Chapter 28

Photosynthetic Functions of Chlorophylls

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Summary

Chlorophylls (Chls) are the signature pigments of photosynthetic organisms and have several distinct functions, including photochemical activity and antenna function. Chls carry out reversible photochemical oxidations and reductions, which determine the basic mechanism of functioning of the photosynthetic reaction center (RC). The light-harvesting function of chlorophylls is based on their ability to absorb light over a wide spectral region. The variety of Chls (and bacteriochlorophylls) that are found in photosynthetic systems is formed by peripheral substitutions and reductions of the molecule's macrocycle. Chls undergo specific adjustments of their absorption properties due to pigment-pigment and pigment-protein interactions. Complexes of RC supplemented with light-harvesting antennas and additional electron transfer proteins are known as photosynthetic units (PSU). Despite the structural variety of the chlorophyll-based photosynthetic antennas known to date, the principles of the antenna design conform to fulfilling its biological function, capturing the energy of sun and conveying it via excitation energy transfer to the reaction center. A number of very different strategies for energy collection, delivery to the RC and regulation can be found in different photosynthetic systems. Chls are also involved in photoprotective processes of excess excitation deactivation in carotenoid-Chl complexes and Chl clusters, accumulation of stress-related Chl binding polypeptides and specific photoprotective electron transfer pathways.

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I. Introduction

Photosynthesis is one of the most important biological processes in the Biosphere. The yearly global net primary production of the biomass in photosynthesis is estimated to be ca. $10.5 \cdot 10^{10}$ tons of carbon, with roughly equal contributions from ocean and terrestrial ecosystems (Field et al., 1998). Since the advent of oxygenic photosynthesis, oxygen, a by-product of the process, has fundamentally changed the biosphere (Blankenship, 2002). This impressive impact of photosynthesis on the Earth highlights a significant role of the molecule of chlorophyll, which sensitizes the whole process of conversion of solar energy into the chemical energy of ATP, a major fuel used by organisms for living, as well as the generation of reductants that reduce CO_2 to sugars. This chapter reviews the major photosynthetic functions of chlorophylls.

II. Structure of Chlorophylls and Their Relevance to Photosynthetic Functions

Chlorophylls (Chls) were naturally selected as photosensitizers that are not consumed in photosynthetic reactions because they have a unique electronic structure. Chlorophylls are tetrapyrroles with a system of coplanar conjugated double bonds that form an aromatic structure with delocalization of electron density in π-orbitals. Structures for all the various chlorophylls can be found in Chapter 1 and are not repeated here. In the tetrapyrrole macrocycle π-orbitals form a ring with shared electron density of 18 electrons around the tetrapyrrole structure (Chapter 23, Steiner and Fowler; Chapter 34, Yerushalmi et al.). This mobile electronic structure leads to a high probability of electronic transitions from the highest occupied molecular orbitals to the lowest unoccupied molecular orbitals and ensures absorption of light in the blue (Soret) region and the green-to-red or near infrared part of the spectrum (Q_x and Q_y absorption bands) (van Amerongen et al., 2000).

In the majority of photosynthetic systems a Mg-ion in the center of the Chl macrocycle is bound via coordinative bonds to four nitrogen atoms of the tetrapyrrole, and to a fifth ligand donated generally by a protein. In derivatives of Chl with Ni or Fe in the center of the macrocycle the energy of the excited singlet state in the molecule dissipates radiationlessly within picoseconds. In proto-photosynthetic organisms biosynthetic pathways adaptively acquired Mg chelatase, an enzyme that inserts Mg into the tetrapyrrole molecule. The only exception is the occurrence of [Zn]-BChl discovered in the aerobic bacterium *Acidiphilium rubrum* (Wakao et al., 1996). This organism lives in an acidic environment in which Mg-BChl is not stable due to loss of metal, with replacement by hydrogen ions to form the bacteriopheophytin (BPhe). While photochemically very similar to BChl, [Zn]-BChl is more resistant to loss of metal and is therefore more suited to this environment.

The presence of the closed shell ions Mg^{2+} or Zn^{2+} in the center of the macrocycle lengthens the lifetime of the excited singlet state in Chl (BChl) to nanoseconds. This time is enough for photosynthetic energy transfer in light-harvesting antennas and storage in reaction centers (see below).

Chlorophylls are planar molecules, however, planarity of the macrocycle might be distorted by alkyl substituents or protein interactions. While suitable to produce red-shifted absorptions, significant distortions by massive side groups would result in a shortening of the excited state lifetime (Fajer, 2000). The variety of (B)Chls that can be found in photosynthetic systems is formed by peripheral substitutions and reductions of the macrocycle that are imposed in a series of biosynthetic modifications of Chls from Mg-protoporphyrin IX (Chl precursor) to Chls (Chapters 13, Yaronskaya and Grimm; 14, Rüdiger; 15, Frigaard et al.). Asymmetry in the π-electron distribution in substituted chlorins correlates with the extent of the low energy shift and the intensity of the Q_y absorption bands (van Amerongen et al., 2000).

A. Overview of Chlorophyll Functions

Chlorophyll-type pigments serve two primary functions in photosynthetic systems. These functions are as light-harvesting antenna pigments and as electron transfer cofactors. It is perhaps surprising that in many cases the same molecules can do both

Abbreviations: A – acceptor of electrons in photosynthetic reaction centers; BChl – bacteriochlorophyll; BPhe – bacteriopheophytin; Chl – chlorophyll; D –secondary donor of electrons in the reaction centers; FMO – Fenna-Matthew-Olson protein; LH – bacterial light-harvesting complex; LHC – light-harvesting complex of oxygenic photosynthesis; P680 (P700, P840, P870) – primary donor of electron in the reaction center (number indicates absorption maximum in nanometers); Phe – pheophytin; PS – Photosystem; PSU – photosynthetic unit; Q – quinone as an electron acceptor in the reaction center; RC – reaction center

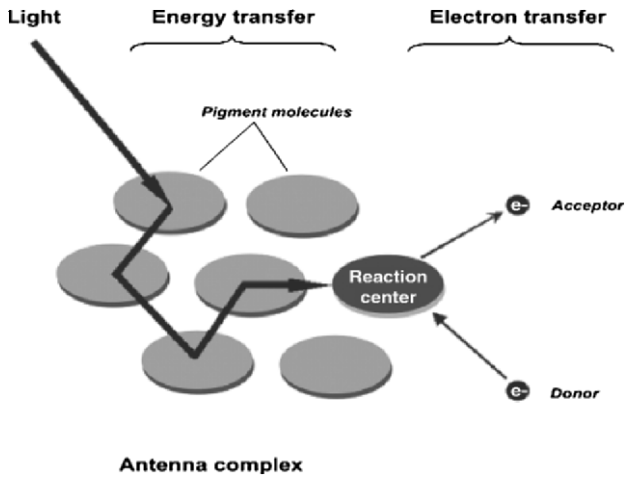

Fig. 1. Schematic depiction of the overall concept of the light harvesting antenna and reaction center. The antenna pigments absorb light and transfer excitation energy to the reaction center where photochemistry takes place. Generally, antennas may serve more than one reaction center.

of these rather different jobs in the process of photosynthesis, as some of the properties that make them suited for one function make them less suited for the other. Some types of chlorophylls are only found as antenna pigments, including Chl *b* and *c*, and BChl *c,d,e*. Other Chls can function as both antenna and electron transfer cofactors, including Chl *a* and *d* and BChl *a, b* and *g*. Some specialized Chls and the demetallated pheophytins, Phe *a*, BPhe *a* and *b* also perform essential functions as electron transfer cofactors (Chapter 4, Kobayashi). Figure 1 shows the overall concept of the light-gathering antenna that feeds energy to the electron transfer complex known as the reaction center (RC).

Functions of chlorophylls that are not related in any way to photosynthesis are extremely rare. The most dramatic is the use of a pigment derived from BChl *d* as a visual pigment in the eye of deep-sea dragon fish (Douglas et al., 1998, 2000). This pigment enables these fish to see in the red region where none of their prey is able to see and therefore gives them an advantage. It is not certain how the dragon fish obtains this pigment, although it is probably derived via the diet and then incorporated into the eye.

III. Chlorophyll-Sensitized Electron Transfer

The primary function of the Chls in reaction centers is photo-induced electron transfer. The ability of (B)Chl to carry out reversible photochemical oxidations and reductions (Krasnovsky reactions) determines the basic mechanism of the photosynthetic RC as an oxido-reductase sensitized by reversible photodisproportionation of Chls. Chls (BChls) possess the redox properties and control of reactivity that are required for reaction centers to extract electrons from a variety of substrates in the environment only when they are bound to protein.

The primary electron donor pigment forms a highly reactive photoexcited singlet state upon photoexcitation, which ejects an electron into the active electron transfer chain. Excited states can act either as strong reductants or strong oxidants (Blankenship and Prince, 1985). In solution, both oxidative and reductive behavior of excited pigments is well documented (Seely, 1978). However, in all known RCs the excited state acts as a strong reductant and the oxidative activity is not observed. The redox potential of the excited state can be estimated using the following simple equation, which relates the redox potential of the ground state of the photoactive pigment and its excitation energy:

$$E_m(P^{\bullet+}/P^*) = E_m(P^{\bullet+}/P) - E(P/P^*) \quad (1)$$

where $E_m(P^{\bullet+}/P^*)$ is the midpoint redox potential of the excited state reaction:

$$P^{\bullet+} + e^- \Leftrightarrow P^* \quad (2)$$

$E_m(P^{\bullet+}/P)$ is the midpoint potential of the ground state reaction:

$$P^{\bullet+} + e^- \Leftrightarrow P \quad (3)$$

and $E(P/P^*)$ is the 0-0 spectroscopic energy of the excited state above the ground state (in units of electron volts):

$$P + h\nu \Leftrightarrow P^* \quad (4)$$

P stands for the photoactive pigment. All the reactions shown are written as reductions, according to custom. The 1.4 to 1.8 eV photon energy and the typical ground state redox potential of 0.5 to 1 V (vs. the normal hydrogen electrode), depending on the type of RC, gives excited state redox potentials of –0.9 to –1.3 V, which makes the excited pigments extremely strong reductants. This is schematically illustrated in Fig. 2.

In order to permit multiple turnovers of the pigments and not have them be sacrificial electron donors, reversible electron transfer reactions are necessary. The neutral ground state of the primary donor regenerates from donation by immediate secondary electron donors. Back transfer of electrons from the primary acceptors to the primary donor is prevented by a chain of ultrafast electron transfer reactions via a system of secondary and tertiary electron acceptors, where in each step the back reaction is order(s) of magnitude slower than forward reaction (Chapter 31, Wachtveitl and Zinth). These rapid reactions serve to spatially separate the oxidized and reduced species and therefore reduce their reactivity. This 'kinetic steering' of the fate of the primary photoproducts is an essential aspect of efficient energy conversion in photosynthetic systems.

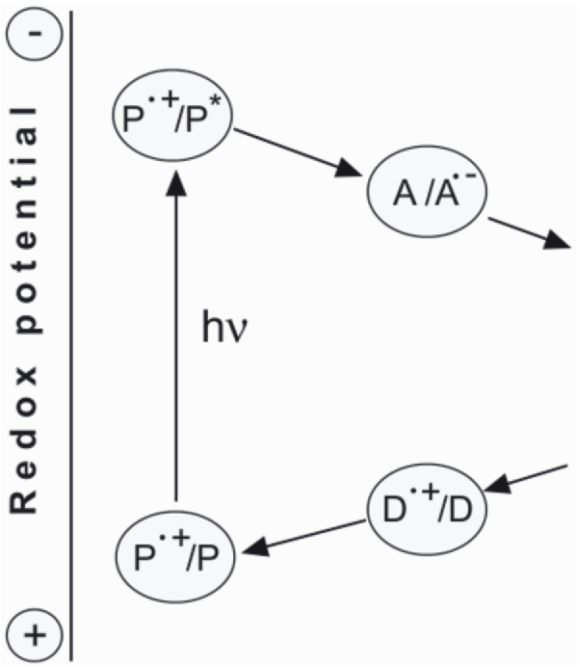

Fig. 2. Energy diagram of basic photochemical reactions in the reaction centers. The ground state and excited state redox reactions are written in the ox/red form according to equations 1–4.

A. Reaction Centers

The reaction center is a pigment-protein complex that is the site of the early electron transfer reactions of photosynthesis. The overall organization of the pigments in the RC is similar in all known photosynthetic organisms. The electron transfer chain starts with the primary donor, in most cases a dimer, and continues in two potential branches of redox cofactors, the closest to the primary donor being another tetrapyrrole pigment.

Depending on the sets of electron donors and acceptors there are two types of the RCs, iron-sulfur type (type I) and pheophytin-quinone type (type II), each adapted to specific secondary electron donors/substrates (sources of electrons). While the two classes of reaction centers have significant differences in the types of redox cofactors that serve as electron acceptors, structural studies strongly suggest that both are descended from a common ancestor (Schubert et al., 1998). Figure 3 shows the arrangement of the electron transfer cofactors in Photosystem I and the purple bacterial reaction center.

Iron-sulfur or type I RCs operate at low redox potentials. A major structural feature of the all known type I RCs is a fusion of the protein that binds core antenna Chls (BChls), with the protein that binds the redox cofactors of the electron transfer (Jordan et al., 2001). This type of RC is found in the anoxygenic green sulfur bacteria (BChl *a*) and heliobacteria (BChl *g*) and in the oxygenic cyanobacteria and chloroplasts (Chl *a*).

Of type I RC's, the structure of the cyanobacterial PS I is known in detail (2.5 Å resolution, Jordan et al., 2001), and recently the structure of the PS I/light-harvesting complex I assembly (PS I-LHCI) from pea has been solved with resolution of 4.4 Å (Ben-Shem et al., 2003). In the PS I RC a primary electron donor,

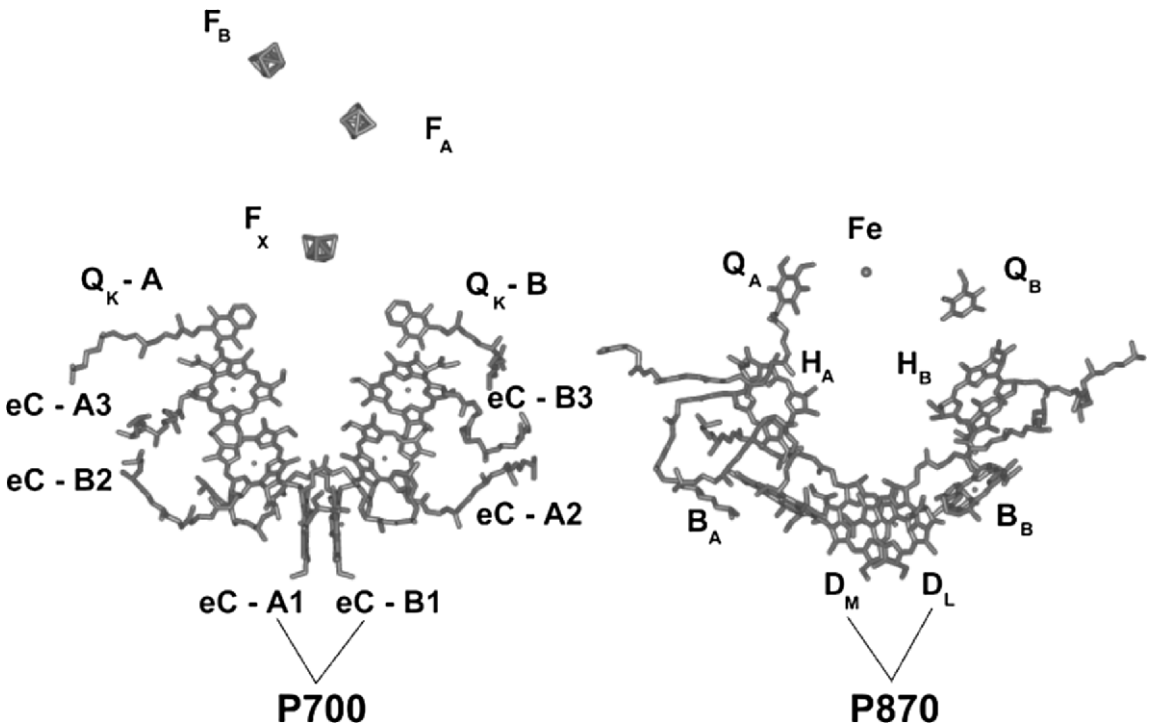

Fig. 3. Redox cofactors of electron transfer in two types of the photosynthetic reaction centers. *Left*: The iron-sulfur (type I) RC. Two branches of potential electron carriers in the PS I include eC-A1-eC-B1, primary donor P700; eC-A2 (eC-B2), accessory Chls; eC-A3 (eC-B3), primary acceptor A_0 and its symmetric counterpart; Q_K-A (Q_K-B), phylloquinones (secondary acceptors); F_X, F_A, F_B, iron-sulfur clusters. Figure produced from Protein Data Bank file 1JB0 with atomic coordinates of the PS I from the cyanobacterium *Synechococcus elongatus* (Jordan et al., 2001). Antenna Chls are not shown. *Right*: Pheophytin-quinone (type II) RC from purple bacteria. Active (A) branch of electron carriers: D_L-D_M, primary donor P870; B_A, 'accessory' BChl; H_A, 'primary' acceptor, bacteriopheophytin; Q_A, secondary acceptor, ubiquinone; Q_B, tertiary acceptor, ubiquinone. Inactive (B) branch of electron carriers: B_B, accessory BChl; H_B, bacteriopheophytin. Figure produced from Protein Data Bank file 1M3X (Camara-Artigas et al., 2002) with atomic coordinates of the RC from the purple bacterium *Rhodobacter sphaeroides.* Both models are shown at the same scale. Note the relative positions of the quinones in two types of the RC. Molecular graphics rendered using Web Lab Viewer from Accelrys, Inc.

P700, is a modified dimer consisting of Chl *a* and its C13[2] epimer, Chl *a*′. The relative positions of interacting Chls in the dimer are fixed by binding to the protein. Pigment-pigment interactions in the dimer and an asymmetric distribution of the H-bonds in the vicinity of P700 is thought to result in red spectral shift of P700 and a highly asymmetric distribution of the spin density over two parts of $P700^{\bullet+}$, respectively (Golbeck, 2003). The photooxidized primary donor $P700^{\bullet+}$ is a strong oxidant, which ensures extraction of the electrons from the substrates via a system of secondary electron donors (cytochromes/plastocyanins). In green sulfur bacteria the oxidized primary donor, $P840^{\bullet+}$, accepts electrons via secondary donors from reduced sulfur compounds.

Upon excitation of the primary donor the redox potential of P700 dramatically decreases to –1.1 V, which guarantees a rapid (picosecond) photoreduction of the primary electron acceptor, a monomeric Chl *a* molecule designated A_0. This molecule also possesses a highly negative redox potential (–1.05 V), and transfers electrons via a chain of consecutive cofactors (phylloquinones and iron-sulfur clusters) with descending redox potentials to ferredoxins on the stromal side of the thylakoid membrane (Chitnis, 2001; Golbeck, 2003). The unique redox properties of monomeric Chl *a* in the PS I type RC may be indispensable in type I RCs because this molecule is thought to function as the primary acceptor in RC from green sulfur bacteria (van de Meent et al., 1992) and a similar molecule is found in heliobacteria (van de Meent et al., 1991). The structural model visualizes the bridging Chls located between P700 and A_0, however, their functional significance

is unknown although their position in the structure undoubtedly indicates their involvement in electron transfer. Earlier, the intermediate function of the accessory BChl has been demonstrated in bacterial RC (Chekalin et al., 1987; Arlt et al., 1993; Chapter 31, Wachtveitl and Zinth).

Acquisition of the sets of the electron donors and acceptors in the type II RC was driven by selective pressure towards efficient extraction of electrons from a broader variety of terminal electron donors including water in PS II from cyanobacteria and chloroplasts. The RC from purple bacteria reveals the elements of common design, which include a BChl *a/b* special pair of the primary donor and two branches of electron transfer cofactors (Allen and Williams, 1998). In contrast to the PS I type RC, a complex of immediate electron acceptors in the type II RC includes BPhe *a/b* with intermediate BChls *a/b* located between the primary donor and the BPhe molecule. Rates of the charge separation between the primary electron donor and the spectroscopically detectable primary acceptor seem to be similar in both types of the RC. Although the secondary electron acceptor in type II RCs is also a quinone (ubiquinone or menaquinone), its redox potential is significantly less negative. Photoreduction of the primary quinone in purple bacterial RCs occurs within 200 ps. Differences in the tertiary electron acceptors (loosely bound Q_B in the type II purple bacterial RCs and PS II versus iron-sulfur clusters in the type I RC) indicate differences in the stabilization of the separated charges in the two types of RCs, as well as the ultimate destination of the electrons. These are soluble ferredoxins (Fd) for type I RCs versus the integral membrane quinone-oxidizing cytochrome bc_1 or b_6f complexes for type II RCs.

Mutagenesis studies of purple bacterial RCs showed that the redox potential of the primary donor could be significantly altered by changes in the protein environment, in particular the hydrogen-bonding pattern (Lin et al., 1994) (Chapter 19, Allen and Williams). This demonstrates an ability of the purple bacterial RCs to withstand active protein modifications without loss in function. In cyanobacteria and chloroplasts this evolutionary potential manifests itself in the form of PS II with a redox potential of the primary electron donor (+1 V in P680) being sufficient to oxidize water (+0.82 V). As confirmed by recent X-ray structure analysis of the PS II from Chl *a* containing cyanobacteria (Zouni et al., 2001; Kamiya and Shen, 2003; Ferreira et al., 2004), the D1 and D2 proteins that form the core of the PS II RC retain the pigment design of the type II RC, however, with some spatial reorganization in the structure of the primary donor and the accessory Chls. This reorganization is likely influenced by changes in the system of secondary electron donors in PS II. The highly reactive photoexcited singlet state of P680* launches an electron transfer to Phe. The resulting cation-radical of P680, an extremely strong oxidant, is capable to oxidize the protein as well as water, so its reactivity must be highly controlled and channeled. The nearby tyrosine residue (Tyr_Z) transfers an electron to $P680^{\bullet+}$ thus mediating water oxidation in the manganese cluster.

IV. Light-harvesting and Energy Transfer

Sunlight is a relatively dilute energy source, such that even in full sun each chlorophyll pigment will absorb only approximately ten photons per second (Blankenship, 2002). To avoid the reaction center idling while it waits for another photon, all photosynthetic organisms contain light-harvesting antennas. Reaction centers supplemented with antennas and additional electron transfer proteins are known as photosynthetic units (PSU). Despite the structural variety of the antennas known to date, the principles of the PSU design conform to fulfilling its biological function, capturing the energy of sun and conveying it safely and efficiently via excitation energy transfer to the reaction center where it is transformed into interchangeable forms used by the cell. Selective pressure during the evolution of the antenna complexes was directed towards fast utilization of the excitation energy before its dissipation or conversion into the long-lived triplet states capable of generating the reactive singlet oxygen species.

The major classes of photosynthetic antennas are peripheral membrane complexes, which are attached to the membrane but largely protrude into the cytoplasm, and integral membrane complexes, which are largely made up of transmembrane pigment-proteins and have most of their mass associated with the membrane. In many cases these integral membrane complexes are directly associated with the reaction center or are even part of the minimal complex, such as in the Type I RCs described above. The light-harvesting complexes closest to the reaction center are often called ‘core’ antennas, while those that are more distant spatially are known as peripheral

antennas. The term peripheral is therefore used in two somewhat different ways to describe antennas. Examples of the peripheral membrane complexes are the phycobilisome found in cyanobacteria and red algae and the chlorosome found in anoxygenic green bacteria (Green and Parson, 2003). Examples of the peripheral antennas that are integral membrane complexes are the light-harvesting complexes 2 (LH2) complexes from purple bacteria and the LHC class of antennas associated with both PS I and II of oxygenic organisms. Figure 4 illustrates the various classes of antenna complexes.

The remarkably different structural forms of the various antenna complexes, including distinct pigment types and organizational principles, strongly suggest that they have evolved independently multiple times in response to both the general need to increase absorption cross section and the specific aspects of the environment of organisms such as light quality or intensity.

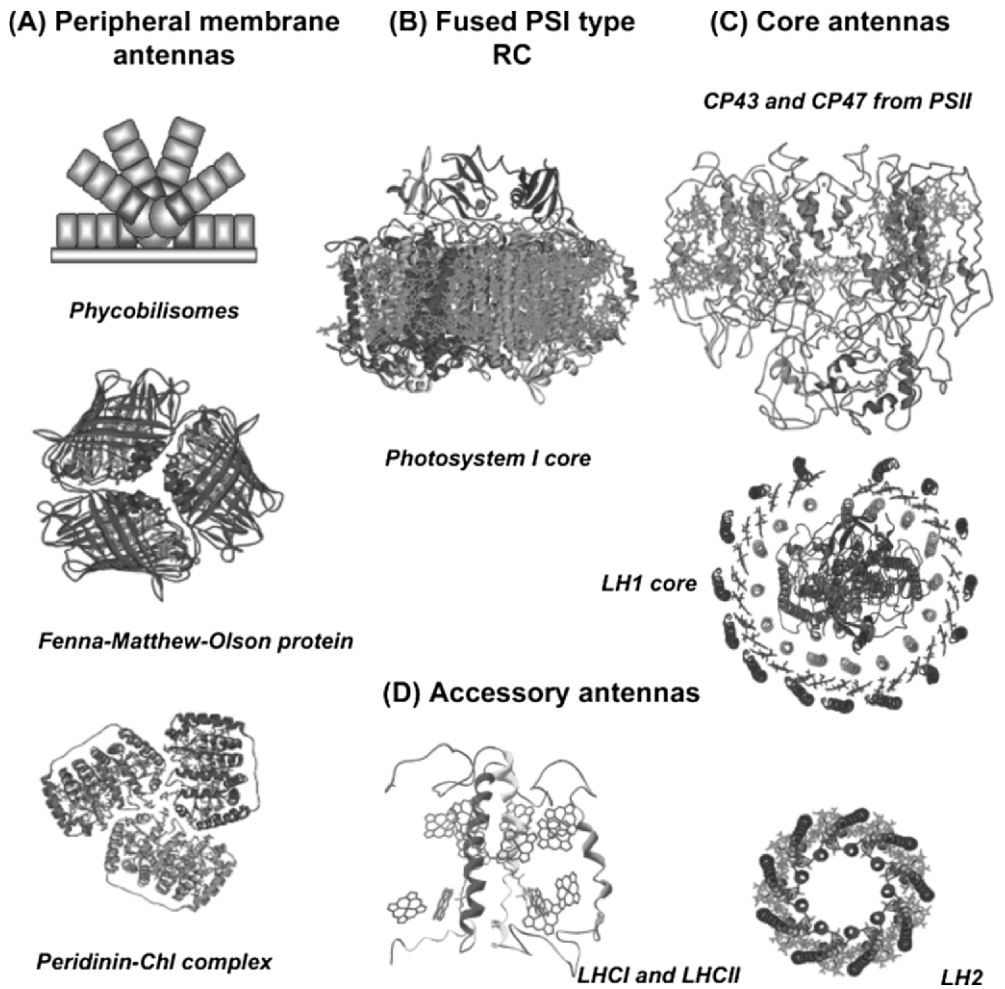

Fig. 4. Classes of light-harvesting antenna complexes. (A) Peripheral antennas: phycobilisomes from cyanobacteria and red algae (schematic); Fenna-Matthew-Olson protein from a green sulfur bacterium *Prosthecochloris aestuarii* (pdb code 4BCL, Tronrud et al., 1986) and peridinin-Chl complex from a dinoflagellate *Amphidinium carterae* (pdb code 1PPR, Hofmann et al., 1996); (B) fused PS I type RC from a cyanobacterium *Synechococcus elongatus* (pdb code 1JB0, Jordan et al., 2001); (C) Core antennas: CP43 and CP47 from the PS II of cyanobacterium *Synechococcus elongatus* (pdb code 1FE1, Zouni et al., 2001) and LH1 core from purple bacterium *Rhodopseudomonas palustris* (1PYH, Roszak et al., 2003); (D) Peripheral antennas: LHCI and LHCII from algae and higher plants (pdb file of LHCII provided by Werner Kühlbrandt, Kühlbrandt et al., 1994) and LH2 from *Rhodopseudomonas acidophila* (pdb code 1KZU, Prince et al., 1997). Molecular graphics rendered using Web Lab Viewer from Molecular Simulations, Inc. See also Color Plates 4 and 5.

A. Spectral Forms and Energy Gradient of Pigments

The protein environment modifies the energies of the excited singlet state transitions of the Chls (BChls) resulting in a heterogeneous absorption spectrum of the PSU. The spectral forms of the pigments within the PSU are distributed so that absorption maxima form a more or less distinct energy gradient directed towards the reaction center. Movement of the excitation from one pigment to another within the peripheral and the core light-harvesting antennas occurs typically on the sub-or low-picosecond time scale. In all known cases of peripheral light-harvesting antennas, the pigments absorb maximally at shorter wavelengths than the primary electron donor in the reaction centers, so that transfer of energy from shorter wavelength pigments to the longer wavelength pigments is accompanied by a loss of energy. This 'funneling' provides a degree of irreversibility to the energy transfer, thereby rapidly transferring the excitations from the distal parts of the peripheral antenna to the membrane and the core antenna and reaction center pigments. In core antennas, this energetic funneling is not always observed to be the case, and sometimes some or even most of the pigments are at a lower energy than the electronic trap, a molecule of the primary donor in the reaction center. In these cases, thermal energy combined with a charge separation that is fast in comparison to the back transfer of excitation energy, is sufficient to ensure efficient excitation trapping. Functional connectivity of pigments in the peripheral antenna and the core antenna in the PSU is determined both by the degree of overlap of spectral forms and the structural coupling of the components of the PSU, including distance and orientation.

Pigment-pigment and pigment-protein interactions are primarily responsible for the distribution of the spectral forms and filling of the energy gradient in the PSU. The primary donors in the RCs absorb at lower energies than the majority of (B)Chl in the PSU. The red spectral shift is caused by the excitonic interactions in a dimer of pigments with all oscillator strength distributed towards the low energy exciton band. Similar interactions occur in the rings of strongly coupled BChls in LH2/LH1 from purple bacteria (Chapter 21, Köhler and Aartsma) or in aggregates of BChl *c/d/e* in chlorosomes (Blankenship and Matsuura, 2003; Chapter 20, deBoer and deGroot). Pigment-pigment interactions in clusters of the Chl *a* antenna network in the PS I core result in appearing of the long-wavelength absorbing Chls that expand the absorption cross section of the complex (Gobets and van Grondelle, 2001). Earlier Trissl (1993) proposed that concentration of the excitation around the PS I reaction center is one of the possible functions of the red pigments.

The protein scaffold fixes the relative orientations of the pigments, thus preventing the excitation on the pigments from quenching, which is observed in concentrated solutions of pigments (Beddard and Porter, 1976). This is one of the most important and yet poorly understood aspects of pigment-protein structure and function. Pigment-protein interactions also affect the site energy of the Q_y absorption bands in Chl (BChl) in light-harvesting antennas. The spectral shifts may be induced by distortions of the macrocycle system by large amino acid side chains or by hydrogen bonding from the protein to the side groups of the porphyrin macrocycle (Fajer, 2000). Structural studies of the LH2 revealed that a loss of the hydrogen bond from an aromatic amino acid to an acetyl group in BChl B850 due to a site-directed mutation correlates with a spectral shift of the 850 nm absorption band to 820 nm and an out-of-plane displacement of the BChl acetyl group, which resulted in a loss of one conjugated double bond in the electronic structure of the BChl (Cogdell et al., 2002).

B. Adaptive Increase of Light-Harvesting Capacity

Photosystems undergo adaptive changes in light-harvesting capacity as a response to variable environmental conditions, including changes in both quality (spectral distribution) and quantity (photon flux) of illumination or other factors such as availability of essential nutrients. Structurally, this can manifest itself as oligomerization of pigment-protein complexes, for example, an increase in number of LH2 rings relative to LH1 in purple bacteria under low light, trimerization of cyanobacterial PS I, formation of rings of the iron-stress-induced proteins around the PS I trimer in aquatic cyanobacteria and oxyphotobacteria, dimerization of PS II in higher plants, LHCII trimerization in the peripheral antennas of green algae and higher plants, etc (see below).

C. Energy Transfer Strategies

A number of very different strategies for energy collection, delivery to the RC and regulation are found in different systems (Chapter 29, Lokstein et al.).

1. Light-Harvesting Capacity Based on Pigment Aggregation in Chlorosomes

A funnel concept fully applies to the PSU in green sulfur bacteria and filamentous anoxygenic phototrophs (Olson, 1998; Blankenship and Matsuura, 2003). Direct aggregation of BChl *c/d/e* in chlorosomes is a simple and metabolically cheap solution for significant increase of light-harvesting capacity and fast energy delivery to the RCs. Despite the dense pigment packing, which can be at a bulk concentration of greater that 1 M, chlorosomes show no signs of concentration quenching. The aggregation results in strong excitonic interactions with the subpicosecond excitation energy delocalization over the aggregate unit. As a result, the absorption spectrum of chlorosomes is significantly red shifted (750 nm relative to absorption of monomers of BChl *c/d/e* at ~ 670 nm), which optimizes the overlap of their fluorescence spectrum with the BChl *a*-carotenoid binding baseplate absorbing at about 800 nm (Montaño et al., 2003). In green sulfur bacteria and green filamentous bacteria the coupling of the chlorosome baseplate with the RC is carried out by different antennas. In green sulfur bacteria with the BChl *a* containing type I RC, this function is provided by a peripheral BChl *a*-binding protein, also known as the Fenna-Matthew-Olson (FMO) protein. Green filamentous bacteria (e.g., *Chloroflexus aurantiacus*) lack the FMO antenna, however, spectral overlap and energy connectivity of the chlorosomes with the type II RC in these organisms are provided by the core light-harvesting antennas, which are analogous to the LH1 in purple bacteria. The strategy of utilizing less protein and more pigments helped these primitive phototrophs to settle in habitats with variable and sometimes extreme conditions, especially low light environments. A schematic structural model of the photosynthetic unit from green sulfur bacteria is shown in Fig. 5. Some of these organism inhabit environments where the light intensity is so low that each pigment absorbs a photon on average once every several hours, making them the champions of photosynthesis at extremely low light intensities.

2. Fast Energy Delocalization and Storage in Light-harvesting Complex 2/Light-harvesting Complex 1/Reaction Center Complex of Purple Bacteria

The LH2-LH1-RC supercomplex in purple bacteria is an example of the light-harvesting strategy based on

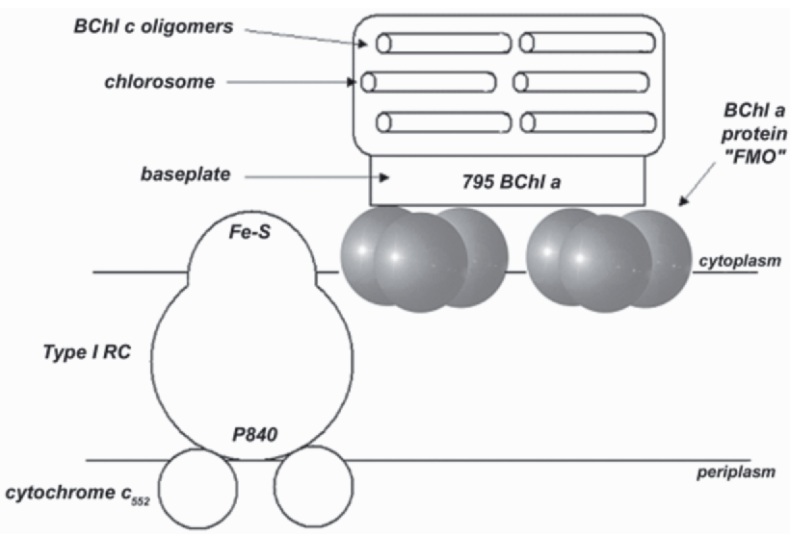

Fig. 5. Schematic model of the photosynthetic unit from the green sulfur bacteria. The peripheral light-harvesting antenna complex of the PSU includes the BChl *c* containing chlorosomes, BChl *a*-carotenoid binding baseplate absorbing at 795–800 nm and BChl *a* containing trimers of the FMO protein. The integral core antenna and the cofactors of the transmembrane electron transfer are fused in the iron-sulfur (Fe-S) type I RC. The primary donor in the RC, a special pair P840, accepts electrons from the cytochromes c_{552} on the periplasmic side of the membrane. Figure modified with permission from Blankenship and Matsuura, 2003. The components of the PSU are not drawn to scale.

the ring structure of the BChl-carrying polypeptides. In photosynthetic membranes of BChl *a/b* containing purple bacteria the geometrical features of the PSU components translate into the physical properties that support biological function (Hu et al., 2002). In the rings (both the peripheral LH2 and the core LH1) the specific structure of the proteins and the arrangement of the chromophores determine the ultrafast delocalization of the excitation energy throughout the ring (Sundström et al., 1999; Cogdell et al., 1999). Two major spectral forms of BChl *a* in LH2, B800 and B850, correspond to absorption of 8–9 BChls in the 'lower' monomeric ring of pigments and 16–18 BChls in the 'upper' ring of strongly coupled pigments, respectively. The architecture of the primary light-harvesting LH1 is similar in structure to LH2, although it is larger to accommodate the RC inside the LH1 ring and lacks the monomeric BChls. The major spectral form of the LH1 is B875 in BChl *a* containing organisms, and B1020 in BChl *b* containing organisms. A ladder of spectral forms such as B800–B850–B875 provides funneling of the energy to the RC (P870). Distances between LH2 and LH1 rings enable a picosecond energy transfer ensured by a good spectral overlap of the fluorescence of B800-B850 complexes (LH2) and B875 in LH1. In turn, the core LH1 complex spectrally overlaps with the RC, which provides efficient energy transfer from the low energy absorbing BChls to the RC. This process takes approximately 35–50 ps and is largely irreversible, so once the excitation has reached the electronic trap in the RC (P870) the probability of escaping back to the antenna is relatively low (10–20%). This transfer-to-trap behavior is determined by the long distance (35–40 Å) between the B875 pigments in LH1 and the pigments in the RC, in combination with the rapid charge separation (~3 ps). The antenna size in the PSU is subject to regulation depending on the light conditions. The final step in the energy transfer into the RC is sometimes slightly uphill, such that the excited state of the LHI antenna is lower in energy than the special pair in the RC. At the temperatures that the organisms live in nature, thermal energy is sufficient to ensure that the excitations are delivered to the RC with little or no loss of efficiency. At cryogenic temperatures, this efficiency is often much reduced due to the fact that the low energy spectral forms of the antennas become irreversible traps. A structural model of the purple bacterial LH2-LH1-RC complex is shown in Fig. 6.

3. Optimized Energy Transfer in the Photosystem II Core Antenna Networks: Speed and 100% Delivery to the Reaction Center

The PS I core in cyanobacteria and chloroplasts of green algae and higher plants is an example of the fused core antenna-reaction center complexes where the Chl *a* antenna network is bound to the same protein as the redox cofactors of the RC (Fromme et al., 2001; Ben-Shem et al., 2003). In this kind of photosynthetic system the relative positions of Chls are optimized for efficient delivery of the excitations to the reaction center. A dense packing of the pigments in the PS I core (Chl *a*/protein ratio of ~1.2) reduces the metabolic cost of the antenna and increases the connectivity of the antenna via random subpicosecond hopping of the excitations in the network (Cogdell and Lindsay, 2000; Byrdin et al., 2002). Optimal connectivity within the network as well as the spectral overlap of antenna pigments with the redox active Chls (primary donor P700) allows equilibration of the excitation energy in the depth of the whole membrane within picoseconds and delivery of the excitation to the primary donor P700 within 20–30 ps (Melkozernov, 2001; Gobets and van Grondelle, 2001). Structural coupling of the Chl antenna network and the redox active Chls in the RC allows for excitations to make multiple visits to the trap before the photochemical quenching due to the primary charge separation in the RC. The PS I excitation dynamics are known as trap-limited when the rate constant for escape of the excitation back to the core antenna is much larger than the intrinsic rate constant for photochemistry, so that multiple visits of the excitation to the trap are the norm. A special feature of the PS I core is clustering of (some of) the pigments. Excitonic interactions in clusters favor further broadening of the absorption spectra, their red shift and better overlap with the absorption spectrum of the primary donor in the reaction center, which leads to efficient energy trapping. Pigments with the strongest pigment-pigment interactions (red pigments) in such an effective network increase the absorption cross-section of the PS I complex and localize the excitation near the RC (Trissl, 1993). (See however the problem of concentration quenching mentioned above).

Some aquatic cyanobacteria have a remarkable adaptation to low light and iron deficiency in the form of a ring of 18 Chl *a* binding CP43-like proteins around the PS I trimers (Bibby et al., 2001a;

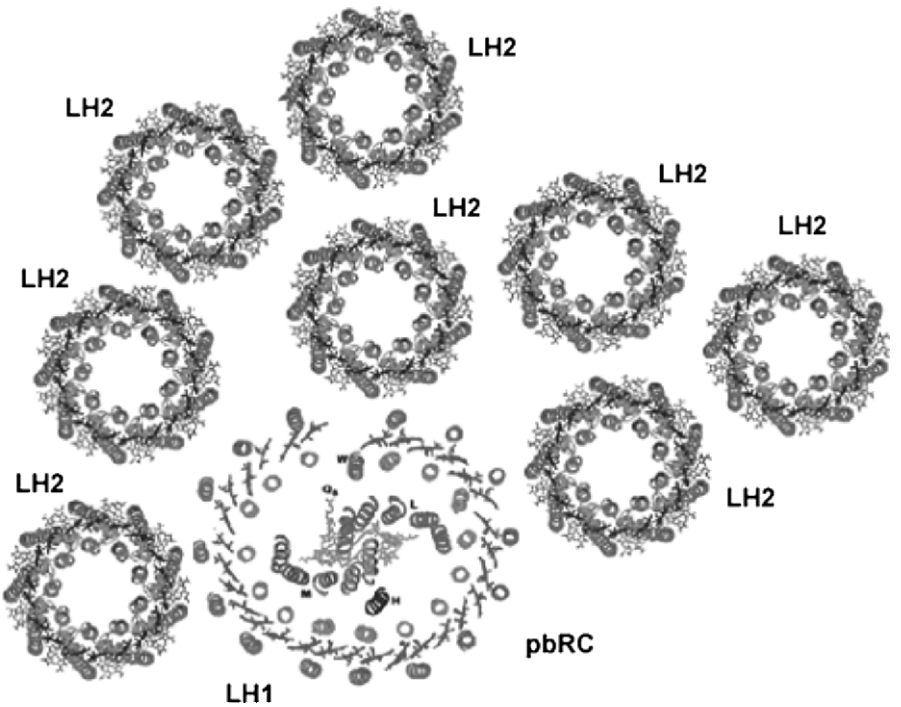

Fig. 6. Structural model of the photosynthetic unit from the purple photosynthetic bacterium *Rhodopseudomonas palustris* (pdb file 1PYH, Roszak et al., 2003*).* See also Color Plate 6.

Boekema et al., 2001). It is likely that the principle of optimal connectivity of pigments and fast delivery of the excitation to the RC holds even for such a large complex based on recent reports of an efficient energy coupling of the iron-stress induced antenna and the PS I trimer (Melkozernov et al., 2003). A model of the cyanobacterial supercomplexes is shown in Fig. 7.

4. Light-harvesting Complex I/Photosystem I and Light-harvesting Complex II/Photosystem II Supercomplexes: Balance of Efficient Energy Delivery and Regulation

In chloroplasts of algae and green plants both PS I and PS II are associated with accessory light-harvesting antennas. Light-harvesting complex I (LHCI) and LHCII are very similar in structure (Kühlbrandt et al., 1994; Ben-Shem et al., 2003) although LHCI complexes form dimers in vitro and possess long wavelength absorbing pigments (red pigments) and the majority of the LHCII complexes form trimers. PS I holocomplex assembles as the PS I monomer associated with four LHCI proteins (Scheller et al., 2001; Ben-Shem et al., 2003). The PS II supercomplex consists of the dimer of the PS II core complexes,

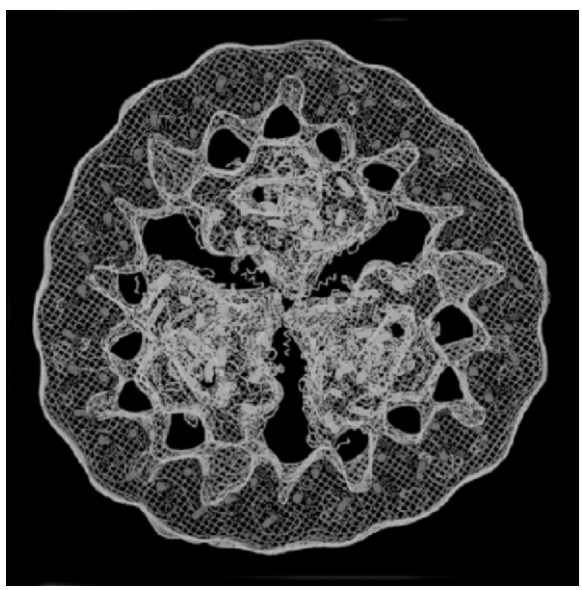

Fig. 7. Structural model of the PS I supercomplex from iron-depleted cyanobacteria. The complex consists of the Photosystem I trimer surrounded by 18 copies of the iron-stress induced CP43-like antenna pigment protein. Figure used with permission from Bibby et al., 2001b. See also Color Plate 6.

several LHCII trimers and monomeric Lhcb antennas associated with both PS II core and LHCII trimers (Boekema et al., 1999; Yakushevska et al., 2001; Barber, 2003). Elementary energy transfer processes in the Chl *a/b* binding LHCI and LHCII are as effective as in the Chl *a*-binding core complexes of PS I and PS II and occur on subpicosecond and picosecond time scales (Melkozernov, 2001; van Amerongen and van Grondelle, 2001). The PS I core belongs to a fast type of the RCs with a photochemical energy trapping occurring within 20–30 ps (see above). Coupling of the PS I core to LHCI significantly slows the time of the excitation delivery to the RC from the periphery of the supercomplex (Melkozernov, 2001). In the PS II core complex (D1/D2-CP43-CP47) the electronic trap in the RC seems to be shallow (Jennings et al., 1996). As a result trapping times are comparable with the excitation delivery times from the peripheral antennas to the PS II core.

In both PS I and PS II supercomplexes the LHC content is flexible and varies with the intensity and spectral properties of the light. Furthermore, the antenna size in both supercomplexes is regulated via physiological State 1 – State 2 transition mechanisms (Wollman, 2001; Haldrup et al., 2001). The time scale of physiological responses in both supercomplexes is seconds, minutes and hours. Striking a balance between the efficient delivery of the excitation to the RC and the regulation of the antenna size appears to be an adaptive strategy for PS I and PS II supercomplexes from green algae and higher plants.

V. Structural Function

In light-harvesting antennas with minimal protein content such as chlorosomes in green sulfur bacteria, the self-assembly of BChl *c/d/e* is determined by the planar structure of the BChl macrocycle, hydrogen bonding between the carbonyl groups and central Mg atoms of neighbors as well as by π-π stacking. Topologically, two-dimensional pigment layers assemble as rod elements. Bundles of rods, which are covered by the lipid envelope, constitute a chlorosome incorporating hundreds of thousands of pigments (Blankenship and Matsuura, 2003; Chapter 20, de Boer and de Groot).

Reaction center complexes only tolerate selective replacements and limited modifications of the pigments (Scheer and Hartwich, 1995). On the contrary, in many light-harvesting antennas the structural role of the protein is balanced with the structural role of the pigments. This is illustrated by successful in vitro reconstitution of the (B)Chl-protein complexes from their components. Oligomers of LH1 antennas from purple bacteria easily dissociate into the monomeric BChl-protein complexes and reassemble with reconstitution of the red-shifted BChl spectral forms resulting from pigment-pigment interactions (Loach and Parkes-Loach, 1995). Successful reconstitution of LH1 from individual components (isolated proteins and purified pigments) demonstrated a stabilizing effect of BChls on the structure of the complexes, which is mediated by affinity of the pigment to the protein ligand, by specific interactions of the BChl's side groups with the protein environment and BChl/BChl interactions (Loach and Parkes-Loach, 1995; Lapouge et al., 2000).

Similar results were obtained for in vitro reconstitution of the Chl *a/b* binding polypeptides from LHCII and LHCI (Paulsen and Schmid, 200; Chapter 26, Paulsen). LHCII apoproteins can be reconstituted with pigments starting from a fully denatured protein (Yang et al., 2003). Chl *b* as well as carotenoids were found to be essential for reconstitution of stable LHCII in vitro because of their exclusive binding to certain binding sites (Chapter 26, Paulsen).

Results of experiments performed in vivo also point to specific structural roles of Chl *a* and Chl *b* in an insertion and assembly of LHCs in thylakoid membranes (Hoober and Eggink, 1999). In higher plants a block in Chl *b* biosynthesis correlates with a decreased LHC antenna. Regulation of the LHC antenna size is suggested to be realized either via gene expression or via reversible enzymatic conversion of Chl *a* and Chl *b* (Rüdiger, 2002; Tanaka and Tanaka, 2000; Chapter 16, Beck and Grimm).

V. Photoprotective Function of Chlorophylls

Free monomeric Chl in solution is dangerous for living photosynthetic (and any other) cells (Chapters 33 and 34, Brandis et al.) cells because the excited singlet state of the Chl molecule easily converts into the relatively stable excited triplet state with a lifetime of 10^{-5}–10^{-3} sec. The triplet state of Chl can induce formation of harmful free radicals and singlet oxygen via photosensitized electron and proton transfer. In photosynthetic systems, production of these deleterious compounds is minimized by mechanisms, which

are still partly unknown. One is the organization in a protein scaffold, which enables excitation energy transfer between neighboring Chls towards the RC. If the excitation is localized on a Chl molecule, its excited triplet state tends to be efficiently quenched by carotenoid molecules, which are commonly located close to Chls in the majority of pigment-protein complexes. The carotenoid triplet state is usually energetically unable to induce formation of reactive oxygen species. Instead, the excitation energy dissipates through the vibrations of the isoprenoid chain of the carotenoid molecule due to overlap of the low-lying excited triplet state and the ground state (see Frank et al., 1999).

A more sophisticated enzymatic xanthophyll cycle in the peripheral antenna of PS II from higher plants and some algae (Niyogi, 2000) involves light-intensity dependent de-epoxidation of carotenoids like violaxanthin, resulting in formation in high light of zeaxanthin, a carotenoid molecule with the energy of its excited S_1 state believed to be sufficiently low to accept energy from the Q_y state of the Chls. This favors a direct protective quenching of the excitation on the Chl molecule (Ma et al., 2003)

In contrast to PS II, PS I quenches excitations at the same rate regardless of whether P700 is oxidized or reduced. Structure of the PS I core with its significant connectivity of pigments predicts that the photoprotective Chl-to-carotenoid channel might be redundant in the PS I core antenna because the ultrafast energy transfer in a well connected antenna leaves little time for formation of Chl triplet states. In the peripheral antenna of the PS I in green algae and higher plants such a channel could work. So far, the xanthophyll cycle has been reported only for the peripheral antenna of the PS II. It is not yet clear whether there is a similar regulatory mechanism that operates in PS I. Although in the PS I core the red pigments are thought to extend the absorption cross-section and concentrate the excitation towards the reaction center, in the peripheral antenna the red pigments might be involved in a photoprotection through localization of the excess excitation energy followed by a nonphotochemical quenching (Melkozernov, 2001). Competition of nonphotochemical trapping in the LHCI and photochemical trapping in the PS I-LHCI complex might be a possible regulatory process, however, the mechanism of this process is unknown. A photoprotective function of the peripherally located red pigments was suggested also for the PS I trimers from the cyanobacterium *Spirulina platensis*, which are characterized by a unique red shifted emission at 760 nm (Karapetyan et al., 1999).

Photoprotective functions of Chls are not limited to deactivation of excess excitation in carotenoid-Chl and Chl clusters. Stress-related Chl binding polypeptides that accumulate in the photosynthetic cells under stress conditions have recently been discovered in many groups of photosynthetic organisms. These polypeptides comprising one, two or three helices show a similarity in sequences with Chl *a/b* binding proteins (Heddad and Adamska, 2002), which suggests their common origin. In addition to Chls, some stress proteins bind carotenoids (luteins, zeaxanthins), which led to a suggestion that the function of an ancient antenna was not associated primarily with light-harvesting but with non-photochemical quenching of the excess energy (Montané and Kloppstech, 2000). The small cab-like polypeptide may function for transient storage of pigments with a regulated release in the sites of the assembly of pigment-protein complexes in membranes.

Chls exhibit their photoprotective functions not only in the antenna complexes but also in the electron transfer pathways. A low quantum yield cyclic electron transfer around P680 discovered in PS II was suggested to function as a photoprotective valve preventing PS II from accumulation of photooxidized P680 and doubly reduced Q_A under high light intensities (photoinhibition) (Hanley et al., 1999). This electron transfer pathway includes plastoquinone Q_B, Cyt *b*559, β-carotene and Chl Z in the core antenna. All the components of this pathway are identified in the structural model of the PS II (Zouni et al., 2001). Additionally, the oxidized Chl Z is an efficient quencher of the excess excitation energy in the vicinity of P680.

In the pheophytin-quinone-type reaction centers from purple bacteria molecular arrangements of the cofactors of electron transfer (BChls and BPhes) and their immediate protein environment favor electron transfer via only the A-branch of electron carriers (P870-B_A-H_A-Q_A, see Fig. 3, right panel). Under specific conditions of excitation with higher energy (blue light) the electron might be transferred from the primary donor via cofactors of the B-side, $BChl_B$ and $BPhe_B$. This pathway is thought to protect the RC from deleterious overexcitation (Lin et al., 2001). Recombination of the charges in the RC may result in formation of the excited triplet state of the primary donor. A carotenoid molecule, which is located in close proximity to the accessory BChl of

the B-branch, is a possible site for the quenching of the BChl triplet state in the reaction center, which is mediated by the 'accessory' BChl-B_B in the 'inactive' branch (Frank, 1999).

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