3.1.3 Chlorophyta

The third group of algae possessing primary plastids is the Chlorophyta. This group of protists is predominantly flagellated with two unequal fl agella. The plastid outer membranes enclose many thylakoid membranes, which are closely positioned to each other over signifi cant portions, but the term **appression** has been reserved for the grana of higher plants. Unlike the plastids of glaucophytes and rhodophytes, the light harvesting system is based on membrane integral proteins. Many of these proteins are in the CAB family of proteins binding Chl *a* and Chl *b* (see Sect. 5). In addition there are a number of other differences: the presence of starch, the close positioning of thylakoid membranes, presence directly in the cytoplasmic compartment, and not enclosed in any other membranes. It should be noted, however, that a CAB-type gene and gene product is found in PSI of rhodophyte plastids (see above), an occurrence which might be explained either by vertical or lateral gene transfer. It should also be pointed out that eukaryotic algae in the chromophyte lineages (chomalveolates) (Fig. 5), which bear secondary plastids, have proteins in the same family as CAB proteins, but they carry Chl *c* not Chl *b* and are referred to as CAC light-harvesting proteins (see below). The genomes of many chlorophyte plastids have been sequenced and range in size from 118 to 204 kb, with 94–107 genes. A number of chlorophyte genomes have been fully sequenced. The size ranges from 12 to 121 Mb, with *Chlamydomonas* being the largest by far. Interestingly, *Chlorella* (*Chlorella variabilis*) turns out to be a member of the Trebouxiaceae, with a history of endosymbiosis, cryptic sex and cryptic/residual flagella,

3.2 Secondary and Tertiary Plastids

It is surmised that all other plastids have arisen by secondary symbiosis, followed in some cases by tertiary symbiosis. Some evidence for this comes for the nucleomorph of cryptophytes, where there are four membranes around the plastid and the nucleomorph, bearing three reduced chromosomes, lies between the two sets of outer membranes. This nucleomorph has been sequenced and indicates that the primary host was a relative of a red alga. The cryptophyte plastid genome of *Guillardia theta* also bears strong similarities to rhodophyte plastids. This is interesting because the primary light- harvesting protein of cryptophytes is quite different, in the alpha subunit from the phycobiliproteins of red algae (see below). In addition they have acquired Chl *c* (see Sect. 3.2.4). For most of the secondary plastids, largely in the chromophyte lineage (also called the chromalveolate branch), no equivalent of the nucleomorph has been found (but it is assumed that these secondary plastids evolved in a similar way by serial endosymbiosis): four outer membranes usually exist, although in the case of dinofl agellates only three are found. A case for tertiary endosymbiosis can also be made out in certain limited instances (e.g. dinoflagellates).

3.2.1 Diatoms (Bacillariophyceae) and Related

Phyla including the Phaeophyceae

The LHCs of diatoms, the fucoxanthin chlorophyll proteins (FCPs) form the most recalcitrant and least understood of all the LHCs. While in other algae it has been relatively easy to assign LHCs to PSI or PSII, this has not been so easy in diatoms. The LHCs of diatoms have been classifi ed into three major groups:

- Group 1, coded by Lhcr genes, is equated with a pure lightharvesting component of PSI.
- Group 2, coded by Lhcf, gives rise to the major fraction of light-harvesting proteins (FCPs).
- Group 3, coded by Lhcx, is similar to the LI818 proteins of other algae, which have a controlling role in nonphotochemical quenching.

The levels of subpopulations of Lhcf change with light conditions: recently two groups have proposed models to describe changes to the thylakoid membranes under low and high light. Nevertheless, it is broadly true that neither state transitions nor spillover of excitation energy from one photosystem to the other have been observed in diatoms. Diatoms have the ability to respond to rapid changes in light intensity as a result of nonphotochemical quenching (NPQ) mechanisms. The situation in diatoms is atypical because they have both the diadinoxanthin cycle (DdC) and the violaxanthin cycle. However, in the diatoms it has recently been shown that there are two NPQ mechanisms, one associated with antenna units attached to PSII and the other associated with antenna units that detach from PSII and remain isolated during high light conditions. None of these NPQ mechanisms would be expected to affect the maximum efficiency of photosynthesis in diatoms, which has been shown

to be high. Diatoms have a unique outer wall formed of silica and arranged in two abutting frustules. The function of the frustules is most probably protection, but LH has been proposed: the frustule has a unique architecture with sculpturing giving rise to small perforations which are the right distance apart to affect coherent laser light at wavelengths of >1000 nm; as a result frustules have become a favorite tool for laser studies; however, to the present time there is no evidence to suggest that there is any connection to photosynthesis and LH. All these unknowns make diatoms one of the most intriguing systems in the field of algal light harvesting today.

3.2.2 Related Phyla

In very general terms the following Phyla fi t into the diatom pattern, having, in addition to Chl *a* , Chl *c 1* and *c 2* , diatoxanthin and diadinoxanthin and Lhc light-harvesting proteins: Phaeophyta, Rhaphidophyta, Chrysophyta, Bolidophyta. The Eustigmatophyta also fall into this group but retain only Chl *a*.

3.2.3 Dinofl agellates

Dinoflagellates evolved within the chromalveolate group ofalgae and gave rise, via *Chromera* to their non-photosynthetic relatives, the apicomplexans, according to modern phylogenetic studies (Fig. 5). In the botanical system they form part of the Chromista (also known as Chromalveolates – see Glossary). In terms of LH, dinofl agellates have a fascinating array of pigments and pigment proteins arranged in a unique Thylakoid membrane and unique plastid. In addition, the molecular biology of the chloroplast is quite unique in dinofl agellates. There are two major types of LHPs in dinoflagellates, (i) a membrane bound protein

(Chl *a* , Chl *c 2* , peridinin protein complex, acpPC) and (ii) a water-soluble peridininchlorophyll protein (PCP) complex. Of these two proteins much more is known on the PCP complex, which has been resolved to a crystal structure of 1.63 ¦ and studied in considerable molecular detail. The gene sequence for PCP was established by Hofmann *et al.* (1996) and has no homology to any other known protein. The crystal structure indicates a protein with the monomer binding two Chl *a* molecules and two peridinin molecules (Fig. 2), essentially as predicted from CD spectra by Song *et al.* (1976). *Peridinin* has an in vivo absorption spectrum extending up to 540 nm and adds greatly to the light absorption capacity of dinoflagellates in the bluegreen region of the spectrum. Clearly PCP significantly augments the light-harvesting capacity of the dinoflagellate CAC proteins and does this at low nitrogen cost and can attain concentrations of up to 50 % of the total peridinin, i.e. 50 % of the concentration of acpPC. The functioning of PCP is still not well resolved. PCP lies in the thylakoid lumen space and is extracted as a soluble protein. However, if it is to function as an effi cient LH protein, it presumably interacts efficiently with adjacent PCPs. This suggests that there may be a semi-crystalline state in vivo, which aids resonance (or even coherent) energy transfer; otherwise energy transfer would be very slow. Recent work on PCP indicates that energy transfer is effi - cient and is directed efficiently into the thylakoid membrane at specific sites.

Earlier it was suggested that there were two types of PCP and that one of these high-salt PCPs might play a role in directing energy into the thylakoid membrane; however, now, the evidenceis less compelling. In hermatypic corals, where coral bleaching occurs, and is triggered via the photosynthetic machinery, there is a definite effect of coral bleaching temperatures (30–34 °C) on the interaction of PCP with the thylakoid membrane, as indicated by changes in the wavelength of fluorescence

emission. Kanazawa *et al.* (2014) have suggested that energy transfer into the membrane is increased by coral bleaching temperatures in Clade C *Symbiodinium* (the most heat sensitive clade of hermatypic corals).

3.2.4 Cryptomonads (Cryptophyceae)

Cryptomonads belong to a phylogenetic group of bifl agellate protists, only a part of which are photosynthetic algae and to which has recently been given the name “Macrobians” (see Keeling 2013) (Fig. 5). Previously this group would have been placed in the Chromista (Chromalveolates). In broad terms, cryptomonads have a secondary plastid that has engulfed an organism with a primary plastid allied to the red algae. However, this cannot be the complete story because the plastid bears an LHC with Chl *a* and Chl *c 2*. In addition the plastid bears phycobiliproteins (PBPs) which are unique and quite different, at least in the alpha chain, from red algal PBPs: these have a beta polypeptide with distinct affinities to red algal PBPs but an alpha chain with no affinity to any other protein; and some of the chromophores of the phycobiliproteins are quite unique. Thus there is much evolutionary history in the production of these PBPs and it is not at all clear that there is any direct, linear descent from red algae. As in dinoflagellates the water soluble PBPs are in the lumen of the thylakoids. Thus photons that are harvested must be passed onto the thylakoid membrane through interaction of these protein units. Recently there has been much excitement with the suggestion from several laboratories that coherent energy transfer may take place, i.e. that the migration of excitation energy is by exciton wave transfer. Exactly how this can take place is being taken up as a challenge by these laboratories.

4 The Need for Light Harvesting Antennae

Figure 1 shows the molecular layout of the reaction centres of photosystem I and photosystem II as currently confi gured from molecular X-ray diffraction studies. The two centres are built around a scaffold, which takes an absorbed photon of light and uses this to drive an electron across the reaction centre (inside to outside) forming a primary reductant and a primary oxidant molecule. From there the primary sites are stabilised by electron transfers to and from secondary sites. And from there an electron transport chain carries out the fixation of energy as ATP and NADPH (by which CO 2 is fixed into organic form). The reaction centres have few pigment molecules and a large number of cofactors and are expensive in terms of light capture and the amount of protein per absorbed photons. It is therefore a second imperative of all photosynthetic systems to build light-harvesting centres with a much greater concentration of pigments per protein molecule. These pigment protein complexes are arranged around the reaction centres and give a density of perhaps up to 1000 pigment molecules per RC. Under these conditions the absorption cross section of the RC increases by 1000 and for Chl molecules this results in a turnover of the RC of about once every 0.1 s under medium light intensities.

Clearly the absorption cross-section will be affected by the pigments that are linked into the light-harvesting pigment protein complexes and the quality of the incident light, e.g. whether this is in full sunlight, or the shade of a forest or the depths of the ocean. Under low light conditions the need for more light harvesting molecules and a greater absorption cross-section is high. Thus it is not surprising that algal and plant systems adjust to lowered light by incorporating a higher density of pigments and thereby increase the absorption cross-section.

Algae also have evolved the ability to place a large number of pigments in their LH antennae. However, surprisingly few have evolved pigments that can absorb in the green region: here phycobiliproteins are the example, *par excellence* ; there are also the oxygenated carotenoids, fucoxanthin and peridinin, which are exceptional in terms of carotenoids by the extension of their absorption spectra out to 550 nm.

5 Light-Harvesting Antennae in Cyanobacteria and Eukaryotic Algae

In Cyanobacteria, the phycobiliproteins (PBPs) are the lightharvesting antenna *par excellence* . However, it seems likely that these are a secondary, although very successful, evolutionary development. PBPs are also water soluble and extrinsic to the thylakoid membrane. It is more likely that they were preceded by membrane intrinsic light-harvesting antennae; of which there are still some in existence. The isiA antenna, containing only Chl *a* (Fig. 6), the pcb antenna, containing Chl *a* + *b* and similar antennae which bind Chls *d* and *f* (see Sect. 2.1) are examples of such antennae, although whether these preceded PBPs is not known. It seems clear too that these pigments if not their antennae were passed on to plastids, although the LHC (CAB/CAC) proteins seem to have evolved from high-light protection proteins (see below).

Fig. 6 Space-fi lling model of the structure of the isiA supercomplex of *Synechococcus* where 18 isiA units surround a trimeric Photosystem 1 reaction centre (See Zhang *et al.* 2010).

In all plastids, except the cyanelles of Glaucophyta, a related family of LHC membrane intrinsic proteins existseach with three membrane-spanning protein helices (Fig. 2). These bind eight Chl *a* and six Chl *b* or *c* and four xanthophyll molecules. In nearly all plastids the LHCs are divided into those attached to PSI (LHCI), and those attached to PSII (LHCII). However it has long been known that LHCII is mobile and can reattach to PSI under conditions where this evens up the activity of the PS. Much is known on the sub-distribution of LHCI and LHCII in different plastids, particularly land plants. In eukaryotic algae most is known for *Chlamydomonas reinhardtii*: 15 LHCII- and 6 LHCI-encoding genes have been identifi ed. Clearly, there is much latitude here for adaptive responses in light harvesting strategies. In eukaryotic algae, LHCII trimers are connected to RCII (a core dimer plus CP43 and CP47) through two monomeric LHCII proteins, CP26 and CP29. PSI complex is monomeric, in contrast to Cyanobacteria (Fig. 6), where it is trimeric; the large number of Chls bound to extra sub-units act as antennae. In addition there are up to six LHCI proteins, bound asymmetrically in a crescent-shaped arc, and, during state transitions (see below), an additional number of LHCIImolecules (see Fig. 8).

6 Control of Energy Supply to PSI and PSII: State Transitions, Absorption Cross-Section Changes and Spillover

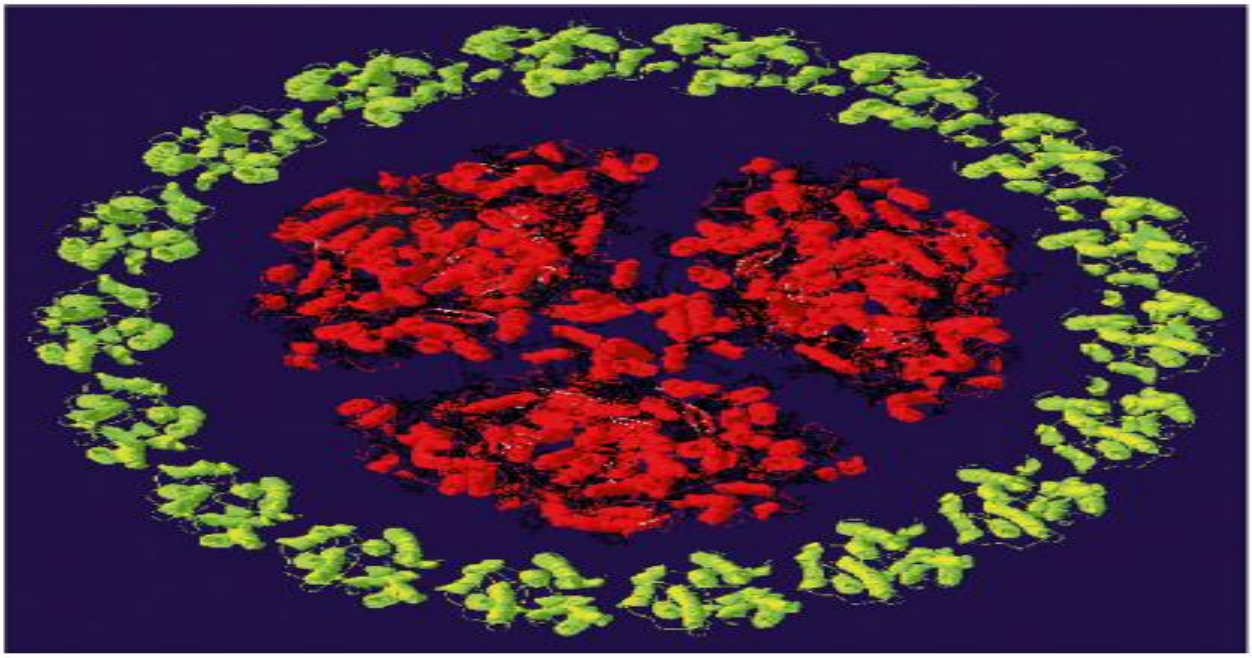
Photosynthetic organisms must often be able to adapt to light intensities that may range over a thousand fold intensity. Such environmental changes may be slow, such as weather– based or seasonal changes. Or they may be fast, ranging from moderately fast, as between dawn and noon time, to very fast

as between clouds and full sunlight, or light fl ecks that occur in forests and in the sea. To meet these circumstances photosynthetic organisms put in place two kinds of adaptive mechanisms: long-term changes brought about by protein

formation to adapt to new levels of pigments and short term changes, which reassign the concentration of pigments already entrained. Here I will concentrate more on the shortterm changes. Long–term changes, which are well known in Cyanobacteria, eukaryotic algae and land plants, have been

dealt with elsewhere (Larkum 2003 ; Falkowski and Raven 2007). The ultimate “aim” of all these mechanisms is to match the energy received by PSI and PSII so that the optimum levels of ATP and NADPH are produced. Note that where

cyclic electron transport (CET) is involved, as it often is, to balance the need of the Calvin-Benson Cycle for more ATPthan NADPH (Lucker and Kramer 2013), there is a need to have PSI operating faster overall than PSII.



6.1 State Transitions

State transitions were one of the fi rst light harvesting mechanisms to be documented and came from the work of Murata (1969 , 1970) who showed in spinach chloroplasts and in red algae that the amount of variable fluorescence (assigned to PSII) was affected by the previous conditions of illumination. Bonaventura and Myers (1989) first defined the phenomenon, albeit in a spillover model, which may be restated, as follows:

- **State I** in which there is an excess of light available to and absorbed by PSI (light I) and a decrease in the amount of excitation energy distributed from the light-harvesting pigments to PSI.
- **State II** in which there is an excess of light available to and absorbed by PSII (light II) and an increase in the amount of excitation energy distributed from the light-harvesting pigments and PSII to PSI. In darkness a State I condition is usually found.
- **State III** from evidence using the red alga *Pyropia* (*Porphyra) perforata*. Illumination of this alga either in state I or state II with light II produced state III in which light energy reaching PSII was decreased with no attendant increase in the energy supply to PSI.

Although there was no change in the distribution of energy between the two photosystems there was a decrease in the overall amount of excitation energy migrating to RCII from the light-harvesting pigments. This phenomenon is probably a photoinhibitory response whereby some phycobilisomes are decoupled under high light to protect the RCs from over-activity. The first physical model of the fluorescence changes in State Transitions came from Butler and coworkers (see Butler 1978) again in terms of a spillover model. It now seems more likely that the changes are effected in terms of re-association of light harvesting

complexes between the two PSs (rather than re-channelling of energy absorbed by one PS to the other, although there is still much debate over this). There is strong evidence that re-association occurs in higher plants and so one must expect a component of fluorescence change in situations which over-excite one photosystem against the other. However it is now known that downregulation of PSII occurs, which is brought about, both by the xanthophyll cycle and by delta pH quenching (see below). State Transitions have been observed in all oxygenic photosynthetic organisms. They are largest in Cyanobacteria and red algae and lowest in tracheophytes (land plants with tracheid vascular tissue). The mechanism of state transitions is likely to be similar to that for changes in optical cross- section of the PSs (see below). However, the two mechanisms may not be identical. State transitions have been a simple way to study short-term changes in energy distribution to PSI and PSII, and have been used extensively for this. Here, I concentrate on what is known from *Chlamydomonas reinhardtii*. In *Chlamydomonas reinhardtii* state transitions involve 80 % mobile LHCII as compared with 15–20 % in *Arabidopsis thaliana*. Work on mutants has revealed a mutant, Stt7, in which State 1 is blocked, is defi cient in LHCII phosphorylation in State 2 and lacks Stt7 protein kinase; this has an equivalent in *Arabidopsis*, STN7. There is also an additional mutant Stt1 (STN8 in *Arabidopsis*). It is likely that in Stt7/STN7 a protein kinase is deficient and cannot phosphorylate LHCII. Thus under State II conditions the mutant is blocked in transferring mobile LHCII from PSII to PSI, i.e. the State Transition is blocked. The arrangement of PSII with LHCII antennae in *C. reinhardtii* was studied by Drop *et al.* (2014a) (see Fig. 8) and in a another study of structural rearrangements during State Transitions by Drop *et al.* (2014b) it was shown that, under State 2 conditions, PSI is able to bind two LHCII trimers that contain all four LHCII types, and one

monomer, most likely CP29, in addition to its Lhca. This structure is the largest PSI complex ever observed, having an antenna size of 340 Chls/P700. Moreover, all PSI-bound Lhcs were efficient in transferring energy to PSI. Interestingly, only LHCII type I, II and IV were phosphorylated when associated with PSI, while LHCII type III and CP29 were not, but CP29 was phosphorylated when associated with PSII in state 2. That study underscores what has become clear from a number of recent studies, that the classical view of phosphorylation of LHCII under reduced PQH conditions is an over simplification. It should be pointed out that the suggested function of State Transitions in bringing about equal activity of the PSs was challenged long ago by Bulté *et al.* (1990), who suggested that the main function of State Transitions might be to balance the production of ATP and NADPH 2. They showed that inhibition of ATP production in intact cells in *Chlamydomonas reinhardtii* led to a transition to State II while an increase of ATP production caused a change to State I. This suggestion seems to have been upheld by later investigations. Recent work on *Chlamydomonas* has shown that CET is controlled by redox chemistry and not by State Transitions; for example State 2 was not required for inducing CET, since anoxic conditions enhanced CET, both in wild type and in an stt7 mutant blocked in State 1 and the PSI-Cytb 6 f supercomplex involved in CET was formed independently of State Transitions.

In the unicellular red alga *Rhodella violacea*, in contrast to *Chlamydomonas*, state transitions were not accompanied by phosphorylation of thylakoid proteins. Also they occurred under conditions where the activity of PSI does not change and it was suggested that ΔpH changes across the thylakoid membrane triggered “state II” quenching possibly through a downregulation process of RCII. State Transitions have been investigated in a number of other algae since the

early work, which was mainly directed to cyanobacteria, green algae and higher plants: the groups investigated include brown algae; *Chromera velia*; chlorophytes (*Dunaliella*); cryptophytes; chrysophytes; *Nannochloropsis* (Eustigmatophyceae); *Pleurochloris* (Xanthophyceae) .In *Pleurochloris* the state transitions were wavelengthindependent. In many cases the extent of State Transitions is much more pronounced in algae than in plastids of higher plants. Furthermore, apart from the streptophyte algae, it appears that there is little lateral heterogeneity in the thylakoids of algae.Thus there is the possibility of energy transfer between the PSs; and therefore further scrutiny of light energy distribution to the PSs in algal plastids is more than justified.