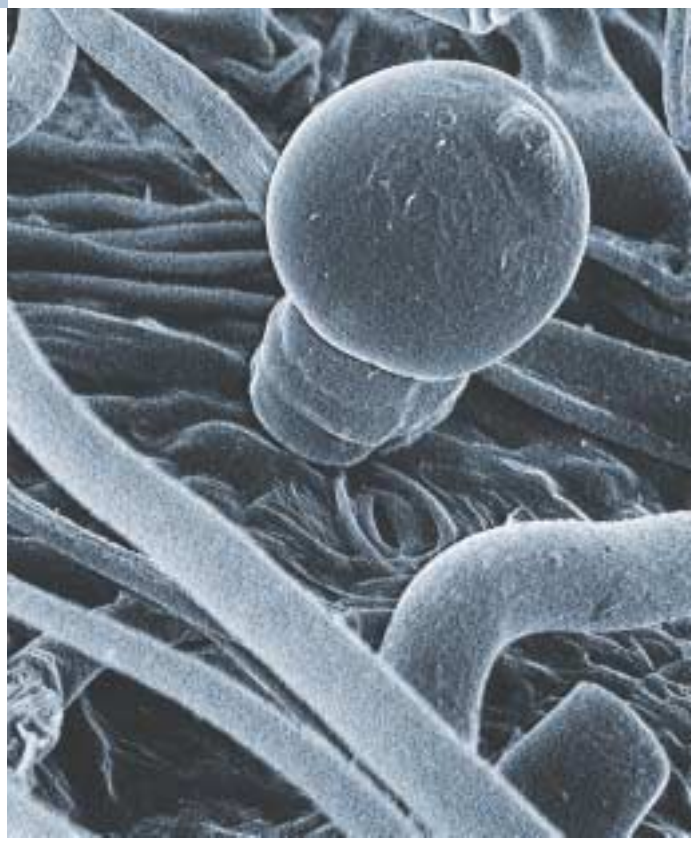
UNIT

III

Biochemistry and Metabolism



Chapter

7

Photosynthesis: The Light Reactions

LIFE ON EARTH ULTIMATELY DEPENDS ON ENERGY derived from the sun. Photosynthesis is the only process of biological importance that can harvest this energy. In addition, a large fraction of the planet's energy resources results from photosynthetic activity in either recent or ancient times (fossil fuels). This chapter introduces the basic physical principles that underlie photosynthetic energy storage and the current understanding of the structure and function of the photosynthetic apparatus (Blankenship 2002).

The term *photosynthesis* means literally "synthesis using light." As we will see in this chapter, photosynthetic organisms use solar energy to synthesize carbon compounds that cannot be formed without the input of energy. More specifically, light energy drives the synthesis of carbohydrates from carbon dioxide and water with the generation of oxygen:

$$\underset{\text{Carbon dioxide}}{6\,CO_2} + \underset{\text{Water}}{6\,H_2O} \rightarrow \underset{\text{Carbohydrate}}{C_6H_{12}O_6} + \underset{\text{Oxygen}}{6\,O_2}$$

Energy stored in these molecules can be used later to power cellular processes in the plant and can serve as the energy source for all forms of life.

This chapter deals with the role of light in photosynthesis, the structure of the photosynthetic apparatus, and the processes that begin with the excitation of chlorophyll by light and culminate in the synthesis of ATP and NADPH.

PHOTOSYNTHESIS IN HIGHER PLANTS

The most active photosynthetic tissue in higher plants is the mesophyll of leaves. Mesophyll cells have many chloroplasts, which contain the specialized light-absorbing green pigments, the **chlorophylls**. In photosynthesis, the plant uses solar energy to oxidize water, thereby releasing oxygen, and to reduce carbon dioxide, thereby forming large carbon compounds, primarily sugars. The complex series of reactions that cul-

minate in the reduction of CO_2 include the thylakoid reactions and the carbon fixation reactions.

The **thylakoid reactions** of photosynthesis take place in the specialized internal membranes of the chloroplast called thylakoids (see Chapter 1). The end products of these thylakoid reactions are the high-energy compounds ATP and NADPH, which are used for the synthesis of sugars in the **carbon fixation reactions**. These synthetic processes take place in the stroma of the chloroplasts, the aqueous region that surrounds the thylakoids. The thylakoid reactions of photosynthesis are the subject of this chapter; the carbon fixation reactions are discussed in Chapter 8.

In the chloroplast, light energy is converted into chemical energy by two different functional units called *photosystems*. The absorbed light energy is used to power the transfer of electrons through a series of compounds that act as electron donors and electron acceptors. The majority of electrons ultimately reduce $NADP^+$ to NADPH and oxidize H_2O to O_2. Light energy is also used to generate a proton motive force (see Chapter 6) across the thylakoid membrane, which is used to synthesize ATP.

GENERAL CONCEPTS

In this section we will explore the essential concepts that provide a foundation for an understanding of photosynthesis. These concepts include the nature of light, the properties of pigments, and the various roles of pigments.

Light Has Characteristics of Both a Particle and a Wave

A triumph of physics in the early twentieth century was the realization that light has properties of both particles and waves. A wave (Figure 7.1) is characterized by a **wavelength**, denoted by the Greek letter lambda (λ), which is the distance between successive wave crests. The **frequency**, represented by the Greek letter nu (ν), is the number of wave crests that pass an observer in a given time. A simple equation relates the wavelength, the frequency, and the speed of any wave:

$$c = \lambda\nu \qquad (7.1)$$

where c is the speed of the wave—in the present case, the speed of light (3.0×10^8 m s^{-1}). The light wave is a transverse (side-to-side) electromagnetic wave, in which

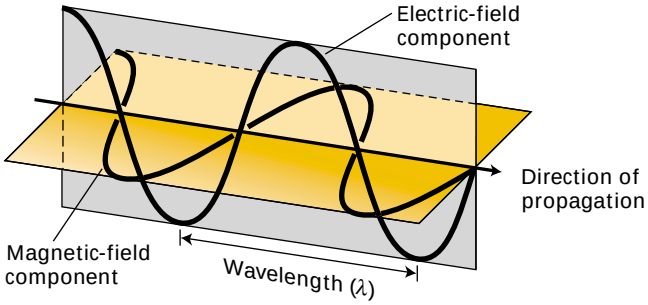


FIGURE 7.1 Light is a transverse electromagnetic wave, consisting of oscillating electric and magnetic fields that are perpendicular to each other and to the direction of propagation of the light. Light moves at a speed of 3×10^8 m s^{-1}. The wavelength (λ) is the distance between successive crests of the wave.

both electric and magnetic fields oscillate perpendicularly to the direction of propagation of the wave and at 90° with respect to each other.

Light is also a particle, which we call a **photon**. Each photon contains an amount of energy that is called a **quantum** (plural *quanta*). The energy content of light is not continuous but rather is delivered in these discrete packets, the quanta. The energy (E) of a photon depends on the frequency of the light according to a relation known as Planck's law:

$$E = h\nu \qquad (7.2)$$

where h is Planck's constant (6.626×10^{-34} J s).

Sunlight is like a rain of photons of different frequencies. Our eyes are sensitive to only a small range of frequencies—the visible-light region of the electromagnetic spectrum (Figure 7.2). Light of slightly higher frequencies (or

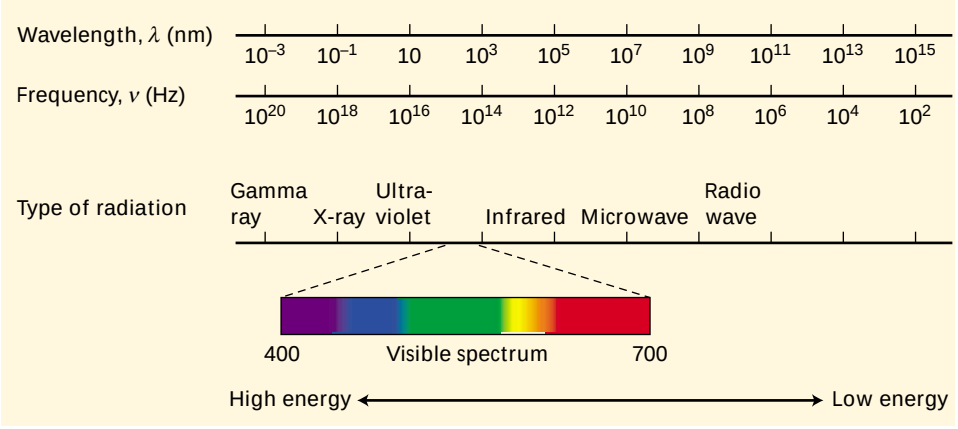


FIGURE 7.2 Electromagnetic spectrum. Wavelength (λ) and frequency (ν) are inversely related. Our eyes are sensitive to only a narrow range of wavelengths of radiation, the visible region, which extends from about 400 nm (violet) to about 700 nm (red). Short-wavelength (high-frequency) light has a high energy content; long-wavelength (low-frequency) light has a low energy content.

shorter wavelengths) is in the ultraviolet region of the spectrum, and light of slightly lower frequencies (or longer wavelengths) is in the infrared region. The output of the sun is shown in Figure 7.3, along with the energy density that strikes the surface of Earth. The absorption spectrum of chlorophyll *a* (curve C in Figure 7.3) indicates approximately the portion of the solar output that is utilized by plants.

An **absorption spectrum** (plural *spectra*) displays the amount of light energy taken up or absorbed by a molecule or substance as a function of the wavelength of the light. The absorption spectrum for a particular substance in a nonabsorbing solvent can be determined by a spectrophotometer as illustrated in Figure 7.4. Spectrophotometry, the technique used to measure the absorption of light by a sample, is more completely discussed in Web Topic 7.1.

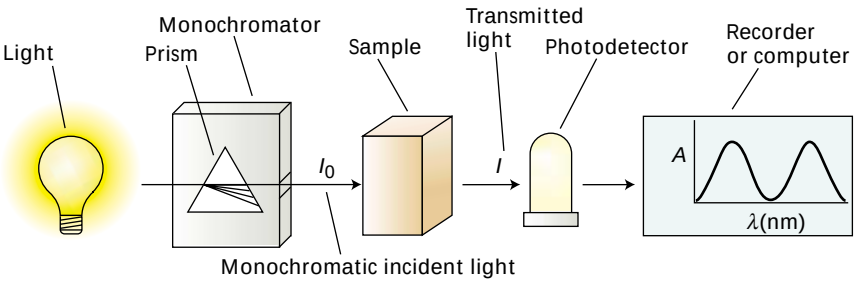


FIGURE 7.4 Schematic diagram of a spectrophotometer. The instrument consists of a light source, a monochromator that contains a wavelength selection device such as a prism, a sample holder, a photodetector, and a recorder or computer. The output wavelength of the monochromator can be changed by rotation of the prism; the graph of absorbance (*A*) versus wavelength (λ) is called a spectrum.

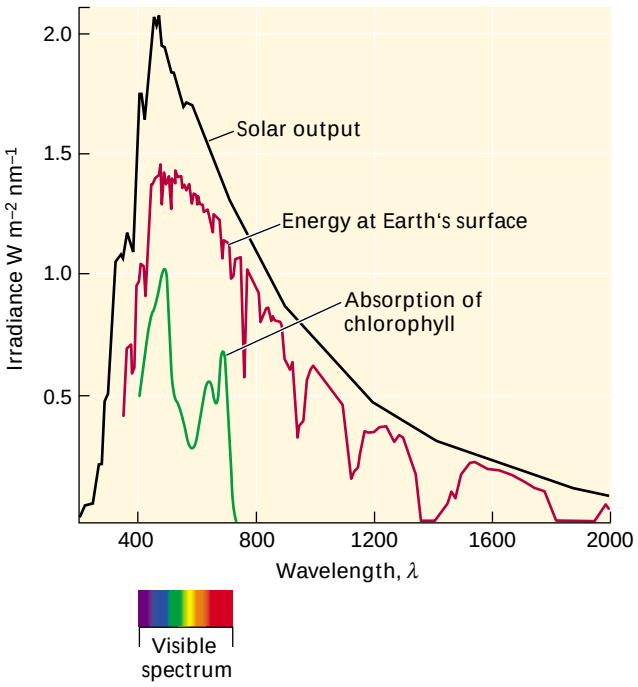


FIGURE 7.3 The solar spectrum and its relation to the absorption spectrum of chlorophyll. Curve A is the energy output of the sun as a function of wavelength. Curve B is the energy that strikes the surface of Earth. The sharp valleys in the infrared region beyond 700 nm represent the absorption of solar energy by molecules in the atmosphere, chiefly water vapor. Curve C is the absorption spectrum of chlorophyll, which absorbs strongly in the blue (about 430 nm) and the red (about 660 nm) portions of the spectrum. Because the green light in the middle of the visible region is not efficiently absorbed, most of it is reflected into our eyes and gives plants their characteristic green color.

When Molecules Absorb or Emit Light, They Change Their Electronic State

Chlorophyll appears green to our eyes because it absorbs light mainly in the red and blue parts of the spectrum, so only some of the light enriched in green wavelengths (about 550 nm) is reflected into our eyes (see Figure 7.3).

The absorption of light is represented by Equation 7.3, in which chlorophyll (Chl) in its lowest-energy, or ground, state absorbs a photon (represented by $h\nu$) and makes a transition to a higher-energy, or excited, state (Chl*):

$$\text{Chl} + h\nu \rightarrow \text{Chl}^* \qquad (7.3)$$

The distribution of electrons in the excited molecule is somewhat different from the distribution in the ground-state molecule (Figure 7.5) Absorption of blue light excites the chlorophyll to a higher energy state than absorption of red light because the energy of photons is higher when their wavelength is shorter. In the higher excited state, chlorophyll is extremely unstable, very rapidly gives up some of its energy to the surroundings as heat, and enters the lowest excited state, where it can be stable for a maximum of several nanoseconds (10^{-9} s). Because of this inherent instability of the excited state, any process that captures its energy must be extremely rapid.

In the lowest excited state, the excited chlorophyll has four alternative pathways for disposing of its available energy.

1. Excited chlorophyll can re-emit a photon and thereby return to its ground state—a process known as **fluorescence**. When it does so, the wavelength of fluorescence is slightly longer (and of lower energy) than the wavelength of absorption because a portion of the excitation energy is converted into heat before the fluorescent photon is emitted. Chlorophylls fluoresce in the red region of the spectrum.
2. The excited chlorophyll can return to its ground state by directly converting its excitation energy into heat, with no emission of a photon.

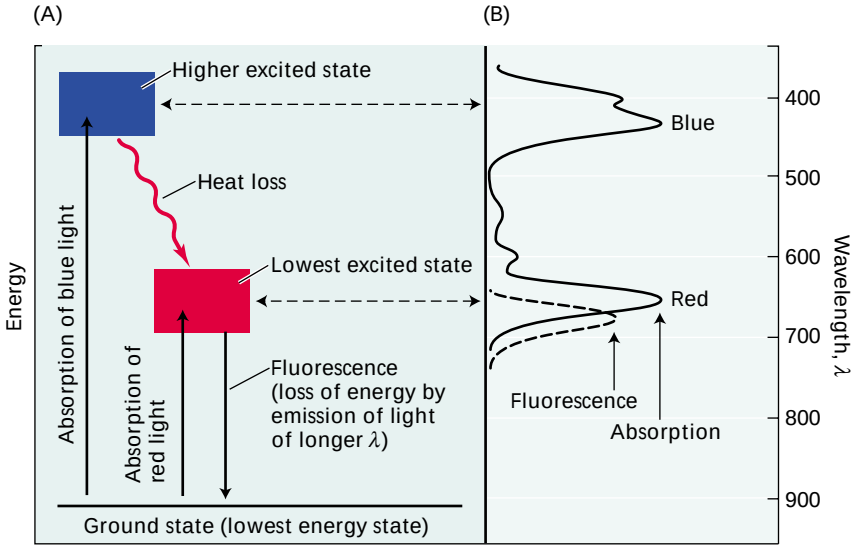
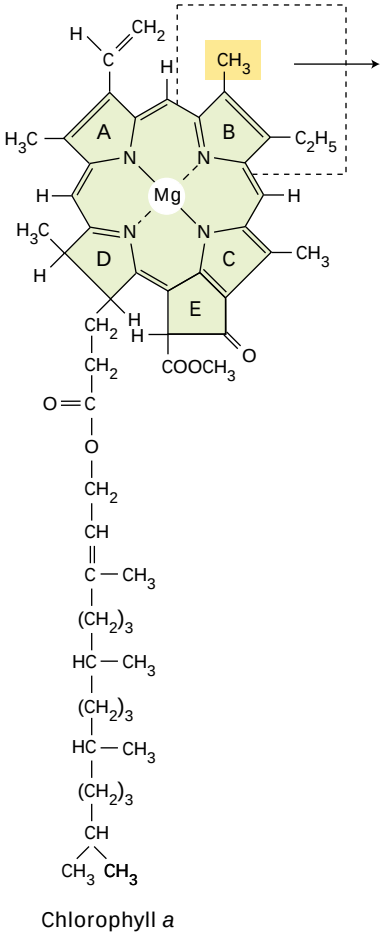
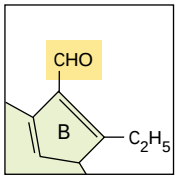


FIGURE 7.5 Light absorption and emission by chlorophyll. (A) Energy level diagram. Absorption or emission of light is indicated by vertical lines that connect the ground state with excited electron states. The blue and red absorption bands of chlorophyll (which absorb blue and red photons, respectively) correspond to the upward vertical arrows, signifying that energy absorbed from light causes the molecule to change from the ground state to an excited state. The downward-pointing arrow indicates fluorescence, in which the molecule goes from the lowest excited state to the ground state while re-emitting energy as a photon. (B) Spectra of absorption and fluorescence. The long-wavelength (red) absorption band of chlorophyll corresponds to light that has the energy required to cause the transition from the ground state to the first excited state. The short-wavelength (blue) absorption band corresponds to a transition to a higher excited state.

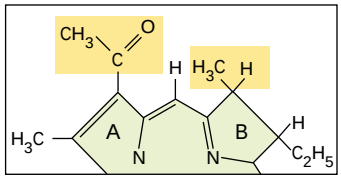
(A) **Chlorophylls**



Chlorophyll *a*

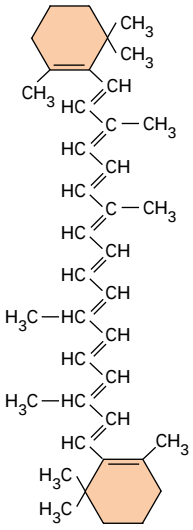


Chlorophyll *b*



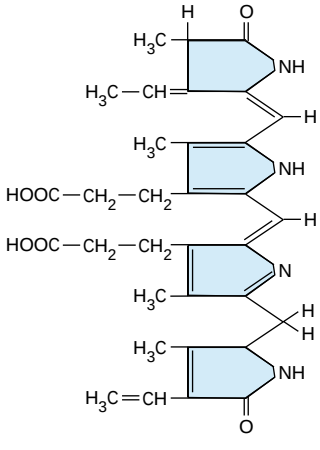
Bacteriochlorophyll *a*

(B) **Carotenoids**



β-Carotene

(C) **Bilin pigments**



Phycoerythrobilin

3. Chlorophyll may participate in **energy transfer**, during which an excited chlorophyll transfers its energy to another molecule.
4. A fourth process is **photochemistry**, in which the energy of the excited state causes chemical reactions to occur. The photochemical reactions of photosynthesis are among the fastest known chemical reactions. This extreme speed is necessary for photochemistry to compete with the three other possible reactions of the excited state just described.

Photosynthetic Pigments Absorb the Light That Powers Photosynthesis

The energy of sunlight is first absorbed by the pigments of the plant. All pigments active in photosynthesis are found in the chloroplast. Structures and absorption spectra of several photosynthetic pigments are shown in Figures 7.6 and 7.7, respectively. The chlorophylls and **bacteriochlorophylls** (pigments found in certain bacteria) are the typical pigments of photosynthetic organisms, but all organisms contain a mixture of more than one kind of pigment, each serving a specific function.

Chlorophylls *a* and *b* are abundant in green plants, and *c* and *d* are found in some protists and cyanobacteria. A number of different types of bacteriochlorophyll have been found; type *a* is the most widely distributed. **Web Topic 7.2** shows the distribution of pigments in different types of photosynthetic organisms.

All chlorophylls have a complex ring structure that is chemically related to the porphyrin-like groups found in hemoglobin and cytochromes (see Figure 7.6A). In addition, a long hydrocarbon tail is almost always attached to the ring structure. The tail anchors the chlorophyll to the hydrophobic portion of its environment. The ring structure contains some loosely bound electrons and is the part of the molecule involved in electron transitions and redox reactions.

The different types of **carotenoids** found in photosynthetic organisms are all linear molecules with multiple conjugated double bonds (see Figure 7.6B). Absorption bands in the 400 to 500 nm region give carotenoids their characteristic orange color. The color of carrots, for example, is due to the carotenoid β-carotene, whose structure and absorption spectrum are shown in Figures 7.6 and 7.7, respectively.

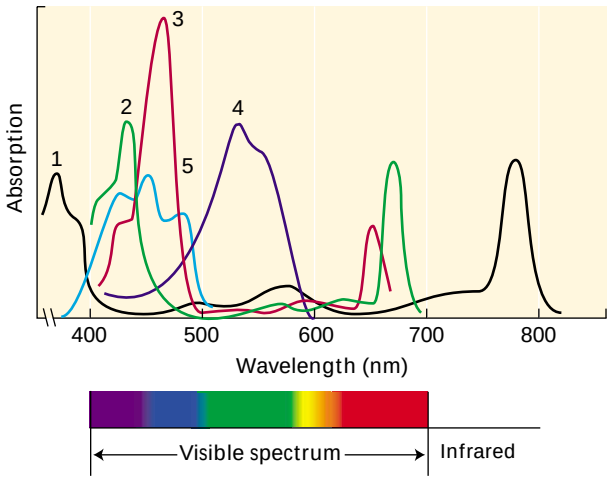


FIGURE 7.7 Absorption spectra of some photosynthetic pigments. Curve 1, bacteriochlorophyll *a*; curve 2, chlorophyll *a*; curve 3, chlorophyll *b*; curve 4, phycoerythrobilin; curve 5, β-carotene. The absorption spectra shown are for pure pigments dissolved in nonpolar solvents, except for curve 4, which represents an aqueous buffer of phycoerythrin, a protein from cyanobacteria that contains a phycoerythrobilin chromophore covalently attached to the peptide chain. In many cases the spectra of photosynthetic pigments in vivo are substantially affected by the environment of the pigments in the photosynthetic membrane. (After Avers 1985.)

Carotenoids are found in all photosynthetic organisms, except for mutants incapable of living outside the laboratory. Carotenoids are integral constituents of the thylakoid membrane and are usually associated intimately with both antenna and reaction center pigment proteins. The light absorbed by the carotenoids is transferred to chlorophyll for photosynthesis; because of this role they are called **accessory pigments**.

KEY EXPERIMENTS IN UNDERSTANDING PHOTOSYNTHESIS

Establishing the overall chemical equation of photosynthesis required several hundred years and contributions by many scientists (literature references for historical developments can be found on the web site). In 1771, Joseph Priestley observed that a sprig of mint growing in air in which a candle had burned out improved the air so that another candle could burn. He had discovered oxygen evolution by plants. A Dutchman, Jan Ingenhousz, documented the essential role of light in photosynthesis in 1779.

Other scientists established the roles of CO_2 and H_2O and showed that organic

◀ **FIGURE 7.6** Molecular structure of some photosynthetic pigments. (A) The chlorophylls have a porphyrin-like ring structure with a magnesium atom (Mg) coordinated in the center and a long hydrophobic hydrocarbon tail that anchors them in the photosynthetic membrane. The porphyrin-like ring is the site of the electron rearrangements that occur when the chlorophyll is excited and of the unpaired electrons when it is either oxidized or reduced. Various chlorophylls differ chiefly in the substituents around the rings and the pattern of double bonds. (B) Carotenoids are linear polyenes that serve as both antenna pigments and photoprotective agents. (C) Bilin pigments are open-chain tetrapyrroles found in antenna structures known as phycobilisomes that occur in cyanobacteria and red algae.

matter, specifically carbohydrate, is a product of photosynthesis along with oxygen. By the end of the nineteenth century, the balanced overall chemical reaction for photosynthesis could be written as follows:

$$6\,CO_2 + 6\,H_2O \xrightarrow{\text{Light, plant}} C_6H_{12}O_6 + 6\,O_2 \qquad (7.4)$$

where $C_6H_{12}O_6$ represents a simple sugar such as glucose. As will be discussed in Chapter 8, glucose is not the actual product of the carbon fixation reactions. However, the energetics for the actual products is approximately the same, so the representation of glucose in Equation 7.4 should be regarded as a convenience but not taken literally.

The chemical reactions of photosynthesis are complex. In fact, at least 50 intermediate reaction steps have now been identified, and undoubtedly additional steps will be discovered. An early clue to the chemical nature of the essential chemical process of photosynthesis came in the 1920s from investigations of photosynthetic bacteria that did not produce oxygen as an end product. From his studies on these bacteria, C. B. van Niel concluded that photosynthesis is a redox (reduction–oxidation) process. This conclusion has been confirmed, and it has served as a fundamental concept on which all subsequent research on photosynthesis has been based.

We now turn to the relationship between photosynthetic activity and the spectrum of absorbed light. We will discuss some of the critical experiments that have contributed to our present understanding of photosynthesis, and we will consider equations for essential chemical reactions of photosynthesis.

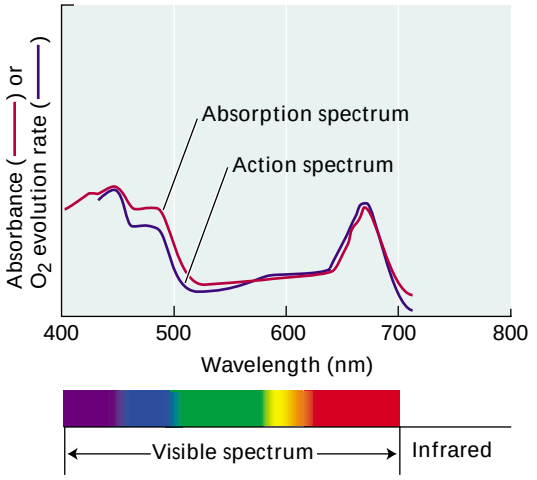


FIGURE 7.8 Action spectrum compared with an absorption spectrum. The absorption spectrum is measured as shown in Figure 7.4. An action spectrum is measured by plotting a response to light such as oxygen evolution, as a function of wavelength. If the pigment used to obtain the absorption spectrum is the same as those that cause the response, the absorption and action spectra will match. In the example shown here, the action spectrum for oxygen evolution matches the absorption spectrum of intact chloroplasts quite well, indicating that light absorption by the chlorophylls mediates oxygen evolution. Discrepancies are found in the region of carotenoid absorption, from 450 to 550 nm, indicating that energy transfer from carotenoids to chlorophylls is not as effective as energy transfer between chlorophylls.

Action Spectra Relate Light Absorption to Photosynthetic Activity

The use of action spectra has been central to the development of our current understanding of photosynthesis. An **action spectrum** depicts the magnitude of a response of a biological system to light, as a function of wavelength. For example, an action spectrum for photosynthesis can be constructed from measurements of oxygen evolution at different wavelengths (Figure 7.8). Often an action spectrum can identify the chromophore (pigment) responsible for a particular light-induced phenomenon.

Some of the first action spectra were measured by T. W. Engelmann in the late 1800s (Figure 7.9). Engelmann used a prism to disperse sunlight into a rainbow that was allowed to fall on an aquatic algal filament. A population of O_2-seeking bacteria was introduced into the system. The

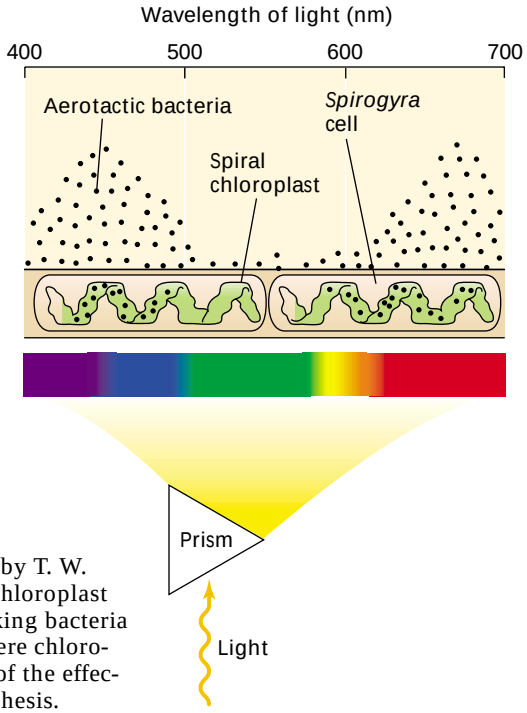


FIGURE 7.9 Schematic diagram of the action spectrum measurements by T. W. Engelmann. Engelmann projected a spectrum of light onto the spiral chloroplast of the filamentous green alga *Spirogyra* and observed that oxygen-seeking bacteria introduced into the system collected in the region of the spectrum where chlorophyll pigments absorb. This action spectrum gave the first indication of the effectiveness of light absorbed by accessory pigments in driving photosynthesis.

bacteria congregated in the regions of the filaments that evolved the most O_2. These were the regions illuminated by blue light and red light, which are strongly absorbed by chlorophyll. Today, action spectra can be measured in room-sized spectrographs in which a huge monochromator bathes the experimental samples in monochromatic light. But the principle of the experiment is the same as that of Engelmann's experiments.

Action spectra were very important for the discovery of two distinct photosystems operating in O_2-evolving photosynthetic organisms. Before we introduce the two photosystems, however, we need to describe the light-gathering antennas and the energy needs of photosynthesis.

Photosynthesis Takes Place in Complexes Containing Light-Harvesting Antennas and Photochemical Reaction Centers

A portion of the light energy absorbed by chlorophylls and carotenoids is eventually stored as chemical energy via the formation of chemical bonds. This conversion of energy from one form to another is a complex process that depends on cooperation between many pigment molecules and a group of electron transfer proteins.

The majority of the pigments serve as an **antenna complex**, collecting light and transferring the energy to the **reaction center complex**, where the chemical oxidation and reduction reactions leading to long-term energy storage take place (Figure 7.10). Molecular structures of some of the antenna and reaction center complexes are discussed later in the chapter.

How does the plant benefit from this division of labor between antenna and reaction center pigments? Even in bright sunlight, a chlorophyll molecule absorbs only a few photons each second. If every chlorophyll had a complete reaction center associated with it, the enzymes that make up this system would be idle most of the time, only occasionally being activated by photon absorption. However, if many pigments can send energy into a common reaction center, the system is kept active a large fraction of the time.

In 1932, Robert Emerson and William Arnold performed a key experiment that provided the first evidence for the cooperation of many chlorophyll molecules in energy conversion during photosynthesis. They delivered very brief (10^{-5} s) flashes of light to a suspension of the green alga *Chlorella pyrenoidosa* and measured the amount of oxygen produced. The flashes were spaced about 0.1 s apart, a time that Emerson and Arnold had determined in earlier work was long enough for the enzymatic steps of the process to be completed before the arrival of the next flash. The investigators varied the energy of the flashes and found that at high energies the oxygen production did not increase when a more intense flash was given: The photosynthetic system was saturated with light (Figure 7.11).

In their measurement of the relationship of oxygen production to flash energy, Emerson and Arnold were surprised to find that under saturating conditions, only one molecule of oxygen was produced for each 2500 chlorophyll molecules in the sample. We know now that several hundred pigments are associated with each reaction center and that each reaction center must operate four times

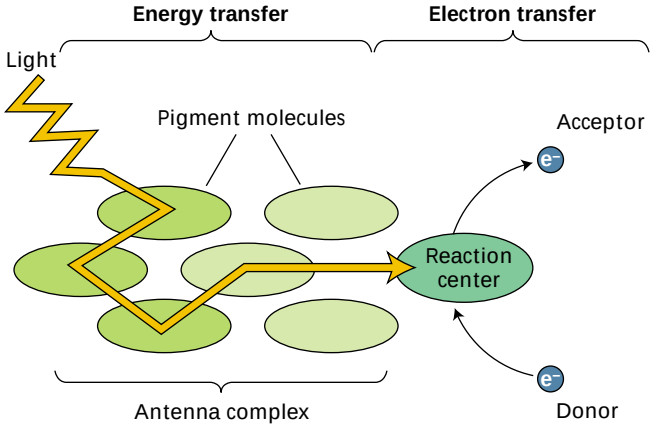


FIGURE 7.10 Basic concept of energy transfer during photosynthesis. Many pigments together serve as an antenna, collecting light and transferring its energy to the reaction center, where chemical reactions store some of the energy by transferring electrons from a chlorophyll pigment to an electron acceptor molecule. An electron donor then reduces the chlorophyll again. The transfer of energy in the antenna is a purely physical phenomenon and involves no chemical changes.

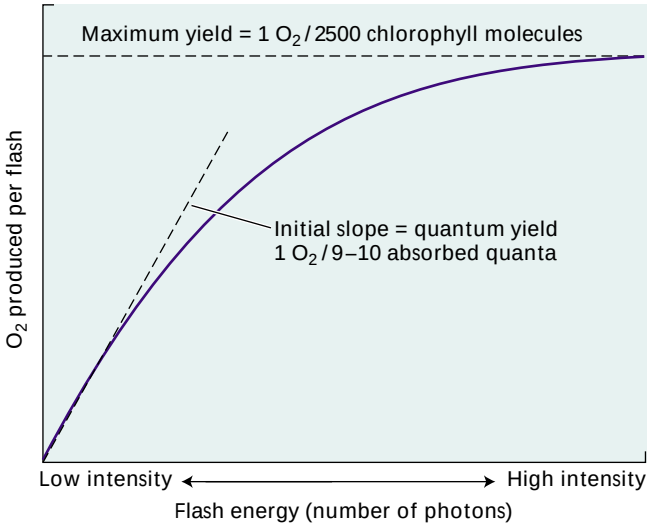


FIGURE 7.11 Relationship of oxygen production to flash energy, the first evidence for the interaction between the antenna pigments and the reaction center. At saturating energies, the maximum amount of O_2 produced is 1 molecule per 2500 chlorophyll molecules.

to produce one molecule of oxygen—hence the value of 2500 chlorophylls per O_2.

The reaction centers and most of the antenna complexes are integral components of the photosynthetic membrane. In eukaryotic photosynthetic organisms, these membranes are found within the chloroplast; in photosynthetic prokaryotes, the site of photosynthesis is the plasma membrane or membranes derived from it.

The graph shown in Figure 7.11 permits us to calculate another important parameter of the light reactions of photosynthesis, the quantum yield. The **quantum yield** of photosynthesis (Φ)is defined as follows:

$$\Phi = \frac{\text{Number of photochemical products}}{\text{Total number of quanta absorbed}} \qquad (7.5)$$

In the linear portion (low light intensity) of the curve, an increase in the number of photons stimulates a proportional increase in oxygen evolution. Thus the slope of the curve measures the quantum yield for oxygen production. The quantum yield for a particular process can range from 0 (if that process does not respond to light) to 1.0 (if every photon absorbed contributes to the process). A more detailed discussion of quantum yields can be found in **Web Topic 7.3**.

In functional chloroplasts kept in dim light, the quantum yield of photochemistry is approximately 0.95, the quantum yield of fluorescence is 0.05 or lower, and the quantum yields of other processes are negligible. The vast majority of excited chlorophyll molecules therefore lead to photochemistry.

The Chemical Reaction of Photosynthesis Is Driven by Light

It is important to realize that equilibrium for the chemical reaction shown in Equation 7.4 lies very far in the direction of the reactants. The equilibrium constant for Equation 7.4, calculated from tabulated free energies of formation for each of the compounds involved, is about 10^{-500}. This number is so close to zero that one can be quite confident that in the entire history of the universe no molecule of glucose has formed spontaneously from H_2O and CO_2 without external energy being provided. The energy needed to drive the photosynthetic reaction comes from light. Here's a simpler form of Equation 7.4:

$$CO_2 + H_2O \xrightarrow{\text{Light, plant}} (CH_2O) + O_2 \qquad (7.6)$$

where (CH_2O) is one-sixth of a glucose molecule. About nine or ten photons of light are required to drive the reaction of Equation 7.6.

Although the photochemical quantum yield under optimum conditions is nearly 100%, the *efficiency* of the conversion of light into chemical energy is much less. If red light of wavelength 680 nm is absorbed, the total energy input (see Equation 7.2) is 1760 kJ per mole of oxygen formed. This amount of energy is more than enough to drive the reaction in Equation 7.6, which has a standard-state free-energy change of +467 kJ mol^{-1}. The efficiency of conversion of light energy at the optimal wavelength into chemical energy is therefore about 27%, which is remarkably high for an energy conversion system. Most of this stored energy is used for cellular maintenance processes; the amount diverted to the formation of biomass is much less (see Figure 9.2).

There is no conflict between the fact that the photochemical quantum efficiency (quantum yield) is nearly 1 (100%) and the energy conversion efficiency is only 27%. The *quantum efficiency* is a measure of the fraction of absorbed photons that engage in photochemistry; the *energy efficiency* is a measure of how much energy in the absorbed photons is stored as chemical products. The numbers indicate that almost all the absorbed photons engage in photochemistry, but only about a fourth of the energy in each photon is stored, the remainder being converted to heat.

Light Drives the Reduction of NADP and the Formation of ATP

The overall process of photosynthesis is a redox chemical reaction, in which electrons are removed from one chemical species, thereby oxidizing it, and added to another species, thereby reducing it. In 1937, Robert Hill found that in the light, isolated chloroplast thylakoids reduce a variety of compounds, such as iron salts. These compounds serve as oxidants in place of CO_2, as the following equation shows:

$$4\,Fe^{3+} + 2\,H_2O \rightarrow 4\,Fe^{2+} + O_2 + 4\,H^+ \qquad (7.7)$$

Many compounds have since been shown to act as artificial electron acceptors in what has come to be known as the Hill reaction. Their use has been invaluable in elucidating the reactions that precede carbon reduction.

We now know that during the normal functioning of the photosynthetic system, light reduces nicotinamide adenine dinucleotide phosphate (NADP), which in turn serves as the reducing agent for carbon fixation in the Calvin cycle (see Chapter 8). ATP is also formed during the electron flow from water to NADP, and it, too, is used in carbon reduction.

The chemical reactions in which water is oxidized to oxygen, NADP is reduced, and ATP is formed are known as the *thylakoid reactions* because almost all the reactions up to NADP reduction take place within the thylakoids. The carbon fixation and reduction reactions are called the *stroma reactions* because the carbon reduction reactions take place in the aqueous region of the chloroplast, the stroma.

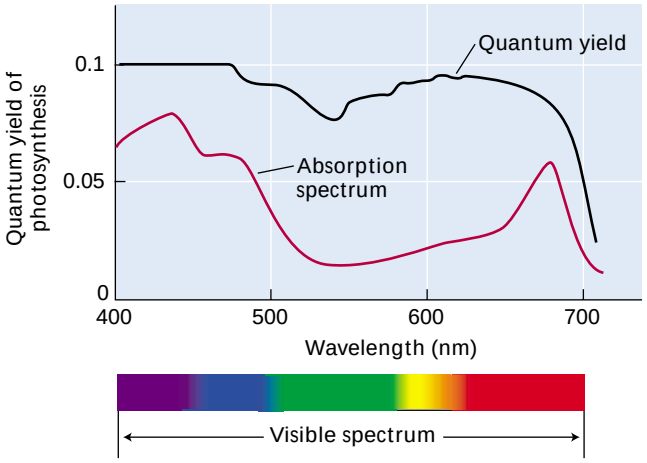


FIGURE 7.12 Red drop effect. The quantum yield of photosynthesis (black curve) falls off drastically for far-red light of wavelengths greater than 680 nm, indicating that far-red light alone is inefficient in driving photosynthesis. The slight dip near 500 nm reflects the somewhat lower efficiency of photosynthesis using light absorbed by accessory pigments, carotenoids.

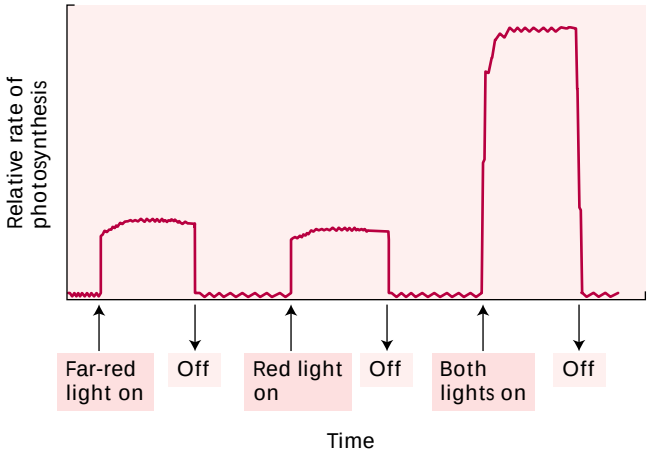


FIGURE 7.13 Enhancement effect. The rate of photosynthesis when red and far-red light are given together is greater than the sum of the rates when they are given apart. The enhancement effect provided essential evidence in favor of the concept that photosynthesis is carried out by two photochemical systems working in tandem but with slightly different wavelength optima.

Although this division is somewhat arbitrary, it is conceptually useful.

Oxygen-Evolving Organisms Have Two Photosystems That Operate in Series

By the late 1950s, several experiments were puzzling the scientists who studied photosynthesis. One of these experiments carried out by Emerson, measured the quantum yield of photosynthesis as a function of wavelength and revealed an effect known as the red drop (Figure 7.12).

If the quantum yield is measured for the wavelengths at which chlorophyll absorbs light, the values found throughout most of the range are fairly constant, indicating that any photon absorbed by chlorophyll or other pigments is as effective as any other photon in driving photosynthesis. However, the yield drops dramatically in the far-red region of chlorophyll absorption (greater than 680 nm).

This drop cannot be caused by a decrease in chlorophyll absorption because the quantum yield measures only light that has actually been absorbed. Thus, light with a wavelength greater than 680 nm is much less efficient than light of shorter wavelengths.

Another puzzling experimental result was the **enhancement effect**, also discovered by Emerson. He measured the rate of photosynthesis separately with light of two different wavelengths and then used the two beams simultaneously (Figure 7.13). When red and far-red light were given together, the rate of photosynthesis was greater than the sum of the individual rates. This was a startling and surprising observation.

These observations were eventually explained by experiments performed in the 1960s (see **Web Topic 7.4**) that led to the discovery that two photochemical complexes, now known as **photosystems I** and **II** (**PSI and PSII**), operate in series to carry out the early energy storage reactions of photosynthesis.

Photosystem I preferentially absorbs far-red light of wavelengths greater than 680 nm; photosystem II preferentially absorbs red light of 680 nm and is driven very poorly by far-red light. This wavelength dependence explains the enhancement effect and the red drop effect. Another difference between the photosystems is that

- Photosystem I produces a strong reductant, capable of reducing $NADP^+$, and a weak oxidant.
- Photosystem II produces a very strong oxidant, capable of oxidizing water, and a weaker reductant than the one produced by photosystem I.

The reductant produced by photosystem II re-reduces the oxidant produced by photosystem I. These properties of the two photosystems are shown schematically in Figure 7.14.

The scheme of photosynthesis depicted in Figure 7.14, called the *Z* (for *zigzag*) *scheme*, has become the basis for understanding O_2-evolving (oxygenic) photosynthetic organisms. It accounts for the operation of two physically and chemically distinct photosystems (I and II), each with its own antenna pigments and photochemical reaction center. The two photosystems are linked by an electron transport chain.

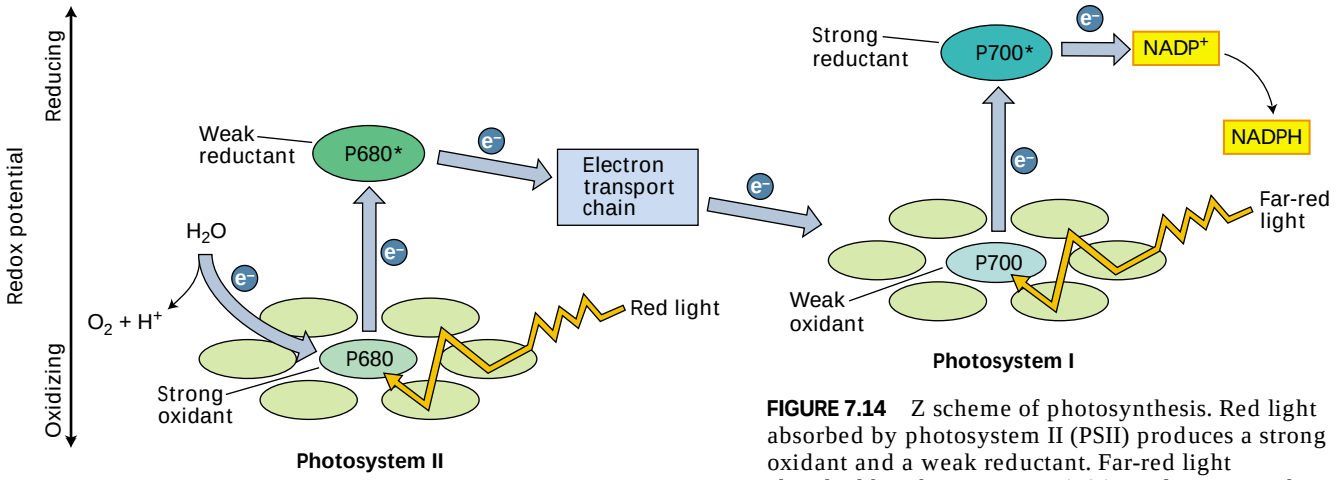


FIGURE 7.14 Z scheme of photosynthesis. Red light absorbed by photosystem II (PSII) produces a strong oxidant and a weak reductant. Far-red light absorbed by photosystem I (PSI) produces a weak oxidant and a strong reductant. The strong oxidant generated by PSII oxidizes water, while the strong reductant produced by PSI reduces $NADP^+$. This scheme is basic to an understanding of photosynthetic electron transport. P680 and P700 refer to the wavelengths of maximum absorption of the reaction center chlorophylls in PSII and PSI, respectively.

ORGANIZATION OF THE PHOTOSYNTHETIC APPARATUS

The previous section explained some of the physical principles underlying photosynthesis, some aspects of the functional roles of various pigments, and some of the chemical reactions carried out by photosynthetic organisms. We now turn to the architecture of the photosynthetic apparatus and the structure of its components.

The Chloroplast Is the Site of Photosynthesis

In photosynthetic eukaryotes, photosynthesis takes place in the subcellular organelle known as the chloroplast. Figure 7.15 shows a transmission electron micrograph of a thin section from a pea chloroplast. The most striking aspect of the structure of the chloroplast is the extensive system of internal membranes known as **thylakoids**. All the chlorophyll is contained within this membrane system, which is the site of the light reactions of photosynthesis.

The carbon reduction reactions, which are catalyzed by water-soluble enzymes, take place in the **stroma** (plural *stromata*), the region of the chloroplast outside the thylakoids. Most of the thylakoids appear to be very closely associated with each other. These stacked membranes are known as **grana lamellae** (singular *lamella*; each stack is called a *granum*), and the exposed membranes in which stacking is absent are known as **stroma lamellae**.

Two separate membranes, each composed of a lipid bilayer and together known as the **envelope**, surround most types of chloroplasts (Figure 7.16). This double-membrane system contains a variety of metabolite transport systems.

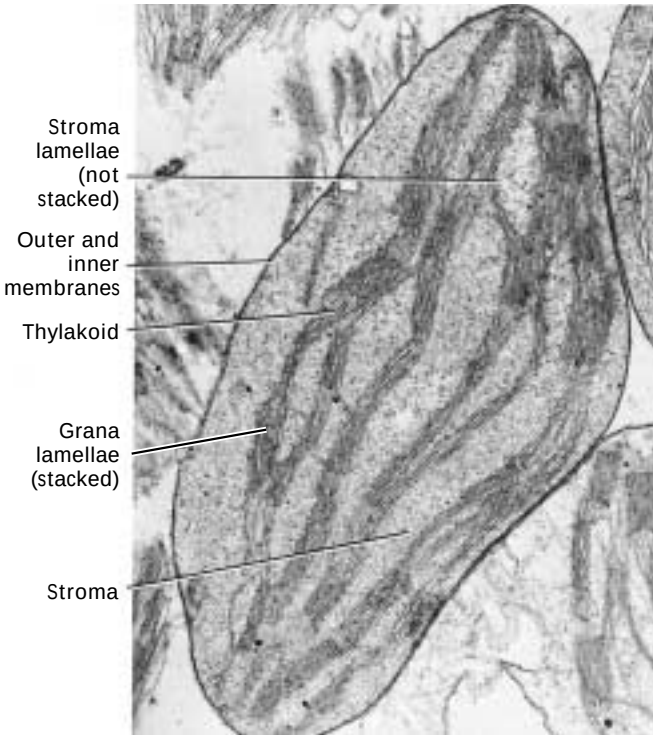


FIGURE 7.15 Transmission electron micrograph of a chloroplast from pea (*Pisum sativum*), fixed in glutaraldehyde and OsO_4, embedded in plastic resin, and thin-sectioned with an ultramicrotome. (14,500×) (Courtesy of J. Swafford.)

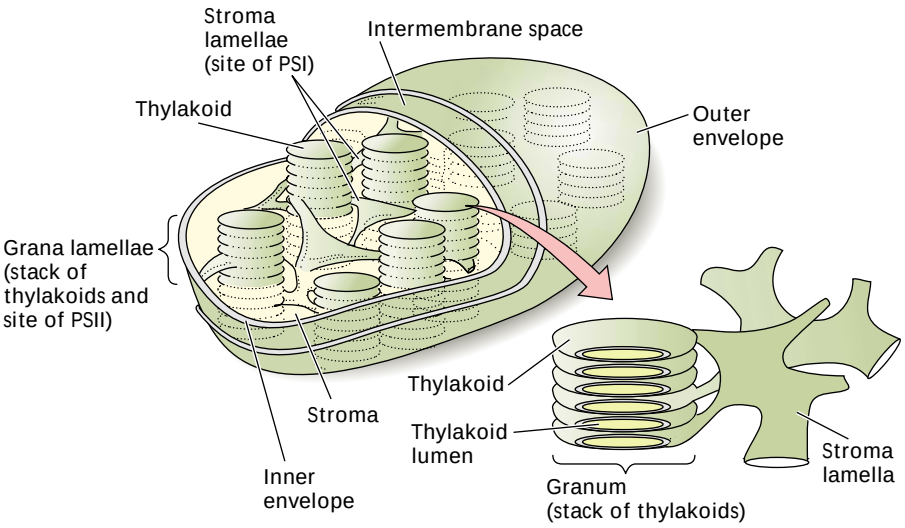


FIGURE 7.16 Schematic picture of the overall organization of the membranes in the chloroplast. The chloroplast of higher plants is surrounded by the inner and outer membranes (envelope). The region of the chloroplast that is inside the inner membrane and surrounds the thylakoid membranes is known as the stroma. It contains the enzymes that catalyze carbon fixation and other biosynthetic pathways. The thylakoid membranes are highly folded and appear in many pictures to be stacked like coins, although in reality they form one or a few large interconnected membrane systems, with a well-defined interior and exterior with respect to the stroma. The inner space within a thylakoid is known as the lumen. (After Becker 1986.)

The chloroplast also contains its own DNA, RNA, and ribosomes. Many of the chloroplast proteins are products of transcription and translation within the chloroplast itself, whereas others are encoded by nuclear DNA, synthesized on cytoplasmic ribosomes, and then imported into the chloroplast. This remarkable division of labor, extending in many cases to different subunits of the same enzyme complex, will be discussed in more detail later in this chapter. For some dynamic structures of chloroplasts see **Web Essay 7.1**.

Thylakoids Contain Integral Membrane Proteins

A wide variety of proteins essential to photosynthesis are embedded in the thylakoid membranes. In many cases, portions of these proteins extend into the aqueous regions on both sides of the thylakoids. These **integral membrane proteins** contain a large proportion of hydrophobic amino acids and are therefore much more stable in a nonaqueous medium such as the hydrocarbon portion of the membrane (see Figure 1.5A).

The reaction centers, the antenna pigment–protein complexes, and most of the electron transport enzymes are all integral membrane proteins. In all known cases, integral membrane proteins of the chloroplast have a unique orientation within the membrane. Thylakoid membrane proteins have one region pointing toward the stromal side of the membrane and the other oriented toward the interior portion of the thylakoid, known as the *lumen* (see Figures 7.16 and 7.17).

The chlorophylls and accessory light-gathering pigments in the thylakoid membrane are always associated in a noncovalent but highly specific way with proteins. Both antenna and reaction center chlorophylls are associated with proteins that are organized within the membrane so as to optimize energy transfer in antenna complexes and electron transfer in reaction centers, while at the same time minimizing wasteful processes.

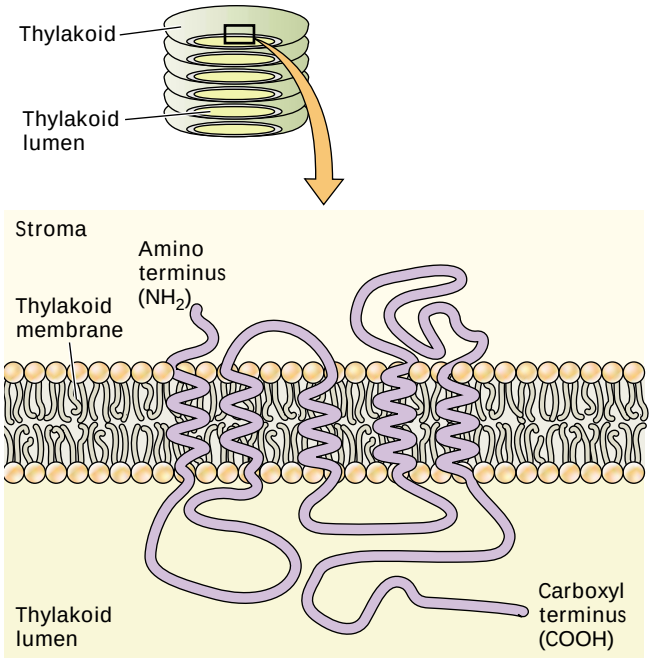
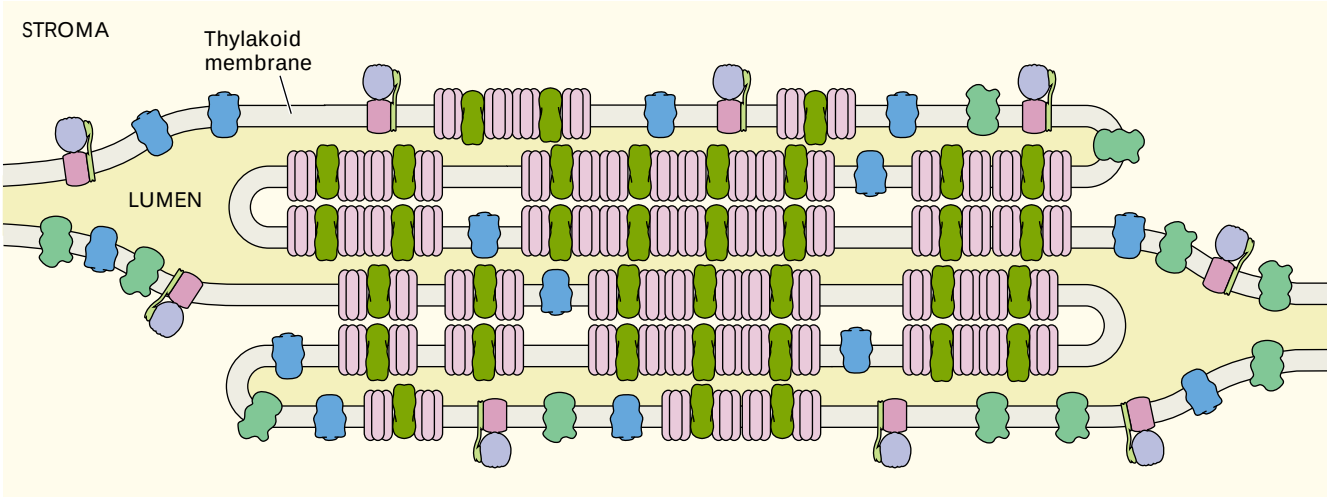


FIGURE 7.17 Predicted folding pattern of the D1 protein of the PSII reaction center. The hydrophobic portion of the membrane is traversed five times by the peptide chain rich in hydrophobic amino acid residues. The protein is asymmetrically arranged in the thylakoid membrane, with the amino (NH_2) terminus on the stromal side of the membrane and the carboxyl (COOH) terminus on the lumen side. (After Trebst 1986.)



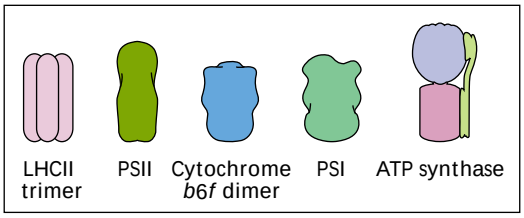


FIGURE 7.18 Organization of the protein complexes of the thylakoid membrane. Photosystem II is located predominantly in the stacked regions of the thylakoid membrane; photosystem I and ATP synthase are found in the unstacked regions protruding into the stroma. Cytochrome b_6f complexes are evenly distributed. This lateral separation of the two photosystems requires that electrons and protons produced by photosystem II be transported a considerable distance before they can be acted on by photosystem I and the ATP-coupling enzyme. (After Allen and Forsberg 2001.)

Photosystems I and II Are Spatially Separated in the Thylakoid Membrane

The PSII reaction center, along with its antenna chlorophylls and associated electron transport proteins, is located predominantly in the grana lamellae (Figure 7.18) (Allen and Forsberg 2001).

The PSI reaction center and its associated antenna pigments and electron transfer proteins, as well as the coupling-factor enzyme that catalyzes the formation of ATP, are found almost exclusively in the stroma lamellae and at the edges of the grana lamellae. The cytochrome b_6f complex of the electron transport chain that connects the two photosystems (see Figure 7.21) is evenly distributed between stroma and grana.

Thus the two photochemical events that take place in O_2-evolving photosynthesis are spatially separated. This separation implies that one or more of the electron carriers that function between the photosystems diffuses from the grana region of the membrane to the stroma region, where electrons are delivered to photosystem I.

In PSII, the oxidation of two water molecules produces four electrons, four protons, and a single O_2 (see Equation 7.8). The protons produced by this oxidation of water must also be able to diffuse to the stroma region, where ATP is synthesized. The functional role of this large separation (many tens of nanometers) between photosystems I and II is not entirely clear but is thought to improve the efficiency of energy distribution between the two photosystems (Trissl and Wilhelm 1993; Allen and Forsberg 2001).

The spatial separation between photosystems I and II indicates that a strict one-to-one stoichiometry between the two photosystems is not required. Instead, PSII reaction centers feed reducing equivalents into a common intermediate pool of soluble electron carriers (plastoquinone), which will be described in detail later in the chapter. The PSI reaction centers remove the reducing equivalents from the common pool, rather than from any specific PSII reaction center complex.

Most measurements of the relative quantities of photosystems I and II have shown that there is an excess of photosystem II in chloroplasts. Most commonly, the ratio of PSII to PSI is about 1.5:1, but it can change when plants are grown in different light conditions.

Anoxygenic Photosynthetic Bacteria Have a Reaction Center Similar to That of Photosystem II

Non-O_2-evolving (anoxygenic) organisms, such as the purple photosynthetic bacteria of the genera *Rhodobacter* and *Rhodopseudomonas*, contain only a single photosystem. These simpler organisms have been very useful for detailed structural and functional studies that have contributed to a better understanding of oxygenic photosynthesis.

Hartmut Michel, Johann Deisenhofer, Robert Huber, and coworkers in Munich resolved the three-dimensional structure of the reaction center from the purple photosynthetic bacterium *Rhodopseudomonas viridis* (Deisenhofer and Michel 1989). This landmark achievement, for which a Nobel Prize was awarded in 1988, was the first high-reso-

lution, X-ray structural determination for an integral membrane protein, and the first structural determination for a reaction center complex (see Figures 7.5.A and 7.5.B in **Web Topic 7.5**). Detailed analysis of these structures, along with the characterization of numerous mutants, has revealed many of the principles involved in the energy storage processes carried out by all reaction centers.

The structure of the bacterial reaction center is thought to be similar in many ways to that found in photosystem II from oxygen-evolving organisms, especially in the electron acceptor portion of the chain. The proteins that make up the core of the bacterial reaction center are relatively similar in sequence to their photosystem II counterparts, implying an evolutionary relatedness.

ORGANIZATION OF LIGHT-ABSORBING ANTENNA SYSTEMS

The antenna systems of different classes of photosynthetic organisms are remarkably varied, in contrast to the reaction centers, which appear to be similar in even distantly related organisms. The variety of antenna complexes reflects evolutionary adaptation to the diverse environments in which different organisms live, as well as the need in some organisms to balance energy input to the two photosystems (Grossman et al. 1995; Green and Durnford 1996).

Antenna systems function to deliver energy efficiently to the reaction centers with which they are associated (van Grondelle et al. 1994; Pullerits and Sundström 1996). The size of the antenna system varies considerably in different organisms, ranging from a low of 20 to 30 bacteriochlorophylls per reaction center in some photosynthetic bacteria, to generally 200 to 300 chlorophylls per reaction center in higher plants, to a few thousand pigments per reaction center in some types of algae and bacteria. The molecular structures of antenna pigments are also quite diverse, although all of them are associated in some way with the photosynthetic membrane.

The physical mechanism by which excitation energy is conveyed from the chlorophyll that absorbs the light to the reaction center is thought to be **resonance transfer**. By this mechanism the excitation energy is transferred from one molecule to another by a nonradiative process.

A useful analogy for resonance transfer is the transfer of energy between two tuning forks. If one tuning fork is struck and properly placed near another, the second tuning fork receives some energy from the first and begins to vibrate. As in resonance energy transfer in antenna complexes, the efficiency of energy transfer between the two tuning forks depends on their distance from each other and their relative orientation, as well as their pitches or vibrational frequencies.

Energy transfer in antenna complexes is very efficient: Approximately 95 to 99% of the photons absorbed by the antenna pigments have their energy transferred to the reaction center, where it can be used for photochemistry. There is an important difference between energy transfer among pigments in the antenna and the electron transfer that occurs in the reaction center: Whereas energy transfer is a purely physical phenomenon, electron transfer involves chemical changes in molecules.

The Antenna Funnels Energy to the Reaction Center

The sequence of pigments within the antenna that funnel absorbed energy toward the reaction center has absorption maxima that are progressively shifted toward longer red wavelengths (Figure 7.19). This red shift in absorption maximum means that the energy of the excited state is somewhat lower nearer the reaction center than in the more peripheral portions of the antenna system.

As a result of this arrangement, when excitation is transferred, for example, from a chlorophyll *b* molecule absorbing maximally at 650 nm to a chlorophyll *a* molecule absorbing maximally at 670 nm, the difference in energy between these two excited chlorophylls is lost to the environment as heat.

For the excitation to be transferred back to the chlorophyll *b*, the energy lost as heat would have to be resupplied. The probability of reverse transfer is therefore smaller simply because thermal energy is not sufficient to make up the deficit between the lower-energy and higher-energy pigments. This effect gives the energy-trapping process a degree of directionality or irreversibility and makes the delivery of excitation to the reaction center more efficient. In essence, the system sacrifices some energy from each quantum so that nearly all of the quanta can be trapped by the reaction center.

Many Antenna Complexes Have a Common Structural Motif

In all eukaryotic photosynthetic organisms that contain both chlorophyll *a* and chlorophyll *b*, the most abundant antenna proteins are members of a large family of structurally related proteins. Some of these proteins are associated primarily with photosystem II and are called **light-harvesting complex II** (**LHCII**) proteins; others are associated with photosystem I and are called *LHCI* proteins. These antenna complexes are also known as **chlorophyll *a/b* antenna proteins** (Paulsen 1995; Green and Durnford 1996).

The structure of one of the LHCII proteins has been determined by a combination of electron microscopy and electron crystallography (Figure 7.20) (Kühlbrandt et al. 1994). The protein contains three α-helical regions and binds about 15 chlorophyll *a* and *b* molecules, as well as a few carotenoids. Only some of these pigments are visible in the resolved structure. The structure of the LHCI proteins has not yet been determined but is probably similar to that of the LHCII proteins. All of these proteins have significant sequence similarity and are almost certainly descendants of a common ancestral protein (Grossman et al. 1995; Green and Durnford 1996).

Light absorbed by carotenoids or chlorophyll *b* in the LHC proteins is rapidly transferred to chlorophyll *a* and

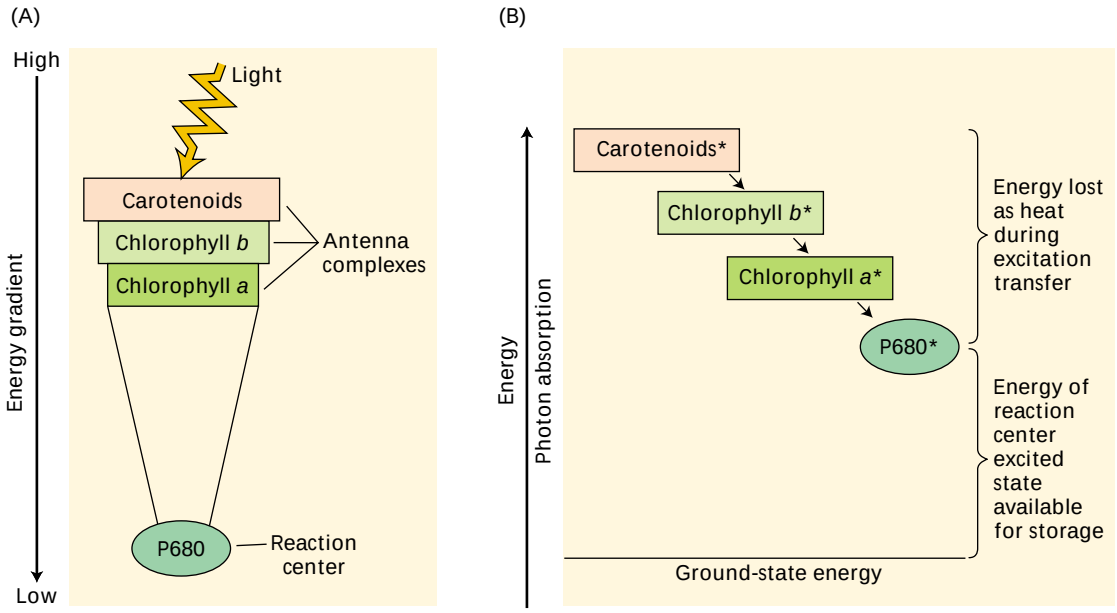


FIGURE 7.19 Funneling of excitation from the antenna system toward the reaction center. (A) The excited-state energy of pigments increases with distance from the reaction center; that is, pigments closer to the reaction center are lower in energy than those farther from the reaction center. This energy gradient ensures that excitation transfer toward the reaction center is energetically favorable and that excitation transfer back out to the peripheral portions of the antenna is energetically unfavorable. (B) Some energy is lost as heat to the environment by this process, but under optimal conditions almost all the excitations absorbed in the antenna complexes can be delivered to the reaction center. The asterisks denote an excited state.

then to other antenna pigments that are intimately associated with the reaction center. The LHCII complex is also involved in regulatory processes, which are discussed later in the chapter.

MECHANISMS OF ELECTRON TRANSPORT

Some of the evidence that led to the idea of two photochemical reactions operating in series was discussed earlier in this chapter. Here we will consider in detail the chemical reactions involved in electron transfer during photosynthesis. We will discuss the excitation of chlorophyll by light and the reduction of the first electron acceptor, the flow of electrons through photosystems II and I, the oxidation of water as the primary source of electrons, and the reduction of the final electron acceptor ($NADP^+$). The chemiosmotic mechanism that mediates ATP synthesis will be discussed in detail later in the chapter (see "Proton Transport and ATP Synthesis in the Chloroplast").

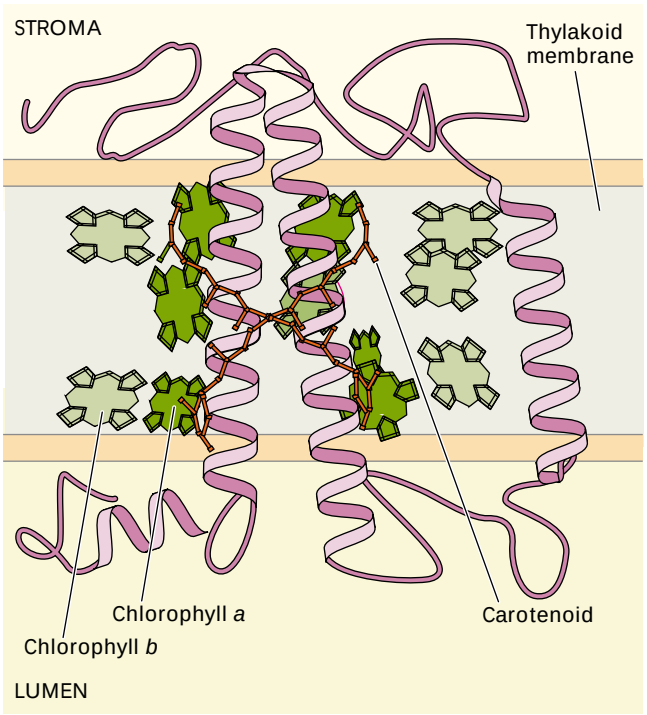


FIGURE 7.20 Two-dimensional view of the structure of the LHCII antenna complex from higher plants, determined by a combination of electron microscopy and electron crystallography. Like X-ray crystallography, electron crystallography uses the diffraction patterns of soft-energy electrons to resolve macromolecule structures. The antenna complex is a transmembrane pigment protein, with three helical regions that cross the nonpolar part of the membrane. Approximately 15 chlorophyll *a* and *b* molecules are associated with the complex, as well as several carotenoids. The positions of several of the chlorophylls are shown, and two of the carotenoids form an X in the middle of the complex. In the membrane, the complex is trimeric and aggregates around the periphery of the PSII reaction center complex. (After Kühlbrandt et al. 1994.)

Electrons Ejected from Chlorophyll Travel Through a Series of Electron Carriers Organized in the "Z Scheme"

Figure 7.21 shows a current version of the Z scheme, in which all the electron carriers known to function in electron flow from H_2O to $NADP^+$ are arranged vertically at their midpoint redox potentials (see **Web Topic 7.6** for further detail). Components known to react with each other are connected by arrows, so the Z scheme is really a synthesis of both kinetic and thermodynamic information. The large vertical arrows represent the input of light energy into the system.

Photons excite the specialized chlorophyll of the reaction centers (P680 for PSII, and P700 for PSI), and an electron is ejected. The electron then passes through a series of electron carriers and eventually reduces P700 (for electrons from PSII) or $NADP^+$ (for electrons from PSI). Much of the following discussion describes the journeys of these electrons and the nature of their carriers.

Almost all the chemical processes that make up the light reactions of photosynthesis are carried out by four major protein complexes: photosystem II, the cytochrome b_6f complex, photosystem I, and the ATP synthase. These four integral membrane complexes are vectorially oriented in the thylakoid membrane to function as follows (Figure 7.22):

- Photosystem II oxidizes water to O_2 in the thylakoid lumen and in the process releases protons into the lumen.
- Cytochrome b_6f receives electrons from PSII and delivers them to PSI. It also transports additional protons into the lumen from the stroma.
- Photosystem I reduces $NADP^+$ to NADPH in the stroma by the action of ferredoxin (Fd) and the flavoprotein ferredoxin–NADP reductase (FNR).
- ATP synthase produces ATP as protons diffuse back through it from the lumen into the stroma.

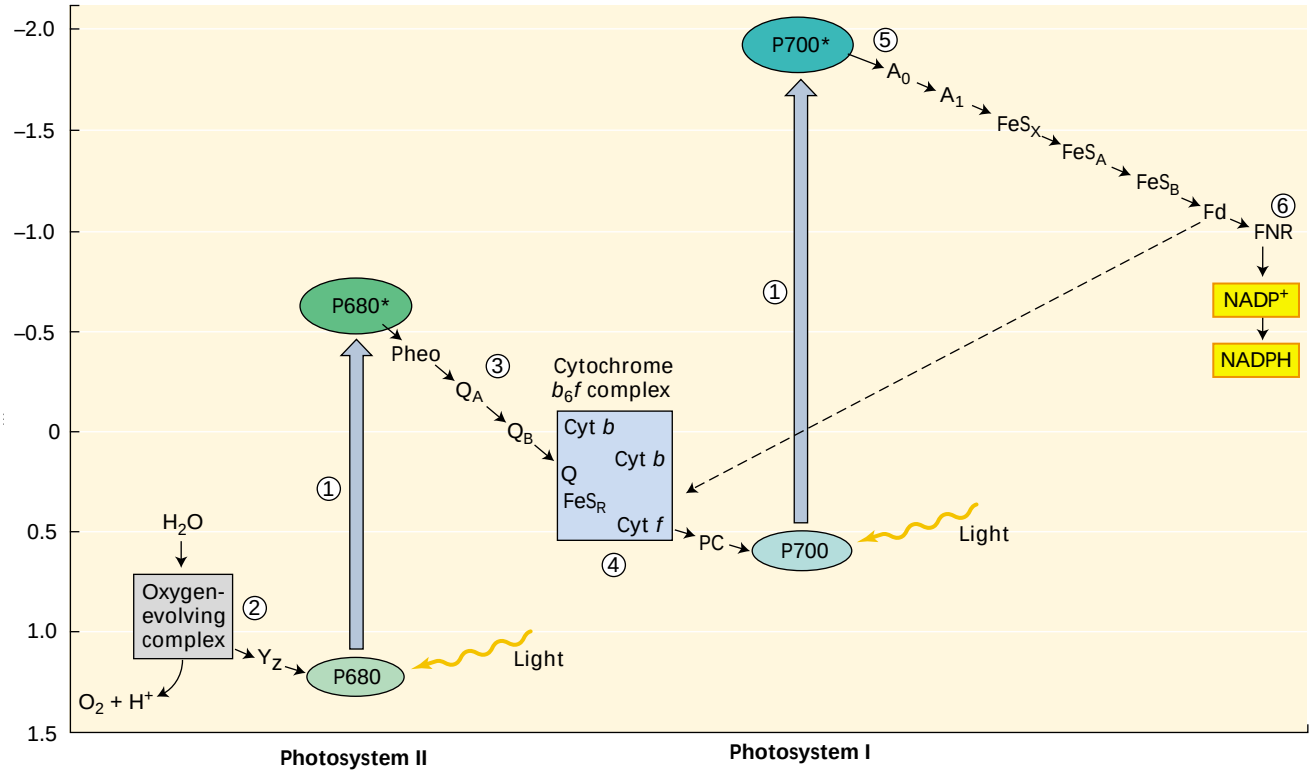


FIGURE 7.21 Detailed Z scheme for O_2-evolving photosynthetic organisms. The redox carriers are placed at their midpoint redox potentials (at pH 7). (1) The vertical arrows represent photon absorption by the reaction center chlorophylls: P680 for photosystem II (PSII) and P700 for photosystem I (PSI). The excited PSII reaction center chlorophyll, P680*, transfers an electron to pheophytin (Pheo). (2) On the oxidizing side of PSII (to the left of the arrow joining P680 with P680*), P680 oxidized by light is re-reduced by Y_z, that has received electrons from oxidation of water. (3) On the reducing side of PSII (to the right of the arrow joining P680 with P680*), pheophytin transfers electrons to the acceptors Q_A and Q_B, which are plastoquinones. (4) The cytochrome b_6f complex transfers electrons to plastocyanin (PC), a soluble protein, which in turn reduces $P700^+$ (oxidized P700). (5) The acceptor of electrons from P700* (A_0) is thought to be a chlorophyll, and the next acceptor (A_1) is a quinone. A series of membrane-bound iron–sulfur proteins (FeS_X, FeS_A, and FeS_B) transfers electrons to soluble ferredoxin (Fd). (6) The soluble flavoprotein ferredoxin–NADP reductase (FNR) reduces $NADP^+$ to NADPH, which is used in the Calvin cycle to reduce CO_2 (see Chapter 8). The dashed line indicates cyclic electron flow around PSI. (After Blankenship and Prince 1985.)

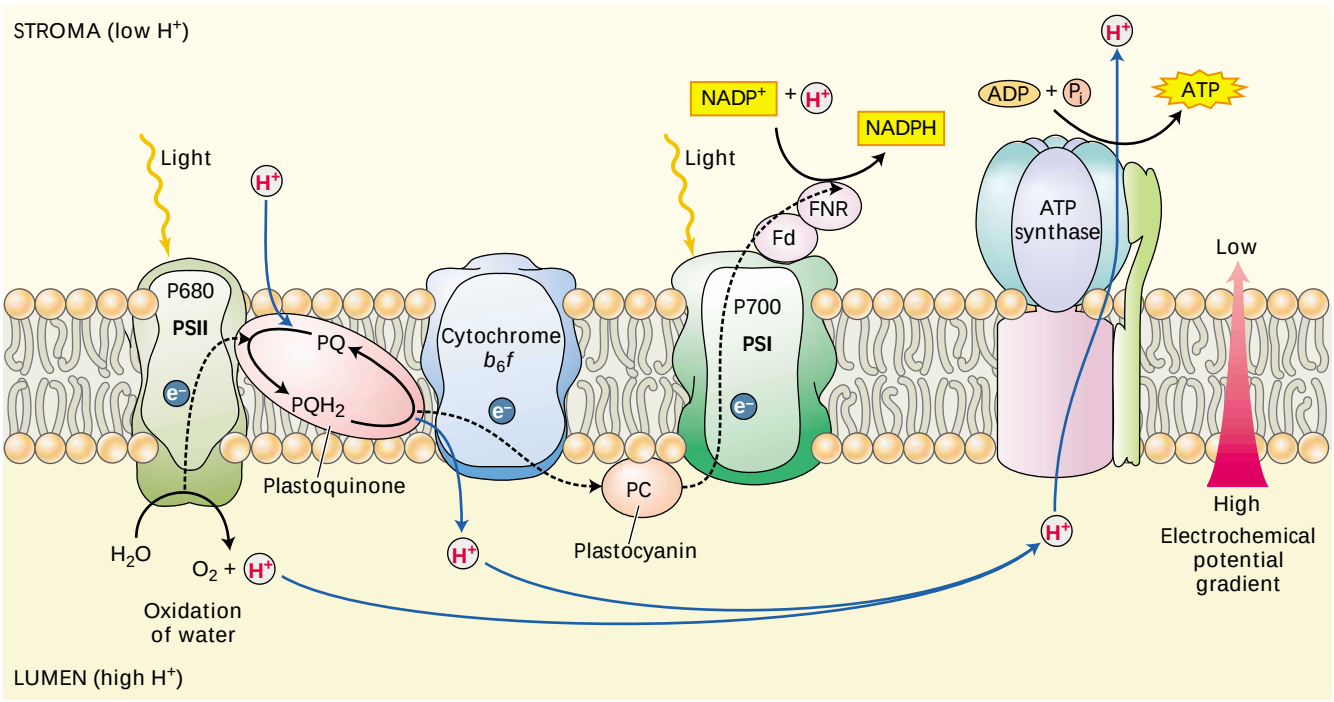


FIGURE 7.22 The transfer of electrons and protons in the thylakoid membrane is carried out vectorially by four protein complexes. Water is oxidized and protons are released in the lumen by PSII. PSI reduces $NADP^+$ to NADPH in the stroma, via the action of ferredoxin (Fd) and the flavoprotein ferredoxin–NADP reductase (FNR). Protons are also transported into the lumen by the action of the cytochrome b_6f complex and contribute to the electrochemical proton gradient. These protons must then diffuse to the ATP synthase enzyme, where their diffusion down the electrochemical potential gradient is used to synthesize ATP in the stroma. Reduced plastoquinone (PQH_2) and plastocyanin transfer electrons to cytochrome b_6f and to PSI, respectively. Dashed lines represent electron transfer; solid lines represent proton movement.

Energy Is Captured When an Excited Chlorophyll Reduces an Electron Acceptor Molecule

As discussed earlier, the function of light is to excite a specialized chlorophyll in the reaction center, either by direct absorption or, more frequently, via energy transfer from an antenna pigment. This excitation process can be envisioned as the promotion of an electron from the highest-energy filled orbital of the chlorophyll to the lowest-energy unfilled orbital (Figure 7.23). The electron in the upper orbital is only loosely bound to the chlorophyll and is easily lost if a molecule that can accept the electron is nearby.

The first reaction that converts electron energy into chemical energy—that is, the primary photochemical event—is the transfer of an electron from the excited state of a chlorophyll in the reaction center to an acceptor molecule. An equivalent way to view this process is that the absorbed photon causes an electron rearrangement in the reaction center chlorophyll, followed by an electron transfer process in which part of the energy in the photon is captured in the form of redox energy.

Immediately after the photochemical event, the reaction center chlorophyll is in an oxidized state (electron deficient, or positively charged) and the nearby electron acceptor mol-

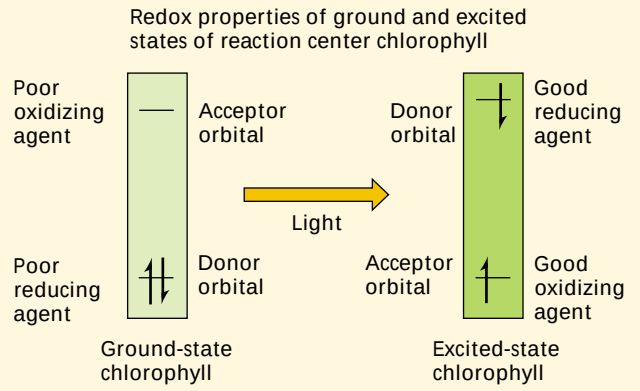


FIGURE 7.23 Orbital occupation diagram for the ground and excited states of reaction center chlorophyll. In the ground state the molecule is a poor reducing agent (loses electrons from a low-energy orbital) and a poor oxidizing agent (accepts electrons only into a high-energy orbital). In the excited state the situation is reversed, and an electron can be lost from the high-energy orbital, making the molecule an extremely powerful reducing agent. This is the reason for the extremely negative excited-state redox potential shown by P680* and P700* in Figure 7.21. The excited state can also act as a strong oxidant by accepting an electron into the lower-energy orbital, although this pathway is not significant in reaction centers. (After Blankenship and Prince 1985.)

ecule is reduced (electron rich, or negatively charged). The system is now at a critical juncture. The lower-energy orbital of the positively charged oxidized reaction center chlorophyll shown in Figure 7.23 has a vacancy and can accept an electron. If the acceptor molecule donates its electron back to the reaction center chlorophyll, the system will be returned to the state that existed before the light excitation, and all the absorbed energy will be converted into heat.

This wasteful *recombination* process, however, does not appear to occur to any substantial degree in functioning reaction centers. Instead, the acceptor transfers its extra electron to a secondary acceptor and so on down the electron transport chain. The oxidized reaction center of the chlorophyll that had donated an electron is re-reduced by a secondary donor, which in turn is reduced by a tertiary donor. In plants, the ultimate electron donor is H_2O, and the ultimate electron acceptor is $NADP^+$ (see Figure 7.21).

The essence of photosynthetic energy storage is thus the initial transfer of an electron from an excited chlorophyll to an acceptor molecule, followed by a very rapid series of secondary chemical reactions that separate the positive and negative charges. These secondary reactions separate the charges to opposite sides of the thylakoid membrane in approximately 200 picoseconds (1 picosecond = 10^{-12} s).

With the charges thus separated, the reversal reaction is many orders of magnitude slower, and the energy has been captured. Each of the secondary electron transfers is accompanied by a loss of some energy, thus making the process effectively irreversible. The quantum yield for the production of stable products in purified reaction centers from photosynthetic bacteria has been measured as 1.0; that is, every photon produces stable products, and no reversal reactions occur.

Although these types of measurements have not been made on purified reaction centers from higher plants, the measured quantum requirements for O_2 production under optimal conditions (low-intensity light) indicate that the values for the primary photochemical events are very close to 1.0. The structure of the reaction center appears to be extremely fine-tuned for maximal rates of productive reactions and minimal rates of energy-wasting reactions.

The Reaction Center Chlorophylls of the Two Photosystems Absorb at Different Wavelengths

As discussed earlier in the chapter, PSI and PSII have distinct absorption characteristics. Precise measurements of absorption maxima were made possible by optical changes in the reaction center chlorophylls in the reduced and oxidized states. The reaction center chlorophyll is transiently in an oxidized state after losing an electron and before being re-reduced by its electron donor.

In the oxidized state, the strong light absorbance in the red region of the spectrum that is characteristic of chlorophylls is lost, or **bleached**. It is therefore possible to monitor the redox state of these chlorophylls by time-resolved optical absorbance measurements in which this bleaching is monitored directly (see **Web Topic 7.1**).

Using such techniques, Bessel Kok found that the reaction center chlorophyll of photosystem I absorbs maximally at 700 nm in its reduced state. Accordingly, this chlorophyll is named **P700** (the P stands for *pigment*). H. T. Witt and coworkers found the analogous optical transient of photosystem II at 680 nm, so its reaction center chlorophyll is known as **P680**. Earlier, Louis Duysens had identified the reaction center bacteriochlorophyll from purple photosynthetic bacteria as **P870**.

The X-ray structure of the bacterial reaction center (see Figures 7.5.A and 7.5.B in **Web Topic 7.5**) clearly indicates that P870 is a closely coupled pair or dimer of bacteriochlorophylls, rather than a single molecule. The primary donor of photosystem I, P700, is a dimer of chlorophyll *a* molecules. Photosystem II also contains a dimer of chlorophylls, although the primary donor, P680, may not reside entirely on these pigments. In the oxidized state, reaction center chlorophylls contain an unpaired electron. Molecules with unpaired electrons often can be detected by a magnetic-resonance technique known as **electron spin resonance** (**ESR**). ESR studies, along with the spectroscopic measurements already described, have led to the discovery of many intermediate electron carriers in the photosynthetic electron transport system.

The Photosystem II Reaction Center Is a Multisubunit Pigment–Protein Complex

Photosystem II is contained in a multisubunit protein supercomplex (Figure 7.24) (Barber et al. 1999). In higher plants, the multisubunit protein supercomplex has two complete reaction centers and some antenna complexes. The core of the reaction center consists of two membrane proteins known as D1 and D2, as well as other proteins, as shown in Figure 7.25 (Zouni et al. 2001).

The primary donor chlorophyll (P680), additional chlorophylls, carotenoids, pheophytins, and plastoquinones (two electron acceptors described in the following section) are bound to the membrane proteins D1 and D2. These proteins have some sequence similarity to the L and M peptides of purple bacteria. Other proteins serve as antenna complexes or are involved in oxygen evolution. Some, such as cytochrome b_{559}, have no known function but may be involved in a protective cycle around photosystem II.

Water Is Oxidized to Oxygen by Photosystem II

Water is oxidized according to the following chemical reaction (Hoganson and Babcock 1997):

$$2\ H_2O \rightarrow O_2 + 4\ H^+ + 4\ e^- \qquad (7.8)$$

This equation indicates that four electrons are removed from two water molecules, generating an oxygen molecule and four hydrogen ions. (For more on oxidation–reduction reactions, see Chapter 2 on the web site and **Web Topic 7.6**.)

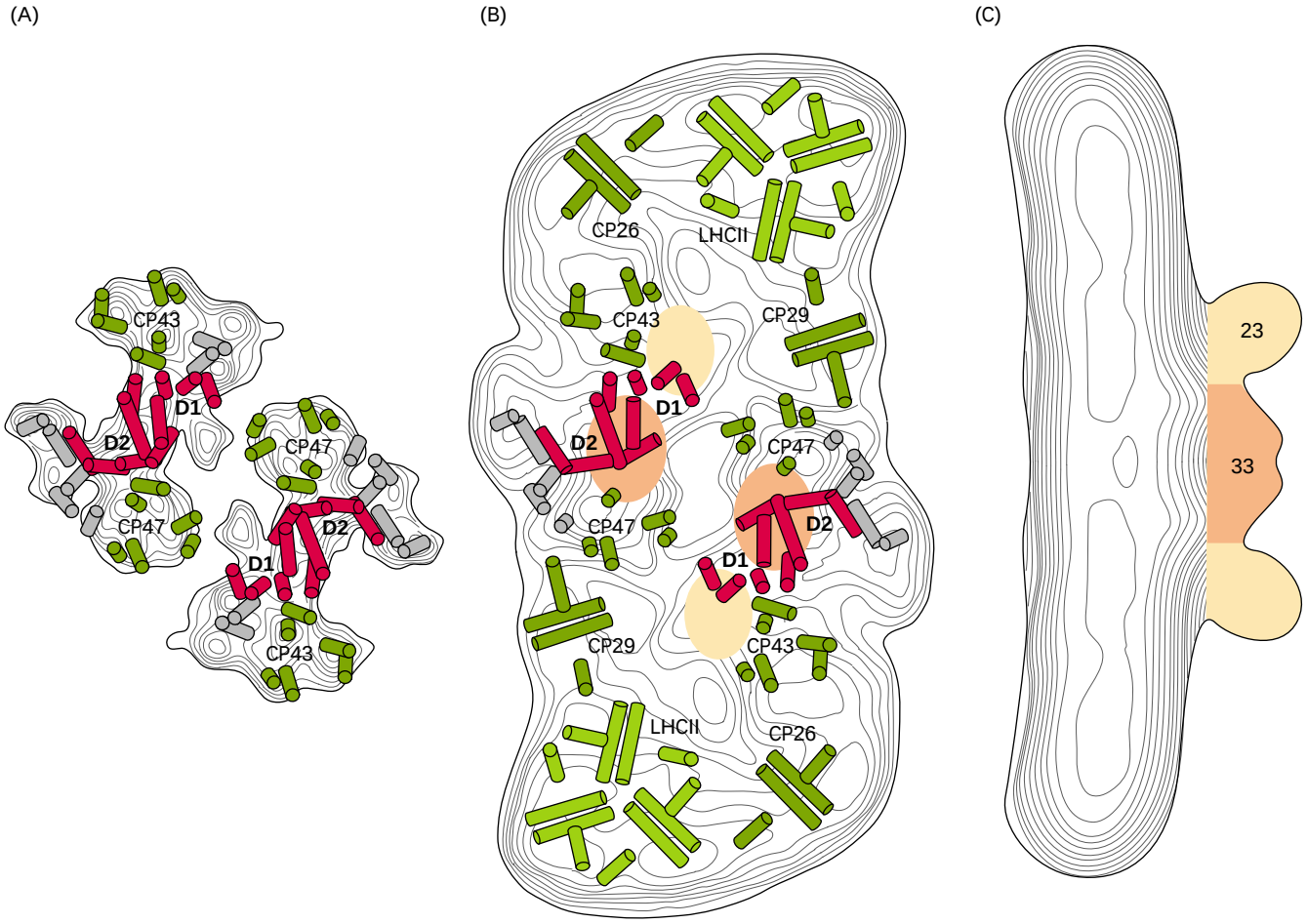


FIGURE 7.24 Structure of dimeric multisubunit protein supercomplex of photosystem II from higher plants, as determined by electron microscopy. The figure shows two complete reaction centers, each of which is a dimeric complex. (A) Helical arrangement of the D1 and D2 (red) and CP43 and CP47 (green) core subunits. (B) View from the lumenal side of the supercomplex, including additional antenna complexes, LHCII, CP26 and CP29, and extrinsic oxygen-evolving complex, shown as orange and yellow circles. Unassigned helices are shown in gray. (C) Side view of the complex illustrating the arrangement of the extrinsic proteins of the oxygen-evolving complex. (After Barber et al. 1999.)

Water is a very stable molecule. Oxidation of water to form molecular oxygen is very difficult, and the photosynthetic oxygen-evolving complex is the only known biochemical system that carries out this reaction. Photosynthetic oxygen evolution is also the source of almost all the oxygen in Earth's atmosphere.

The chemical mechanism of photosynthetic water oxidation is not yet known, although many studies have provided a substantial amount of information about the process (see **Web Topic 7.7** and Figure 7.26). The protons produced by water oxidation are released into the lumen of the thylakoid, not directly into the stromal compartment (see Figure 7.22). They are released into the lumen because of the vectorial nature of the membrane and the fact that the oxygen-evolving complex is localized on the interior surface of the thylakoid. These protons are eventually transferred from the lumen to the stroma by translocation through ATP synthase. In this way the protons released during water oxidation contribute to the electrochemical potential driving ATP formation.

It has been known for many years that manganese (Mn) is an essential cofactor in the water-oxidizing process (see Chapter 5), and a classic hypothesis in photosynthesis research postulates that Mn ions undergo a series of oxidations—which are known as *S states,* and are labeled S_0, S_1, S_2, S_3, and S_4 (see **Web Topic 7.7**)—that are perhaps linked to H_2O oxidation and the generation of O_2 (see Figure 7.26). This hypothesis has received strong support from a variety of experiments, most notably X-ray absorption and ESR studies, both of which detect the manganese directly (Yachandra

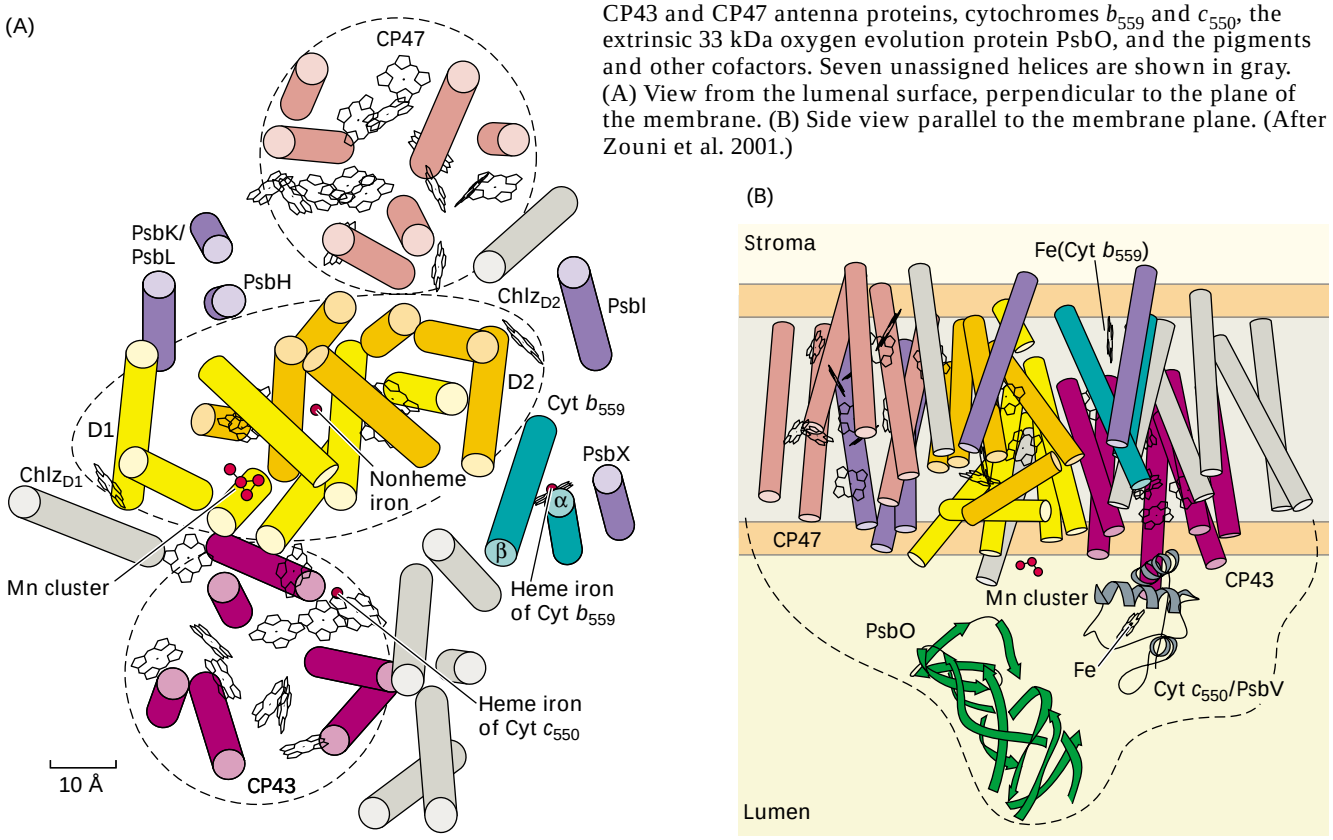


FIGURE 7.25 Structure of the photosystem II reaction center from the cyanobacterium *Synechococcus elongatus*, resolved at 3.8 Å. The structure includes the D1 and D1 core reaction center proteins, the CP43 and CP47 antenna proteins, cytochromes b_{559} and c_{550}, the extrinsic 33 kDa oxygen evolution protein PsbO, and the pigments and other cofactors. Seven unassigned helices are shown in gray. (A) View from the lumenal surface, perpendicular to the plane of the membrane. (B) Side view parallel to the membrane plane. (After Zouni et al. 2001.)

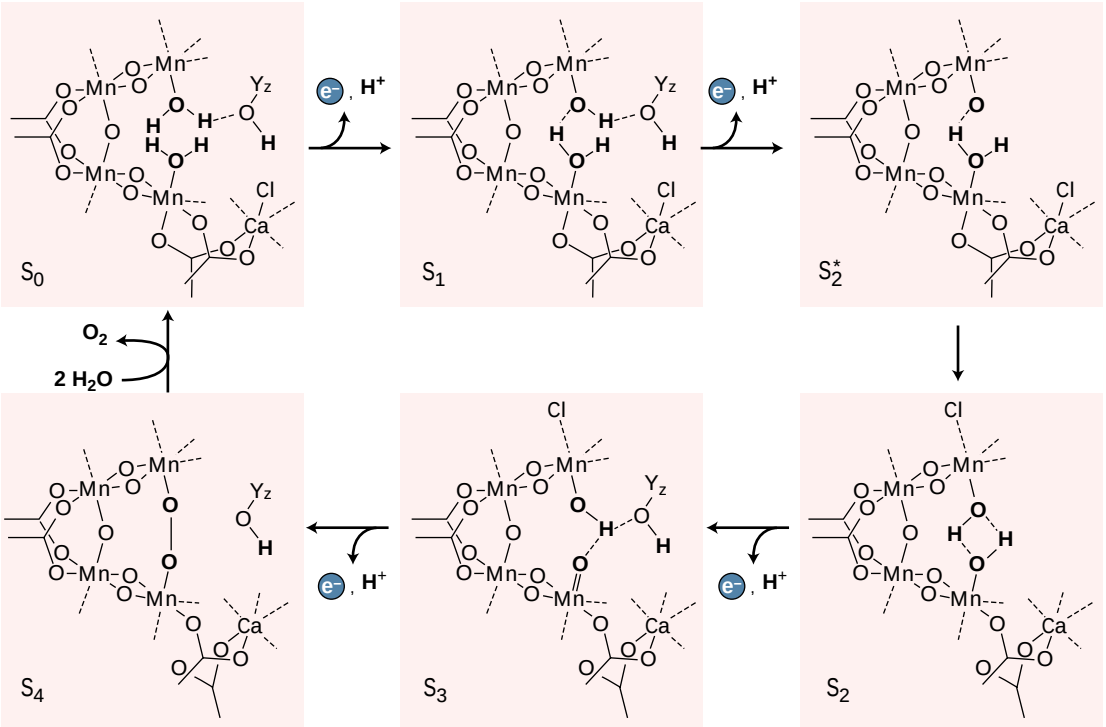


FIGURE 7.26 Model of the S state cycle of oxygen evolution in PSII. Successive stages in the oxidation of water via the Mn oxygen-evolving complex are shown. Y_z is a tyrosine radical that is an intermediate electron carrier between P680 and the Mn cluster. (After Tommos and Babcock 1998.)

et al. 1996). Analytical experiments indicate that four Mn ions are associated with each oxygen-evolving complex. Other experiments have shown that Cl^- and Ca^{2+} ions are essential for O_2 evolution (see Figure 7.26 and **Web Topic 7.7**).

One electron carrier, generally identified as Y_z, functions between the oxygen-evolving complex and P680 (see Figures 7.21 and 7.26). To function in this region, Y_z needs to have a very strong tendency to retain its electrons. This species has been identified as a radical formed from a tyrosine residue in the D1 protein of the PSII reaction center.

Pheophytin and Two Quinones Accept Electrons from Photosystem II

Evidence from spectral and ESR studies indicates that pheophytin acts as an early acceptor in photosystem II, followed by a complex of two plastoquinones in close proximity to an iron atom. **Pheophytin** is a chlorophyll in which the central magnesium atom has been replaced by two hydrogen atoms. This chemical change gives pheophytin chemical and spectral properties that are slightly different from those of chlorophyll. The precise arrangement of the carriers in the electron acceptor complex is not known, but it is probably very similar to that of the reaction center of purple bacteria (for details, see Figure 7.5.B in **Web Topic 7.5**).

Two plastoquinones (Q_A and Q_B) are bound to the reaction center and receive electrons from pheophytin in a sequential fashion (Okamura et al. 2000). Transfer of the two electrons to Q_B reduces it to Q_B^{2-}, and the reduced Q_B^{2-} takes two protons from the stroma side of the medium, yielding a fully reduced **plastohydroquinone** (QH_2) (Figure 7.27). The plastohydroquinone then dissociates from the reaction center complex and enters the hydrocarbon portion of the membrane, where it in turn transfers its electrons to the cytochrome b_6f complex. Unlike the large protein complexes of the thylakoid membrane, hydroquinone is a small, nonpolar molecule that diffuses readily in the nonpolar core of the membrane bilayer.

Electron Flow through the Cytochrome b_6f Complex Also Transports Protons

The **cytochrome b_6f complex** is a large multisubunit protein with several prosthetic groups (Cramer et al. 1996; Berry et al. 2000). It contains two *b*-type hemes and one *c*-type heme (**cytochrome *f***). In *c*-type cytochromes the heme is covalently attached to the peptide; in *b*-type cytochromes the chemically similar protoheme group is not covalently attached (Figure 7.28). In addition, the complex contains a **Rieske iron–sulfur protein** (named for the scientist who discovered it), in which two iron atoms are bridged by two sulfur atoms.

The structures of cytochrome *f* and the related cytochrome bc_1 complex have been determined and suggest a mechanism for electron and proton flow. The precise way by which electrons and protons flow through the cytochrome b_6f complex is not yet fully understood, but a mechanism known as the **Q cycle** accounts for most of the observations. In this mechanism, plastohydroquinone (QH_2) is oxidized, and one of the two electrons is passed along a linear electron transport chain toward photosystem I, while the other electron goes through a cyclic process that increases the number of protons pumped across the membrane (Figure 7.29).

In the linear electron transport chain, the oxidized Rieske protein ($\mathbf{FeS_R}$) accepts an electron from plastohydroquinone (QH_2) and transfers it to cytochrome *f* (see Figure 7.29A). Cytochrome *f* then transfers an electron to the blue-colored copper protein plastocyanin (PC), which in turn reduces oxidized P700 of PSI. In the cyclic part of the process (see Figure 7.29B), the plastosemiquinone (see Figure 7.27) transfers its other electron to one of the *b*-type hemes, releasing both of its protons to the lumenal side of the membrane.

The *b*-type heme transfers its electron through the second *b*-type heme to an oxidized quinone molecule, reducing it to the semiquinone form near the stromal surface of

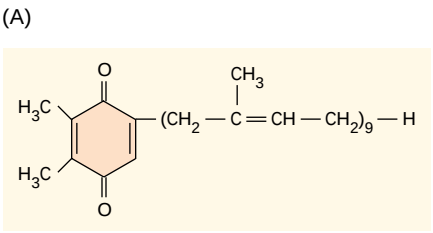


FIGURE 7.27 Structure and reactions of plastoquinone that operate in photosystem II. (A) The plastoquinone consists of a quinoid head and a long nonpolar tail that anchors it in the membrane. (B) Redox reactions of plastoquinone. The fully oxidized quinone (Q), anionic semiquinone ($Q^{\bullet-}$), and reduced hydroquinone (QH_2) forms are shown; R represents the side chain.

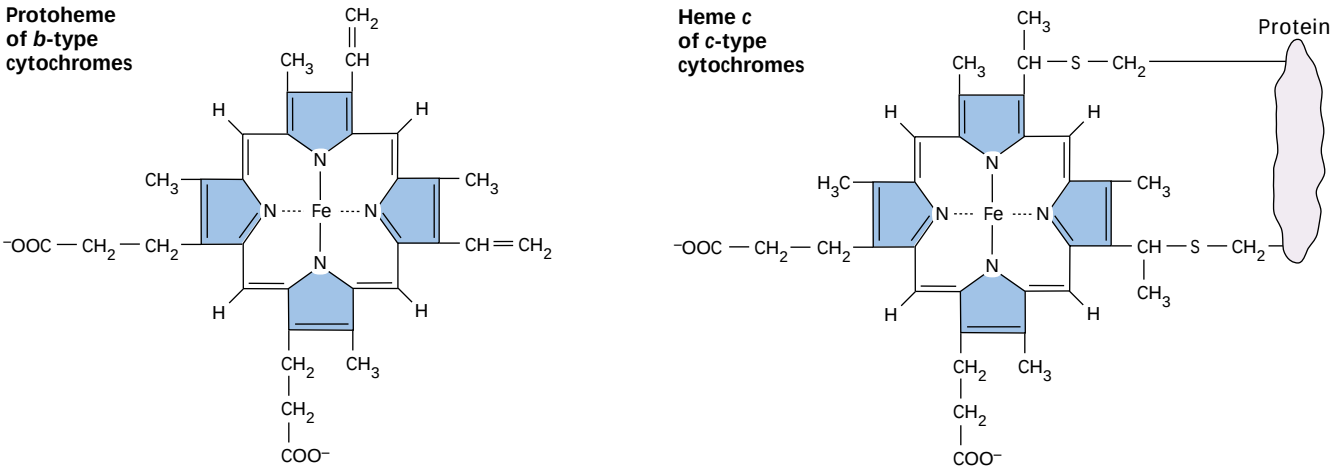


FIGURE 7.28 Structure of prosthetic groups of *b*- and *c*-type cytochromes. The protoheme group (also called protoporphyrin IX) is found in *b*-type cytochromes, the heme *c* group in *c*-type cytochromes. The heme *c* group is covalently attached to the protein by thioether linkages with two cysteine residues in the protein; the protoheme group is not covalently attached to the protein. The Fe ion is in the 2+ oxidation state in reduced cytochromes and in the 3+ oxidation state in oxidized cytochromes.

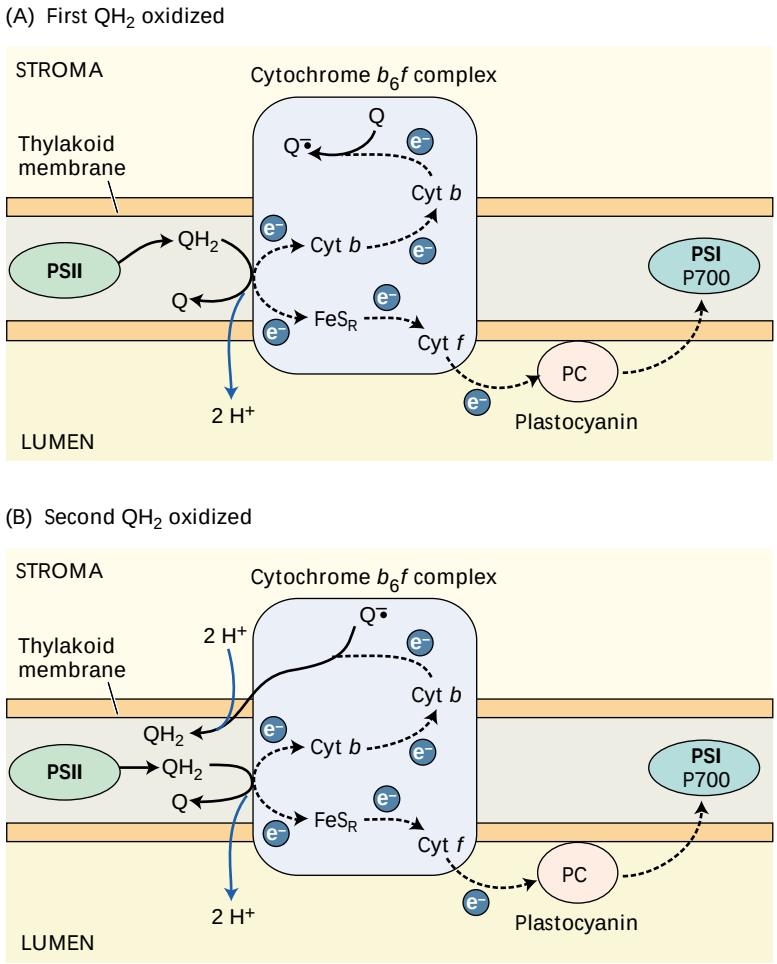


FIGURE 7.29 Mechanism of electron and proton transfer in the cytochrome b_6f complex. This complex contains two *b*-type cytochromes (Cyt *b*), a *c*-type cytochrome (Cyt *c*, historically called cytochrome *f*), a Rieske Fe–S protein (FeS_R), and two quinone oxidation–reduction sites. (A) The noncyclic or linear processes: A plastohydroquinone (QH_2) molecule produced by the action of PSII (see Figure 7.27) is oxidized near the lumenal side of the complex, transferring its two electrons to the Rieske Fe–S protein and one of the *b*-type cytochromes and simultaneously expelling two protons to the lumen. The electron transferred to FeS_R is passed to cytochrome *f* (Cyt *f*) and then to plastocyanin (PC), which reduces P700 of PSI. The reduced *b*-type cytochrome transfers an electron to the other *b*-type cytochrome, which reduces a quinone (Q) to the semiquinone ($Q^{\bullet}$) state (see Figure 7.27). (B) The cyclic processes: A second QH_2 is oxidized, with one electron going from FeS_R to PC and finally to P700. The second electron goes through the two *b*-type cytochromes and reduces the semiquinone to the plastohydroquinone, at the same time picking up two protons from the stroma. Overall, four protons are transported across the membrane for every two electrons delivered to P700.

the complex. Another similar sequence of electron flow fully reduces the plastoquinone, which picks up protons from the stromal side of the membrane and is released from the b_6f complex as plastohydroquinone.

The net result of two turnovers of the complex is that two electrons are transferred to P700, two plastohydroquinones are oxidized to the quinone form, and one oxidized plastoquinone is reduced to the hydroquinone form. In addition, four protons are transferred from the stromal to the lumenal side of the membrane.

By this mechanism, electron flow connecting the acceptor side of the PSII reaction center to the donor side of the PSI reaction center also gives rise to an electrochemical potential across the membrane, due in part to H^+ concentration differences on the two sides of the membrane. This electrochemical potential is used to power the synthesis of ATP. The cyclic electron flow through the cytochrome *b* and plastoquinone increases the number of protons pumped per electron beyond what could be achieved in a strictly linear sequence.

Plastoquinone and Plastocyanin Carry Electrons between Photosystems II and I

The location of the two photosystems at different sites on the thylakoid membranes (see Figure 7.18) requires that at least one component be capable of moving along or within the membrane in order to deliver electrons produced by photosystem II to photosystem I. The cytochrome b_6f complex is distributed equally between the grana and the stroma regions of the membranes, but its large size makes it unlikely that it is the mobile carrier. Instead, plastoquinone or plastocyanin or possibly both are thought to serve as mobile carriers to connect the two photosystems.

Plastocyanin is a small (10.5 kDa), water-soluble, copper-containing protein that transfers electrons between the cytochrome b_6f complex and P700. This protein is found in the lumenal space (see Figure 7.29). In certain green algae and cyanobacteria, a *c*-type cytochrome is sometimes found instead of plastocyanin; which of these two proteins is synthesized depends on the amount of copper available to the organism.

The Photosystem I Reaction Center Reduces $NADP^+$

The PSI reaction center complex is a large multisubunit complex (Figure 7.30) (Jordan et al. 2001). In contrast to PSII, a core antenna consisting of about 100 chlorophylls is a part of the PSI reaction center, P700. The core antenna and P700 are bound to two proteins, PsaA and PsaB, with molecular masses in the range of 66 to 70 kDa (Brettel 1997; Chitnis 2001; see also **Web Topic 7.8**).

The antenna pigments form a bowl surrounding the electron transfer cofactors, which are in the center of the complex. In

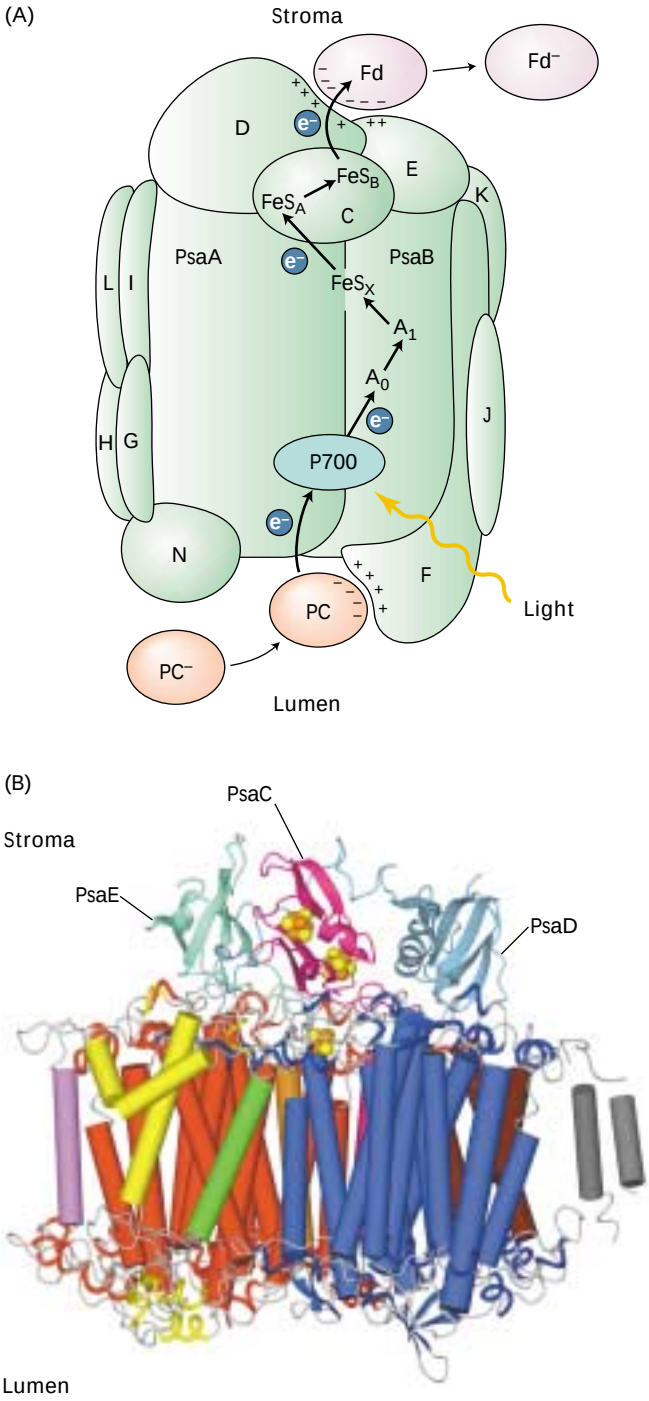


FIGURE 7.30 Structure of photosystem I. (A) Structural model of the PSI reaction center. Components of the PSI reaction center are organized around two major proteins, PsaA and PsaB. Minor proteins PsaC to PsaN are labelled C to N. Electrons are transferred from plastocyanin (PC) to P700 (see Figures 7.21 and 7.22) and then to a chlorophyll molecule, A_0, to phylloquinone, A_1, to the FeS_X, FeS_A, and FeS_B Fe–S centers, and finally to the soluble iron–sulfur protein, ferrodoxin (Fd). (B) Side view of one monomer of PSI from the cyanobacterium *Synechococcus elongatus*, at 2.5 Å resolution. The stromal side of the membrane is at the top, and the lumenal side is at the bottom of the figure. Transmembrane α-helices of PsaA and PsaB are shown as blue and red cylinders, respectively. (A after Buchanan et al. 2000; B from Jordan et al. 2001.)

their reduced form, the electron carriers that function in the acceptor region of photosystem I are all extremely strong reducing agents. These reduced species are very unstable and thus difficult to identify. Evidence indicates that one of these early acceptors is a chlorophyll molecule, and another is a quinone species, phylloquinone, also known as vitamin K_1.

Additional electron acceptors include a series of three membrane-associated iron–sulfur proteins, or bound ferredoxins, also known as **Fe–S centers FeS_X**, **FeS_A**, and **FeS_B** (see Figure 7.30). Fe–S center X is part of the P700-binding protein; centers A and B reside on an 8 kDa protein that is part of the PSI reaction center complex. Electrons are transferred through centers A and B to **ferredoxin** (**Fd**), a small, water-soluble iron–sulfur protein (see Figures 7.21 and 7.30). The membrane-associated flavoprotein **ferredoxin–NADP reductase** (**FNR**) reduces $NADP^+$ to NADPH, thus completing the sequence of noncyclic electron transport that begins with the oxidation of water (Karplus et al. 1991).

In addition to the reduction of $NADP^+$, reduced ferredoxin produced by photosystem I has several other functions in the chloroplast, such as the supply of reductants to reduce nitrate and the regulation of some of the carbon fixation enzymes (see Chapter 8).

Cyclic Electron Flow Generates ATP but no NADPH

Some of the cytochrome b_6f complexes are found in the stroma region of the membrane, where photosystem I is located. Under certain conditions **cyclic electron flow** from the reducing side of photosystem I, through the b_6f complex and back to P700, is known to occur. This cyclic electron flow is coupled to proton pumping into the lumen, which can be utilized for ATP synthesis but does not oxidize water or reduce $NADP^+$. Cyclic electron flow is especially important as an ATP source in the bundle sheath chloroplasts of some plants that carry out C_4 carbon fixation (see Chapter 8).

Some Herbicides Block Electron Flow

The use of herbicides to kill unwanted plants is widespread in modern agriculture. Many different classes of herbicides have been developed, and they act by blocking amino acid, carotenoid, or lipid biosynthesis or by disrupting cell division. Other herbicides, such as DCMU (dichlorophenyl-dimethylurea) and paraquat, block photosynthetic electron flow (Figure 7.31). DCMU is also known as diuron. Paraquat has acquired public notoriety because of its use on marijuana crops.

Many herbicides, DCMU among them, act by blocking electron flow at the quinone acceptors of photosystem II, by competing for the binding site of plastoquinone that is normally occupied by Q_B. Other herbicides, such as paraquat, act by accepting electrons from the early acceptors of photosystem I and then reacting with oxygen to form superoxide, O_2^-, a species that is very damaging to chloroplast components, especially lipids.

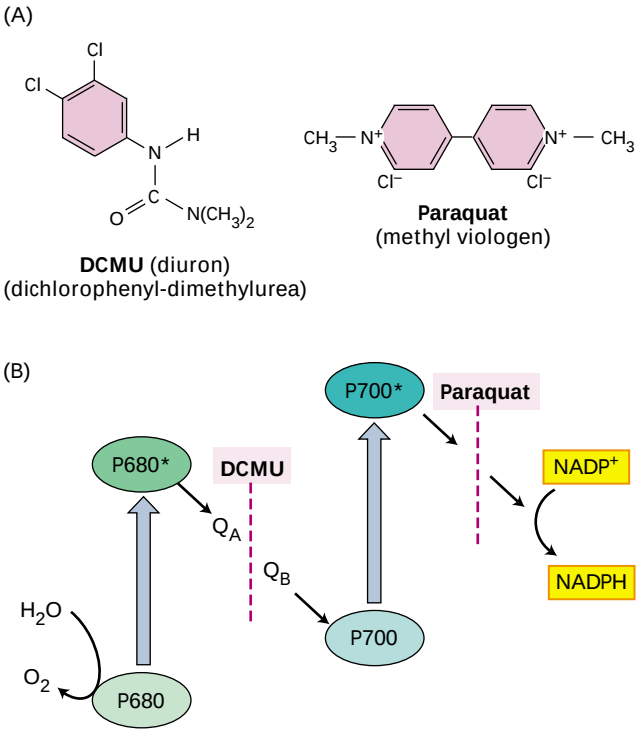


FIGURE 7.31 Chemical structure and mechanism of action of two important herbicides. (A) Chemical structure of dichlorophenyl-dimethylurea (DCMU) and methyl viologen (paraquat), two herbicides that block photosynthetic electron flow. DCMU is also known as diuron. (B) Sites of action of the two herbicides. DCMU blocks electron flow at the quinone acceptors of photosystem II, by competing for the binding site of plastoquinone. Paraquat acts by accepting electrons from the early acceptors of photosystem I.

PROTON TRANSPORT AND ATP SYNTHESIS IN THE CHLOROPLAST

In the preceding sections we learned how captured light energy is used to reduce $NADP^+$ to NADPH. Another fraction of the captured light energy is used for light-dependent ATP synthesis, which is known as **photophosphorylation**. This process was discovered by Daniel Arnon and his coworkers in the 1950s. In normal cellular conditions, photophosphorylation requires electron flow, although under some conditions electron flow and photophosphorylation can take place independently of each other. Electron flow without accompanying phosphorylation is said to be **uncoupled**.

It is now widely accepted that photophosphorylation works via the **chemiosmotic mechanism**, first proposed in the 1960s by Peter Mitchell. The same general mechanism drives phosphorylation during aerobic respiration in bacteria and mitochondria (see Chapter 11), as well as the transfer of many ions and metabolites across membranes (see Chapter 6). Chemiosmosis appears to be a unifying aspect of membrane processes in all forms of life.

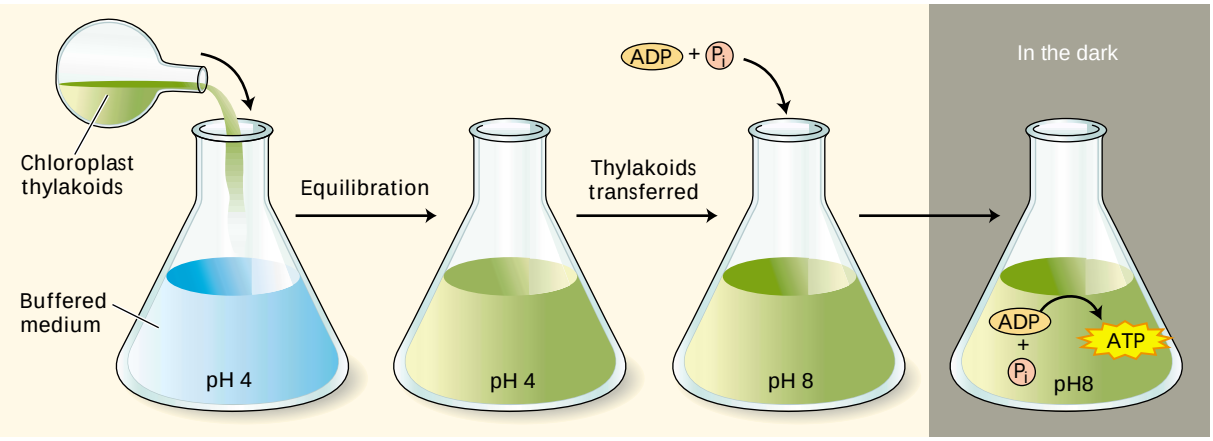


FIGURE 7.32 Summary of the experiment carried out by Jagendorf and coworkers. Isolated chloroplast thylakoids kept previously at pH 8 were equilibrated in an acid medium at pH 4. The thylakoids were then transferred to a buffer at pH 8 that contained ADP and P_i. The proton gradient generated by this manipulation provided a driving force for ATP synthesis in the absence of light. This experiment verified a prediction of the chemiosmotic theory stating that a chemical potential across a membrane can provide energy for ATP synthesis.

In Chapter 6 we discussed the role of ATPases in chemiosmosis and ion transport at the cell's plasma membrane. The ATP used by the plasma membrane ATPase is synthesized by photophosphorylation in the chloroplast and oxidative phosphorylation in the mitochondrion. Here we are concerned with chemiosmosis and transmembrane proton concentration differences used to make ATP in the chloroplast.

The basic principle of chemiosmosis is that ion concentration differences and electric-potential differences across membranes are a source of free energy that can be utilized by the cell. As described by the second law of thermodynamics (see Chapter 2 on the web site for a detailed discussion), any nonuniform distribution of matter or energy represents a source of energy. Differences in **chemical potential** of any molecular species whose concentrations are not the same on opposite sides of a membrane provide such a source of energy.

The asymmetric nature of the photosynthetic membrane and the fact that proton flow from one side of the membrane to the other accompanies electron flow were discussed earlier. The direction of proton translocation is such that the stroma becomes more alkaline (fewer H^+ ions) and the lumen becomes more acidic (more H^+ ions) as a result of electron transport (see Figures 7.22 and 7.29).

Some of the early evidence supporting a chemiosmotic mechanism of photosynthetic ATP formation was provided by an elegant experiment carried out by André Jagendorf and coworkers (Figure 7.32). They suspended chloroplast thylakoids in a pH 4 buffer, and the buffer diffused across the membrane, causing the interior, as well as the exterior, of the thylakoid to equilibrate at this acidic pH. They then rapidly transferred the thylakoids to a pH 8 buffer, thereby creating a pH difference of 4 units across the thylakoid membrane, with the inside acidic relative to the outside.

They found that large amounts of ATP were formed from ADP and P_i by this process, with no light input or electron transport. This result supports the predictions of the chemiosmotic hypothesis, described in the paragraphs that follow.

Mitchell proposed that the total energy available for ATP synthesis, which he called the **proton motive force** (Δp), is the sum of a proton chemical potential and a transmembrane electric potential. These two components of the proton motive force from the outside of the membrane to the inside are given by the following equation:

$$\Delta p = \Delta E - 59(\mathrm{pH_i} - \mathrm{pH_o}) \qquad (7.9)$$

where ΔE is the transmembrane electric potential, and $\mathrm{pH_i} - \mathrm{pH_o}$ (or ΔpH) is the pH difference across the membrane. The constant of proportionality (at 25°C) is 59 mV per pH unit, so a transmembrane pH difference of 1 pH unit is equivalent to a membrane potential of 59 mV.

Under conditions of steady-state electron transport in chloroplasts, the membrane electric potential is quite small because of ion movement across the membrane, so Δp is built almost entirely by ΔpH. The stoichiometry of protons translocated per ATP synthesized has recently been found to be four H^+ ions per ATP (Haraux and De Kouchkovsky 1998).

In addition to the need for mobile electron carriers discussed earlier, the uneven distribution of photosystems II and I, and of ATP synthase at the thylakoid membrane (see Figure 7.18), poses some challenges for the formation of ATP. ATP synthase is found only in the stroma lamellae and at the edges of the grana stacks. Protons pumped

across the membrane by the cytochrome b_6f complex or protons produced by water oxidation in the middle of the grana must move laterally up to several tens of nanometers to reach ATP synthase.

The ATP is synthesized by a large (400 kDa) enzyme complex known by several names: **ATP synthase**, **ATPase** (after the reverse reaction of ATP hydrolysis), and **CF_o–CF_1** (Boyer 1997). This enzyme consists of two parts: a hydrophobic membrane-bound portion called CF_o and a portion that sticks out into the stroma called CF_1 (Figure 7.33).

CF_o appears to form a channel across the membrane through which protons can pass. CF_1 is made up of several peptides, including three copies of each of the α and β peptides arranged alternately much like the sections of an orange. Whereas the catalytic sites are located largely on the β polypeptide, many of the other peptides are thought to have primarily regulatory functions. CF_1 is the portion of the complex that synthesizes ATP.

The molecular structure of the mitochondrial ATP synthase has been determined by X-ray crystallography (Stock et al. 1999). Although there are significant differences between the chloroplast and mitochondrial enzymes, they have the same overall architecture and probably nearly identical catalytic sites. In fact, there are remarkable similarities in the way electron flow is coupled to proton translocation in chloroplasts, mitochondria, and purple bacteria (Figure 7.34). Another remarkable aspect of the mechanism of the ATP synthase is that the internal stalk and probably much of the CF_o portion of the enzyme rotate during catalysis (Yasuda et al. 2001). The enzyme is actually a tiny molecular motor (see **Web Topics 7.9 and 11.4**).

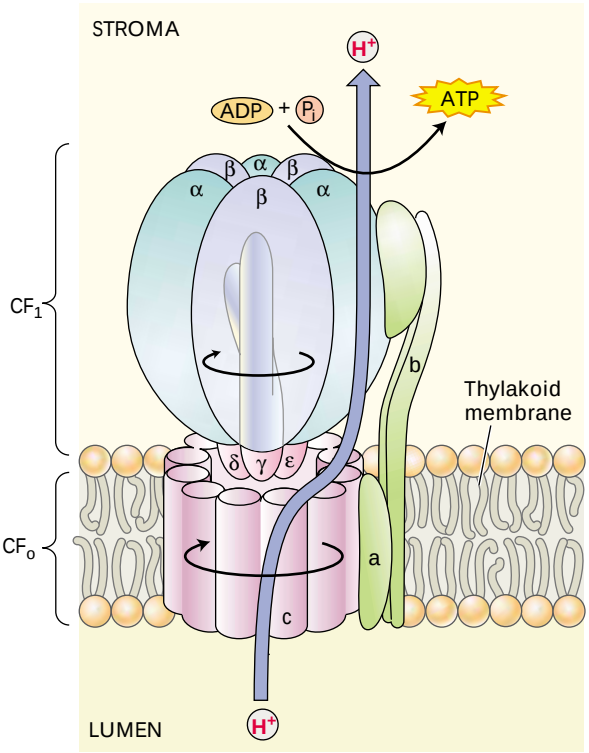


FIGURE 7.33 Structure of ATP synthase. This enzyme consists of a large multisubunit complex, CF_1, attached on the stromal side of the membrane to an integral membrane portion, known as CF_o. CF_1 consists of five different polypeptides, with a stoichiometry of α_3, β_3, γ, δ, ε. CF_o contains probably four different polypeptides, with a stoichiometry of a, b, b′, c_{12}.

REPAIR AND REGULATION OF THE PHOTOSYNTHETIC MACHINERY

Photosynthetic systems face a special challenge. They are designed to absorb large amounts of light energy and process it into chemical energy. At the molecular level, the energy in a photon can be damaging, particularly under unfavorable conditions. In excess, light energy can lead to the production of toxic species, such as superoxide, singlet oxygen, and peroxide, and damage can occur if the light energy is not dissipated safely (Horton et al. 1996; Asada 1999; Müller et al. 2001). Photosynthetic organisms therefore contain complex regulatory and repair mechanisms. Some of these mechanisms regulate energy flow in the antenna system, to avoid excess excitation of the reaction centers and ensure that the two photosystems are equally driven. Although very effective, these processes are not entirely fail-safe, and sometimes toxic compounds are produced. Additional mechanisms are needed to dissipate these compounds—in particular, toxic oxygen species.

Despite these protective and scavenging mechanisms, damage can occur, and additional mechanisms are required to repair the system. Figure 7.35 provides an overview of the several levels of the regulation and repair systems.

Carotenoids Serve as Photoprotective Agents

In addition to their role as accessory pigments, carotenoids play an essential role in **photoprotection**. The photosynthetic membrane can easily be damaged by the large amounts of energy absorbed by the pigments if this energy cannot be stored by photochemistry; this is why a protection mechanism is needed. The photoprotection mechanism can be thought of as a safety valve, venting excess energy before it can damage the organism. When the energy stored in chlorophylls in the excited state is rapidly dissipated by excitation transfer or photochemistry, the excited state is said to be **quenched**.

If the excited state of chlorophyll is not rapidly quenched by excitation transfer or photochemistry, it can react with molecular oxygen to form an excited state of oxygen known as **singlet oxygen** ($^1O_2$*). The extremely reactive singlet oxygen goes on to react with and damage many cellular components, especially lipids. Carotenoids exert their photoprotective action by rapidly quenching the excited state of chlorophyll. The excited state of carotenoids does not have

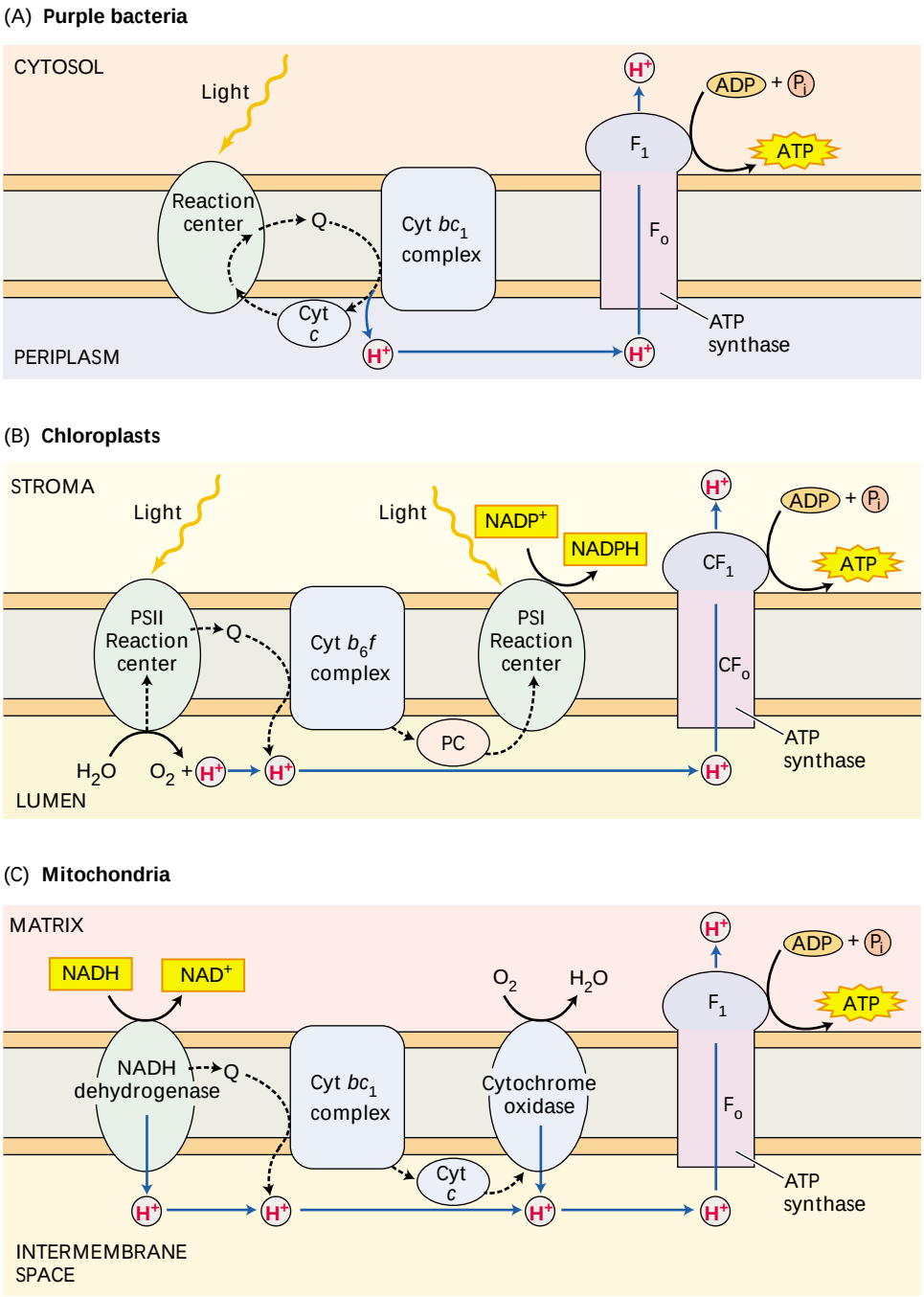


FIGURE 7.34 Similarities of photosynthetic and respiratory electron flow in bacteria, chloroplasts, and mitochondria. In all three, electron flow is coupled to proton translocation, creating a transmembrane proton motive force (Δp). The energy in the proton motive force is then used for the synthesis of ATP by ATP synthase. (A) A reaction center (RC) in purple photosynthetic bacteria carries out cyclic electron flow, generating a proton potential by the action of the cytochrome bc_1 complex. (B) Chloroplasts carry out noncyclic electron flow, oxidizing water and reducing $NADP^+$. Protons are produced by the oxidation of water and by the oxidation of PQH_2 (Q) by the cytochrome b_6f complex. (C) Mitochondria oxidize NADH to NAD^+ and reduce oxygen to water. Protons are pumped by the enzyme NADH dehydrogenase, the cytochrome bc_1 complex, and cytochrome oxidase. The ATP synthases in the three systems are very similar in structure.

sufficient energy to form singlet oxygen, so it decays back to its ground state while losing its energy as heat.

Mutant organisms that lack carotenoids cannot live in the presence of both light and molecular oxygen—a rather difficult situation for an O_2-evolving photosynthetic organism. For non-O_2-evolving photosynthetic bacteria, mutants that lack carotenoids can be maintained under laboratory conditions if oxygen is excluded from the growth medium.

Recently carotenoids were found to play a role in nonphotochemical quenching, which is a second protective and regulatory mechanism.

Some Xanthophylls Also Participate in Energy Dissipation

Nonphotochemical quenching, a major process regulating the delivery of excitation energy to the reaction center, can be thought of as a "volume knob" that adjusts the flow of

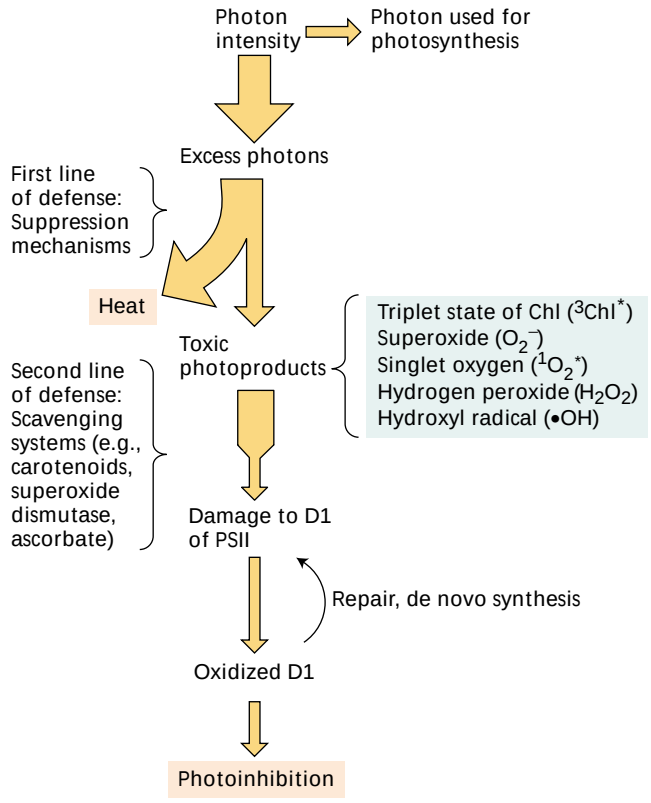


FIGURE 7.35 Overall picture of the regulation of photon capture and the protection and repair of photodamage. Protection against photodamage is a multilevel process. The first line of defense is suppression of damage by quenching of excess excitation as heat. If this defense is not sufficient and toxic photoproducts form, a variety of scavenging systems eliminate the reactive photoproducts. If this second line of defense also fails, the photoproducts can damage the D1 protein of photosystem II. This damage leads to photoinhibition. The D1 protein is then excised from the PSII reaction center and degraded. A newly synthesized D1 is reinserted into the PSII reaction center to form a functional unit. (After Asada 1999.)

excitations to the PSII reaction center to a manageable level, depending on the light intensity and other conditions. The process appears to be an essential part of the regulation of antenna systems in most algae and plants.

Nonphotochemical quenching is the quenching of chlorophyll fluorescence (see Figure 7.5) by processes other than photochemistry. As a result of nonphotochemical quenching, a large fraction of the excitations in the antenna system caused by intense illumination are quenched by conversion into heat (Krause and Weis 1991). Nonphotochemical quenching is thought to be involved in protecting the photosynthetic machinery against overexcitation and subsequent damage.

The molecular mechanism of nonphotochemical quenching is not well understood, although it is clear that the pH of the thylakoid lumen and the state of aggregation of the antenna complexes are important factors. Three carotenoids, called **xanthophylls**, are involved in nonphotochemical quenching: violaxanthin, antheraxanthin, and zeaxanthin (Figure 7.36).

In high light, violaxanthin is converted into zeaxanthin, via the intermediate antheraxanthin, by the enzyme violaxanthin de-epoxidase. When light intensity decreases, the process is reversed. Binding of protons and zeaxanthin to light-harvesting antenna proteins is thought to cause conformational changes that lead to quenching and heat dissipation (Demmig-

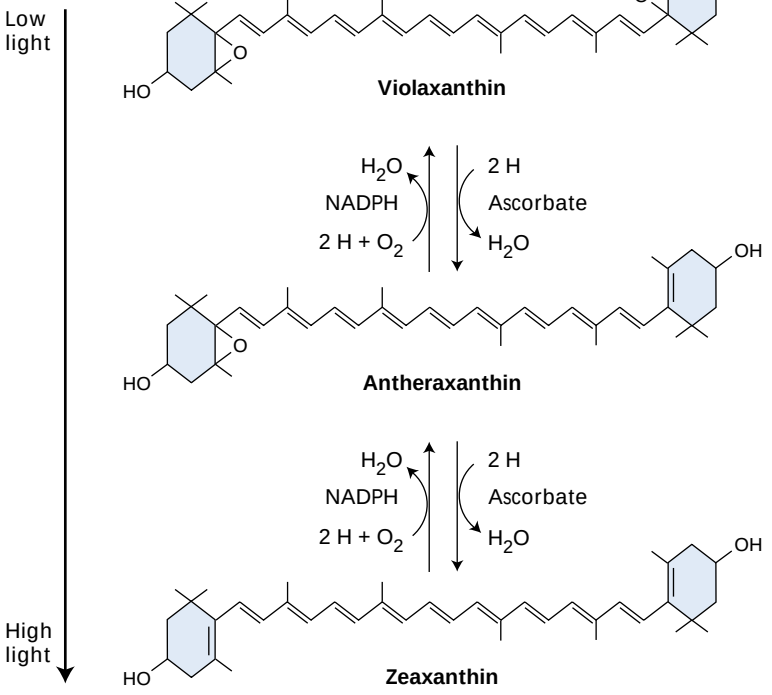


FIGURE 7.36 Chemical structure of violaxanthin, antheraxanthin, and zeaxanthin. The highly quenched state of photosystem II is associated with zeaxanthin, the unquenched state with violaxanthin. Enzymes interconvert these two carotenoids, with antheraxanthin as the intermediate, in response to changing conditions, especially changes in light intensity. Zeaxanthin formation uses ascorbate as a cofactor, and violaxanthin formation requires NADPH. (After Pfündel and Bilger 1994.)

Adams and Adams 1992; Horton et al. 1996). Nonphotochemical quenching appears to be preferentially associated with a peripheral antenna complex of photosystem II, the PsbS protein (Li et al. 2000).

The Photosystem II Reaction Center Is Easily Damaged

Another effect that appears to be a major factor in the stability of the photosynthetic apparatus is photoinhibition, which occurs when excess excitation arriving at the PSII reaction center leads to its inactivation and damage (Long et al. 1994). **Photoinhibition** is a complex set of molecular processes, defined as the inhibition of photosynthesis by excess light.

As will be discussed in detail in Chapter 9, photoinhibition is reversible in early stages. Prolongued inhibition, however, results in damage to the system such that the PSII reaction center must be disassembled and repaired (Melis 1999). The main target of this damage is the D1 protein that makes up part of the PSII reaction center complex (see Figure 7.24). When D1 is damaged by excess light, it must be removed from the membrane and replaced with a newly synthesized molecule. The other components of the PSII reaction center are not damaged by excess excitation and are thought to be recycled, so the D1 protein is the only component that needs to be synthesized.

Photosystem I Is Protected from Active Oxygen Species

Photosystem I is particularly vulnerable to damage from active oxygen species. The ferredoxin acceptor of PSI is a very strong reductant that can easily reduce molecular oxygen to form superoxide (O_2^-). This reduction competes with the normal channeling of electrons to the reduction of $NADP^+$ and other processes. Superoxide is one of a series of active oxygen species that can be very damaging to biological membranes. Superoxide formed in this way can be eliminated by the action of a series of enzymes, including superoxide dismutase and ascorbate peroxidase (Asada 1999).

Thylakoid Stacking Permits Energy Partitioning between the Photosystems

The fact that photosynthesis in higher plants is driven by two photosystems with different light-absorbing properties poses a special problem. If the rate of delivery of energy to PSI and PSII is not precisely matched and conditions are such that the rate of photosynthesis is limited by the available light (low light intensity), the rate of electron flow will be limited by the photosystem that is receiving less energy. In the most efficient situation, the input of energy would be the same to both photosystems. However, no single arrangement of pigments would satisfy this requirement because at different times of day the light intensity and spectral distribution tend to favor one photosystem or the other (Trissl and Wilhelm 1993; Allen and Forsberg 2001).

This problem can be solved by a mechanism that shifts energy from one photosystem to the other in response to different conditions. Such a regulating mechanism has been shown to operate in different experimental conditions. The observation that the overall quantum yield of photosynthesis is nearly independent of wavelength (see Figure 7.12) strongly suggests that such a mechanism exists.

Thylakoid membranes contain a protein kinase that can phosphorylate a specific threonine residue on the surface of LHCII, one of the membrane-bound antenna pigment proteins described earlier in the chapter (see Figure 7.20). When LHCII is not phosphorylated, it delivers more energy to photosystem II, and when it is phosphorylated, it delivers more energy to photosystem I (Haldrup et al. 2001).

The kinase is activated when plastoquinone, one of the electron carriers between PSI and PSII, accumulates in the reduced state. Reduced plastoquinone accumulates when PSII is being activated more frequently than PSI. The phosphorylated LHCII then migrates out of the stacked regions of the membrane into the unstacked regions (see Figure 7.18), probably because of repulsive interactions with negative charges on adjacent membranes.

The lateral migration of LHCII shifts the energy balance toward photosystem I, which is located in the stroma lamellae, and away from photosystem II, which is located in the stacked membranes of the grana. This situation is called *state 2*. If plastoquinone becomes more oxidized because of excess excitation of photosystem I, the kinase is deactivated and the level of phosphorylation of LHCII is decreased by the action of a membrane-bound phosphatase. LHCII then moves back to the grana, and the system is in *state 1*. The net result is a very precise control of the energy distribution between the photosystems, allowing the most efficient use of the available energy.

GENETICS, ASSEMBLY, AND EVOLUTION OF PHOTOSYNTHETIC SYSTEMS

Chloroplasts have their own DNA, mRNA, and protein synthesis machinery, but some chloroplast proteins are encoded by nuclear genes and imported into the chloroplast. In this section we will consider the genetics, assembly, and evolution of the main chloroplast components.

Chloroplast, Cyanobacterial, and Nuclear Genomes Have Been Sequenced

The complete chloroplast genomes of several organisms have been sequenced. Chloroplast DNA is circular and ranges in size from 120 to 160 kilobases. The chloroplast genome contains coding sequences for approximately 120 proteins. Some of these DNA sequences code for proteins that are yet to be characterized. It is uncertain whether all these genes are transcribed into mRNA and translated into protein, but it seems likely that some chloroplast proteins remain to be identified.

The complete genome of the cyanobacterium *Synechocystis* (strain PCC 6803) and the higher plant *Arabidopsis* have been sequenced, and genomes of important crop plants such as rice and maize have been completed (Kotani and Tabata 1998; Arabidopsis Genome Initiative 2000). Genomic data for both chloroplast and nuclear DNA will provide new insights into the mechanism of photosynthesis, as well as many other plant processes.

Chloroplast Genes Exhibit Non-Mendelian Patterns of Inheritance

Chloroplasts and mitochondria reproduce by division rather than by **de novo synthesis**. This mode of reproduction is not surprising, since these organelles contain genetic information that is not present in the nucleus. During cell division, chloroplasts are divided between the two daughter cells. In most sexual plants, however, only the maternal plant contributes chloroplasts to the zygote. In these plants the normal Mendelian pattern of inheritance does not apply to chloroplast-encoded genes because the offspring receive chloroplasts from only one parent. The result is **non-Mendelian**, or **maternal**, **inheritance**. Numerous traits are inherited in this way; one example is the herbicide resistance trait discussed in **Web Topic 7.10**.

Many Chloroplast Proteins Are Imported from the Cytoplasm

Chloroplast proteins can be encoded by either chloroplastic or nuclear DNA. The chloroplast-encoded proteins are synthesized on chloroplast ribosomes; the nucleus-encoded proteins are synthesized on cytoplasmic ribosomes and then transported into the chloroplast. Many nuclear genes contain introns—that is, base sequences that do not code for protein. The mRNA is processed to remove the introns, and the proteins are then synthesized in the cytoplasm.

The genes needed for chloroplast function are distributed in the nucleus and in the chloroplast genome with no evident pattern, but both sets are essential for the viability of the chloroplast. Some chloroplast genes are necessary for other cellular functions, such as heme and lipid synthesis. Control of the expression of the nuclear genes that code for chloroplast proteins is complex, involving light-dependent regulation mediated by both phytochrome (see Chapter 17) and blue light (see Chapter 18), as well as other factors (Bruick and Mayfield 1999; Wollman et al. 1999).

The transport of chloroplast proteins that are synthesized in the cytoplasm is a tightly regulated process (Chen and Schnell 1999). For example, the enzyme rubisco (see Chapter 8), which functions in carbon fixation, has two types of subunits, a chloroplast-encoded large subunit and a nucleus-encoded small subunit. Small subunits of rubisco are synthesized in the cytoplasm and transported into the chloroplast, where the enzyme is assembled.

In this and other known cases, the nucleus-encoded chloroplast proteins are synthesized as precursor proteins containing an N-terminal amino acid sequence known as a **transit peptide**. This terminal sequence directs the precursor protein to the chloroplast, facilitates its passage through both the outer and the inner envelope membranes, and is then clipped off. The electron carrier plastocyanin is a water-soluble protein that is encoded in the nucleus but functions in the lumen of the chloroplast. It therefore must cross three membranes to reach its destination in the lumen. The transit peptide of plastocyanin is very large and is processed in more than one step.

The Biosynthesis and Breakdown of Chlorophyll Are Complex Pathways

Chlorophylls are complex molecules exquisitely suited to the light absorption, energy transfer, and electron transfer functions that they carry out in photosynthesis (see Figure 7.6). Like all other biomolecules, chlorophylls are made by a biosynthetic pathway in which simple molecules are used as building blocks to assemble more complex molecules (Porra 1997; Beale 1999). Each step in the biosynthetic pathway is enzymatically catalyzed.

The chlorophyll biosynthetic pathway consists of more than a dozen steps (see **Web Topic 7.11**). The process can be divided into several phases (Figure 7.37), each of which can be considered separately, but which in the cell are highly coordinated and regulated. This regulation is essential because free chlorophyll and many of the biosynthetic intermediates are damaging to cellular components. The damage results largely because chlorophylls absorb light efficiently, but in the absence of accompanying proteins, they lack a pathway for disposing of the energy, with the result that toxic singlet oxygen is formed.

The breakdown pathway of chlorophyll in senescent leaves is quite different from the biosynthetic pathway (Matile et al. 1996). The first step is removal of the phytol tail by an enzyme known as chlorophyllase, followed by removal of the magnesium by magnesium de-chelatase. Next the porphyrin structure is opened by an oxygen-dependent oxygenase enzyme to form an open-chain tetrapyrrole.

The tetrapyrrole is further modified to form water-soluble, colorless products. These colorless metabolites are then exported from the senescent chloroplast and transported to the vacuole, where they are permanently stored. The chlorophyll metabolites are not further processed or recycled, although the proteins associated with them in the chloroplast are subsequently recycled into new proteins. The recycling of proteins is thought to be important for the nitrogen economy of the plant.

Complex Photosynthetic Organisms Have Evolved from Simpler Forms

The complicated photosynthetic apparatus found in plants and algae is the end product of a long evolutionary sequence. Much can be learned about this evolutionary

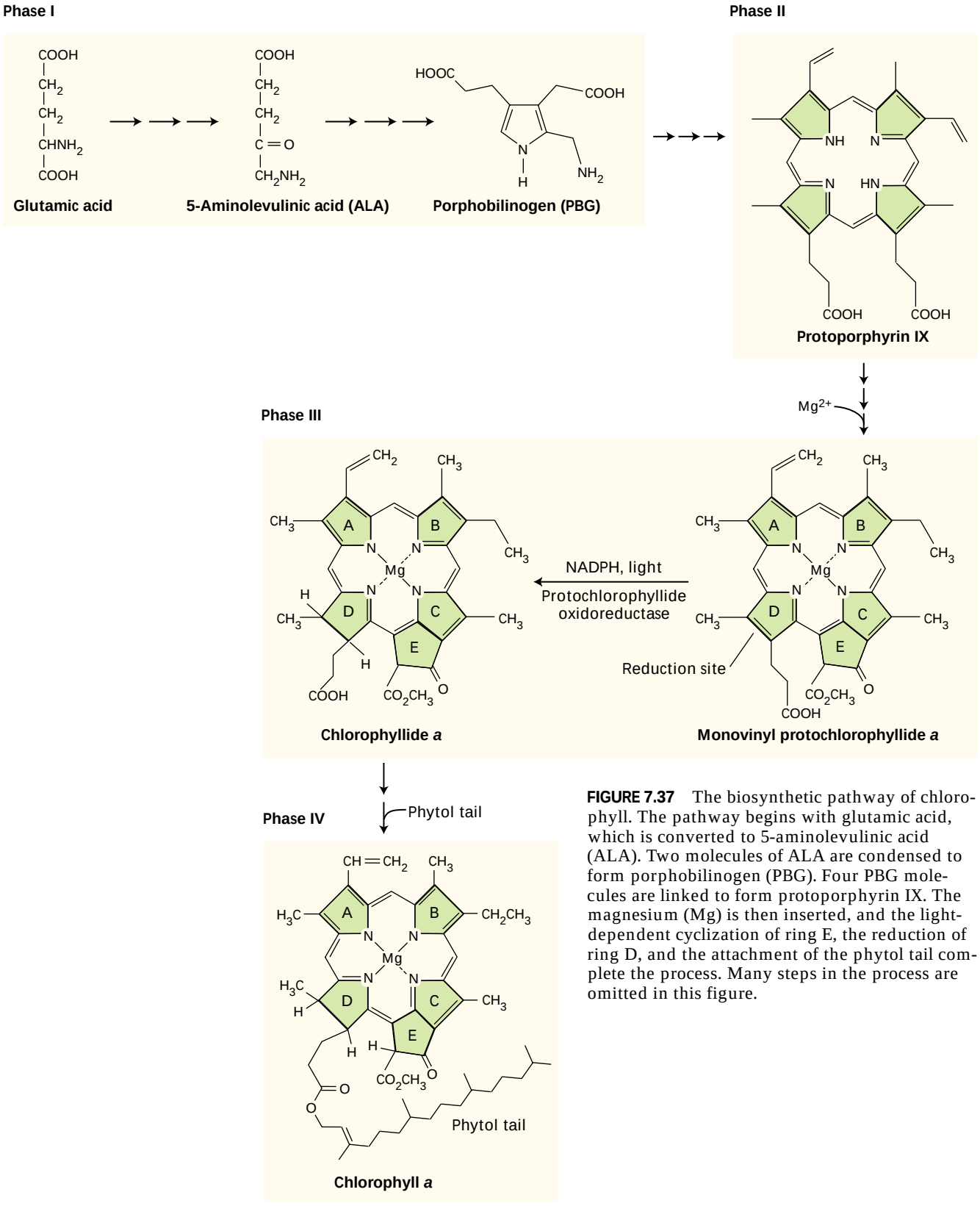


FIGURE 7.37 The biosynthetic pathway of chlorophyll. The pathway begins with glutamic acid, which is converted to 5-aminolevulinic acid (ALA). Two molecules of ALA are condensed to form porphobilinogen (PBG). Four PBG molecules are linked to form protoporphyrin IX. The magnesium (Mg) is then inserted, and the light-dependent cyclization of ring E, the reduction of ring D, and the attachment of the phytol tail complete the process. Many steps in the process are omitted in this figure.

process from analysis of simpler prokaryotic photosynthetic organisms, including the anoxygenic photosynthetic bacteria and the cyanobacteria.

The chloroplast is a semiautonomous cell organelle, with its own DNA and a complete protein synthesis apparatus. Many of the proteins that make up the photosynthetic apparatus, as well as all the chlorophylls and lipids, are synthesized in the chloroplast. Other proteins are imported from the cytoplasm and are encoded by nuclear genes. How did this curious division of labor come about? Most experts now agree that the chloroplast is the descendant of a symbiotic relationship between a cyanobacterium and a simple nonphotosynthetic eukaryotic cell. This type of relationship is called **endosymbiosis** (Cavalier-Smith 2000).

Originally the cyanobacterium was capable of independent life, but over time much of its genetic information needed for normal cellular functions was lost, and a substantial amount of information needed to synthesize the photosynthetic apparatus was transferred to the nucleus. So the chloroplast was no longer capable of life outside its host and eventually became an integral part of the cell.

In some types of algae, chloroplasts are thought to have arisen by endosymbiosis of eukaryotic photosynthetic organisms (Palmer and Delwiche 1996). In these organisms the chloroplast is surrounded by three and in some cases four membranes, which are thought to be remnants of the plasma membranes of the earlier organisms. Mitochondria are also thought to have originated by endosymbiosis in a separate event much earlier than chloroplast formation.

The answers to other questions related to the evolution of photosynthesis are less clear. These include the nature of the earliest photosynthetic systems, how the two photosystems became linked, and the evolutionary origin of the oxygen evolution complex (Blankenship and Hartman 1998; Xiong et al. 2000).

SUMMARY

Photosynthesis is the storage of solar energy carried out by plants, algae, and photosynthetic bacteria. Absorbed photons excite chlorophyll molecules, and these excited chlorophylls can dispose of this energy as heat, fluorescence, energy transfer, or photochemistry. Light is absorbed mainly in the antenna complexes, which comprise chlorophylls, accessory pigments, and proteins and are located at the thylakoid membranes of the chloroplast.

Photosynthetic antenna pigments transfer the energy to a specialized chlorophyll–protein complex known as a reaction center. The reaction center contains multisubunit protein complexes and hundreds or, in some organisms, thousands of chlorophylls. The antenna complexes and the reaction centers are integral components of the thylakoid membrane. The reaction center initiates a complex series of chemical reactions that capture energy in the form of chemical bonds.

The relationship between the amount of absorbed quanta and the yield of a photochemical product made in a light-dependent reaction is given by the quantum yield. The quantum yield of the early steps of photosynthesis is approximately 0.95, indicating that nearly every photon that is absorbed yields a charge separation at the reaction center.

Plants and some photosynthetic prokaryotes have two reaction centers, photosystem I and photosystem II, that function in series. The two photosystems are spatially separated: PSI is found exclusively in the nonstacked stroma membranes, PSII largely in the stacked grana membranes. The reaction center chlorophylls of PSI absorb maximally at 700 nm, those of PSII at 680 nm. Photosystems II and I carry out noncyclic electron transport, oxidize water to molecular oxygen, and reduce $NADP^+$ to NADPH. It is energetically very difficult to oxidize water to form molecular oxygen, and the photosynthetic oxygen-evolving system is the only known biochemical system that can oxidize water, thus providing almost all the oxygen in Earth's atmosphere. The photooxidation of water is modeled by the five-step S state mechanism. Manganese is an essential cofactor in the water-oxidizing process, and the five S states appear to represent successive oxidized states of a manganese-containing enzyme.

A tyrosine residue of the D1 protein of the PSII reaction center functions as an electron carrier between the oxygen-evolving complex and P680. Pheophytin and two plastoquinones are electron carriers between P680 and the large cytochrome $b_6 f$ complex. Plastocyanin is the electron carrier between cytochrome $b_6 f$ and P700. The electron carriers that accept electrons from P700 are very strong reducing agents, and they include a quinone and three membrane-bound iron–sulfur proteins known as bound ferredoxins. The electron flow ends with the reduction of $NADP^+$ to NADPH by a membrane-bound, ferrodoxin–NADP reductase.

A portion of the energy of photons is also initially stored as chemical-potential energy, largely in the form of a pH difference across the thylakoid membrane. This energy is quickly converted into chemical energy during ATP formation by action of an enzyme complex known as the ATP synthase. The photophosphorylation of ADP by the ATP synthase is driven by a chemiosmotic mechanism. Photosynthetic electron flow is coupled to proton translocation across the thylakoid membrane, and the stroma becomes more alkaline and the lumen more acidic. This proton gradient drives ATP synthesis with a stoichiometry of four H^+ ions per ATP. NADPH and ATP formed by the light reactions provide the energy for carbon reduction.

Excess light energy can damage photosynthetic systems, and several mechanisms minimize such damage. Carotenoids work as photoprotective agents by rapidly quenching the excited state of chlorophyll. Changes in the phosphorylated state of antenna pigment proteins can

change the energy distribution between photosystems I and II when there is an imbalance between the energy absorbed by each photosystem. The xanthophyll cycle also contributes to the dissipation of excess energy by nonphotochemical quenching.

Chloroplasts contain DNA and encode and synthesize some of the proteins that are essential for photosynthesis. Additional proteins are encoded by nuclear DNA, synthesized in the cytosol, and imported into the chloroplast. Chlorophylls are synthesized in a biosynthetic pathway involving more than a dozen steps, each of which is very carefully regulated. Once synthesized, proteins and pigments are assembled into the thylakoid membrane.

Web Material

Web Topics

7.1 Principles of Spectrophotometry
Spectroscopy is a key technique to study light reactions.

7.2 The Distribution of Chlorophylls and Other Photosynthetic Pigments
The content of chlorophylls and other photosynthetic pigments varies among plant kingdoms.

7.3 Quantum Yield
Quantum yields measure how effectively light drives a photobiological process.

7.4 Antagonistic Effects of Light on Cytochrome Oxidation
Photosystems I and II were discovered in some ingenious experiments.

7.5 Structures of Two Bacterial Reaction Centers
X-ray diffraction studies resolved the atomic structure of the reaction center of photosystem II.

7.6 Midpoint Potentials and Redox Reactions
The measurement of midpoint potentials is useful for analyzing electron flow through photosystem II.

7.7 Oxygen Evolution
The S state mechanism is a valuable model for water splitting in PSII.

7.8 Photosystem I
The PSI reaction is a multiprotein complex.

7.9 ATP Synthase
The ATP synthase functions as a molecular motor.

7.10 Mode of Action of Some Herbicides
Some herbicides kill plants by blocking photosynthetic electron flow.

7.11 Chlorophyll Biosynthesis
Chlorophyll and heme share early steps of their biosynthetic pathways.

Web Essay

7.1 A novel view of chloroplast structure
Stromules extend the reach of the chloroplasts.

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