated with LHCI and PSI and *lhcb* genes for proteins associated with LHCII. LHCI and LHCII are multimers of these Lhc proteins. All Lhc proteins have a similar tertiary structure composed of three membrane-spanning helices (**FIGURE 1.21**). They have significant amino acid similarity, particularly in the regions that bind the pigments; these include, in most cases, 12 to 15 chlorophylls and 2 to 4 carotenoids.

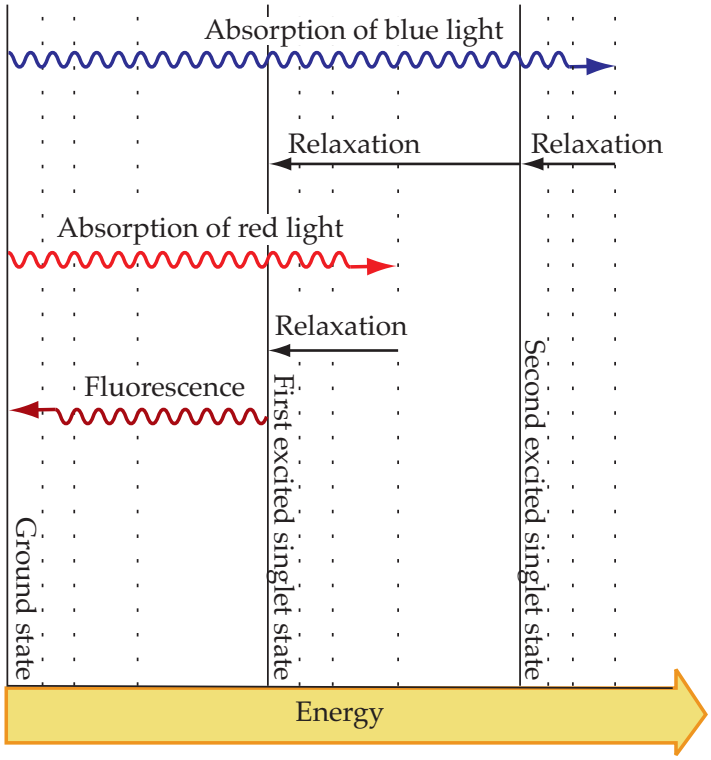


FIGURE 1.20 Molecular orbitals of chlorophyll. The diagram shows that excitation with blue light sends an electron to the second excited singlet state that quickly relaxes to the first excited singlet state. The energy is lost as heat. The first excited singlet state corresponds to the excitation due to red light. Basically, only energy from this state, corresponding to red light absorption, is available to drive photosynthesis. (*Adapted from*: Buchanan, et al., *Biochemistry and Molecular Biology of Plants*, Wiley-Blackwell, 2002. Pg. 573, Fig. 12.3.)

State Transitions

The number of LHCIs associated with PSI appears to be constant, whereas the LHCIIs associated with PSII depend on environmental conditions. Plants grown in high light (PAR > 400) usually have fewer LHCIIs than those grown under low light conditions. In general, PSI is more efficient at electron transfer than PSII. The D1 protein in PSII is also vulnerable to light-induced damage and must be constantly synthesized and replaced. The amount and positioning of LHCII is therefore controlled at several levels. The regulation of the amount of Lhcb polypeptides has been shown to require both transcriptional and translational controls. A more rapid regulation, however, occurs by the relocation of the LHCII within the thylakoid membrane, a process referred to as "state transitions."

In the normal functioning of the Z-scheme, there is an equal distribution of light to both photosystems; this is referred to as state 1. Under conditions when more light energy is funneled into PSII than can be used for electron transfer, the plastoquinone pool will be highly reduced. This triggers movement of the LHCIIs away from PSII, and energy is then mainly directed into PSI, referred to as state 2. This lateral movement of LHCII refers to its redistribution in the thylakoid membranes of vascular plants with their elaborate architecture of granal and stromal thylakoids.

As shown in **FIGURE 1.22**, the PSI–LHCI complexes are mainly located in the stromal

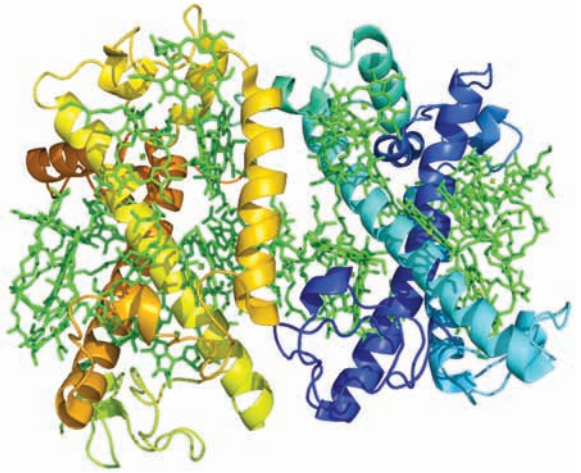


FIGURE 1.21 Structure of the LHCII from spinach. The crystal structure shows a helical molecule with 14 chlorophylls and 4 carotenoids, all colored green. The pigments are not covalently bound to the protein. The LHCs are integral membrane proteins; the helices and attached pigments span the lipid bilayer of the thylakoid membranes. (Structure from Protein Data Bank, 1rwt. Z. Liu, H. Yan, K. Wang, T. Kuang, J. Zhang, L. Gui, X. An, and W. Chang, *Nature* 428 [2004]: 287–292.)

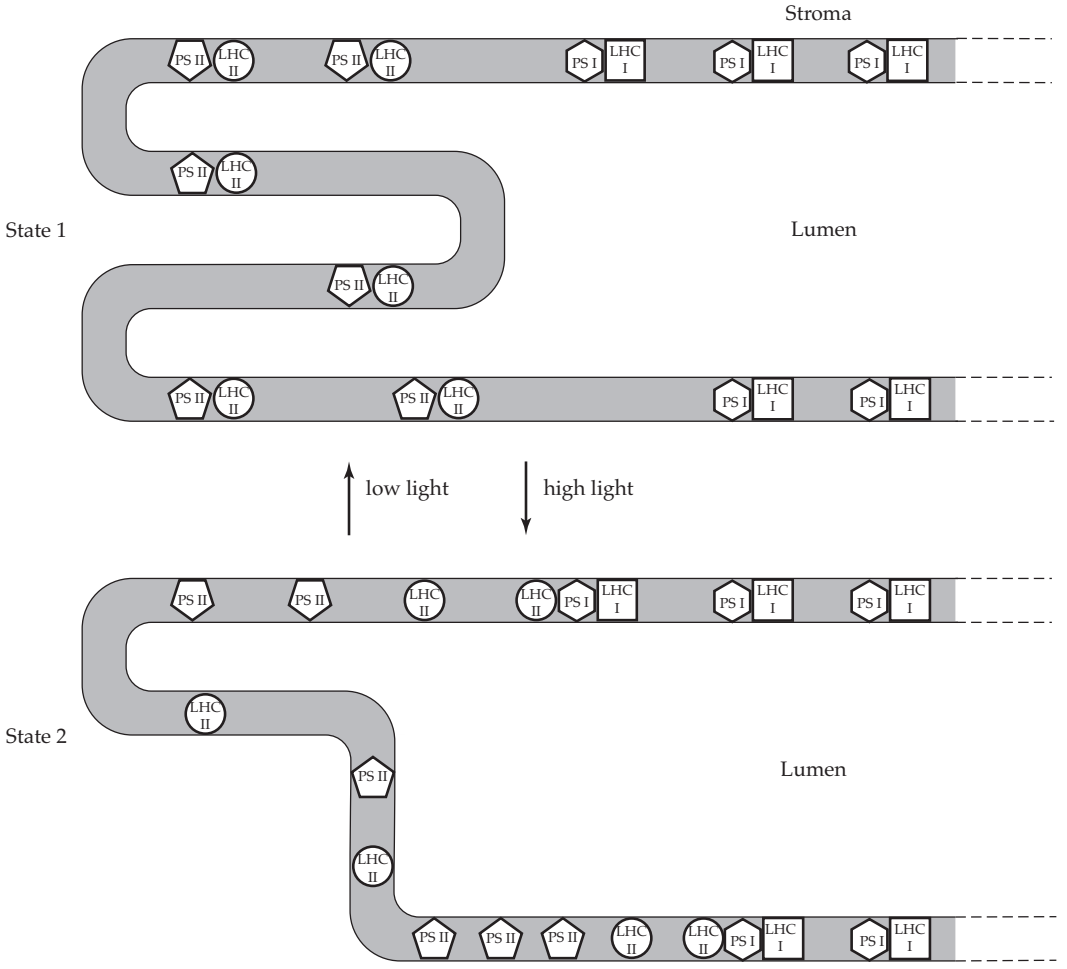


FIGURE 1.22 State transitions. The four integral membrane components of the light reaction are not distributed randomly in the thylakoids. In angiosperms, PSII is mainly located in the granal stacks, whereas PSI is found in the single, stromal portion of the thylakoids. The amount of LHCI associated with PSI is constant, but the association of LHCII with PSII is variable. In low to moderate light conditions, LHCII is associated with PSII in the grana; this is referred to as state 1. Under high light conditions, LHCII is phosphorylated and moves away from PSII and can associate with PSI. More light energy is then channeled into PSI as shown in state 2. State transitions may also be accompanied by changes in the stacking of the thylakoids.

thylakoids, whereas PSII–LHCII complexes are associated with the granal stacks. In state 2 a reorganization occurs in which LHCII is no longer closely associated with PSII and can form a complex with PSI. This may also involve a reorientation of the membrane stacks in addition to a physical movement of the proteins in the thylakoids. A loosening of the thylakoid structure in state 2 could also facilitate replacement of the D1 protein without total disruption of the PSII complex. The state 2 arrangement funnels more energy into PSI and promotes cyclic electron transport. In this state, ferredoxin that is reduced by PSI donates electrons to the cytochrome b_6f complex by a reversal of the ferredoxin-NADP reductase reaction. NADPH can also serve as a cyclic electron donor via a membrane-bound NADPH dehydrogenase. Cyclic electron transport ensures continued production of a proton gradient in the lumen and ATP synthesis without NADP reduction.

What promotes this movement of LHCII and/or membrane reorganization? It has been shown that one of the proteins of the LHCII can be phosphorylated on a threonine residue near its N-terminus by a thylakoid-associated kinase. This protein kinase is activated by redox conditions in the chloroplast, such as a highly reduced plastoquinone pool and elevated pH in the stroma. A phosphorylated LHCII is electrostatically repelled by PSII and moves out of its immediate vicinity, constituting a state 2 configuration with more energy directed into PSI. A change in the chloroplast redox state, as in darkness, inactivates the kinase. A phosphatase removes the Pi from LHCII, allowing it to reassociate with PSII and return to the state 1 configuration.

1.11 Nonphotochemical Quenching

Dissipation of excess light energy can also occur by a process called nonphotochemical quenching (NPQ) during which light energy is converted to radiation that is not active in driving photochemistry. Nonphotochemical quenching is facilitated by specific carotenoids organized into a pathway called the xanthophyll cycle (**FIGURE 1.23**). This cycle is triggered by excess acidification of the

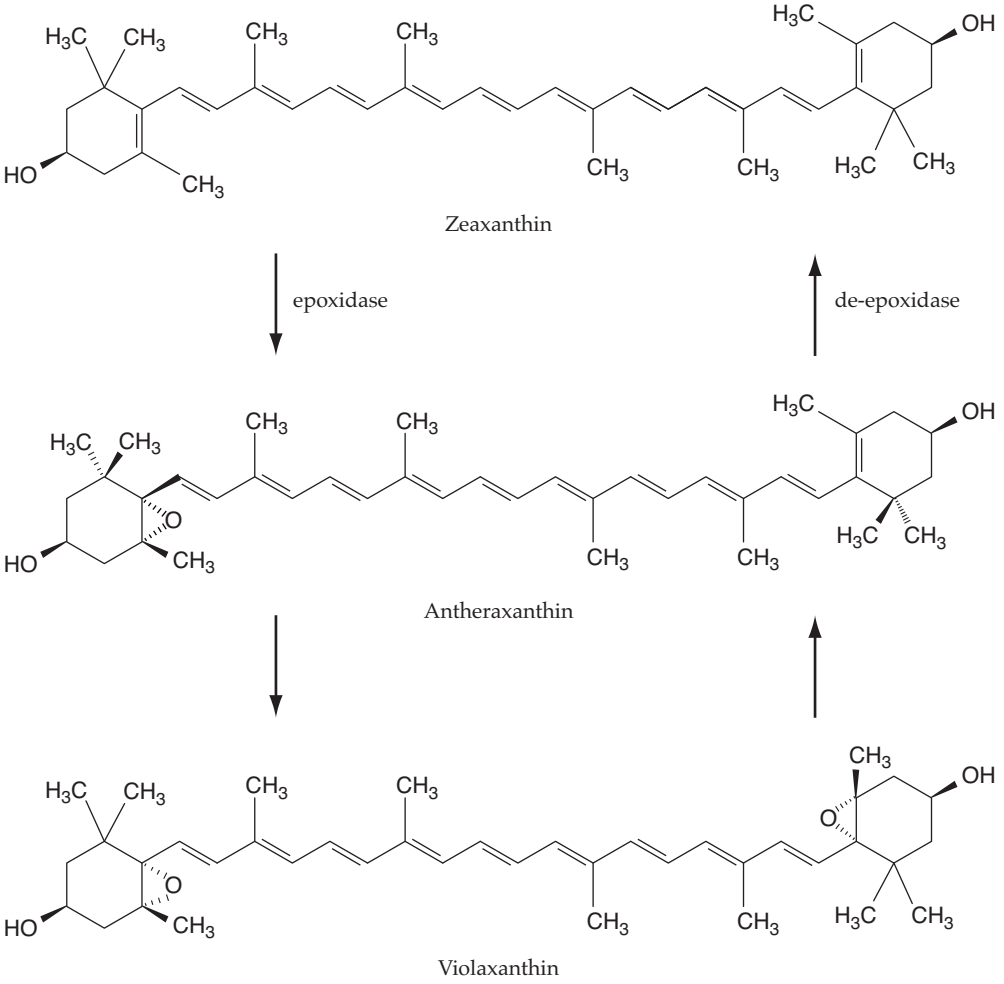


FIGURE 1.23 Xanthophyll cycle. Excess light energy can be dissipated as heat via processes called nonphotochemical quenching (NPQ). The best-characterized mechanism is the de-epoxidation of violaxanthin. The de-epoxidase enzyme is located in the thylakoid lumen and is activated at low pH; it catalyzes a transition from violaxanthin to zeaxanthin. The additional conjugation of the zeaxanthin molecule puts it at a lower energy level than the epoxide carotenoids, which accounts for the heat lost in these reactions.

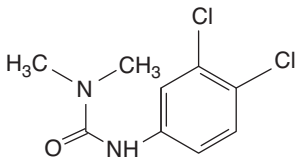
thylakoid lumen. The low pH activates violaxanthin de-epoxidase, a water-soluble enzyme found in the lumen, which catalyzes the de-epoxidation of violaxanthin to zeaxanthin as shown in Figure 1.23. Zeaxanthin has a higher degree of conjugation and is therefore at a lower energy state. Epoxidation conversely requires energy, and thus the cycle serves to absorb excess light energy and uses it to alter chemical bonds and produce heat.

1.12 Photosynthesis Inhibitors: Herbicides

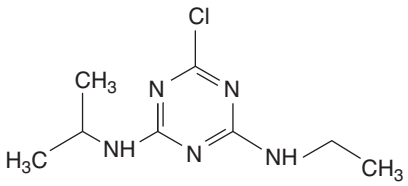
It is a common practice in biochemical research to use inhibitors that are specific for the reaction or pathway being investigated. Several types of inhibitors have been used to study the light reaction, some of which are also used commercially as herbicides. The substituted ureas and triazines (**FIGURE 1.24**) block electron transport in PSII. They compete for the quinone B binding site on the D1 protein. Some plants, such as maize, are resistant to triazines because they have an effective system to detoxify the herbicide. Both compounds are presumably specific for PSII and thus should pose no toxicity to animals or other nontarget organisms. However, atrazine, at high concentrations, inhibits mitochondrial electron transport. In addition, because of their chlorine substituents, the triazines are particularly resistant to microbial decay. In temperate environments, atrazine may accumulate to toxic levels in drinking water supplies.

Paraquat (Figure 1.24) inhibits electron transport from PSI. It can accept an electron from PSI and inhibit reduction of ferredoxin and NADP. In addition, it can transfer an electron to oxygen, causing the formation of superoxide and hydroxyl radicals that damage the photosynthetic membranes (see Appendix 5). This dual effect makes paraquat an extremely effective herbicide. However, it also causes the formation of oxygen radicals in many other organisms. Use of paraquat as a herbicide is banned in the United States because of its potential for causing DNA damage and cancer in humans and other animals.

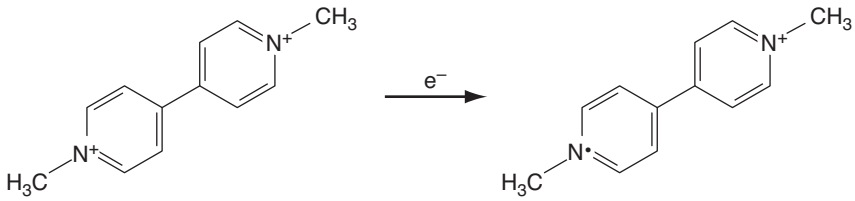
FIGURE 1.24 Herbicides that inhibit the light reaction. DCMU and atrazine compete with oxidized quinone B at its binding site on the D1 protein in PSII. Paraquat will accept an electron from PSI and inhibit reduction of ferredoxin. The paraquat radical will also initiate chemical reactions that can cause membrane damage.



Dichlorophenyl dimethyl urea (DCMU, Diuron)



Atrazine



Paraquat

CHAPTER SUMMARY

Photosynthesis is the essential process that supports most life on our planet. Both bacterial and green plant photosynthesis are characterized by similar processes:

a. Capture of light energy by antenna pigments
b. Transfer of the light energy to RCs
c. Using the light energy to free an electron from the RC and transfer it to cofactors that are then used to reduce CO_2
d. Using light energy to generate a proton gradient that can be used to synthesize ATP.

$$2NADP + Pi + ADP + 2H_2O \xrightarrow{\text{Light}} 2NADPH + 2H^+ + ATP + O_2$$

The overall light reaction shown above is common to organisms that perform oxygen-evolving photosynthesis. This reaction requires two RCs that work in series and

are embedded in thylakoid membranes. A precise orientation of the components in the membrane is essential for their function.

PSI consists of several antenna pigments that transfer excitation energy to the RC that contains two chlorophyll *a* molecules, P_{700}. P_{700} is oxidized, and the electron is transferred through a series of pigments to an Fe-S subunit in the RC. This subunit then reduces a peripheral membrane protein, ferredoxin, located on the stromal side of the thylakoid membrane. Ferredoxin, in turn, donates electrons to NADP. P_{700}^+ is reduced by plastocyanin, another peripheral membrane protein located on the lumenal side of the thylakoid membrane. Plastocyanin is reduced by a series of electron transfer events that originate in PSII. PSII also consists of several antenna pigments that transfer excitation energy to a pair of chlorophyll molecules (P_{680}) in the RC core. P_{680} is oxidized, and an electron is transferred to quinone B. Two photo events are required to reduce the quinone to the quinol, which then leaves the RC and diffuses into to the thylakoid lipid bilayer where it is reoxidized by a cytochrome b_6f complex. This complex reduces plastocyanin and also pumps protons into the thylakoid lumen. P_{680}^+ is reduced by a 4Mn-1Ca cluster in the RC that oxidizes H_2O and transfers electrons sequentially to P_{680}. The protons generated by the oxidation of water remain in the lumen. The proton gradient that builds up in the lumen supplies the energy for ATP synthesis via an integral membrane complex, the chloroplast ATP synthase. The NADPH and ATP generated in the light reaction are used to drive CO_2 fixation in the chloroplast stroma, as described in Chapter 2. The oxygen generated in the light reaction is released from the cell but is essential for all organisms, including plants, which also catabolize carbohydrates by aerobic pathways described in Chapter 3.

REFERENCES FOR FURTHER INFORMATION

Allen, J.P., Seng, C., & Larson, C. (2009). Structures of proteins and cofactors: X-ray crystallography. *Photosyn. Res.* **102:**231–240.

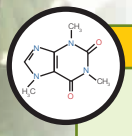
Amunts, A. & Nelson, N. (2008). Functional organization of a plant photosystem I: Evolution of a highly efficient photochemical machine. *Plant Physiol. & Biochem.* **46:**228–237.

Blankenship, R.E. (2002). History and early developments in photosynthesis, In: *Molecular Mechanisms of Photosynthesis*, Oxford, UK: Blackwell Science, pp. 26–41.

Boyer, P.D. (2002). A research journey with ATP synthase. *J. Biol. Chem.* **277:**39045–39061.

Kargul, J. & Barber, J. (2008). Photosynthetic acclimation: Structural reorganization of light harvesting antenna—role of redox-dependent phosphorylation of major and minor chlorophyll a/b binding proteins. *FEBS J.* **275:**1056–1068.

Nelson, N. & Yocum, C.E. (2006). Structure and function of photosystems I and II. *Annu. Rev. Plant Biol.* **57:**521–565.



PLANT BIOCHEMISTRY *on the web*

Visit the *Plant Biochemistry* student companion Web site at http://biology.jbpub.com/gleason/plantbiochemistry/ for additional online study resources on these topics.