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# Photosystem II

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Photosystem II is a specialized protein complex that uses light energy to oxidize water, resulting in the release of molecular oxygen into the atmosphere, and to reduce plastoquinone, which is released into the hydrophobic core of the photosynthetic membrane. All oxygenic photosynthetic organisms, which include plants, algae and some bacteria, depend on photosystem II to extract electrons from water that are eventually used to reduce carbon dioxide in the carbon reduction cycle. The complex is composed of a central reaction centre in which electron transport occurs, and a peripheral antenna system which contains chlorophyll and other pigment molecules that absorb light.

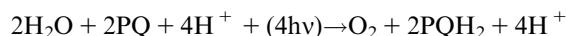
## Introduction

Photosynthetic organisms use light energy to produce organic molecules (Ort and Whitmarsh, 2001). In plants, algae and some types of bacteria, the photosynthetic process depends on photosystem II, a membrane-bound protein complex that removes electrons from water and transfers them to plastoquinone, a specialized organic molecule. Because the removal of electrons from water results in the release of molecular oxygen into the atmosphere, this photosystem II-dependent process is known as oxygenic photosynthesis. Photosystem II is the only protein complex known to oxidize water and release molecular oxygen. A more ancient form of photosynthesis occurs in some bacteria that are unable to oxidize water and therefore do not release oxygen. There is fossil evidence that photosystem II-containing organisms evolved about three billion years ago and that oxygenic photosynthesis converted the earth's atmosphere from a highly reducing anaerobic state to the oxygen-rich air surrounding us today (Des Marais, 2000). By releasing oxygen into the atmosphere, photosystem II enabled the evolution of cellular respiration and thus profoundly affected the diversity of life.

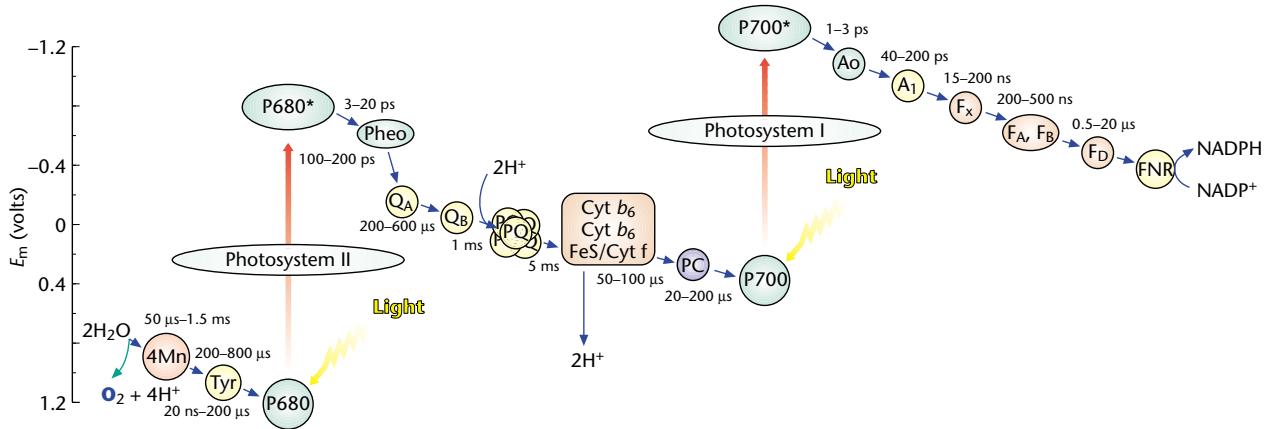
Oxygenic photosynthesis depends on two reaction centre protein complexes, photosystem II and photosystem I, which are linked by the cytochrome *bc* complex and small mobile electron carriers (Whitmarsh and Govindjee, 1999) (Figure 1). Photosystem II, the cytochrome *bc* complex and photosystem I are embedded in the thylakoid membrane and operate in series to transfer electrons from water to nicotinamide-adenine dinucleotide phosphate (NADP<sup>+</sup>). The energy necessary to move electrons from water to NADP<sup>+</sup> is provided by light, which is captured by the photosystem II and photosystem I antenna systems. In plants and algae the thylakoid membranes are located inside chloroplasts, which are subcellular organelles. In oxygenic bacteria the thylakoids are located inside the plasma membrane.

Chloroplasts originated from photosynthetic bacteria invading a nonphotosynthetic cell. In both chloroplasts and bacteria, thylakoid membranes form vesicles that define an inner and outer aqueous space. Light-driven electron transfer through the photosystem II and photosystem I reaction centres provides the energy for creating a proton electrochemical potential across the thylakoid membrane. The energy stored in the proton electrochemical gradient drives a membrane-bound adenosine triphosphate (ATP) synthase that produces ATP. Overall, the light-driven electron transport reactions, occurring through the thylakoid membrane, provide NADPH and ATP for the production of carbohydrates, the final product of oxygenic photosynthesis.

Photosystem II uses light energy to drive two chemical reactions: the oxidation of water and the reduction of plastoquinone (Govindjee and Coleman, 1990; Nugent, 2001). The primary photochemical reaction of photosystem II results in separating a positive and a negative charge within the reaction centre and is governed by Einstein's law of photochemistry: one absorbed photon drives the transfer of one electron. This means that four photochemical reactions are needed to remove four electrons from water, which results in the release of one molecule of dioxygen and four protons, and in the reduction of two molecules of plastoquinone:



This chapter focuses on the composition, structure and operation of photosystem II. For brevity we describe what we know without explaining the experimental results that underlie our knowledge. The references given at the end of



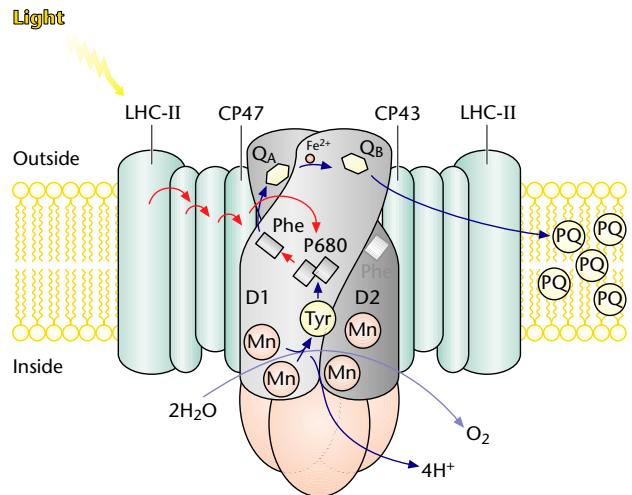
**Figure 1** The Z scheme showing the pathway of oxygenic photosynthetic electron transport. The vertical scale shows the equilibrium midpoint potential ( $E_m$ ) of the electron transport components. Approximate electron transfer times are shown for several reactions. Mn, tetranuclear manganese cluster; Tyr, tyrosine-161 on D1 protein ( $Y_2$ ); P680, reaction centre chlorophyll *a* of photosystem II; P680\*, excited electronic state of P680; Pheo, pheophytin;  $Q_A$ , a bound plastoquinone;  $Q_B$ , a plastoquinone that binds and unbinds from photosystem II; PQ, a pool of mobile plastoquinone molecules; the brown box is a protein complex containing cytochrome  $b_6$  (Cyt  $b_6$ ), iron-sulfur protein (FeS) and cytochrome *f* (Cyt *f*); PC, plastocyanin; P700, reaction centre chlorophyll *a* of photosystem I; P700\*, excited electronic state of P700; Ao, a special chlorophyll *a* molecule;  $A_1$ , vitamin K;  $F_x$ ,  $F_A$ ,  $F_B$ , iron sulfur centres;  $F_D$ , ferredoxin; FNR, ferredoxin–NADP reductase;  $NADP^+$ , nicotinamide–adenine dinucleotide phosphate. For details see Whitmarsh and Govindjee (1999).

the article are an entryway into the vast literature on photosystem II that extends back more than half a century.

## Organization, Composition and Structure

Photosystem II is embedded in the thylakoid membrane, with the oxygen-evolving site near the inner aqueous phase and the plastoquinone reduction site near the outer aqueous phase (Figure 2), an orientation that enables the oxidation–reduction chemistry of the reaction centre to contribute to the proton electrochemical gradient across the thylakoid membrane. In chloroplasts, the photosystem II and photosystem I complexes are distributed in different regions of the thylakoid membrane. Most of the photosystem II complexes are located in the stacked membranes (grana), whereas the photosystem I complexes are located in the stromal membranes. It is not clear why two reaction centres that operate in series are spatially separated in eukaryotic organisms. In prokaryotes, the thylakoid membranes do not form grana and the photosystem II and I complexes appear to be intermixed. In eukaryotes the photosystem II complex is densely packed in the thylakoid membrane, with average centre to centre distances of a few hundred angstroms. One square centimetre of a typical leaf contains about 30 trillion photosystem II complexes.

Although photosystem II is found in prokaryotic cyanobacteria (e.g. *Synechocystis* spp.) and prochlorophytes (e.g. *Prochloron* spp.), in eukaryotic green algae (e.g. *Chlamydomonas* spp.), red algae (e.g. *Porphyridium* spp.), yellow-green algae (e.g. *Vaucheria*) and brown algae



**Figure 2** Schematic drawing of the photosystem II antenna system and reaction centre in the thylakoid membrane. D1 and D2 are the core proteins of photosystem II reaction centre. Photosystem II uses light energy to remove electrons from water, resulting in the release of oxygen and protons. The electrons from water are transferred via redox cofactors in the protein complex to form reduced plastoquinone.  $(Mn)_4$ , manganese cluster involved in removing electrons from water; P680, reaction centre chlorophyll of photosystem II; Pheo, pheophytin;  $Q_A$ , a bound plastoquinone;  $Q_B$ , plastoquinone that binds and unbinds from photosystem II; Tyr, a tyrosine residue in photosystem II ( $Y_2$ ); PQ, a pool of mobile plastoquinone molecules. The antenna complexes are: LHC-II, peripheral light-harvesting complex of photosystem II; CP47 and CP43, chlorophyll–protein complexes of 47 and 43 kDa, respectively.

(e.g. *Fucus* spp.) and all higher plants (e.g. *Arabidopsis* spp.), extensive research indicates that its structure and function are remarkably similar in these diverse organisms.

Photosystem II is arranged as a central reaction centre core surrounded by a light-harvesting antenna system. The reaction centre is the site of primary charge separation and of the subsequent electron transfer reactions that oxidize water and reduce plastoquinone. The antenna system consists of protein complexes that contain light-harvesting molecules (chlorophyll and other accessory pigments) which serve to capture light energy and deliver it to the reaction centre.

In eukaryotic organisms the light-harvesting protein complexes are organized into an inner antenna system located close to the reaction centre and a peripheral antenna system composed of pigment proteins known as light-harvesting complex II (LHC-II). Excluding the peripheral light-harvesting complexes, the reaction centre complex contains more than 20 different polypeptides. A list of the genes encoding the known polypeptides, together with the molecular weights and putative functional roles of the proteins, is shown in **Table 1**. Most of the photosystem II polypeptides are membrane-bound integral proteins. The exceptions are a few peripheral membrane proteins that are located in the lumen.

Photosystem II contains at least nine different redox components (chlorophyll, pheophytin, plastoquinone, tyrosine, manganese, iron, cytochrome *b559*, carotenoid and histidine) which have been shown to undergo light-induced electron transfer. However, only five of these redox components are known to be involved in transferring electrons from H<sub>2</sub>O to the plastoquinone pool: the water-oxidizing manganese cluster (Mn)<sub>4</sub>, the amino acid tyrosine (Y<sub>Z</sub>), the reaction centre chlorophyll (P680), pheophytin and two plastoquinone molecules, Q<sub>A</sub> and Q<sub>B</sub> (**Figures 1 and 2**). At the heart of photosystem II is a heterodimer protein complex composed of the D1 and D2 polypeptides; this complex coordinates the key redox components of photosystem II (including P680 plus four additional chlorophyll molecules, two pheophytin molecules, Q<sub>A</sub> and Q<sub>B</sub>) and provides ligands for the (Mn)<sub>4</sub> cluster. In addition to these components, the photosystem II reaction centre core together with the inner antenna proteins (CP43 and CP47) binds about 45 molecules of chlorophyll *a*, five or six carotenoids, one nonhaem iron, one calcium, one or more chloride, and one or two bicarbonate ions. Cyanobacteria have an additional redox component, cytochrome *c550*, located on the luminal side of photosystem II. All photosystem II complexes contain cytochrome *b559*, which is a *b*-haem composed of two polypeptides. Despite numerous studies, there is disagreement in the literature concerning whether there is one or two *b*-haems in each photosystem II reaction centre (Whitmarsh and Pakrasi, 1996).

After decades of effort, the inner core of photosystem II has finally been crystallized and its three-dimensional structure determined to 3.8-Å resolution by Witt, Saenger and coworkers (Zouni *et al.*, 2001) (**Figure 3**). While this resolution does not provide the position of most of the

atoms that make up the reaction centre, an atomic resolution of photosystem II should be available in the near future. Current research is guided by photosystem II structural models that were developed using the atomic structure of the reaction centre in purple bacteria, together with biochemical and spectroscopic data (**Figure 4a**) (e.g. Xiong *et al.*, 1998). These homology-based models all suffer from the fact that reaction centres of purple bacteria do not oxidize water.

As revealed by Zouni *et al.* (2001), the photosystem II reaction centre core is 100 Å across (in the plane of the membrane) and extends about 10 Å into the stromal aqueous phase and about 55 Å into the lumen. At the centre of the reaction centre are the D1 and D2 polypeptides, which combine to provide the scaffolding for the electron carriers, locating them at precise distances from one another (**Figure 4b**). The rate of electron transfer from one redox site to another is controlled by several factors, including the distance between the components (Moser *et al.*, 1992). In addition to positioning the electron carriers, the photosystem II polypeptides provide multiple pathways for proton transfer from the outer water phase to the Q<sub>B</sub> site. Note that the D1 and D2 polypeptides form a symmetrical central structure which appears to provide two potential electron transport pathways through the reaction centre. However, as shown in **Figure 2**, only one pathway is active.

## Light Capture: The Antenna System

Oxygenic photosynthesis is driven mainly by visible light (wavelength from 400 to 700 nm), which is absorbed by chlorophyll and other pigments (e.g. carotenoids) that are anchored in the thylakoid membrane to the light-harvesting proteins (**Table 2**). Chlorophyll absorbs light mainly in the blue and red regions of the spectrum due to a cyclic tetrapyrrole in which the nitrogens of the pyrroles are coordinated to a central magnesium ion. Plants (and many types of algae) contain two types of chlorophyll, *a* and *b*, which differ by a single group on one of the pyrrole rings. Typically about 200–250 chlorophyll and 40–60 carotenoid molecules serve a single reaction centre. Carotenoids are linear polyenes that serve as accessory pigments in the antenna system, absorbing light in the blue and green spectral region. In addition, carotenoids protect the photosynthetic apparatus from damage caused by excess light in a process known as downregulation (see below), as well as quenching relatively stable, excited states of chlorophyll known as triplet states which lead to oxidative damage of components in the thylakoid membrane (Frank *et al.*, 1999).

The light-harvesting protein complexes (LHC-II complexes) are made up of a family of related proteins that bind chlorophyll *a*, chlorophyll *b* and carotenoids. The structure

**Table 1** Photosystem II genes, proteins and putative roles (excluding antenna light-harvesting complex II)<sup>a</sup>

| Gene <sup>b</sup> | Protein                   | Mass (kDa) <sup>c</sup> | Integral or peripheral <sup>d</sup> | Comments   |
|-------------------|---------------------------|-------------------------|-------------------------------------|--|
| <i>psbA</i> (c)   | D1                        | 39                      | I (5)                               | D1 (and D2) form the reaction centre core that binds most of the PS-II electron transport components; Q <sub>B</sub> binds to D1 |
| <i>psbB</i> (c)   | CP47                      | 56                      | I (6)                               | Binds antenna chlorophyll <i>a</i>   |
| <i>psbC</i> (c)   | CP43                      | 47                      | I (6)                               | Binds antenna chlorophyll <i>a</i>   |
| <i>psbD</i> (c)   | D2                        | 39                      | I (5)                               | D2 (and D1) form the reaction centre core that binds most of the PS-II electron transport components; Q <sub>A</sub> binds to D2 |
| <i>psbE</i> (c)   | α subunit Cyt <i>b559</i> | 9.3                     | I (1)                               | Binds <i>b</i> -haem; involved in photoprotection  |
| <i>psbF</i> (c)   | β subunit Cyt <i>b559</i> | 4.5                     | I (1)                               | Binds <i>b</i> -haem; may be involved in photoprotection   |
| <i>psbH</i> (c)   | PsbH                      | 7.8                     | I (1)                               | Unknown function   |
| <i>psbI</i> (c)   | PsbI                      | 4.2                     | I (1)                               | Unknown function   |
| <i>psbJ</i> (c)   | PsbJ                      | 4.2                     | I (0)                               | Unknown function   |
| <i>psbK</i> (c)   | PsbK                      | 4.3                     | I (1)                               | Unknown function   |
| <i>psbL</i> (c)   | PsbL                      | 4.5                     | I (0)                               | Unknown function   |
| <i>psbM</i> (c)   | PsbM                      | 4                       | I                                   | Unknown function   |
| <i>psbN</i> (c)   | PsbN                      | 4.7                     | I (1)                               | Unknown function   |
| <i>psbO</i> (n)   | PsbO (MSP)                | 27                      | P (0)                               | Involved in regulating oxygen evolution  |
| <i>psbP</i> (n)   | PsbP                      | 20                      | P (0)                               | Involved in oxygen evolution; eukaryote specific   |
| <i>psbQ</i> (n)   | PsbQ                      | 17                      | P (0)                               | Involved in oxygen evolution; eukaryote specific   |
| <i>psbR</i> (n)   | PsbR                      | 10                      | I (0 or 1)                          | Unknown function; eukaryote specific   |
| <i>psbS</i> (n)   | PsbS                      | 22                      | I (4)                               | Binds chlorophyll; involved in downregulation  |
| <i>psbT</i> (c)   | PsbT                      | 3.8                     | P (1)                               | Unknown function; eukaryote specific   |
| <i>psbU</i>       | PsbU                      | 10                      | P                                   | Unknown function; prokaryote specific  |
| <i>psbV</i>       | Cyt <i>c550</i>           | 12                      | P (0)                               | Binds <i>c</i> -haem; prokaryote specific  |
| <i>psbW</i> (n)   | PsbW                      | 6                       | I (1)                               | Involved in PS-II dimerization; eukaryote specific   |
| <i>psbX</i> (c)   | PsbX                      | 4                       | I (1)                               | Unknown function   |
| <i>psbY</i> (c)   | PsbY                      | 3                       | I                                   | Unknown function   |
| <i>psbZ</i> (c)   | PsbZ                      | 11                      | I                                   | Unknown function   |

<sup>a</sup>The authors thank Drs H.P. Pakrasi and K.-H. Rhee (Rhee, 2001) for information contributing to this table.

<sup>b</sup>For eukaryotic organisms, the letter in parentheses indicates whether nuclear (n) or chloroplast (c) gene is encoded.

<sup>c</sup>Mass calculated from amino acid sequence.

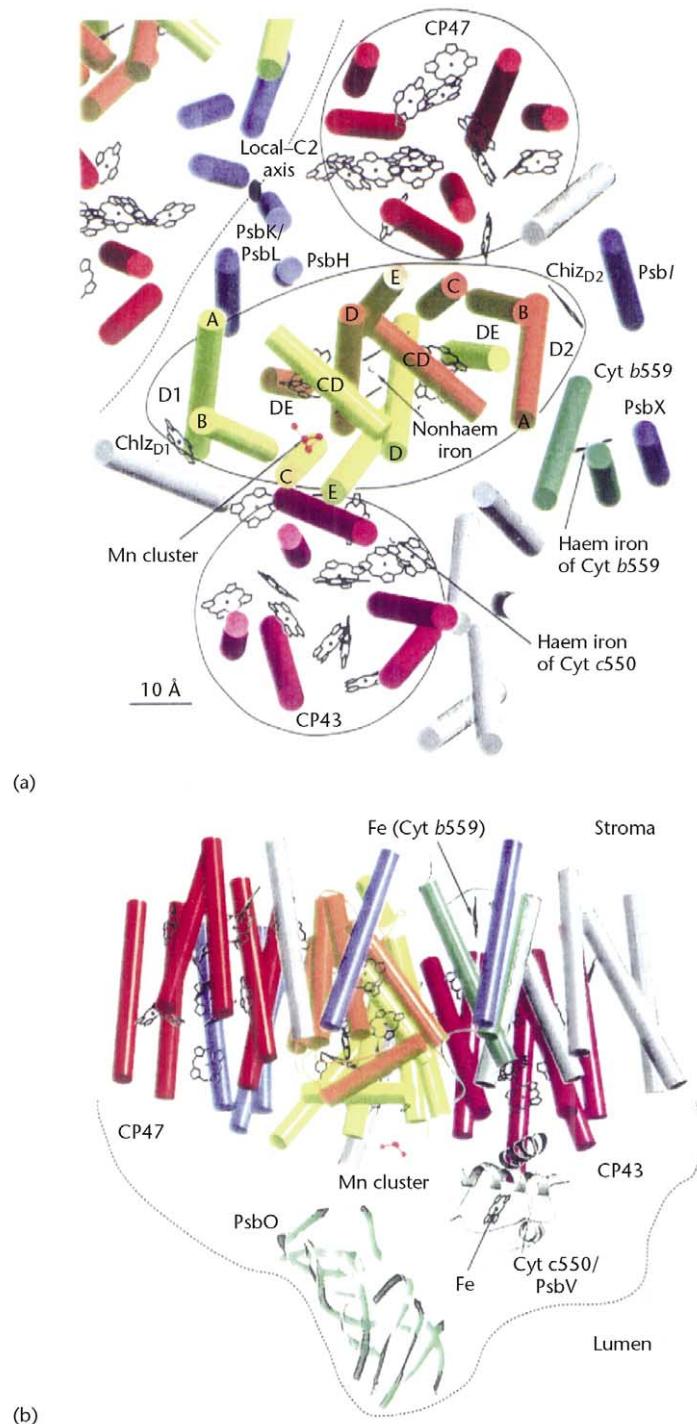
<sup>d</sup>Number of α helices is given in parentheses.

I, integral; P, peripheral; Cyt, cytochrome; PS, photosystem; MSP, manganese-stabilizing protein.

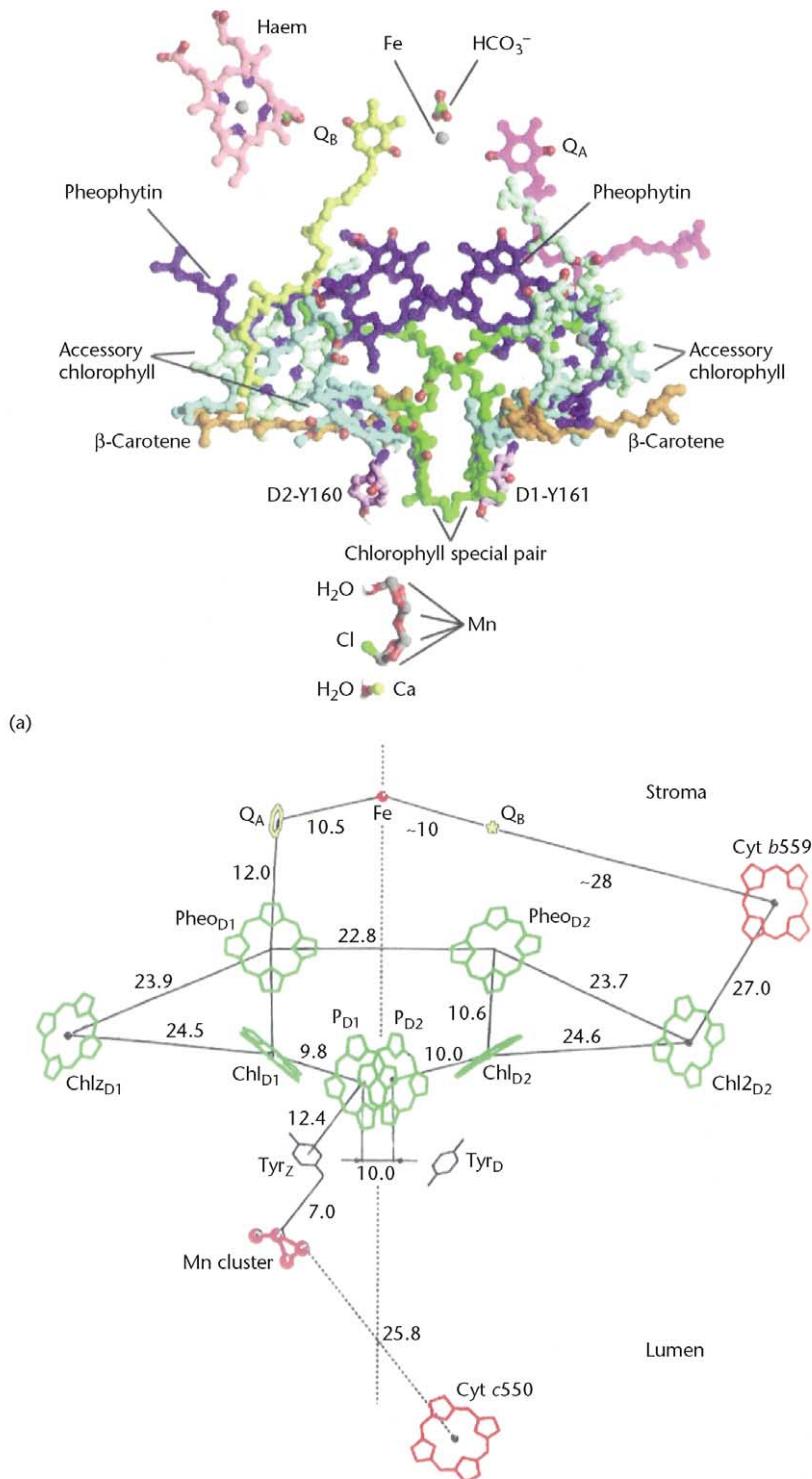
of one of the LHC-II complexes has been determined by electron crystallography (Kühlbrandt *et al.*, 1994). The complex forms a trimer in the membranes, with each subunit binding seven molecules of chlorophyll *a*, five molecules of chlorophyll *b* and two carotene molecules.

Photosynthesis starts with the absorption of a photon by an antenna molecule, which causes a rapid ( $10^{-15}$  s)

transition from the electronic ground state to an excited state. Within  $10^{-13}$  s, the electronic excited state decays by vibrational relaxation to the first excited singlet state. These excited electron states are short lived, and the fate of the excitation energy in the antenna system is guided by the structure of the protein–pigment complexes. Because of the proximity of other antenna molecules with the same or similar electron energy levels, the excited state energy has a



**Figure 3** Organization of *Synechococcus elongatus* photosystem II  $\alpha$  helices determined by X-ray crystallography. (a) Viewed from above the thylakoid membrane. (b) Viewed in the plane of the membrane. Abbreviations are as in Figure 1 and Table 1. From Zouni *et al.* (2001); reproduced with permission from H. T. Witt.



**Figure 4** Organization of photosystem II cofactors. (a) Determined by modelling. Abbreviations are as in **Figure 1**, except D1-Y161 is Tyr (Y<sub>Z</sub>) and D2-Y160 is a tyrosine in D2 (Y<sub>D</sub>). From Xiong *et al.* (1998). (b) Organization of *Synechococcus elongatus* photosystem II cofactors determined by X-ray crystallography. The numbers represent centre to centre distances in angstroms. From Zouni *et al.* (2001); reproduced with permission from H. T. Witt.

**Table 2** Distribution of chlorophyll in photosystem II

| Protein  | No. of molecules                        |
|--|---|
| Reaction centre proteins (D1/D2)                   | 6 Chl <i>a</i>                          |
| Inner antenna proteins                             |   |
| CP47   | 13 Chl <i>a</i>                         |
| CP43   | 13 Chl <i>a</i>                         |
| CP24 + CP26 + CP29                                 | 21 Chl <i>a</i> + 6 Chl <i>b</i>        |
| Outer antenna proteins                             |   |
| One tightly bound + one medium-bound LH-IIb trimer | 42 Chl <i>a</i> + 30 Chl <i>b</i>       |
| Loosely bound LH-IIb + other LHs                   | Approx. 100 Chl ( <i>a</i> + <i>b</i> ) |
| Photosystem II (reaction centre + antenna system)  | Approx. 230 Chl ( <i>a</i> + <i>b</i> ) |

Chl, chlorophyll; CP, chlorophyll-binding protein; LH, light-harvesting complex.

high probability of being transferred to a neighbouring molecule by a process known as Förster resonance energy transfer (Lakowicz, 1999).

The transfer of excitation energy between chlorophyll molecules is due to interaction between the transition dipole moments of the donor and acceptor molecules. The probability of transfer falls off quickly as the distance between the pigments increases (the rate is proportional to  $R^{-6}$ , where  $R$  is the distance between the transition dipoles), and depends strongly on the overlap of the emission spectrum of the donor molecule and the absorption spectrum of the acceptor molecule, as well as the relative orientation of the donor and acceptor chromophores.

As shown in **Figure 5**, resonance energy transfer enables excitation energy to migrate over the antenna system. Because the first excited singlet state of chlorophyll *a* is lower than that of chlorophyll *b* or the carotenoids, excitation energy is rapidly localized on the chlorophyll *a* molecules. As a consequence, the energy that escapes the antenna system as fluorescence comes almost entirely from chlorophyll *a*.

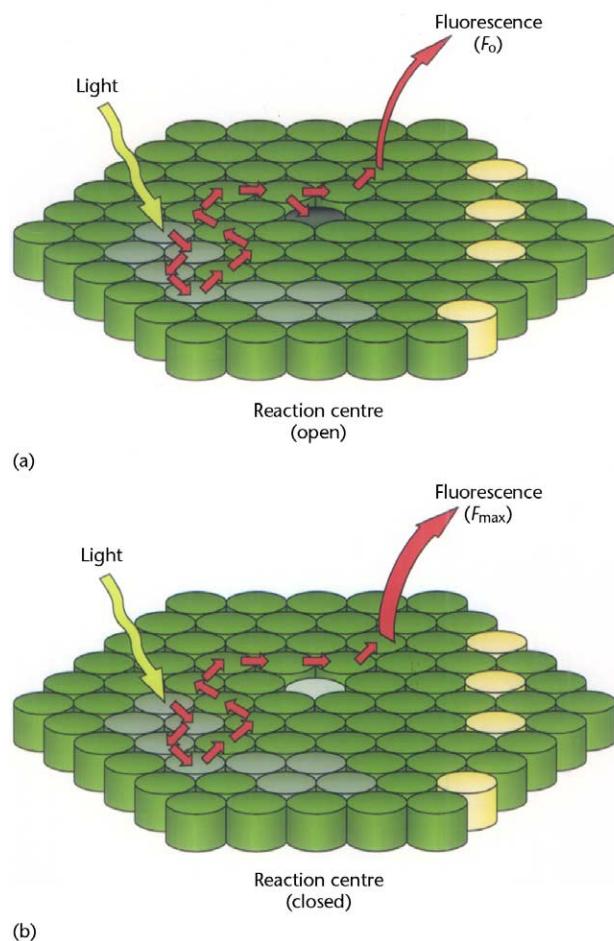
During the migration process, the excitation energy is either trapped by a reaction centre, converted into heat or released as photons. Photosynthetic antenna systems are designed to be very efficient at getting the excited state energy to a reaction centre. Measurements of photosynthesis at low light intensities show that over 90% of absorbed photons can be trapped by a reaction centre and promote primary charge separation. However, environmental conditions may impose limitations on photosynthesis that significantly slow the rate of electron transport, which greatly increases the fraction of absorbed light energy that goes into heat and fluorescence. Measurements of chlorophyll fluorescence provide a noninvasive method for monitoring photosynthetic performance *in vivo* (see below).

## Primary Photochemistry: The Reaction Centre

The primary photochemical reaction of photosystem II depends on electron transfer from P680 to pheophytin (Pheo), creating the charge separated state:  $P680^+ / Pheo^-$  (Dekker and van Grondelle, 2000). This primary photochemical reaction differs from subsequent electron transfer reactions in that the equilibrium redox midpoint potential of the primary donor (P680) is lower in energy than that of the primary electron acceptor (Pheo). As a consequence, electron transfer from P680 to Pheo can occur only if P680 is in an excited electronic state (denoted by  $P680^*$ ), which is created either by excitation energy from the antenna system or by direct absorption of a photon by the reaction centre.

Subsequent electron transfer steps prevent the primary charge separation from recombining by transferring the electron within 200 ps from  $Pheo^-$  to  $Q_A$  (**Figures 1, 2 and 4**). From  $Q_A^-$ , the electron is transferred to another plastoquinone molecule bound at the  $Q_B$  site. After two photochemical turnovers,  $Q_B$  becomes fully reduced ( $PQH_2$ ), after which it unbinds from photosystem II and is released into the thylakoid membrane. While the electron removed from P680 is rapidly sent away, an electron from a tyrosine residue ( $Y_Z$ ) on the D1 polypeptide reduces  $P680^+$ . Electrons for the reduction of  $Y_Z$  are extracted from the water-oxidizing complex, which includes the  $(Mn)_4$  cluster. The rate of electron transfer from  $Y_Z$  to  $P680^+$  ranges from 20 ns to 200  $\mu$ s, depending on the redox state of components involved in water oxidation.

Although photochemistry in photosystem II leads to charge separation between P680 and Pheo, the steps leading to this relatively stable state are complicated and not well understood. As shown in **Figure 4b**, P680 is



**Figure 5** Schematic drawing showing excitation energy transfer from one chlorophyll molecule to another in the antenna system. Green cylinders represent chlorophylls *a* and *b*, and yellow cylinders represent carotenoids; the dark cylinder in (a) represents an open reaction centre and the grey cylinder in (b) represents a closed reaction centre.

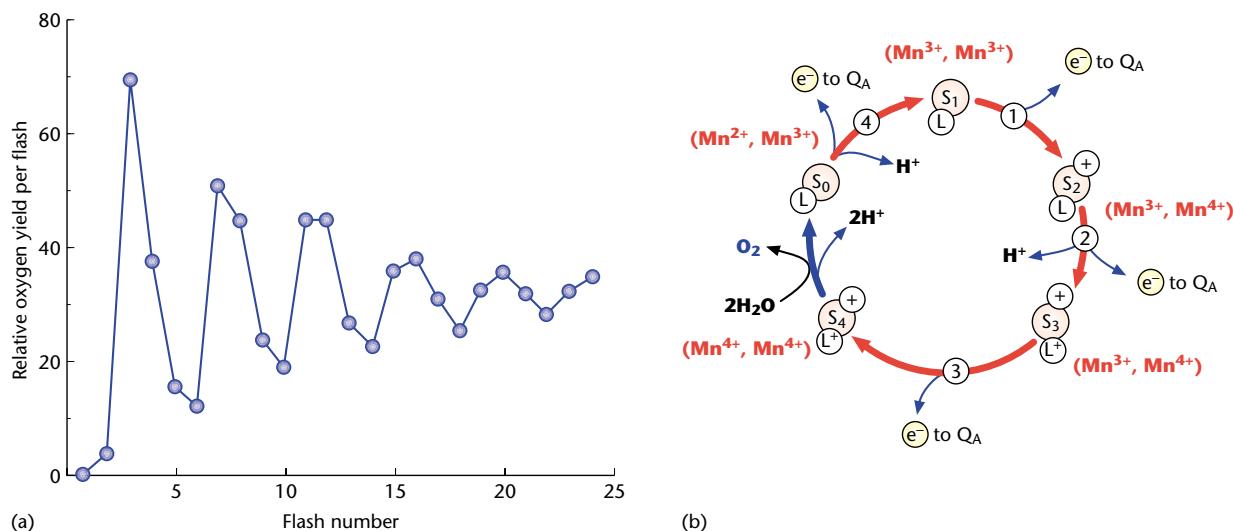
surrounded by four chlorophyll and two pheophytin molecules. Owing to the close proximity of these molecules, and the fact that the electronic energy levels of the six chlorophyll molecules are quite similar, excitation energy within the reaction centre equilibrates between the chlorophyll and pheophytin molecules before charge separation. The result appears to be that charge separation can occur between different chromophores in the reaction centre (Dekker and van Grondelle, 2000). Despite uncertainty in the early events of the photochemical reaction, ultrafast spectroscopic measurements indicate that  $P680^+ / Pheo^-$  is created within 8 ps (Greenfield *et al.*, 1997).

## Oxidation of Water: The Source of Molecular Oxygen

In 1969, Pierre Joliot and coworkers published a paper in which they measured the amount of oxygen during successive single-turnover light flashes (Joliot *et al.*, 1969). Using algae that were adapted to darkness, they found that the yield of oxygen plotted as a function of the flash number exhibited a periodicity of 4 (Figure 6a). This is a classical experiment because it demonstrated that each photosystem II complex operates independently, and that four photochemical reactions are required for the release of one molecule of oxygen (Joliot and Kok, 1975). The periodicity of 4 was readily explained by the chemistry of water oxidation, but the observation that the maximum oxygen yield occurred on the third rather than the fourth flash, and that the modulation disappeared after several cycles, revealed an unexpected level of complexity in the mechanism of water oxidation.

Based on the results of the Joliot *et al.* experiments, Kok and coworkers (1970) developed an elegant model of water oxidation by photosystem II. In this model, the oxygen-evolving complex of the reaction centre can exist in five states, labelled  $S_0$ ,  $S_1$ ,  $S_2$ ,  $S_3$  and  $S_4$  (Figure 6b). A photochemical reaction, which removes one electron, advances the oxygen-evolving complex to the next higher S state. The result is the creation of four oxidizing equivalents in the water-oxidizing complex. Substrate water is bound in separate sites in the complex through the  $S_3$  state and does not involve the formation of symmetrical peroxo intermediates (Hillier and Wydrzynski, 2000). Then, either through sequential steps or through a concerted reaction in the  $S_3$ – $S_4$ – $S_0$  transition, the oxidation of water results. The net reaction results in the release of one oxygen molecule, the release of four protons into the lumen, and the sequential transfer of four electrons through the reaction centre to the plastoquinone pool.

To account for the maximum oxygen yield on the third flash, Kok *et al.* (1970) suggested that in the dark-adapted algae most of the oxygen-evolving complexes are in the  $S_1$  state, rather than  $S_0$ . Thus, after three flashes the  $S_4$  state is reached and oxygen is released. Noting that some oxygen was released on the fourth flash, the Kok model assumes that in the dark-adapted sample the ratio of  $S_1 : S_0$  is 3 : 1. To explain the loss of periodicity as the flash number increased, the model assumes that in some photosystem II complexes the light flash fails to advance the S state (misses), while in others the light flash promotes an advance of two states (double hits). This remarkably insightful model successfully explained the flash dependence of oxygen evolution, and continues to guide research into the mechanism of water oxidation and oxygen release by photosystem II.



**Figure 6** The oxygen cycle. (a) Oxygen yield from photosystem II as a function of flash number (oxygen cycle) (see Joliot and Kok, 1975). (b) One of the current models of the steps in oxygen evolution in photosystem II. See text or Joliot and Kok (1975) for details.

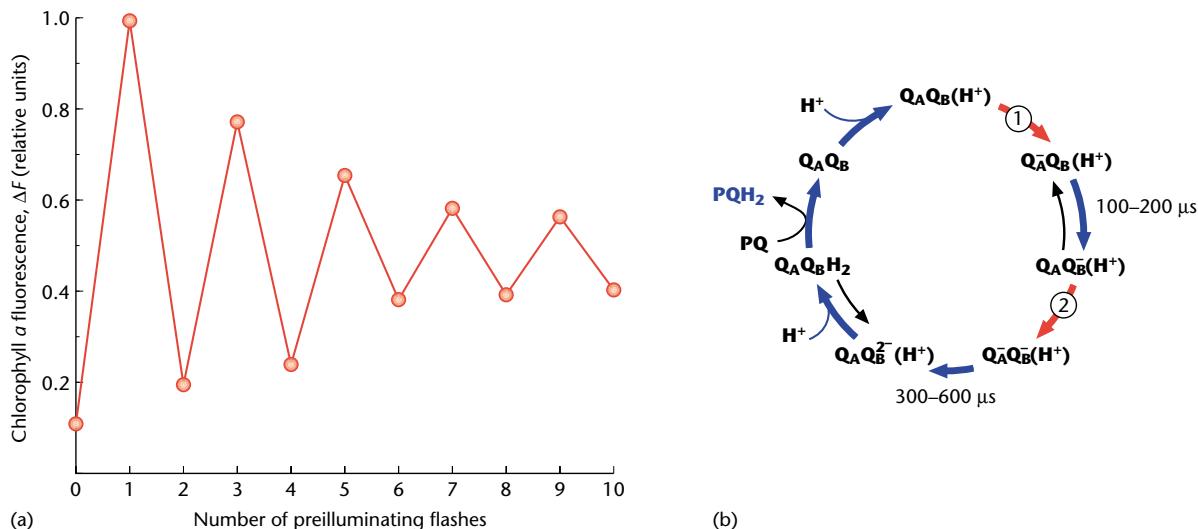
Oxygen evolution occurs on the lumenal side of the D1 and D2 proteins, and involves four manganese ions, several extrinsic polypeptides, including a 33-kDa protein known as the manganese-stabilizing protein (MSP), a 23-kDa protein (PsbP) and a 17-kDa protein (PsbQ), as well as calcium, chloride and bicarbonate ions (van Rensen *et al.*, 1999). In addition to these components, numerous studies have shown a dependence of oxygen evolution on cytochrome *b559* and CP47. In cyanobacteria the 23- and 17-kDa polypeptides are absent, while a *c*-type cytochrome (cytochrome *c550*) and a 10-kDa polypeptide are present on the lumenal side of the reaction centre.

A cluster of four manganese ions lies at the heart of the oxygen-evolving site of photosystem II (Figure 4b). Although it appears that all four manganese ions are needed for efficient oxygen evolution, there is a suggestion that only two of them undergo redox changes associated with water oxidation. Extended X-ray absorption fine-structure analysis indicates that in the transition from the S<sub>2</sub> to S<sub>3</sub> state manganese does not change oxidation state, leading to the suggestion that a redox active ligand is oxidized during this transition ('L' in Figure 6b). There is evidence that the ligand may be a histidine on the D1 polypeptide. Proton release during water oxidation has been measured by monitoring pH changes in the lumen. As shown in Figure 6b, protons are released during the advancement of the S states in a 1:0:1:2 pattern during the S<sub>0</sub>-S<sub>1</sub>, S<sub>1</sub>-S<sub>2</sub>, S<sub>2</sub>-S<sub>3</sub> and S<sub>3</sub>-(S<sub>4</sub>)-S<sub>0</sub> transitions, respectively (Fowler, 1977). The observation that the protons released into the lumen appear to come from amino acids near the water oxidation site indicates that this pattern may not reveal proton release associated with the catalytic steps involved in water oxidation.

Although Cl<sup>-</sup> and Ca<sup>2+</sup> are needed for water oxidation, their role is not known. One possibility is that the negative charge of the Cl<sup>-</sup> ions serves to stabilize the water-oxidizing complex during the accumulation of positive charge. Based on the close proximity of Cl<sup>-</sup> and Ca<sup>2+</sup> to the Mn cluster, both ions have been proposed to stabilize the (Mn)<sub>4</sub> cluster for efficient water oxidation. Ca<sup>2+</sup> may play a role in gating the access of water to the catalytic site. In addition, bicarbonate has been recently shown to play an important role on the water oxidation process, although the molecular mechanism remains unknown (Klimov and Baranov, 2001).

## Reduction of Plastoquinone: The Two-electron Gate

Plastoquinone plays a key role in photosynthesis by linking electron transport to proton transfer across the photosynthetic membrane. In the photosystem II complex, two plastoquinone molecules work in tandem, with one molecule permanently bound at the Q<sub>A</sub> site and another molecule bound at the Q<sub>B</sub> site. Once plastoquinone at the Q<sub>B</sub> site has been fully reduced by the addition of two electrons and two protons, it unbinds from the reaction centre and is released into the thylakoid membrane. The reduction of plastoquinone at the Q<sub>B</sub> site is known as the two-electron gate, because two photochemical reactions are needed for the formation and release of plastoquinol (Velthuys and Ames, 1974) (Figure 7a). Numerous compounds have been discovered that inhibit photosynthetic electron transport by binding at or near the Q<sub>B</sub> site, thereby preventing access of plastoquinone to the site



**Figure 7** The two-electron gate. (a) Chlorophyll *a* fluorescence from photosystem II as a function of flash number showing the two-flash dependence. (b) Steps in the two-electron reduction of plastoquinone at the  $Q_B$  site of photosystem II. See text for details.

(Wraight, 1981). A few of these compounds (e.g. atrazine) are used as commercial herbicides.

The pathway of electrons from P680 to  $Q_B$  is shown in **Figures 1** and **2**. The reduction of plastoquinone requires two photochemical reactions. In the first reaction an electron is transferred from  $Q_A^-$  to  $Q_B$  within 100–200  $\mu$ s, producing the state  $Q_A^-/Q_B^-$  (**Figure 7b**). In the second reaction an electron is transferred from  $Q_A^-$  to  $Q_B^-$  within 400–600  $\mu$ s, producing the state  $Q_A^-Q_B^-$ , which rapidly takes up protons, producing  $PQH_2$ . Protons involved in the reduction of plastoquinone come from the outer water phase and are delivered by branched pathways through the protein that include amino acids near the  $Q_B$  site. There is evidence that bicarbonate ions also play a role by binding near the  $Q_B$  site. Reduced plastoquinone unbinds from the  $Q_B$  site and enters the hydrophobic core of the membrane, which allows an oxidized plastoquinone molecule to bind to the site so that the cycle can be repeated.

## Photosystem II Contributes to a Proton Electrochemical Potential that Drives ATPase

The production of ATP in photosynthesis depends on the conversion of redox free energy into a proton electrical chemical potential, which is made up of a pH difference ( $\Delta$ pH) and an electrical potential difference ( $\Delta\Psi$ ) across the thylakoid membrane, and is given by the following equation:

$$\Delta\mu_H^+ = F\Delta\Psi - 2.3RT\Delta\text{pH}$$

where  $F$  is the Faraday constant,  $R$  is the gas constant, and  $T$  is the temperature in kelvin. Photosystem II contributes to this proton potential energy by: (1) the release of protons during the oxidation of water by photosystem II into the lumen; (2) the uptake of protons from the stromal phase associated with the reduction of plastoquinone at the  $Q_B$  site – this reaction is the first half of a proton-transporting mechanism that is completed by the oxidation of plastoquinol by the cytochrome *bf* complex, which releases the protons initially taken up by photosystem II into the lumen; and (3) the creation of an electrical potential across the membrane due to electron transfer through the photosystem II reaction centre from water to plastoquinone.

## Downregulation: Energy is Diverted Away from the Reaction Centre when there is Excess Light

Although photosynthesis can be very efficient, environmental conditions typically impose severe limitations on both rate and efficiency. One of the most common stress situations for a photosynthetic organism is the absorption of more light than they can use for carbon reduction. Excess light can drive inopportune electron transfer reactions, which may cause both long-term and short-term damage to photosystem II, impairing photosynthetic productivity.

Photosynthetic organisms have evolved different strategies to avoid injury due to excess light. One of the dominant

protective mechanisms in plants and algae is known as downregulation or nonphotochemical quenching: this is a dynamic regulation of excitation energy transfer pathways within the antenna system that diverts excitation energy into heat before it reaches the reaction centre (Demmig-Adams *et al.*, 1996). It is not unusual for half of the absorbed quanta to be directed from the antenna array and converted into heat. Under such conditions the efficiency of photosynthetic light energy conversion can be reduced by more than 50%. It is not known why plants respond to excess light by downregulating light capture rather than increasing photosynthetic capacity.

## Secondary Electron Transfer Reactions in Photosystem II Protect Against Photodamage

Photosystem II is susceptible to damage by excess light. This is not surprising in view of the fact that photosystem II must switch between various high-energy states that involve powerful oxidants required for the oxidation of water, and strong reductants for the reduction of plastoquinone. In saturating light, a single reaction centre can have an energy throughput of  $600 \text{ eV s}^{-1}$ , which is equivalent to 60 000 kW per mole of photosystem II. To avoid damage, photosystem II contains redox components that appear to serve as safety valves by accepting or donating electrons at opportune times. For example, it has been proposed that cytochrome *b559* serves to deactivate a rarely formed, but highly damaging, redox state of photosystem II, by accepting an electron from pheophytin when forward electron transfer is overloaded (Whitmarsh and Pakrasi, 1996).

## Inactive Photosystem II: A Significant Proportion of Reaction Centres do not Work *In Vivo*

Although most photosystem II reaction complexes work efficiently to oxidize water and reduce plastoquinone, a number of *in vivo* assays have shown that a significant proportion are unable to transfer electrons to the plastoquinone pool at physiologically significant rates. Experiments using higher plants, algae and cyanobacteria indicate that photosynthetically inactive photosystem II complexes are a common feature of oxygenic organisms. For example, in healthy spinach leaves, *in vivo* measurements show that 30% of the photosystem II complexes are inactive (Chylla and Whitmarsh, 1989). Inactive photosystem II centres are impaired at the  $Q_A$  site, which is reoxidized approximately 1000 times slower than in active

centres. In addition, the antenna system serving inactive photosystem II complexes is approximately half the size of that serving each active complex. Membrane fractionation studies indicate that stromal membranes are enriched in inactive photosystem II centres, but they are also present in granal membranes. These differences, between the antenna size and membrane distribution of active and inactive centres, are shared by photosystems  $II_z$  and  $II_\beta$ , which are defined by their relative antenna size. It is not known why plants contain photosystem II reaction centre complexes that do not contribute to energy transduction, but it has been estimated that inactive centres could reduce the quantum efficiency of photosynthesis by as much as 10%.

## Fluorescence: Monitoring Photosystem II Activity *In Vivo*

Measurements of chlorophyll fluorescence provide a noninvasive technique for monitoring photosynthetic processes in plants, algae and cyanobacteria. One of the primary applications is determining the activity of photosystem II reaction centres *in vivo*. The technique relies on the observation that the yield of chlorophyll fluorescence depends in large part on the capacity of photosystem II to carry out a stable charge separation between P680, the primary donor, and  $Q_A$ , the primary quinone acceptor of the reaction centre. When  $Q_A$  is oxidized, the reaction centre is able to utilize the light energy harvested by the antenna system for charge separation and the fraction of excitation lost to fluorescence is low, giving rise to low fluorescence yields (Figure 5). In contrast, when  $Q_A$  is reduced, the reaction centre is unable to undergo stable charge separation and the fraction of excitation lost to fluorescence is high, giving rise to the maximum fluorescence yield.

Measurements of the chlorophyll fluorescence emission from leaves provide data for calculating photochemical yields under physiological conditions (Schreiber *et al.*, 1998). The recent introduction of highly sensitive charge-coupled device cameras has enabled instrumentation that images chlorophyll fluorescence in cells, leaves and plants (Nedbal *et al.*, 2000; Holub *et al.*, 2000). The next stage in development is to use remote sensing to measure chlorophyll fluorescence dynamics for crops, forests, grasslands and aquatic photosynthesis.

## Summary

Photosystem II is a multiunit chlorophyll–protein complex that uses light energy to transfer electrons from water to plastoquinone. It is located in the thylakoid membrane and is made up of an antenna system, which captures light, and

a reaction centre core, which uses the light energy to drive electron and proton transfer. The antenna system is composed of protein complexes that contain pigment molecules, chlorophyll *a* and *b* plus accessory molecules, which capture light by converting photon energy to excitation energy. The reaction centre contains carriers that create a pathway for electrons from water to plastoquinone. These carriers include a cluster of four manganese ions, a tyrosine ( $Y_Z$ ) residue, a specialized pair of chlorophyll molecules (P680), pheophytin, a permanently bound plastoquinone ( $Q_A$ ) and a plastoquinone that binds reversibly to photosystem II at the  $Q_B$  site.

The primary photochemical reaction leads to the formation of a charge-separated state in which P680, a specialized pair of chlorophyll molecules, is oxidized and a pheophytin molecule is reduced. Oxidized P680 serves to remove electrons from water, while reduced pheophytin provides the electrons for the reduction of plastoquinone. Four consecutive photochemical reactions lead to the oxidation of two water molecules, which results in the release of one dioxygen molecule.

The structure of the photosystem II reaction centre is just becoming available, which means that the long-time goal of understanding the molecular mechanism of water oxidation is finally within reach.

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