Light Reactions of Photosynthesis

4

Key concepts

- Early cells evolved chlorophyll photosystems capable of capturing light energy to supply the energy needs of the cell.

- Charge separation occurs at a chlorophyll molecule in the light reaction center of the photosystems, with the reduction of an acceptor molecule, leaving the chlorophyll in an oxidized state.

- Light harvesting chlorophyll proteins extend the antenna area for collecting photons and channeling energy to the photosystems.

- Two photosystems evolved: one uses water to supply electrons that reduce the oxidized chlorophyll (with the loss of oxygen), the other uses electrons derived from reduced plastocyanin supplied from the first center to reduce $NADP^+$ to NADPH.

- Electron flow between the two centers occurs via a chain of intermediates assisted by a cytochrome protein complex.

- The splitting of water releases protons into the lumen of the photosynthetic lamellae, and more protons are pumped in during the successive reduction and oxidation steps of the intermediates, with the hydrogen atom carrier plastoquinone playing a crucial part.

- The high proton concentration gradient across the lamella membrane is used to drive a molecular turbine, ATP synthase, which generates ATP.

- Mechanisms exist that balance light energy input into the two photosystems and protect the cell from the consequences of the capture of excess light energy.

Bacteria evolved the basic photochemical pathways found in plants today

Photosynthesis has been pivotal in the development of complex life forms on this planet. This process converts light energy into chemical energy that is used to support cellular processes and to provide the basic raw materials from which cell structures are made. In addition, photosynthesis releases oxygen from water as a by-product. The oxygen has accumulated in the atmosphere, transforming the early anoxic conditions on our planet and enabling the evolution of terrestrial life forms. The metabolism of eukaryotes is invariably aerobic. This chapter will concentrate on the photochemical

processes involved in photosynthesis, while Chapter 5 will deal with the subsequent chemical events involved in carbon fixation from atmospheric carbon dioxide. First, the basic features of the photochemical processes found in plants and their evolutionary origins will be reviewed. The key to the success of photosynthesis lies in the rapid separation of electrically charged products after an initial light reaction and the subsequent efficient formation of stable products that can be used to drive anabolic reactions (molecule-building reactions) in the cell. The section on plastids in Chapter 3 should be consulted before reading this chapter.

In the initial evolution of life and cellular entities, sources of chemical energy were exploited to facilitate the synthesis of more complex molecules. Initially, relatively simple chemical molecules that were available in the anoxic environment of that time served this purpose. Analogous activities are found today in organisms living in the vicinity of marine hydrothermal vents and other geothermal environments. As with all biological systems, relatively simple single chemical steps evolved, and were combined to give sequential reactions (pathways) that conferred a selective advantage in the evolutionary struggle for resources, and hence survival. It is probable that these cells evolved a high level of capability, providing the forerunners of today's organisms.

Over time (hundreds of millions of years), the chemical energy sources became depleted, providing a strong selective advantage for the evolution of organisms capable of using alternative energy sources. One pervasive energy source is sunlight. Light energy at more energetic, shorter, ultraviolet (UV) wavelengths probably promoted the synthesis of carbon and nitrogen compounds from simple precursors, such as carbon dioxide, methane, water, and ammonia. These compounds would then have been utilized by living organisms. Although the high energy quanta of UV light are favorable for generating small molecules, they are far too energetic to be used by cells for this purpose. Instead cells arose containing pigment molecules that could safely absorb and utilize light energy in the slightly longer wavelength, visible part of the spectrum; this gave them a survival advantage over their competitors.

Specifically, bacteria evolved that could use the energy trapped from light to form reduced compounds and high energy intermediates, which were then utilized in anabolic processes. The earliest such organisms used chemical sources of reducing power, for example purple photosynthetic bacteria used ferrous ions (Fe^{2+}), which were oxidized to insoluble ferric (Fe^{3+}) salts. These were deposited as iron bands in sediments. Green sulfur bacteria used hydrogen sulfide (H_2S) as a source of electrons. Elemental sulfur was discarded as a waste product leading to the creation of the large deposits of sulfur that are mined today. These organisms probably evolved early in the history of the evolution of life, about 3×10^9 years ago. They left recognizable signals in the geological record consistent with carbon fixation (reduction of atmospheric carbon dioxide to organic molecules).

These early photosynthetic systems were not sustainable on a planet-wide scale, because of limitations in the local availability of appropriate chemicals, such as H_2S. Evolution of variant forms that could utilize other electron donors was therefore favored. About 3.5×10^9 years ago cyanobacteria evolved that were able to utilize water (H_2O). Water is chemically similar to H_2S, and, as a source of electrons, it is clearly far more readily available on this planet. Chlorophyll evolved as the light-absorbing pigment, and the energy trapped was used to power ATP synthesis, while NADPH was made as the stable reduced end product. Oxygen was released as a waste product and started to accumulate in the atmosphere about 2.7×10^9 years ago.

The appearance of free oxygen, a very reactive molecule, led to dramatic chemical changes on this planet. Soluble ferrous iron salts were oxidized to insoluble ferric compounds that were laid down in vast beds of iron ore, mined today for the steel industry. Accumulation of molecular oxygen in the atmosphere shielded living organisms from the damaging effects of UVC. In addition, some of this oxygen was photochemically converted to ozone forming a layer in the upper atmosphere that provided protection against UVB radiation for the emerging life forms below. Loss of UV radiation at the surface of the planet meant that abiotic photochemical production of organic compounds was greatly reduced; therefore, the conditions that had led to the initial evolution of life were irreversibly changed.

Organisms unable to cope with the presence of oxygen, a strong oxidant, were forced to live in anaerobic environments, such as deep muds, where similar forms can be found today. Some, the aerobic organisms, evolved mechanisms to take advantage of the presence of oxygen to power oxidation processes that released all the available chemical energy in organic compounds. These were oxidized to water and carbon dioxide in a process that we call cellular respiration (Chapter 6).

How does photosynthesis fundamentally work? Trapping of photons by chlorophyll molecules raises some of their electrons to a higher energy level. These would naturally return to their ground state condition within nanoseconds (10^{-9} s), releasing the energy as heat and photons emitted as fluorescence at a longer (lower energy level) wavelength. The trick evolved by photosynthetic organisms is to move this high energy electron to an acceptor molecule on a much faster, picosecond (10^{-12} s), time scale. The acceptor molecule is thus reduced and the chlorophyll is left in a photo-oxidized state.

Two sets of reactions and components (two photosystems) evolved in cyanobacteria to take advantage of this momentary charge separation. In one photosystem, electrons had to be supplied to the oxidized chlorophyll, to replenish (reduce) the pigment molecule for the next photon excitation event, and to minimize the chance of a backflow from the acceptor molecule. This was achieved by the evolution of a water-splitting, oxygen-evolving, center that sequentially provided electrons to a pathway culminating at the chlorophyll molecule. Then the acceptor molecule had to rapidly pass on an electron, again to minimize the risk of backflow, and to enable the acceptor to receive the next electron from chlorophyll. These two sets of reactions were kept physically separated, to avoid short-circuiting the whole system, by arranging them in a linear sequence across the width of a lipid bilayer membrane.

This electron flow from chlorophyll to acceptor resulted in the formation of a stable reduced form of a copper-containing protein called plastocyanin. Plastocyanin is a relatively large molecule, whose reducing power is inaccessible for cellular metabolism. However, its utility lies in the relative ease with which the reduced form can donate electrons (much easier than water). This allows a second light-trapping chlorophyll photosystem, also located across a membrane, to pump these electrons along a carrier chain to eventually reach and reduce NADPH. NADPH is a relatively small molecule that can readily pass on its reducing power in cellular metabolism. Two systems are required because a single light reaction does not yield enough energy to extract electrons from water and move them through the entire pathway to the formation of NADPH.

Crucially, the photosynthetic membranes enclose a lumen, an internal space separated from the stroma outside. Both of these light reactions, and some of

the intermediate steps, result in the accumulation of protons (H$^+$) within the lumen. The concentration gradient of protons established across this membrane is then used to drive a molecular turbine, located in these membranes, which synthesizes ATP.

It is probable that the two photosystems evolved independently. The oxygen-evolving photosystem could have been derived from purple and green non-sulfur bacteria, while the NADPH-forming photosystem probably originated from green sulfur bacteria. These were combined in the cyanobacteria (presumably by lateral gene transfer) which were the first organisms to use the oxidation of water to power ATP and NADPH formation with the release of free oxygen (Figure 4.1).

Symbiotic association between these bacteria and eukaryotes led to the evolution of the eukaryotic plant cell with chloroplasts. Today the dual photochemical

Figure 4.1 Possible evolutionary pathways for the two photosystems (RCI and RCII) and pigments found in cyanobacteria (bacterial chlorophylls bch*a* and bch*g*). Lateral transfer of genetic material for both pigment synthesis and photosynthetic membrane protein complexes must have occurred at several stages. (Reprinted with permission from the *Annual Review of Plant Biology*, Volume 53, © 2003 by Annual Reviews, www.annualreviews.org.)
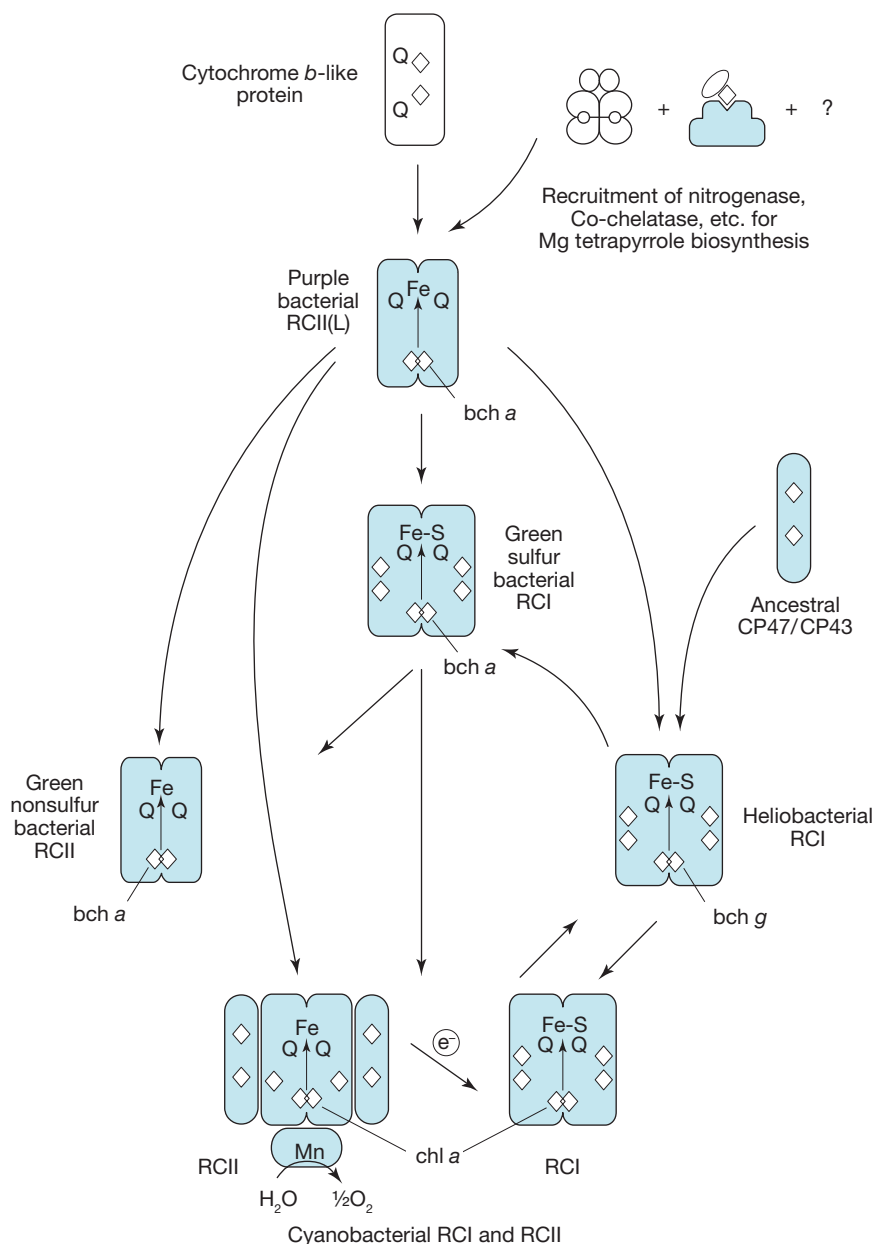
system of terrestrial, aquatic, and marine plants traps a significant proportion of the solar energy striking the planet, using it to form stable chemical products that have supported the existence and evolution of most of the life forms found on Earth today.

Remarkably, comparison of the structures of the two photosystems from higher plants with those from cyanobacteria show that over one thousand million years of evolution has resulted in very few changes. Clearly, the supposed primitive ancestors of today's plants had, in fact, evolved a truly efficient process that has stood the test of time. This account concentrates on the structure and function of higher plant photosystems as far as they are known at present. The major advance that has occurred during evolution is the development of light harvesting chlorophyll (LHC) proteins that greatly increase the area of the receiving antenna for each photosystem. This development has characterized the few changes that are found in the photosystems of modern land plants. Some polypeptides have been lost to make way for the effective docking of these new chlorophyll proteins, and others have evolved to assist the docking process. Across the range of land plants there is a remarkably small level of variation in the structure of their photosystems and the amino acid sequences of their constituent proteins. In addition to chlorophylls, other pigments, i.e. carotenoids, have been added to these proteins, which extend the range of light wavelengths that can be trapped for photosynthesis. These developments have ensured that plant leaves are able to intercept and utilize a high proportion of the visible light energy that is incident on the leaf surface.

In summary, photosynthesis starts with two linked photochemical systems that trap light energy. Water is split to release oxygen and electrons, which are ultimately used to make the reduced form of NADPH. Along the way protons (H^+) are accumulated in the grana lumen, creating a charge separation across the photosynthetic membrane that drives the ATP synthesis system.

When placed in their physical context (Figure 4.2) the two photosystems are seen to lie in the plane of the photosynthetic membrane. Both pass electrons via carrier intermediates from the lumen side to the stromal side of the membrane. The first photosystem, called Photosystem II (PSII; because it was the second to be discovered), releases protons and oxygen within the granal lumen. PSII reduces the small molecule plastoquinone (PQ) to plastoquinol (PQH_2), which is formed on the stromal side of the membrane. PQH_2 is a small lipophyllic molecule that diffuses in the plane of the membrane to a transmembrane cytochrome $b6$ complex where it enters the Q cycle. This cycle produces reduced plastocyanin on the lumen side while also pumping protons from the stroma into the lumen. Reduced plastocyanin donates
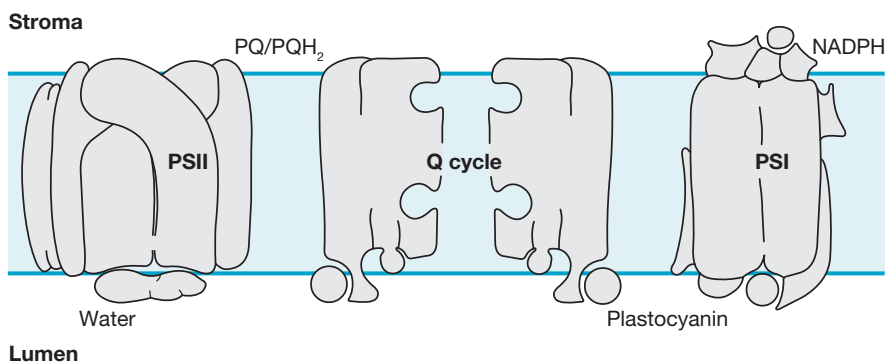

Stroma

PQ/PQH$_2$ NADPH

PSII   Q cycle   PSI

Water   Plastocyanin

Lumen

Figure 4.2 Photosystem II and Photosystem I are positioned in the photosynthetic membrane.

electrons to the second photosystem, Photosystem I (PSI; discovered first), which ultimately leads to the formation of reduced NADPH.

These reactions can be considered in terms of the redox potentials of the component redox couples, as each component can exist in either the reduced or the oxidized states. These drive the reactions energetically downhill from more negative potentials to more positive ones. A plot of the standard redox potentials of the components (Figure 4.3) shows that the pathway from water to reduced PSII goes from +1.1 to –0.7 electron volts (eV). The potentials of the PSII to plastocyanin pathway decline from –0.7 to +0.5 eV. PSI is boosted to –1.3 eV on light activation, and then the pathway declines to –0.4 eV with the formation of reduced NADPH. This plot is referred to as the Z scheme because of its characteristic shape, and was first proposed by Eugene Rabinowitch in 1945 and confirmed by his experiments in 1956 and 1957; and by the work of Robin Hill with Derek and Fay Bendall. Rabinowitch is also famous for his role in the development of the atomic bomb and for his campaign to the American Government for the development of peaceful uses for atomic energy. We will return to the Z scheme in more detail in a later section.

The following sections provide details of the structure and function of the two photosystems, the cytochrome complex, and the ATP-generating complex (ATP synthase). It must be emphasized that there is much that we still do not understand about photosynthesis. This is a hot research area with many papers being published each year exploring such basic issues as the molecular structure of the components, their functions, and the kinetics of the processes involved. The usual introductory textbook account of photosynthesis is a simplification that, in places, hides uncertainty and ignores fascinating, if perplexing, details.

Figure 4.3 The Z scheme summarizing the electron flow from water to NADPH plotted on the redox potential scale. The components are plotted according to the redox potentials of the component redox couples.
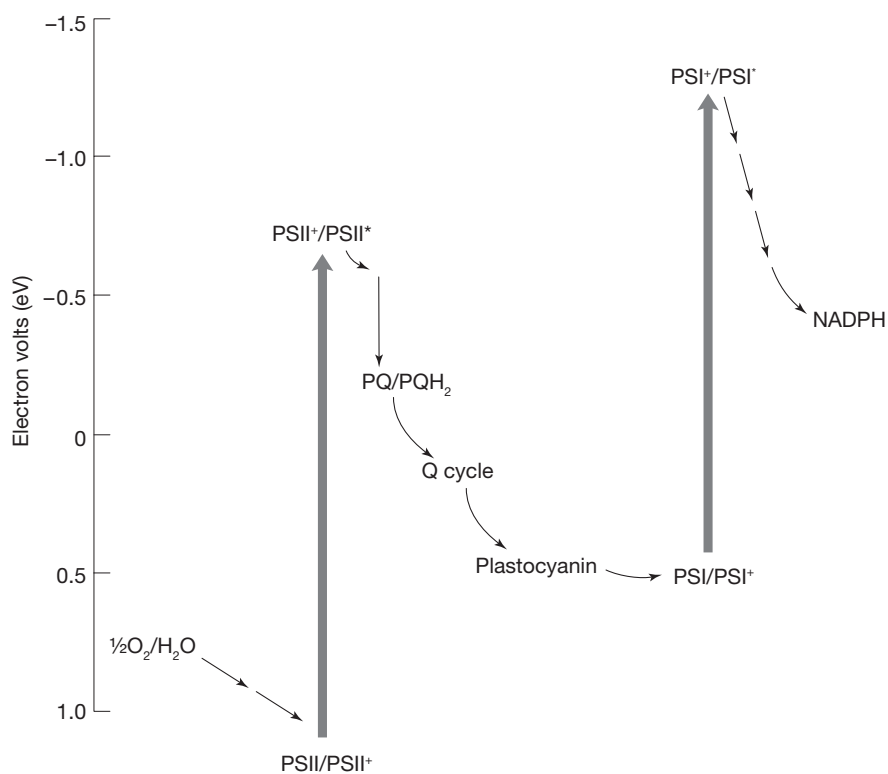
Chlorophyll captures light energy and converts it to a flow of electrons

Chlorophyll pigments have absorption maxima in the blue and red bands of the visible light spectrum and thus reflect and transmit green light. Engelmann and Sachs first discovered the dependence of photosynthesis on chlorophyll and Emerson and Arnold (in 1932) deduced that several hundred molecules of chlorophyll (a photosynthetic unit) are required for the production of one molecule of oxygen. Later, 1939, Robin Hill, working with isolated chloroplasts, demonstrated the direct connection between the light reaction steps and the release of molecular oxygen. This Hill reaction was extensively studied in both isolated chloroplasts and in whole algal cells by Otto Warburg. Eventually (1957) Emerson's work revealed that two photosystems, bearing chlorophylls of slightly different absorption properties, are involved in the photosynthetic process.

Evolution has favored the conservation of a very precise molecular configuration, so we must assume that all elements of the chlorophyll molecule are of critical importance for its functioning. Each chlorophyll molecule (Figure 4.4, see Chapter 12 for synthesis of this pigment) has a hydrophobic phytol tail attached to a hydrophilic head, which is a tetrapyrrole ring (porphyrin, more properly termed chlorin) containing a single magnesium atom (analogous heme rings contain an iron atom).

Small variations in the head pyrroles lead to formation of different chlorophyll molecules with slightly different light absorption characteristics (Figure 4.5). Analysis of chlorophyll extracts from leaves shows that chlorophyll *a* is the main form present (main absorption peak 665 nm), with chlorophyll *b* making up about 15% of the total. Chlorophyll *b* has a formyl (–CHO) group on the porphyrin ring in place of the methyl (–CH$_3$) group in this position in chlorophyll *a*. In some algal groups chlorophyll *b* is replaced by chlorophyll *c* (lacks a phytol tail) or chlorophyll *d* (has a formyl group in the ring in place of the vinyl group found in chlorophyll *a*). Chlorophyll *d* has its main absorption peak at longer wavelengths than chlorophyll *a*, beyond the visible light spectrum in the infrared (700 nm instead of 665 nm). It is found in cyanobacteria that grow as epiphytes under the fronds of red algae, and in other places, such as the undersides of didemnid ascidians of coral reefs. These are habitats where visible light wavelengths have been depleted leaving a light spectrum relatively rich in near infra-red radiation. In these cyanobacteria chlorophyll *d* replaces chlorophyll *a* in at least one of the photosystem reaction centers.

The phytol tail is a hydrophobic terpene chain 20 carbons long. The tails are identical in all forms of chlorophyll and in bacteriochlorophyll. The tails associate with a series of specific membrane proteins located in the thylakoid and stroma lamellae (frets) of chloroplasts (and with the internal membranes of cyanobacteria). Hence nearly all chlorophyll molecules are part of specific membrane protein complexes.

Two of these membrane protein complexes are the light reacting centers, Photosystems II and I (PSII and PSI) first detected by Emerson in 1957. PSI is involved in the second light reaction in the electron flow sequence. Most of the chlorophyll molecules are associated with proteins of the LHC protein complexes. These occupy most of the internal membrane surface area in the chloroplast, providing an efficient light-trapping system. See Box 4.1 for details of the structure of LHC protein complexes.
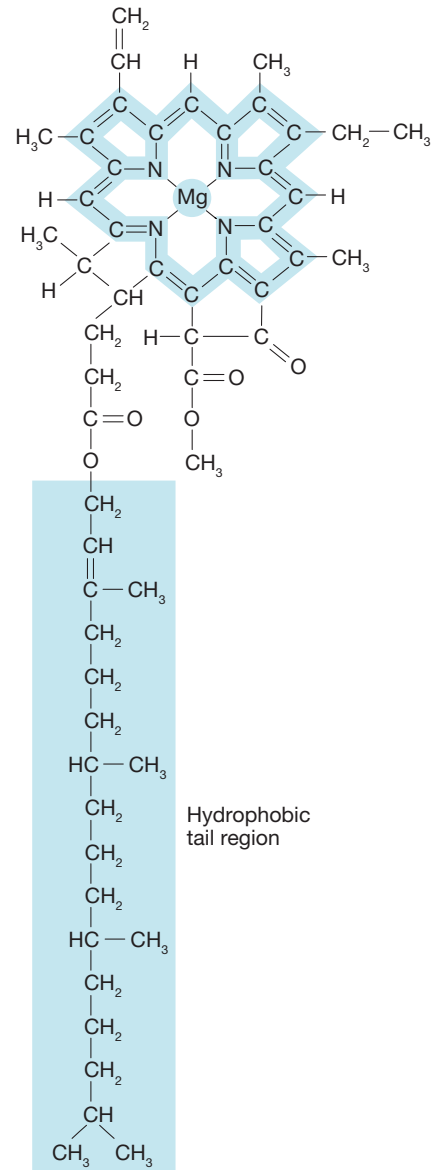

Figure 4.4 Structure of chlorophyll *a*. The tetrapyrrole porphyrin head has a single magnesium atom at its center. Electrons are shared between the atoms of this ring, making it less difficult for one to be temporarily lost from the structure. The hydrophobic phytol tail associates with specific sites in the membrane proteins. (From Alberts et al, Molecular Biology of the Cell 4th edition, New York, NY: Garland Science, 2002.)

Figure 4.5 Different pigments have different light absorption spectra, as shown here for chlorophyll *a*, chlorophyll *b* (main chlorophyll *a* peak shifted to blue green, minor peak shifted to shorter red wavelengths) and a carotene (absorbs only at blue–green end). A combination of these pigments provides a leaf with a fairly broad absorption spectrum. This is closely matched by the action spectrum showing that the energy absorbed at each wavelength is efficiently utilized in photosynthesis. Deviations from close matching of action and absorption spectra are attributable principally to some inactive absorption by carotenoids and the red drop wavelength around 700 nm, where PSII is not activated by monochromatic light at this wavelength. (Reprinted with permission from *Cell and Molecular Biology: Concepts and Experiments,* Gerald Karp, 2003. Copyright John Wiley & Sons Limited. Reproduced with permission.)
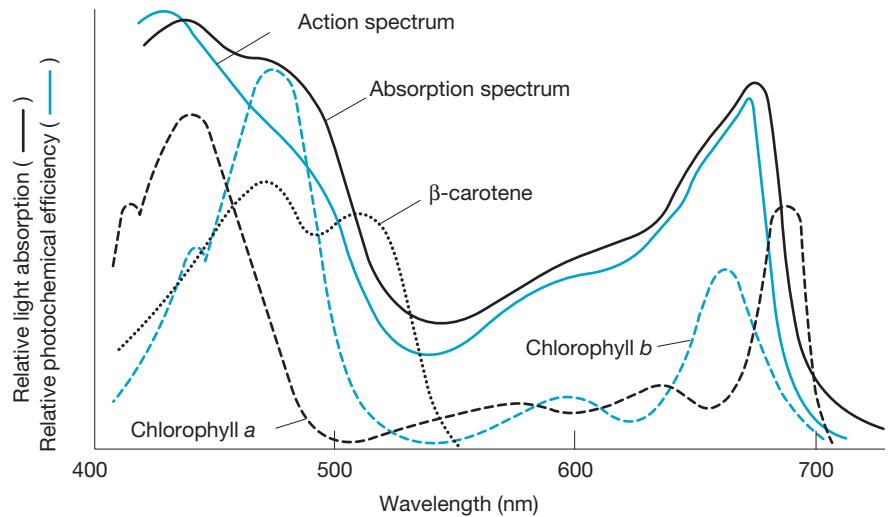
Light photons from the sun possess different energy levels, depending on the frequency or wavelength. Shorter wavelength photons (blue, 450 nm) have quanta with 1.5 times the energy level of longer wavelength photons (red, 675 nm). When a chlorophyll molecule absorbs a red wavelength photon, an electron in the porphyrin head is raised to a higher energy level (an excited singlet state, state 1). Blue light photons will raise an electron to a still higher energy level (state 2), but this state is very unstable and the electron rapidly descends to the state 1 level, with the loss of energy as heat. In free chlorophyll molecules, electrons would return from the excited state 1 to the ground state on a nanosecond (10^{-9} s) time-scale, losing energy by emitting a photon (fluorescence) at a lower energy (longer wavelength) level. When bound in a membrane protein, the excited electron is passed on to an acceptor molecule on a picosecond (10^{-12} s) time-scale. Excited state 1 singlet electrons may be temporarily unable to return to the ground state, so they are trapped at a slightly lower energy level in the triplet state. In this state the spin orientation of electrons is aligned (parallel), in contrast to state 1 electrons, which have opposite (antiparallel) spins. When these triplet electrons decay to the ground state they emit phosphorescence. This occurs later than fluorescence and is at a slightly longer wavelength than fluorescence, reflecting the lower amount of energy released. Delayed fluorescence can also take place, but in the living chloroplast fluorescence is very low compared with isolated chlorophyll in extracts, because so many excited electrons are trapped and do not return to the ground state.

The energy from excited state 1 electrons can be passed on to other pigment molecules (energy transfer), or used to reduce an acceptor molecule, converting the energy gained from light into a chemical product (photochemistry). Only a small fraction of the chlorophyll molecules is directly involved in photochemistry, the conversion of light energy to chemical energy, by reducing an acceptor molecule. As this event happens rapidly (about 1 ps) it is not a rate-limiting step. Acquisition of the appropriate photon is a much slower event, so a large number of chlorophyll molecules collaborate as an antenna, trapping photons and passing their energy on by resonance energy transfer to the core chlorophyll molecules. This ensures a high throughput of electrons to the acceptor. In fact the placement of chlorophyll molecules in the antenna molecules is so precise that there is almost 100% quantum efficiency of trapping.

Box 4.1 Structure of light-harvesting chlorophyll protein complexes and their role in photosynthesis

Most of the chlorophyll molecules in a plant are associated with light-harvesting chlorophyll protein complexes (LHC). These are divided into two main classes, those associated with Photosystem I (PSI), which are known as LHCI, and those predominantly associated with Photosystem II (PSII), termed LHCII. These proteins are very similar to each other. Both have four helices (A, B, C, and D); with A and B being closely associated transmembrane helices, C also lying perpendicular and D lying parallel to the membrane. LHCI chlorophyll pigments have absorption maxima at longer wavelengths than those of LHCII.

LHCI protein molecules contain polypeptides from a family of four 25 kDa proteins, Lhca 1–4. These bind a total of 56 chlorophylls with an a–b ratio of 3.5:1. LHC1 680 consists of homodimers of Lhca 2 and 3, while LHC1 730 consists of heterodimers of Lhca 1 and 4. Light of up to 750 nm can be used to oxidize the P700 reaction center. This high concentration of pigment molecules leads to red-shifting of their absorption spectra, enabling light harvesting to occur in dense vegetation canopies where the remaining light is richer in longer wavelengths (above 680 nm).

There have been many attempts to define the relationship between these four proteins and PSI. Some models, partly based on examination of bacterial photosystems, have four dimers (eight molecules) of Lhca associated with each PSI complex. Recent models based on higher plant material have one molecule each of all four. These form a semicircular array around one side of the PSI complex, in the sequence Lhca1 with Lhca4, and Lhca2 with Lhca3. These molecules are arranged to maximize the contact between their chlorophylls and PSI, with the D helix of one pointing towards the C helix of the next around the semi-circle from Lhca1 to Lhca3.

The main link to PSI (Box 4.4) appears to be via Lhca1 to PsaG, with the C helix interacting with two helices in PsaG. There is a weaker link from Lhca3 to PsaK at the opposite side of the semicircle (Figure 1). This arrangement facilitates changes in the precise composition of the array. There are slight differences between the four polypeptides and their chlorophylls, which affect their light absorption properties, so different combinations are optimal for different environmental conditions.

Lhca polypeptides bind 14 chlorophyll *a* or chlorophyll *b* molecules. Chlorophyll molecules also act as linkers, both between the Lhca polypeptides (two between each pair in a dimer, one between the dimers) and between these polypeptides and PSI (one on each, facing the reaction center). These have distinctive absorption spectra and serve to pass excitation energy between the polypeptides and to PSI. The absorption peaks of LHCI are at slightly shorter wavelengths (680–700 nm) than PSI (700 nm), facilitating energy flow to the reaction center.
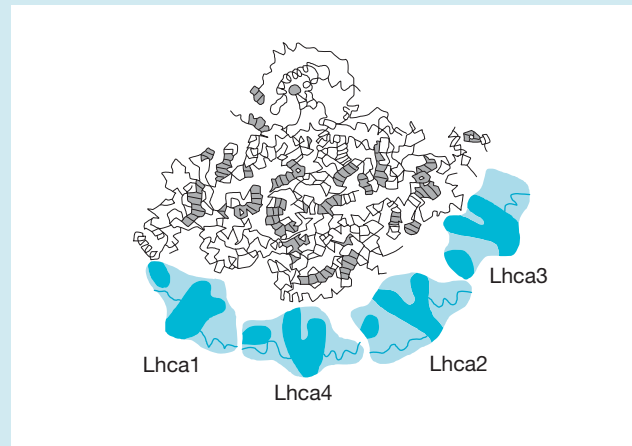

Figure 1 Four LHCI proteins form a semi-circle around one side of the PSI complex. This arrangement leaves room for LHCII trimers to bind to the other side of PSI under certain conditions. (Reprinted with permission from J. P. Dekker: *Biochimica et Biophysica Acta* 1706: 12–39, Figures 2A Page 14 and 4A, Page 18; by J.P. Dekker and E.J. Boekema, 2005.)

The LHCII complex contains the major chlorophyll and protein component of internal chloroplast membranes, accounting for more than half of all the membrane proteins and chlorophyll pigments in the plant. This major LHCII protein exists as a trimer of polypeptides, taken from the three polypeptides Lhcb1, Lhcb2, and Lhcb3. Trimers can be of homopolymers of Lhcb1, or heteropolymers of any combination of two or all three polypeptides. These major LHCII polypeptides are not present in equimolar proportions, there is twice as much Lhcb1 as of the other two combined.

Each major LHCII polypeptide consists of eight chlorophyll *a* and six chlorophyll *b* molecules, four carotenoid molecules, two complex lipids and a polypeptide chain that has three membrane-spanning α-helices. Eight chlorophylls are arranged as a layer towards the stromal side of each major polypeptide, and six towards the lumen side. Two central carotenoids are essential for the folding of LHCII polypeptides into a stable complex. A third carotenoid is associated with the chlorophyll *b* molecules. These three carotenoids serve to extend the absorption spectrum of LHCII into the blue–green part of the light spectrum, channeling the excitation energy received to the chlorophylls. The fourth carotenoid is associated with the xanthophyll cycle and the nonphotochemical quenching processes. The lipid phosphatidylglycerol is the only significant phospholipid of thylakoid membranes and is associated with LHCII. An unusual *trans*-fatty acid, 16:1Δ3, is a component of phosphatidylglycerol.

Box 4.1 Structure of light-harvesting chlorophyll protein complexes and their role in photosynthesis (continued)

In the trimer the three sets of eight chlorophylls on the stromal side form two concentric rings. The inner six are chlorophyll *a*, acting to transfer energy between monomers, while the outer 18 have alternating three chlorophyll *a* and three chlorophyll *b* around the circle. These serve to collect energy from a broad spectrum and pass it on to the P680 reaction center. Chlorophylls on the lumen side pass their collected energy on to these rings on the stromal side.

There are also three minor LHCII proteins, Lhcb4, Lhcb5, and Lhcb6, which do not polymerize with each other, but form bridges between PSII and the major LHCII components. Lhcb4 and Lhcb6 lie side by side, binding to PSII on one side and to a LHCII trimer on the other (Figure 2). This trimer (M) is less strongly bound to the PSII–LHCII supercomplex than the adjacent trimer (S), which binds directly to the PSII surface beside Lhcb4. Lhcb5 is associated with this strongly bound trimer and the PSII surface. Each supercomplex consists of two PSII complexes (the PSII dimer) and four LHCII trimers, two strongly bound and two more weakly bound. Overall analysis of the total chlorophyll protein complexes shows a ratio of eight trimers per PSII dimer, so it is assumed that only half the trimers are bound specifically to the supercomplexes and the remainder are loosely associated in the surrounding membrane. As PSII is located in the appressed granal membranes, it is thought that supercomplexes in adjacent membranes lie one above the other, permitting excitation energy to flow between LHCII proteins on adjacent membranes of neighboring thylakoids.

Light harvesting by LHCII is assisted by the carotenoids, absorbing at 450–500 nm, passing energy to the chlorophylls, which absorb at 660–680 nm. This absorption
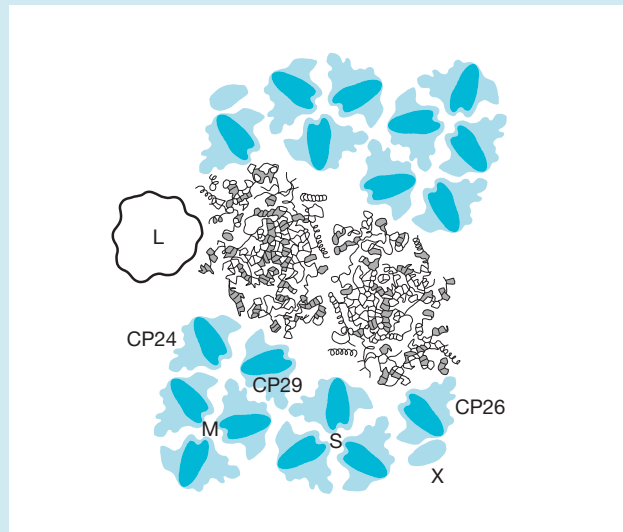

Figure 2 Major LHCII trimers (M and S) and minor proteins Lhcb4 (CP29), Lhcb5 (CP26), Lhcb6 (CP24) bound to a PSII dimer. The S trimer is strongly bound, while the M trimer shows medium binding. L indicates a possible position for a weakly bound trimer. (Reprinted with permission from J.P. Dekker: *Biochimica et Biophysica Acta* 1706: 12–39, by J.P. Dekker and E.J. Boekema, 2005.)

range is at shorter wavelengths than the PSII reaction center (680 nm), so creating a downhill energy flow from the LHC to the reaction center (shorter wavelength light is more energetic).

Carotenoids extend the spectral range of light that can be utilized in photosynthesis

Carotenoids are long chain pigments that absorb blue and green light (Figure 4.5), leaving yellow, orange, and red colors seen in such plant tissues as carrot roots (for details of carotenoid synthesis see Chapter 12). There are two types of carotenoids, carotenes, and xanthophylls. Each is a chain of 40 carbon atoms with alternating single and double bonds. Carotenes have only hydrogen atoms attached to this carbon backbone, but xanthophylls have one atom of oxygen at each end of the molecule. For example, zeaxanthin is the same as β carotene but with a hydroxyl group at each end. Carotenoids are incorporated into the chlorophyll/protein complexes where they perform two functions. They extend the range of wavelength energies (Figure 4.5) that can contribute to photosynthesis by passing on absorbed energy to neighboring chlorophylls. The xanthophyll fucoxanthin in brown seaweeds and diatoms is especially efficient in harvesting blue and green light and passing energy to chlorophylls. Also they provide protection for the reaction centers, dissipating excess energized electrons as heat and preventing the formation of damaging reactive oxygen species (see section Nonphotochemical quenching and the xanthophyll cycle).

Photosystem II splits water to form protons and oxygen, and reduces plastoquinone

PSII is located in the membranes of thylakoids making up the granal stacks. It consists of a core dimer of chlorophyll protein molecules that spans the membrane bilayer (Box 4.2). PSII uses light energy to remove electrons from water releasing protons and oxygen. The electrons are then used to reduce PQ to PQH_2. The core chlorophyll *a* in PSII has a maximum light absorption peak at 680 nm, and so is called P680 (pigment 680). This passes an electron on to an acceptor chlorophyll-like molecule (pheophytin, lacks magnesium), called A_0. The first photochemical reaction thus leaves the P680 molecule in an oxidized state ($P680^+$) and the acceptor in a reduced state (A_0^-). Two sets of reactions rapidly regenerate the $P680/A_0$ pair in time for the next light reaction.

All these electron transfer steps in photosynthesis share a common feature. They involve loss of an electron from one component, leaving it in an oxidized state indicated by a plus charge sign; and the gain of the electron by another component, leaving it in a reduced state, indicated by a minus sign. These components lie close to each other in a chain, and each has a redox potential that is slightly higher (more positive) than the previous member of the chain. This dictates the direction of flow of the charge along the chain. Typically, chain members are small molecules or atoms of metallic elements that can exist in a number of valency states. Iron would be a common example, existing in either the reduced state Fe^{2+}, or the oxidized state Fe^{3+}. We shall also meet others, such as copper and manganese. These atoms are held, either singly or in defined clusters, in an organic matrix created by polypeptides. Using iron as an example again, it is often held in place by cross-linking to sulfur atoms of cysteine residues as in Rieske proteins (2Fe–2S) and other non-heme iron proteins that carry electrons. This arrangement allows the gain or loss of an electron to be shared between the coordinately linked atoms.

In one set of reactions, $P680^+$ is reduced by the movement of an electron from an adjacent tyrosine molecule (TyrZ) in the polypeptide chain of the D1 protein of the PSII complex (Box 4.2). The oxidized tyrosine is in turn reduced by the provision of electrons from the oxygen-evolving center.

Electrons are donated from water to replenish those lost by the tyrosine molecule. Two water molecules are split simultaneously to yield one oxygen atom (O_2), four protons (H^+), and four electrons. However, tyrosine can only accept one electron at a time. So the oxygen-evolving center has evolved as a charge accumulation device, consisting of four manganese atoms held in a protein matrix with one atom each of calcium and chlorine (Box 4.2). This center sequentially provides single electrons to the tyrosine molecule, until four electrons have been donated, one from each manganese atom. Then two molecules of water are split simultaneously to replenish the four electrons donated to tyrosine. The protons are released into the lumen of the thylakoid/stroma-lamellar system. Later we will see how these electrons are utilized in ATP synthesis (see section ATP synthase utilizes the proton motive force to generate ATP).

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

In the dark, the ground state of the oxygen-evolving center is always (+), so that, on illumination, only three photochemical events are needed to bring about the evolution of the first oxygen molecule. Thereafter one is formed on every fourth event. This was first observed by P. Joliot and coworkers in 1969, who illuminated a suspension of algal cells that had been kept in the dark

Author query
Do you need to add anything to link this paragraph to the 2nd paragraph on page 78? They are separated by two pages of Box 4.2

Box 4.2 Structure of Photosystem II

Photosystem II (PSII) is a major membrane protein complex consisting of over 20 polypeptides and about 250 chlorophyll molecules. It is located in the grana stack thylakoid membranes, where it protrudes on each side of the plane of the membrane, by about 1 nm on the stromal side and 5.5 nm on the lumen side. The boundary lipids associated with the complex provide a specific membrane environment for the hydrophobic photosystem proteins.

Fourteen of the 20 polypeptides are integral membrane proteins with a total of 36 transmembrane α-helices. The complex is bilaterally symmetrical, with a dimer of very similar proteins, D1 and D2, in the center (Figure 1). Each polypeptide has five transmembrane helices and this pair of proteins is flanked by a further pair of strongly related proteins, CP43 (adjacent to D1) and CP47 (adjacent to D2). CP43 and CP47 each consist of six transmembrane helices. CP43 has a large loop on the lumen side that is essential for completing the water splitting manganese ion cluster (Figure 2). One of the manganese ions is bound to a CP43 glutamine residue.

D1 and D2 each contain three chlorophyll molecules, while CP43 contains 14 and CP47 contains 16 chlorophyll molecules. The majority of these chlorophylls are ligated to fully conserved histidine residues in each protein. The heads of the chlorophylls lie in flat planes near each face of the membrane, but each protein has one chlorophyll located midway across the membrane, which acts as a bridge between the chlorophylls on the opposite faces. Two xanthophyll (lutein) molecules are embedded in the complex. This structure has been determined by a combination of spectroscopy and X-ray crystallography of isolated components. Functional analysis has been aided by site-directed mutagenesis, in which individual amino acids in specific proteins have been replaced and the resultant effects on photosynthetic efficiency measured.

PSII exists as a dimer surrounded by LHCII trimers to form a supercomplex. The LHCII chlorophyll proteins act as an antenna that receives photon excitation energy and passes it on to the CP43/47 chlorophylls (Box 4.1).

The P680 reaction center consists of a pair of chlorophyll molecules, one on each of the central dimer polypeptides, D1/D2, that lie very closely together (Figure 1). One of these, on D1, is the recipient of excitation energy from CP43/47 at 680 nm, forming the reduced compound P680$^+$. This in turn reduces pheophytin on D1 and then the first plastoquinone molecule, at Q_A over on D2, where it is tightly bound. It is important to note that the redox potential of the Q_A/Q_A^- is sufficiently positive relative to P680 that it decreases the probability of charge recombination (backflow). Thus part of the energy received by the reaction center is lost as stabilizing energy to decrease the probability of backflow and increase the probability of forward electron transfer down a redox potential gradient.

Q_B is the corresponding site back on D1, where plastoquinol is loosely bound, so that when plastoquinone is reduced and protonated to plastoquinol it is released. The Q_B site lies to the stromal side of the membrane. It is in the form of an elbow-shaped cavity or tunnel opening on to the stromal surface of PSII at one end and to the adjacent membrane matrix at the other. The tunnel is lipophilic, lined with the phytol tails of chlorophylls, together with some lipid groups and carotenoids. This allows the plastoquinol/plastoquinone to diffuse in and out of the photosystem, from the membrane, without encountering the aqueous phase of the stroma.

There is also an alternative route for electrons on the stromal side of PSII. They can be fed back to reduce P680$^+$ in a cyclic flow from Q_B via cytochrome b_{559}. This provides an important safety route for disposing of excess electrons that would otherwise lead to the formation of damaging intermediates, such as active oxygen species (Box 4.6).

On the inner, luminal side of D1 lies the tyrosine residue (Tyr161) that donates electrons to P680$^+$. This step is closely associated with a histidine amino acid (D1-His190) to which it donates a proton, leaving a tyrosyl residue that can oxidize the oxygen evolving center. The corresponding amino acid on D2 (D2-Tyr160, associated with D2-His189)
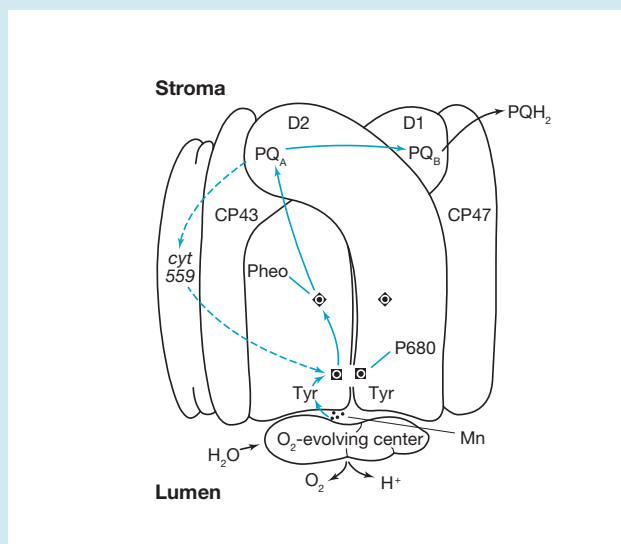

Figure 1 Photosystem II showing the main components discussed in this box. The solid blue arrows show the pathway of electrons from manganese in the oxygen-evolving center to P680, pheophytin (Pheo, A$_0$) and on to the site PQ$_B$ and the formation of plastoquinol (PQH$_2$). The dashed blue arrow shows a possible cyclic route for electrons from PQ$_A$ back to P680 through cytochrome 559. Black arrows indicate small molecules that diffuse towards or away from the photosystem. Black lines label components.

Box 4.2 Structure of Photosystem II (continued)

is oxidized by P680, but is not involved in water oxidation. This oxidation seems to bias electron transfer to the D1 side of the reaction center.

The oxygen evolving center consists of three extrinsic membrane proteins. These are closely bound to the projecting chains of D1 and D2 on the luminal side of PSII and serve to shield the core of the center. The core consists of four manganese atoms, a calcium atom, and a chlorine atom (Figure 2). The manganese atoms provide electrons to Tyr$_Z$, shifting progressively from state III to state IV as four electrons are lost from the cluster. The calcium and chlorine atoms provide a chemical environment that assists with the combining of oxygen atoms from water to make oxygen molecules.

The most likely arrangement of these atoms has three of the manganese atoms lying on a flat plane at the corners of the base of an imaginary triangular pyramid, with a calcium atom at its apex. In this pyramid, the calcium-manganese distances are all similar (0.34 nm), but greater than that for two of the manganese–manganese distances (0.27 nm). The third side of the manganese triangle is longer, about the same as the calcium–manganese distance (0.33 nm). The fourth manganese atom is linked by an oxygen atom to one of the manganese atoms, and lies in about the same plane. This cluster of cations is interlinked by oxygen bridges and stabilized by interactions with carboxylate groups on the adjacent polypeptide chains.

The calcium atom is linked by oxygen bridges to two of the manganese atoms and to chlorine (Figure 3). Calcium is highly electrophilic and would facilitate the withdrawal of electrons from oxygen in a water molecule and the release of protons. One of the water molecules destined for splitting is probably bound to one of the manganese atoms while the other is held within the coordination sphere of the calcium atom. Possibly this manganese atom is oxidized to state V, deprotonating the bound water molecule and leaving the oxygen atom open to forming an O=O bond with the oxygen from water bound to the calcium via a nucleophilic attack.
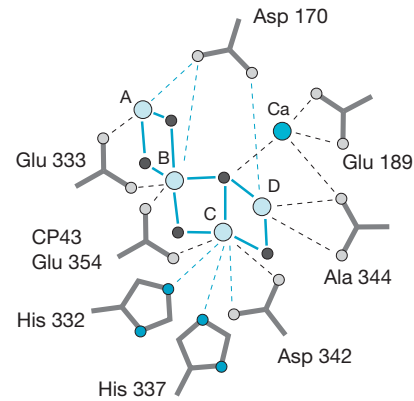

Figure 2 A proposed model of calcium and manganese (light blue) atoms in the oxygen-evolving center. Bonds from manganese to bridging oxo are in solid blue color. Bonds from manganese and calcium to amino acids are dotted. (Reprinted with permission from K. Sauer: *Science* 314: 821, Figure 4C, 11/03.2006. Reprinted with permission from AAAS (American Association for the Advancement of Science).)

This cluster of metal ions is ligated to four adjacent amino acids, three on D1 and one on CP43. Other groups provide stability via hydrogen bonds, trapped water molecules, and possibly a bicarbonate ion. All these provide an environment that can stabilize the four rounds of reduction induced by Tyr$_Z$ and then form a σ-bond between the oxygen atoms of two positioned water molecules leading to O$_2$ formation. The structure also has to provide an exit pathway for the H$^+$ protons from this water oxidation site to the lumen.
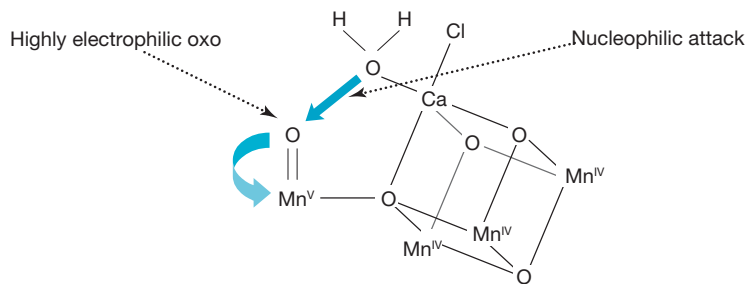

Figure 3 Model for the involvement of calcium and chlorine in holding a water molecule in position so that the oxygen atom can be released and used to form molecular oxygen. Note that the adjacent manganese atom is reduced from Mn^{IV} to Mn^V. (Reprinted with permission from Elsevier Limited, from *Current Opinion in Structural Biology*, "Structure of photosytem II and molecular architecture of the oxygen-evolving centre." Volume 14, Number 4, Page 7, 2004.)

with 20 µs flashes of light with 0.3-s dark intervals. This was interpreted by B Kok and coworkers (1970) as a cycle of successive oxidations, called the S-cycle. The ground (dark) state is S_1, and the center passes to S_2, S_3, and finally S_4 states when two water molecules are split (Box 4.2).

In the other set of reactions, A_0^- pheophytin is rapidly (picoseconds) oxidized to A_0 by passing an electron on to the first of two PQ molecules (at site Q_A, Box 4.2) and then, via an iron atom to the next PQ at site Q_B. PQ requires two electrons to become fully reduced. At Q_A, PQ is tightly bound and only undergoes single reduction events to the semiquinone state before being reoxidized by the PQ at Q_B. Each Q_B molecule undergoes two successive reduction events to become fully reduced. It then takes up two protons from the stromal side of the membrane to form PQH_2, which is released from the site and leaves the PSII complex. It is replaced by a new PQ molecule binding to the Q_B site. The PQH_2 diffuses in the lipid bilayer, acting as a mobile carrier of hydrogen atoms.

$$PQ + 2e^- + 2H^+ \longrightarrow PQH_2$$
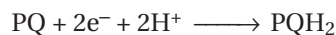
The $P680^+/A_0^-$ pair regeneration steps are relatively slow (millisecond timescale), so that a second light reaction cannot effectively occur inside 1 ms. However, the first fairly stable end product, PQ at Q_A, is formed in less than a nanosecond, so the electron flow from the excited state of P680 is maintained.

The Q cycle uses plastoquinol to reduce plastocyanin and transport protons into the lumen

The PQH_2 formed by PSII is the substrate for the Q cycle on the integral transmembrane protein complex, cytochrome b_6f. The cycle regenerates PQ for PSII, reduces a protein electron carrier, plastocyanin, and pumps more protons from the stroma into the lumen of the grana and stroma membrane systems.

As each plastocyanin reduction requires only a single electron, the Q cycle ensures that removal of two electrons from PQH_2 is achieved safely in two steps, with one electron going to reduce plastocyanin and the other being recycled to reduce a further PQ molecule (Box 4.3).

The cytochrome b_6f complex contains two cytochromes, cytochrome b_6 and cytochrome f, linked by an iron–sulfur Rieske protein. There are two Q (quinol) binding sites on cytochrome b_6. Electrons taken from PQH_2 at the binding site Q_p (also called Q_o) on the luminal side of the cytochrome are moved via two iron heme groups to reduce PQ at the other binding site Q_n (also called Q_i) on the stromal side, to form more PQH_2.

PQH_2 is oxidized in two steps to PQ (Box 4.3, Figure 1) at the Q_p site. The first step forms plastosemiquinone, with the release of an electron that reduces plastocyanin.

In the second step, plastosemiquinone is oxidized to PQ with the release of an electron that is passed via a heme molecule to the first step of reducing a further PQ molecule at the Q_n site. Another PQH_2 molecule is oxidized in the same two steps at the Q_p site. This forms a further reduced plastocyanin molecule and completes the reduction of plastosemiquinone to PQH_2 at the Q_n site, which is fed back into the cycle.

Proton pumping is a very important feature of the Q cycle. Oxidation of the PQH_2 releases protons taken up from the stroma, during PQH_2 formation on PSII, into the thylakoid lumen. PQH_2 synthesis at Q_n also takes up protons

Box 4.3 Structure and operation of cytochrome b_6f

In the unicellular green alga *Chlamydomonas*, cytochrome b_6f exists as a dimer, each monomer being composed of four large polypeptides, cytochrome b_6, cytochrome *f*, an iron–sulfur Rieske protein, and subunit IV, and four small polypeptides (Figure 1). In the domains that project into the lumen side of the membrane are found a heme in cytochrome *f* and an Fe_2S_2 center in the Rieske protein. The cytochrome b_6f complex has two Q sites (quinol binding sites) and two heme groups within the depth of the membrane and a recently discovered heme group towards the stromal side, near the Q_n site. This heme may act as the acceptor for electrons from PSI during cyclic photophosphorylation. A chlorophyll *a* molecule and a carotenoid molecule are also present, whose functions are unknown. The chlorophyll tail lies near the Q_n site and the carotenoid near the Q_p site. In common with other membrane protein complexes on the photosynthetic membrane, there has been very strong conservation of the cytochrome b_6f proteins and the positions of their hemes and other cofactors from their prokaryote ancestors to single-celled eukaryote algae and on to higher plants.

The dimer arrangement is such that the Q sites face each other in a protected environment. The two opposing cytochrome *f* proteins form a cavity which protects the Rieske proteins (FeS in Figure 1). These Rieske proteins serve as a safety gate preventing the two electrons from plastoquinol entering the iron–sulfur center simultaneously. Each single electron donated to the heme during semiquinone formation at Q_p induces the Rieske protein to move away from cytochrome b_6 to cytochrome *f*, transferring the electron to the *c*-heme. It then returns to Q_p. This movement prevents the accumulation of the dangerous semiquinone intermediate, as reduction of plastoquinone only occurs when the *b*-heme of cytochrome b_6 is in the oxidized state and is therefore immediately able to oxidize the semiquinone to quinone. The Rieske protein oscillates in the cavity as electrons flow through the system. Inevitably it is not a perfect system and in time single electrons can pass to molecular oxygen, which is destructive.
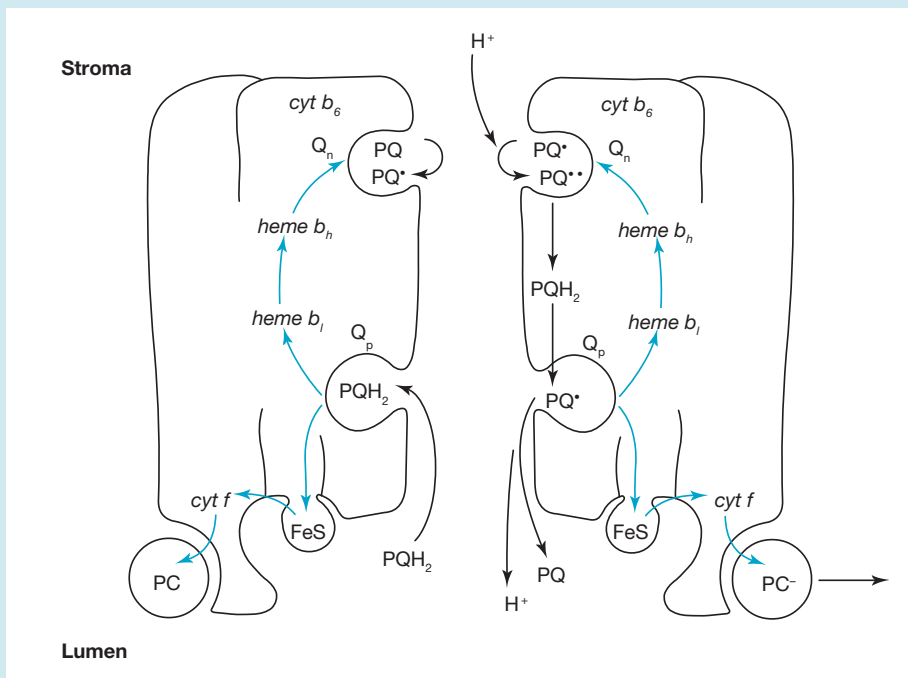

Figure 1 Cytochrome (cyt) b_6f dimer, with the Q_n and Q_p sites facing each other. Blue arrows show the pathways of electrons, black arrows show the net movements of diffusing components. In the left-hand cyt b_6f, the first step in oxidation of plastoquinol at Q_p is shown, with formation of the semiquinone ($PQ^\bullet$) at Q_n. The second step, in the right-hand cyt b_6f, shows formation of plastoquinone, PQ, at the Q_p site, while PQH_2 is formed at the Q_n site. Note the uptake of protons (H^+) from the stroma side during PQ reduction and their release into the lumen during oxidation. The FeS head oscillates in a cavity during electron transfer to plastocyanin (PC).

on the stromal side, while its oxidation at Q_p releases more protons on the lumen side. The Q cycle therefore acts as a proton pump, generating a transmembrane electrochemical H^+ gradient. As we shall see later, this pump can be operated independently of PSII by taking electrons directly from PSI, a process called cyclic photophosphorylation because it leads to enhanced ATP generation without concomitant oxidation of H_2O and reduction of $NADP^+$ to NADPH.

In summary, for every two molecules of PQH_2 supplied by PSII, four molecules of reduced plastocyanin are formed, four protons are released on the lumen side, and one molecule of PQH_2 is formed with the uptake of two protons on the stroma side. Both protons will eventually end up on the lumen side as this new PQH_2 is oxidized in the same cycle.

Oxidized plastocyanin, a copper-containing protein, is reduced ($Cu^{2+} \rightarrow Cu^+$) by electron flow through an iron–sulfur cluster and cytochrome f, also on the lumen side. The plastocyanin diffuses in the thylakoid lumen and serves as an electron donor for PSI.

$$PQH_2 + 2Cu^{2+} \longrightarrow PQ + 2Cu^+ + 2H^+$$
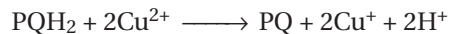
The Q cycle appears to present a paradox, in that the same products are cycled and remade while pumping protons into the lumen. The driving force is the downhill electrochemical gradient from PQH_2 to plastocyanin (Figure 4.3). This has driven the evolution of the cytochrome b_6f complex that takes advantage of the gradient in this step of the pathway to greatly increase the efficiency of the overall photochemical process. For each electron transported to plastocyanin, two H^+ are transferred into the lamellar lumen. So for the four electrons released from each water oxidation step, four protons are added to the lumen at the oxygen-releasing center, and eight protons are added from the Q cycle. As these were taken up from the stroma side during PQH_2 formation, the Q cycle generates a net movement of protons into the granal lumen that are utilized in ATP formation and are crucial prerequisites for this process.

Photosystem I takes electrons from plastocyanin and reduces ferredoxin, which is used to make NADPH and other reduced compounds

PSI is located in the stroma lamellae membranes. Like PSII, it also has a dimer of membrane-spanning chlorophyll proteins at its core (Box 4.4). The reaction center consists of a dimer of chlorophyll a molecules, one on each protein molecule. These exist in a different protein environment from those in PSII, and have an absorption maximum of about 700 nm, and so the center is called P700. When energy is passed to this center from the PSI antenna chlorophyll molecules, P700 is energized and a free electron is passed on to the first acceptor molecule, A_0, another chlorophyll molecule. This in turn reduces A_1, a phylloquinone molecule, which oxidizes A_0^- back to A_0.

The reaction center is located close to the lumen side of the PSI complex, so that $P700^+$ can be reduced back to P700 by direct interaction with reduced plastocyanin diffusing from the cytochrome b_6f complex. The plastocyanin docks directly on to PSI, passing one electron from its copper atom to $P700^+$.

The pathway from A_1 is via a series of three further reduction–oxidation steps that moves the electron through three iron–sulfur complexes, known as F_X, F_A, and F_B, in the order that they are reduced. As P700 is located on the lumen side of PSI, the linear arrangement of the acceptor molecules and iron–sulfur complexes across the width of the membrane ensures a rapid charge separation. The result is that the reduced F_B lies close to the stroma surface. Here it reduces a bound ferredoxin protein molecule, a small water-soluble iron–sulfur protein.

Reduced ferredoxin is capable of reducing a variety of molecules via suitable reductases. Usually this will be $NADP^+$, which requires two electrons and a

Box 4.4 Photosystem I

Photosystem I (PSI) is an integral membrane protein complex located on the stroma lamellae lying between the grana stacks. The structure of PSI has been strongly conserved over the past 1 billion years since plants evolved from symbiotic associations between cyanobacteria and eukaryote cells. Comparison of the higher plant structure with the equivalent structure in cyanobacteria reveals minimal alterations. This is very unusual and suggests that evolution had produced a near-perfect structure long before eukaryotic plants evolved. Only three chlorophyll molecules have been lost, there are 93 in higher plants, against 96 in the prokaryote. Two of these were in small polypeptides that were deleted as part of a suite of changes that accommodated the close binding of LHC1 antenna polypeptides, which are not present in cyanobacteria.

At the core of higher plant PSI is a dimer of homologous 80 kD protein molecules, PsaA and PsaB (Figure 1). These have 11 transmembrane sequences and they bind most of the electron carriers utilized in PSI. The transmembrane sequences have strong homologies to the D1/D2 and CP43/CP47 pairs in PSII. The six transmembrane helices of CP43/CP47 are homologous to the six transmembrane helices at the N-terminal end of the PsaA/PsaB proteins, and the five helices of the D1/D2 proteins are homologous to the five helices at the C-terminal end of these proteins.

The PSI dimer core protein probably evolved by fusion of the two genes for the PSII core proteins. Depending on the species examined, there are about 13 other protein subunits associated with this PSI core pair. These provide binding sites for electron carriers on the luminal and stromal sides of the membrane complex. They also provide binding sites for light harvesting chlorophyll protein complexes (LHC1, Box 4.1).

P700 is a dimer of two chlorophylls, one on each of PsaA and PsaB at the luminal side of PSI (Figure 2 and Plate 4.1). These chlorophylls are not identical, the PsaA one is chlorophyll a, while the one on PsaB is chlorophyll a', a C132-epimer of chlorophyll a. Their tetrapyrrole rings lie perpendicular to the plane of the membrane, with the heads and magnesium atoms stabilized by coordination with specific histidine residues and hydrogen bonds. Photoactivation yields an unpaired electron that is shared almost equally between the two molecules.

Both PsaA and PsaB carry A_0 (a chlorophyll a molecule) and A_1 (a phylloquinone molecule). Each A_0 chlorophyll is bound by axial ligands to sulfur atoms of methionine residues on the respective PsaA and PsaB proteins. Further stability is provided by the hydroxyl groups of adjacent tyrosine molecules. Both pathways appear to oxidize P700 during photosynthesis, though their properties are not identical. Both phylloquinones can reduce F_X. This is in contrast to PSII, where despite the presence of some components on both core proteins, electron flow is limited to the pathway on only one of them.

Excitation energy for P700 is derived from chlorophyll a and b molecules in the PSI complex and associated LHCI proteins. Binding sites for LHCI complexes are provided by PsaG and PsaK, which lie on opposite sides of the molecule (Box 4.1). Those for LHCII complexes are provided by PsaH (Box 4.1), which lies on the side opposite the necklace of LHCI. The core PSI complex contains 93 chlorophyll molecules, with 79 in the central dimer. Therefore, unlike PSII, these two proteins contain both antenna and reaction center chlorophylls, creating a hard-wired system on one molecule for gathering photons and for effecting charge separation.
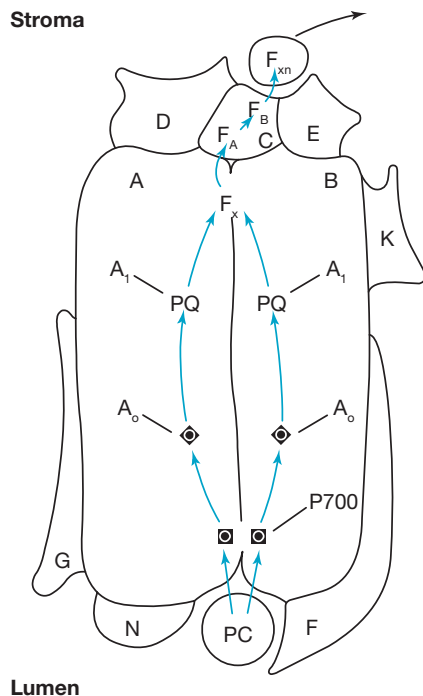

Figure 1 Photosystem I structure showing the arrangement of protein subunits (lettered A to N). The solid blue arrows show the pathway of electrons from plastocyanin (PC) to P700 and on to the formation of ferredoxin (Fxn). The black arrow indicates ferredoxin diffusing away from the photosystem. Black lines label components. The P700, A_0, A_1 pathway probably exists in parallel on both PsaA and PsaB subunits, combining at the single F_X site.

Box 4.4 Photosystem I (continued)

The three F-sites are each clusters of four iron atoms linked to four sulfur atoms. The FX cluster is ligated by two cysteine residues from PsaA and PsaB. The F_A and F_B clusters (both 4Fe–4S) are bound to the PsaC protein via eight of the nine conserved cysteine residues in that protein.

The oxidized reaction center (P700+) is reduced by the Cu^+-plastocyanin molecule produced by cytochrome b_6f. Plastocyanin diffuses from the granal membranes to the stroma lamellae. On the luminal side of PSI, P700 lies close to a hydrophobic surface of PsaA and PsaB, which interacts with the hydrophobic surface of plastocyanin. Docking is assisted by the PsaF protein, through the lysine-rich N-terminal region. PsaN may also be involved. The net surface charge on PSI in this location is therefore positive, and interacts with negative charges on the reduced plastocyanin. This brings the reduced copper atom into close proximity with two tryptophan residues on the PsaA/PsaB proteins, which mediate electron transfer to P700+. Docking/undocking of plastocyanin, a relatively large molecule, is quite rapid. The reduced form is strongly bound, and the transfer of the electron to P700+ fast (10–20 μs), while the oxidized form is rapidly released. Diffusion of this protein is a limiting factor in the functioning of P700. Plastocyanin is 3–4 nm across, and has to move in the granal lumen, which is about 4 nm in width, restricting the rate of reduction of P700+. This can be shown by reducing the grana width osmotically, which

reduces the photosynthetic electron flow through P700 (see Box 4.5 for further discussion).

The Z scheme pathway for photoelectron flow, as commonly presented here and elsewhere, fails to take account of the distinction between granal and stroma membranes. A further consideration is the distinction between appressed granal membranes and granal rim membranes exposed to the stroma. PSII is located primarily in the appressed membranes, and PSI in the rim and stromal membranes. Granal rim membrane PSI is juxtaposed to PSII and the cytochromes that yield reduced plastocyanin, but stromal membrane PSI (about 70% of the total) is relatively remote from PSII electron carriers. Diffusion of plastocyanin over these long distances could significantly reduce the rate of electron flow in the system. Even with the usual stoichiometry of two PSII centers for each PSI center and four to five plastocyanin molecules for each PSI reaction center, there seems to be a bottleneck at this step in the models, leading to a 10 ms transfer time observed between cytochrome b_6f and PSI.

On the stromal side of PSI, ferredoxin is reduced. This is an iron-rich protein that docks with PSI via electrostatic charges provided by PsaD. Following electron transfer, dissociation is mediated by Arg 39 on PsaE, which provides a positive charge limiting the lifetime of the ferredoxin-PSI complex.

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proton to yield NADPH, a process first discovered by Daniel Arnon in 1962. Arnon had previously (1951) noted that isolated chloroplasts could reduce NADP to NADPH and later (1954) found that ATP is produced at the same time. The reduction of NADP by reduced ferredoxin is mediated by ferredoxin-NADP+ reductase, a flavoprotein enzyme.

$$2Fd_{reduced} + H^+ + NADP^+ \longrightarrow 2Fd_{oxidized} + NADPH$$
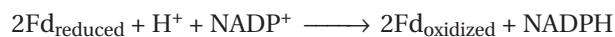
Note that the proton is removed from the stromal side of the membrane, enhancing the transmembrane proton gradient. Two electrons are needed for each NADPH molecule formed, so the splitting of two molecules of water at PSII provides enough electrons to form two molecules of NADPH. This stoichiometry is not exact, because other molecules can be reduced by ferredoxin, as it is not a closed system. The ferredoxin can diffuse to other reductases and reduce other molecules in the appropriate redox potential range. These include nitrite, which is used to produce amino acids (Chapter 8), and sulfate, which is reduced to sulfydryl in cysteine (Chapter 8). It can also donate electrons to the Q cycle (see section above, The Q cycle uses plastoquinol to reduce plastocyanin and transport protons into the lumen).

Thus P700 is capable of pumping electrons from plastocyanin to achieve reduction of NADP+ (Figure 4.6). The path from A_0 even involves a slight uphill segment from F_A to F_B, though this is overcome in the overall gradient (Figure 4.7).
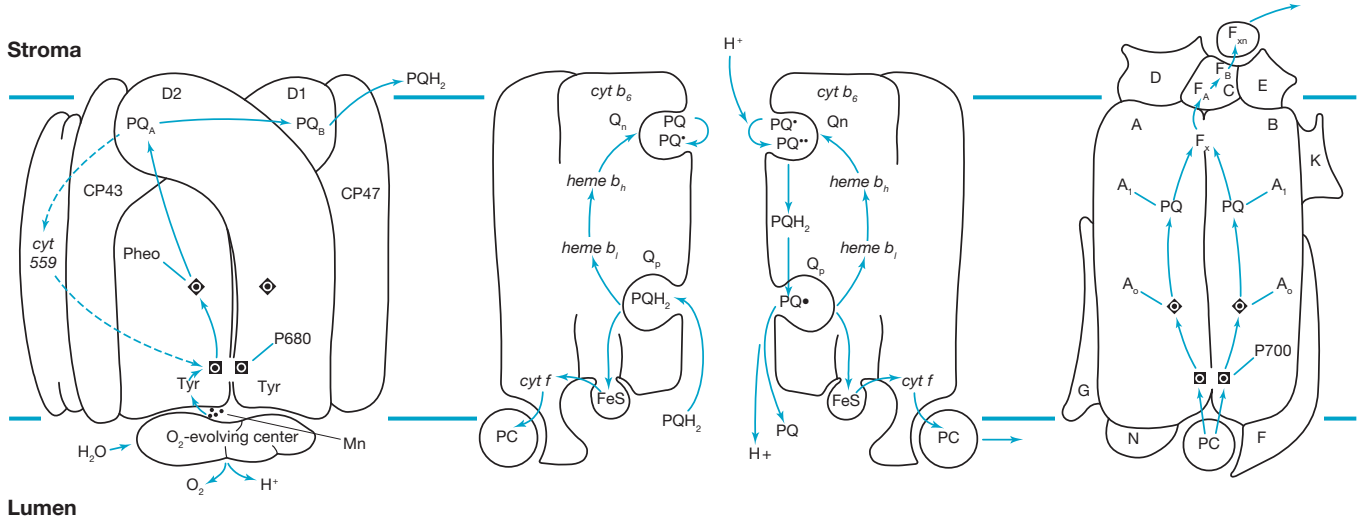
Stroma

Lumen

ATP synthase utilizes the proton motive force to generate ATP

The activity of the photosystems results in a net increase in the proton content of the lumen relative to the bulk phase of the stroma. Increases within the lumen result from water splitting at PSII and PQH_2/PQ conversions in the Q cycle of cytochrome b_6f. Synthesis of NADPH leads to loss of protons in the stroma and a concentration difference of protons across the membrane, which could exist as a pH gradient or a membrane polarization gradient. This leads to a proton motive force that is the sum of the pH difference across the membrane (proton concentration difference) and the electrical potential component arising from the charge difference across the membrane. The proton motive force drives a flow of protons through a transmembrane enzyme complex generating

Figure 4.6 Summary of photochemical reactions and linking electron flow pathways. The light reactions in PSII generate PQH_2, which diffuses through the membrane to the cytochrome b_6f complex where it is oxidized back to PQ in the Q cycle. Protons are taken up from the stroma in the process and released into the lumen. Reduced plastocyanin (PC) is formed and moves to PSI, where it passes its electrons to the oxidized chlorophyll. The light reactions in PSI generate NADPH. Details of the components are presented in Boxes 4.2, 4.3, and 4.4.
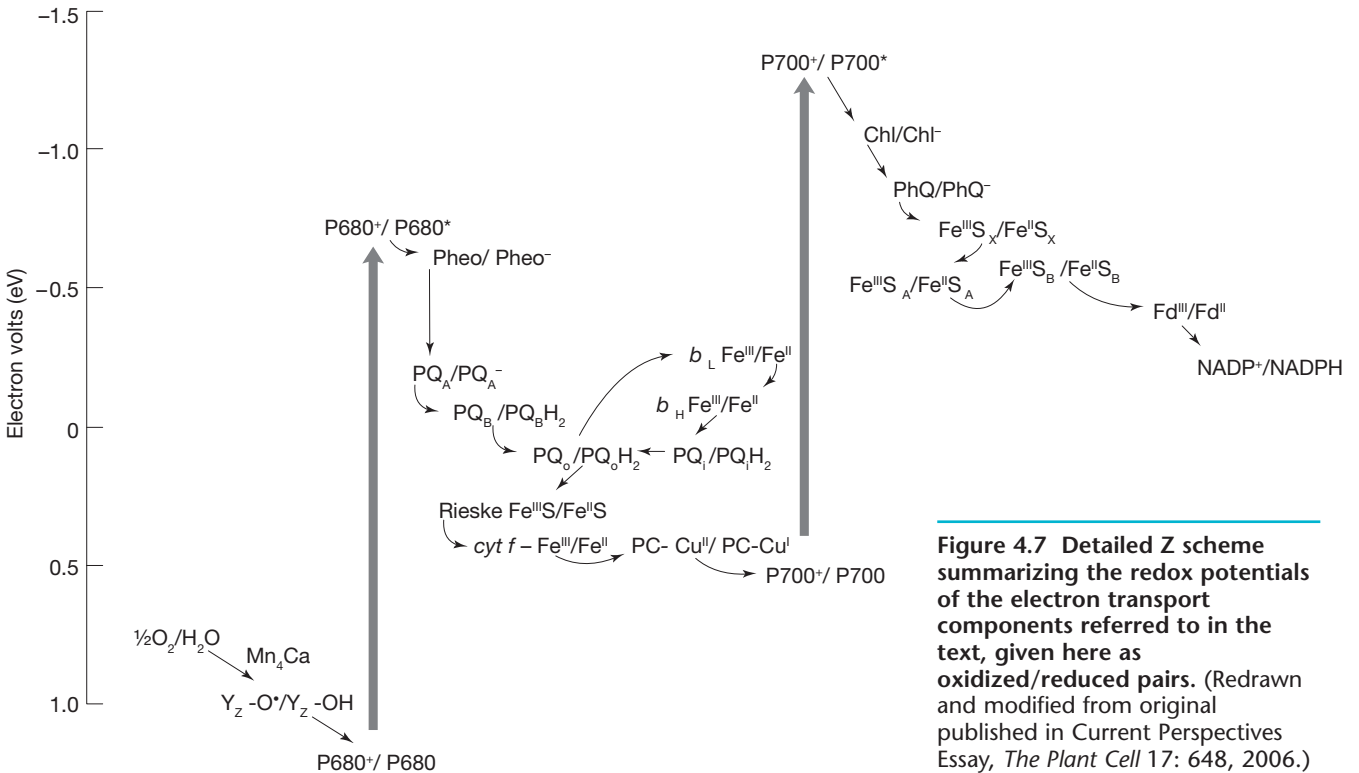

Figure 4.7 Detailed Z scheme summarizing the redox potentials of the electron transport components referred to in the text, given here as oxidized/reduced pairs. (Redrawn and modified from original published in Current Perspectives Essay, *The Plant Cell* 17: 648, 2006.)

Box 4.5 Structure of chloroplast ATP synthase

Chloroplast ATP synthase consists of an integral membrane protein complex, CF_0, attached by a stalk on the stromal side of the lamellar membrane to a large hydrophilic complex, CF_1. Protons flow across the membrane through the CF_0 complex. This induces deformations in the CF_1 complex that results in ATP synthesis.

Microscopy has been used to visualize the components using negative staining, cryomicroscopy and immunolabeling. CF_1 consists of a hexagonal ring of alternating α- and β-subunits (Figure 1). The stalk consists of one large subunit, γ, that is in contact with one of the three α/β pairs and CF_0, and ε, a smaller subunit whose placement is less clear.

CF_0 consists of a ring of subunits (called III) each composed of two transmembrane helices with a loop out to the stromal side. There is a variable number of III subunits in the ring between species and between chloroplast and mitochondrial synthases. Based on microscopy, it is reasonably certain that there are 14 in spinach CF_0. The cylindrical ring so formed is tapered, and is 5.9 nm in diameter on the lumen side and 7.4 nm diameter on the stroma side, in contact with the CF_1 complex. In addition, CF_0 has one each of subunits I, II, and IV which provide a link between the outside edge of the ring and the CF_1 hexamer. Subunit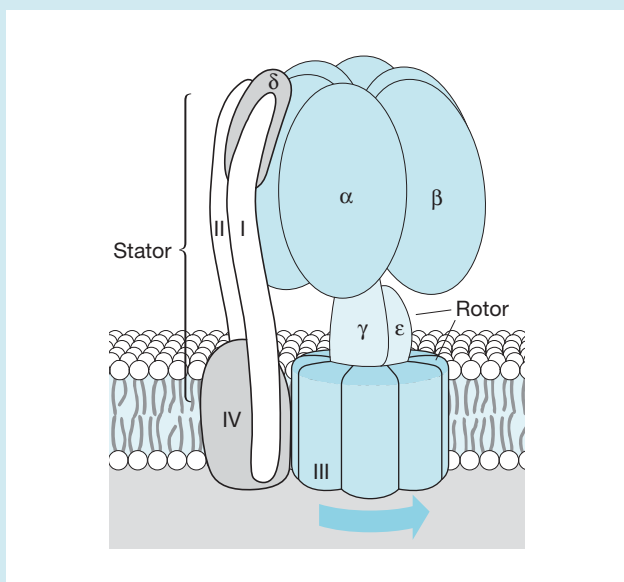 I has a single membrane spanning α helix and a large polar domain that extends out to the CF_1 hexamer. I and II may function as a dimer and are involved in binding to the α/β pairs, III binds to ε.

The β subunits are strongly conserved across all ATP synthases. Other polypeptides are less conserved with ε the least conserved of all the polypeptides. The α subunits are chemically equivalent, but differ in their properties due to differences in their local environment induced by other subunits. There are six nucleotide-binding sites at the interfaces of the α and β subunits, which can be divided up into three distinct classes, although sites within a class can differ. These differences are due to an asymmetric interaction of the hexamer with the γ subunit. The P-loop motif GXXXGKT/S is found on the β subunits with a Glu residue. These bind di- and triphosphates. The noncatalytic sites on the α subunits do not have Glu residues.

Much of the information on ATP synthesis is derived from studies on mitochondrial synthases (MF_1MF_0, see Chapter 6). These show that three of the sites are catalytically active and three are nonactive. In CF1 there are two tightly bound ADP sites (N1 and N4), and two bind ATP or AMP-PNP tightly in presence of magnesium ions (N2 and N5). The third site (probably at N1) tightly binds Mg-ATP, but hydrolyzes it to ADP. N3 and N6 bind di- and triphosphates in a freely dissociable manner.

In the dark, ADP is bound to the sites, and on illumination it is released initially as the proton gradient becomes established, either from N1 or N4. If CF_1 activity is analogous to that of MF_1, then the active catalytic sites would be N1 and N3 and probably N6. While ATP is synthesized in the light, CF_1 can reverse the action in the dark and have ATP hydrolysis driving a proton pump that places protons in the lumen.

The three catalytic sites alternate their properties in a cycle that progresses between three states: loose binding, tight binding, and unbound or open during one cycle of activity (Figure 2). ATP is present at the tight binding site from which it is released by a conformational change in the site, to create the open state to which ADP and Pi become attached, and then loosely bound.

The only way that all three β subunits can participate in catalysis is if the properties of each change during the cycle. As interactions with γ are responsible for asymmetry around the hexamer, it follows that the CF_1 complex must rotate γ during catalysis. Obtaining convincing evidence for rotation has not been easy, and early claims for success were heavily criticized, but it is now accepted that rotation occurs. The conformation of successive β subunits is altered as they come into contact with the γ shaft on each turn of the ring. CF_0 subunits I and II act as a stator, holding the CF_1 hexamer in place relative to the CF_0 ring as the γ and ε shaft is rotated.

Figure 1 Chloroplast ATP synthase, showing the membrane bound CF_0 and the stromal CF_1 units.
Electron flow leads to rotation of the III subunits of CF_0, which turns an off-center axle against the α and β subunits of CF_1. These are held in place by a stator linked to the CF_0 complex.

Author query for 2nd column of text, 2nd paragraph
Both single-letter abbreviations for amino acids and three-letter abbreviations are used in the same sentence. Would it be better to stick to one version, or to write out Glu (and give single letter abbreviation)?

Box 4.5 Structure of chloroplast ATP synthase (continued)

Rotation is powered by the interaction of protons with the III subunit ring in the membrane. These feed through a channel one at a time, each proton ratcheting the ring around by one III subunit. Each turn of the ring completes a single turn of γ, which releases three ATP molecules from CF_1. The number of protons required to complete one turn depends crucially on the number of III subunits in the ring. Originally this was estimated from the known stoichiometry of ATP synthesis and proton utilization as 10–12 subunits. In fact, visualization of the CF_1 from spinach using atomic force microscopy shows that there are unequivocally 14 III subunits in each ring, so 14 protons are required for a complete cycle. Therefore in spinach each ATP molecule needs 4.67 protons for its synthesis.
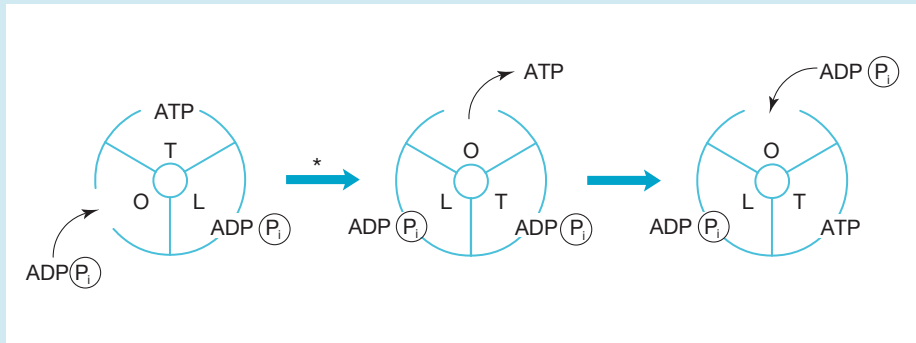

Figure 2 Diagram of CF_1 function. Three sites rotate clockwise around a central axis. As they rotate, their properties progress from tight (T) when ATP is formed and held (first diagram), to open (O), when ATP is released (first step, one-third rotation) and then ADP and P_i are bound (second step, no rotation), to loosely bound (L), when ADP and P_i are held at the site, returning to the tight condition at the end of one complete rotation.

ATP from ADP on the stromal side of the membrane. This enzyme was called coupling factor in the earlier literature, but is now more usefully labeled ATP synthase (Box 4.5). Chloroplast ATP synthase is structurally and functionally similar to mitochondrial and bacterial ATP synthases (Chapter 6).

The flow of protons is not linked directly to the synthesis of ATP from ADP and P_i. The synthase consists of a series of binding sites for ADP and P_i, and for ATP (Box 4.5). The proton gradient brings about conformational changes in the subunits binding the newly formed ATP molecule that release it from the enzyme complex. The precise number of protons required depends on the exact structure of the synthase. Using atomic force microscopy, it has been found that one molecule of ATP requires 4.67 protons for its production.

Cyclic photophosphorylation generates ATP independently of water oxidation and NADPH formation

The oxidation of two water molecules at PSII provides sufficient reductants, linked through PSI, to reduce two molecules of $NADP^+$ to NADPH and release 12 protons into the lumen. Therefore, the Z pathway alone produces slightly less than 1.5 ATP per NADPH. However, the carbon dioxide fixation cycle requires 1.5 moles of ATP for each mole of NADPH (Chapter 5). For this and other reasons additional ATP synthesis is required. The ATP deficit is made up by PSI complexes that are able to function without the involvement of PSII. Electrons from ferredoxin are diverted through the Q cycle on cytochrome b_6f to reduce plastocyanin and $P700^+$ (Figure 4.8). This is known as cyclic photophosphorylation, and it operates at the expense of NADPH synthesis. It

Figure 4.8 Cyclic photophosphorylation depends on diverting reduced ferredoxin (Fxn) to reduce plastoquinone (PQ) on cytochrome b_6f. This results in uptake of protons (H^+) from the stroma, and their release into the lumen as the plastoquinol (PQH_2).
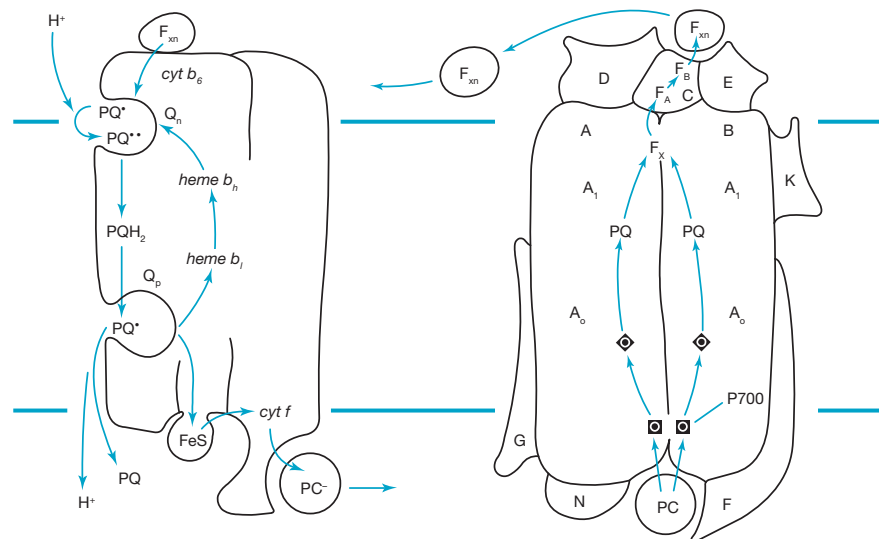
results in additional PQH_2 synthesis and a net proton flow into the luminal space, which is used for ATP synthesis. This mechanism operates to balance the output of ATP and NADPH for carbon dioxide fixation (Chapter 5). Notice that cytochrome b_6f and the Q cycle are involved in two distinct pathways, the Z scheme in association with PSII, and cyclic photophosphorylation in association with PSI. Hence this cytochrome is found both in the grana and the stroma membranes.

Regulation of electron flow pathways in response to fluctuating light levels

This section explores some of the issues that affect the whole electron flow pathway from water to NADPH. Leaves in a vegetation canopy are subjected to a great range of solar radiation levels and light quality. Heavy cloud cover severely reduces the level of irradiance, so the remaining photons have to be trapped and used efficiently. In contrast, noon on a clear day will deliver very high radiation levels to the leaves at the top of the canopy. The photosynthetic system will become light-saturated. There is, however, a limit to the rate at which energy can be utilized in the production of reduced carbon compounds, mainly because of limitations on the rate of carbon dioxide diffusing into the leaf. Under these conditions the antenna chlorophylls will become saturated and deliver very high flows of excitation energy to the reaction centers that cannot be dissipated along the normal electron transport route. If these conditions are accompanied by even a temporary drought, the stomata will close, so there will be even less carbon dioxide to act as an acceptor for the reducing power generated. PSII polypeptides, especially D1, can suffer damage from oxidation by unreduced $P680^+$ and oxygen radicals. Damaged reaction centers can continue to trap light energy, which exacerbate the problem if they cannot pass on the charge to PQ. Specific proteases are on hand to excise damaged components, principally polypeptide D1. Such repairs lead to a delay in resuming full photosynthetic activity while new component synthesis is activated and replacement achieved.

In contrast, leaves in the understory will receive light that is depleted in the main absorption wavebands of chlorophyll *a*, and so these will have to use particular *in vivo* forms of chlorophyll and accessory pigments (carotenoids) to boost their photosynthetic output. Such leaves also develop internal

anatomy and chloroplast grana organization to maximize light harvesting in their environment (shade leaves). For these leaves transient increases in light intensity, e.g. from sun flecks, can also be very damaging.

High light levels expose the cellular components to all the risks posed by photosynthesis. Significant levels of light energy end up trapped in component molecules. Excess irradiation can saturate the electron flow system leading to accumulation of molecules in the excited triplet state. This leads to formation of reactive superoxides, peroxides, and aldehydes, which have the potential to destroy the proteins and lipids in the chloroplast. Plants have evolved systems to remove these dangerous radicals and to prevent or reduce their formation in the first place. We will see that both the removal of radicals and the prevention of their formation involve many other molecules and enzymes, all working within the close confines of the thylakoid and fret membrane systems. It has therefore proved difficult to study each system in isolation and evaluate its contribution to the overall effect, so our story is incomplete at the present time. Clearly plants possess photosynthetic systems that work in a great range of habitats under a variety of physiological conditions of water and carbon dioxide supply. We might anticipate that the full range of curative and preventative measures will not be present to the same extent in all plants. Here we will describe the main processes that have been unequivocally identified and characterized in a representative number of plant species.

Oxygen molecules are normally stable and pose only a limited risk to the molecular structure of a cell. Singlet oxygen can be generated from these molecules by the input of energy, for example from light energy absorbed by chlorophyll molecules. The normal chlorophyll excited state induced by light absorption lasts for a few nanoseconds, long enough to be passed on to the reaction center. Any delay in this transfer can lead to the formation of a chlorophyll triplet state, which has a lifetime of several microseconds. These chlorophyll molecules can react with oxygen to produce singlet oxygen. This has a greatly increased oxidizing power that attacks any molecule in the vicinity. Typically, the lifetime of such oxygen radicals is about 200 ns, during which time it can diffuse as far as 10 nm. Mechanisms have evolved to rapidly remove these oxygen radicals and to curb their production at the sites of light absorption.

Singlet oxygen can be quenched directly by carotenoids, which are themselves converted to the triplet state, but this energy decays quickly being lost as heat. This can only happen if the carotenoids are close to the site of singlet production, which occurs in the LHCII antenna molecules, for example, but not in the PSII core. The carotenoids present in the PSII complex do serve to quench the P680 if charge recombination occurs to generate triplet P680 (as when the pheophytin at A_0 is not oxidized in time to receive a further reduction from P680). Singlet oxygen can also be quenched by α-tocopherol, a membrane lipid, and again the energy is dissipated as heat.

Scavenging and removal of superoxides, peroxides, and other radicals by dismutases and antioxidants

Oxidized compounds originate at PSI under high light conditions, when there is insufficient NADP to oxidize reduced ferredoxin. The electrons instead reduce molecular oxygen to superoxide, which in turn leads to peroxide formation by combination with protons. Peroxides can interact with reduced ferrous ions (Fe^{2+}) to produce hydroxyl radicals. These are extremely reactive and attack other molecules, sometimes leading to further radical formation so that a chain reaction is set up.

A number of enzymes have evolved to scavenge peroxides, including super-oxide dismutase, ascorbate peroxidase, monodehydroascorbate reductase, and glutathione reductase. Several antioxidants are available to be oxidized in these reactions, such as ascorbate, glutathione, and α-tocopherol (vitamin E). These convert the peroxide to water and oxygen. In effect this sets up a system for water breakdown (at PSII) and synthesis (at PSI), which removes excess energy trapped by the chlorophylls.

ascorbate peroxidase

$2H_2O_2 + \text{ascorbate} \longrightarrow O_2 + 2H_2O + \text{monodehydroascorbate}$

monodehydroascorbate reductase
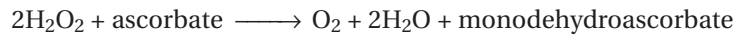
$2\text{monodehydroascorbate} + NADPH \longrightarrow 2\text{ascorbate} + NADP^+$

Peroxide removal by ascorbate. Note that the regeneration of ascorbate leads to consumption of NADPH, removing excess reductant from the system.

Mechanisms for safely returning the levels of trapped high energy states to the ground state

Reducing the exposure of chlorophyll molecules to high light intensities can prevent or reduce singlet oxygen formation. This can be achieved by changing the leaf angle with respect to incident light, and by movement of the chloroplasts to self-shading positions along the side walls of cells. Within the chloroplast there are three basic types of mechanism for coping with high light conditions. Adjustment of the synthesis and amount of the LHCII antenna proteins, movement of the LHCII proteins from PSII to PSI (state transition) and nonphotochemical quenching when electron flow is diverted to heat generating systems.

High light conditions and/or low carbon dioxide concentrations down-regulate expression of *Lhcb* genes. The sensor mechanism is not known. One candidate is the redox potential system, e.g. the level of PQ, but this control seems to be quite loose. Other mechanisms could be the level of reactive oxygen, or the light saturation of precursors of chlorophyll. Clearly this is a slow control mechanism, depending on not replacing chlorophyll proteins lost due to turnover.
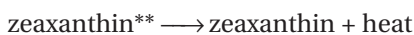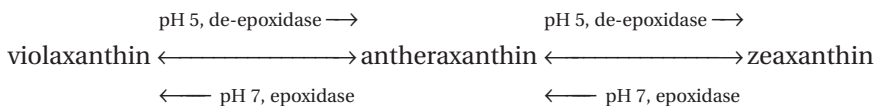
Some relief from excess energy absorption can be made within the PSII complex, by cycling electrons back from PQ via cytochrome *c*-553 to the D1 tyrosine. Another approach that has evolved is to reduce the amount of LHCII antenna proteins associated with PSII. This alters the ratio of light energy received between PSII and PSI by adjusting the extent to which each receives energy from these antenna proteins. As the two photosystems have different absorption spectra, conditions can arise where the energy flow through each is not balanced to meet the requirements of the Z scheme. This could be damaging, as high energy intermediates would accumulate in the system. The LHCII trimers serve as a simple feedback loop that adjusts the amount of antenna chlorophyll providing energy to each photosystem (state transition). If there is excess light energy flowing through PSII, then there is an excess of reduced PQ. This activates a kinase that phosphorylates some of the LHCII trimers. The extra charge causes them to dissociate from the PSII (state 2) complex and migrate towards the stroma lamellae (state 1 transition) where they bind to the PSI complex (state 1), increasing the flow through that system. Conversely, excess PSI activity leads to PQH_2 oxidation, which activates a phosphatase that removes the phosphate group and allows the trimers to return to PSII (state 2 transition).

Under high light conditions, the electron flow from PSII to PSI can be regulated by cytochrome b_6f and plastocyanin. The PQ reoxidation cycle is the slowest step in the photosynthetic electron flow pathway and the number of cytochrome b_6f complexes appears to be controlled, increasing as the flux rate increases. Plastocyanin also shows an increase with flux rate, and in leaves operating efficiently at high photosynthetic rates can reach a ratio of 4–5:1 of PSI. If the leaf's photosynthetic ability is compromised (e.g. by low or zero carbon dioxide availability), then this ratio falls back to below 1. This could limit the flow of electrons into P700. However, electron transfers involving plastocyanin (to PSI and to cytochrome b_6f) are much faster (10–100 times) than the PQ oxidation step, so the only way that reduced levels of plastocyanin could control the overall flux is if its diffusion rate to the PSI site is slow compared with the rate of electron flow.

Nonphotochemical quenching and the xanthophyll cycle

These adjustments to the electron transport system cannot entirely prevent the accumulation of excess energy in photon-trapping chlorophyll molecules. Such excited chlorophylls can return to the ground state by either emitting the energy as fluorescence, or by dissipating it as heat. Molecular mechanisms for removing this trapped energy (quenching the excited state) before it is passed on down the electron transport chain are collectively termed nonphotochemical quenching. The precise details of these molecular mechanisms remain to be discovered. The following account provides a general outline of the mechanisms as far as they are understood at the present time.

In saturating light conditions, chlorophylls in the antenna proteins, which are in equilibrium with the PSII reaction center chlorophylls, are unable to pass their trapped energy on to P680. Such chlorophylls pose a risk, as they are able to activate oxygen to the singlet state. Decay of the excited chlorophylls releases energy as heat and fluorescence. This fluorescence is a temporary response to suddenly increased light levels, which declines as additional resources are developed to convert the energy to heat. A prime candidate for initiation of the controlling mechanisms is the pH of the lumen. Proton generation and pumping during the light reactions lower the lumen pH from about 7 to less than 5, providing a strong signal that can initiate a number of quenching processes based in the lumen. One of these is the activation of an epoxidase that uses electrons to reduce the xanthophyll violaxanthin to antheraxanthin and then to zeaxanthin. The zeaxanthin binds to a protein subunit of LHCII that is protonated at low pH and accepts energy transfer from excited chlorophylls. The zeaxanthin then returns to the ground state, dissipating the energy as heat. In this way excess light energy trapped by chlorophylls is diverted to heat production, away from potentially damaging photochemical events and electron transfers.

$$\text{violaxanthin} \underset{\underset{\text{pH 7, epoxidase}}{\longleftarrow}}{\overset{\overset{\text{pH 5, de-epoxidase} \longrightarrow}{}}{\longleftrightarrow}} \text{antheraxanthin} \underset{\underset{\text{pH 7, epoxidase}}{\longleftarrow}}{\overset{\overset{\text{pH 5, de-epoxidase} \longrightarrow}{}}{\longleftrightarrow}} \text{zeaxanthin}$$

$$\text{LHCII}^{**} + \text{zeaxanthin} \longrightarrow \text{LHCII} + \text{zeaxanthin}^{**}$$

$$\text{zeaxanthin}^{**} \longrightarrow \text{zeaxanthin} + \text{heat}$$

The xanthophyll cycle and nonphotochemical quenching reactions leading to the transfer of excess excitation energy from light harvesting antenna

complexes to zeaxanthin and its release as heat. This is reversed under low light conditions when the pH rises.

Antiquenching is obviously necessary when light levels fall. This is achieved by a different epoxidase, activated at higher pH levels, that converts zeaxanthin back to antheraxanthin and violaxanthin. In this way the xanthophyll cycle is thought to track the pH changes in the lumen that are directly linked to productive photosynthetic activity and regulates the level of nonphotochemical quenching. The importance of xanthophylls and other carotenoids in this protection system can be seen from the fact that, in the absence of carotenoids, photosynthetic systems can only operate in oxygen-free conditions. Also carotenoid-deficient mutants will only survive under very low light conditions; normal light levels lead to bleaching of the leaves.

There is some evidence that a very hydrophobic transmembrane protein, PSbS, is involved in nonphotochemical quenching. It is proposed that PSbS undergoes protonation at low pH, and then binds zeaxanthin into the LHCII/PSII complex facilitating quenching of the excited state molecules. Experiments in which the levels of PSbS protein are altered, or in which the protonation of PSbS at low pH is interfered with, support this hypothesis. However, the location of PSbS remains to be determined, as does the precise way in which quenching is effected.

Summary

Overall PSII and PSI operate to extract electrons from water and reduce $NADP^+$. A proton gradient is produced that is used for ATP generation, and oxygen is released as a by-product. The synthesis of these photosystems is regulated to achieve approximately equal numbers of each. Failure to channel the PSII reducing power can lead to oxygen radical production with harmful consequences for the photosynthetic machinery. This can occur in some unfavorable environmental conditions (e.g. chilling) that damage PSI, leaving the PSII reductants accumulating in the pathway (Box 4.6).

Box 4.6 Herbicides that act through the photosynthetic apparatus

As the photosynthetic electron flow pathway is the only source of energy for growth in a plant, it represents a prime target for chemicals designed to kill plants. DCMU and the herbicide atrazine bind to the Q_B site on PSII, preventing plastoquinol formation. Plastoquinone analogs (dibromothymoquinone) bind to cytochrome b_6f preventing plastoquinol oxidation at the Q_p site. Paraquat is a widely used herbicide that diverts electron flow from ferredoxin to the formation of reactive oxygen species and peroxides that damage the photosynthetic apparatus and ultimately kill the leaf. Perversely the chlorophyll pigments harvest light energy that is used to destroy the plant.

Attacking carotenoid synthesis is an alternative way of killing plants, as this will lead to loss of the nonphotochemical quenching needed to protect photosystems from the effects of relatively high light intensities. Norflurazan is a herbicide of this type and leads to bleaching of leaves that is similar to that seen when carotenoid synthesis mutants are exposed to normal light levels (see Chapter 12).

Further Reading

Evolution of the reaction centers, light harvesting photosystems, and proteins

These papers provide a good overview of the evolution of photosynthesis and would provide useful entry points for the literature both before and after 2002/2004.

De Las Rivas J, Balsera M & Barber J (2004) Evolution of oxygenic photosynthesis: genome-wide analysis of the OEC extrinsic proteins. *Trends Pl. Sci.* 9, 18–25.

Raymond J & Blankenship RE (2004) The evolutionary development of the protein complement of Photosystem II. *Biochim. Biophys. Acta* 1655, 133–139.

Xiong J & Bauer CE (2002) Complex evolution of photosynthesis. *Annu. Rev. Pl. Biol.* 53, 503–521.

Organization of the thylakoid membrane

These two papers provide an overview of photosynthetic membrane components.

Decker JP & Boekema EJ (2005) Supramolecular organization of thylakoid membrane proteins in green plants. *Biochim. Biophys. Acta* 1706, 12–39.

Merchant S & Sawaya MR (2005) The light reactions: a guide to recent acquisitions for the picture gallery. *Pl. Cell* 17, 648–663.

Photosystems

Photosystems have been the focus of research for over 50 years, so there is a very large literature about their structure and function.

Amunts A, Drory O & Nelson N (2007) The structure of a plant photosystem I supercomplex at 3.4Å resolution. *Nature* 447, 58–63.

Blankenship RE (2002) Molecular Mechanisms of Photosynthesis. Blackwell Science Ltd.

Chitnis PR (2001) Photosysytem I: function and physiology. *Annu. Rev. Pl. Physiol. Pl. Mol. Biol.* 52, 593–626.

Diner BA & Rappaport F (2002) Structure, dynamics, and energetics of the primary photochemistry of Photosystem II of oxygenic photosynthesis. *Annu. Rev. Pl. Biol.* 53, 551–580.

Minagawa J & Takahashi Y (2004) Structure, function and assembly of Photosystem II and its light-harvesting proteins. *Photosynthesis Res.* 82, 214–263.

Nelson N & Yocum CF (2006) Structure and function of Photosystems I and II. *Annu. Rev. Pl. Biol.* 57, 521–565.

Water splitting and oxygen evolution

The chemistry of water splitting in photosynthesis is fascinating, how do plants successfully break open this molecule under normal physiological conditions?

Ferreira KN, Iverson TM, Maghlaoui K, Barber J & Iwata S (2004) Architecture of the oxygen-evolving center. *Science* 303, 1831–1838.

Sauer K & Yachandra VK (2004) The water-oxidation process in photosynthesis. *Biochim. Biophys. Acta* 1655, 140–148.

Cytochromes

Processing plastoquinones and producing plastocyanins provides the vital link between PSII and PSI. New information is adding more detail to this part of the photosynthesis story.

Allen JF (2004) Cytochrome b_6f: structure for signaling and vectorial metabolism. *Trends Pl. Sci.* 9, 130–137.

Siegbahn PEM & Blomberg MRA (2004) Important roles of tyrosines in Photosystem II and cytochrome oxidase. *Biochim. Biophys. Acta* 1655, 45–50.

ATP synthase: structure and operation

An amazing molecular turbine has been uncovered that couples a proton gradient to the formation of ATP.

Kramer DM, Cruz JA & Kanazawa A (2003) Balancing the central roles of the thylakoid proton gradient. *Trends Pl. Sci.* 8, 27–32.

McCarty RE, Evron Y & Johnson EA (2000) The chloroplast ATP synthase: a rotary enzyme? *Annu. Rev. Pl. Physiol. Pl. Mol. Biol.* 51, 83–109.

Seelert H, Dencher NA & Müller DJ (2003) Fourteen protomers compose the oligomer III of the proton-rotor in spinach chloroplast ATP synthase. *J. Mol. Biol.* 333, 337–344.

World Wide Web-based information

The following is a useful site providing access to information on all aspects of photosynthesis.

http://porphy.la.asu.edu/photosyn/photoweb/