## 6.2 Absorption Cross-Section Changes

State Transitions is a term that has been applied to changes in light quality, which bring about a change in the activity of PSI and PSII. As shown above these can be understood in general terms as brought about by changes in the oxidationreduction potential of plastoquinone and the resultant phosphorylation/ dephosphorylation of LHCII, although many fine details remain to be worked out. Such changes are largely artificial in that they do not occur naturally, and have been used only to examine the mechanisms behind these changes. A much more natural effect is the short-term change from high light to low light and vice versa, an occurrence which is very normal for an alga undergoing changes in shading due to clouds, diurnal events, sun fl ecks and wind effects, etc. Such changes have become a focus of several recent studies, the most insightful of which have been carried out on *Chlamydomonas reinhardtii*. Several genes have beenidentifi ed which influence the placement of LHCII between PSI and PSII. Also, by analogy with *Arabidopsis*, it appears that the placement of LHCI and LHCII in the two PSs is regulated in different ways when the light intensity changes. The recent demonstration of large changes in the antenna size and distribution of LHCI and LHCII in *C. reinhardtii* indicate the potential of these techniques; the effects of light intensity changes will be awaited with interest. Of course, changes in absorption cross-section in response to light intensity changes is only one mechanism of dealing with increases in light intensity. Another important mechanism is to down-regulate light energy uptake by the process of non-photochemical quenching (NPQ) and this phenomenon is dealt with next. When the flux of photons to PSI and PSII is not equal, one way of effecting equal activity of PSI and PSII, is to reduce the excitation energy of one PS and to increase it to the other.

Initially it was supposed that there was a mechanism (“ **spillover**”), which simply diverted energy from one photosystem to the other – predominantly from PSII to PSI. However, the mechanism now generally proposed is in terms of mobile light-harvesting units, which change the optical cross-section, and also maybe change the spectralproperties, of one or both photosystems. A general mechanism for changes in absorption crosssection in higher plants has been available for over 30 years. The mechanism is thought, in higher plants, to be as follows. Preferential illumination of PSII (**Light 2**) leads to reduction of the plastoquinone (PQ) pool, between the two PSs (**State 2**). Under these conditions, and through the mediation of the Q o site of the cyt b 6 f complex, at least one, and possibly more than one, membrane-bound protein kinase becomes activated leading to the phosphorylation of mobile LHCII and other polypeptides. Phosphorylated LHCII then moves away from the appressed thylakoid regions, to unappressed thylakoids on the outside of grana or in the stroma, where it associates with PSI (Fig. 7). The membrane bound kinase is deactivated in the dark or in **Light 1**(light which preferentially activates PSI and when PQ is oxidised - **State1**) and a latent phosphatase continually reverses the action of the kinase. Evidence for a similar mechanism in the green alga *Dunaliella* has been presented and there is some evidence that it may also exist in dinofl agellates. However, the greatest advance in the area of algae has come over the last two decades from studies of *Chlamydomonas reinhardtii*, which can be transformed and has become the alga of choice, similarly to *Arabidopsis thaliana* in higher plants. This work is largely dealt with in the section on State Transitions (above), but it is obviously connected with changes in absorption cross-section brought about by reassignment of LHCs as a result of short-term rearrangements and long–term production (see Fig. 7). Particularly important here is the realization

that changes are brought about in going from low light to high light (and vice-versa) and that much can be learnt from such an approach. One recent example is for *C. reinhardtii* where changes in the LHCs have been physically mapped onto PSI and PSII; this is possible because through electron microscopy it is possible to carry out electron density scans of light-harvesting particles obtained on developed sucrose density gradients (Fig. 7). What these investigations show is that the standard model of state transitions needs changing. Phosphorylation does not necessarily induce a State II to State I transition. While the general principles of this proposed mechanism have been supported, in the interval there has been much progress in many other areas – both in higher plant and in algal studies. In higher plants it has been shown that there is a specifi c LHCI, which acts to harvest light specifi cally for PSI. Thus changes in crosssectional area of PSI and PSII due to re-association of mobile LHCII can only contribute a small fraction of change in cross-sectional areas (usually <20 %). In higher plants too it has been shown that the PSI subunit H polypeptide is essential for docking of phosphorylated LHCII and in mutants lacking the H subunit phosphorylated LHCII stays associated with PSII – with no changes in cross-sectional area. Furthermore it has been shown that phosphorylation of LHCII is not linearly dependent on the reduction of PQ: the degree of phosphorylation reaches a peak at rather low light intensities (of light which preferentially activates PSII). In algae (other than streptophyte green algae) the distribution of PSI and PSII appears to be much more homogeneous. Here there is less evidence for phosphorylation of LHCs driven by Light 1 and Light 2 (and the involvement of a PQ-driven mechanism). Gibbs and Biggins (1991) argued against such a phosphorylation mechanism in the Chl *c* -containing chrysophyte alga, *Ochromonas*. Allen (1992) gives a review of this early work on

eukaryotic algae. In Cyanobacteria and red algal plastids, the main lightharvesting system is the phycobilisome (PBS) and there has been much debate over whether the attachment of PBS to PSII or PSI is driven by the redox state of PQ involving phosphorylated proteins (for a detailed review see Allen 1992). Certainly there is good evidence that the absorption crosssections of PSI and PSII change in response to Light 1 and Light 2. Many proteins are also phosphorylated in the light. Recent evidence seems to suggest that the PBS physically move between PSII and PSI sites. However, there is certainly evidence for a protective mechanism in red algae whereby energy is directed from one PS to the other in what has been called **spillover** (see below). Almost certainly there are distinct differences in the mechanisms by which short-term accommodation of Light 1 and Light 2 effects changes in the cross-sectional areas of PSI and PSII in cyanobacteria, algae and land plants.

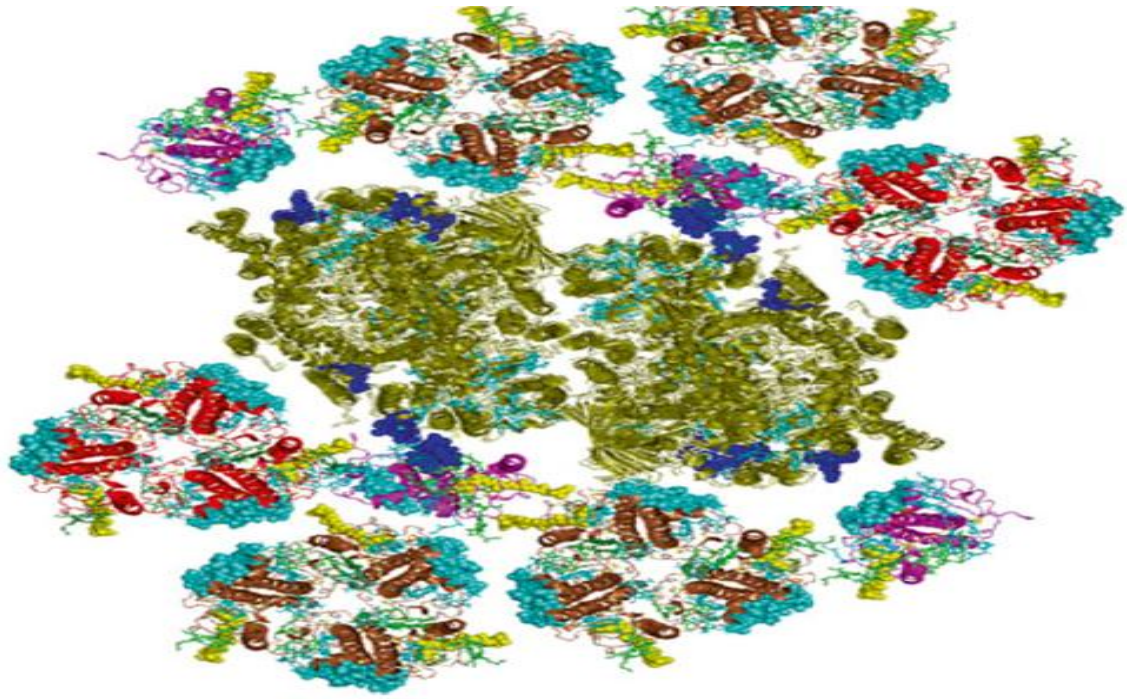

**Fig. 7** The model has been assembled using the crystal structures of the cyanobacterial PSII core, LHCII trimer and CP29. For CP26, the structure of a monomeric LHCII has been used. Proteins of the PSII core (*lime green*), LHCII-S and -M (*brown*), novel LHCII-N (*red*), CP29 and CP26 (*magenta*), Chls *a* (*cyan*), Chls *b* (*green*), neoxanthin (*yellow spheres*), lutein L1 (*orange*), lutein L2 (*dark-yellow sticks*) (Taken from Drop et al. 2014a)

Pursiheimo *et al.* (1998) proposed three categories:

- **Group 1**, Cyanobacteria and red algae, which did not show phosphorylation of any of the photosystem II (PSII) proteins.
- **Group 2,** consisting of some of the remaining eukaryotic algae,mosses, liverworts and ferns, which phosphorylated both the light-harvesting chlorophyll a/b proteins (LHCII) and the PSII core proteins D2 and CP43, but not the D1 protein.
- **Group 3,** where reversible phosphorylation of the D1 protein of PSII was found only in seed plants and was seen as the most recent evolutionary event in the series. In terms of phosphorylation of LHCII they found that Groups 2 and 3 were similar with maximal phosphorylation of LHCII at low light and nearly complete de-phosphorylation at high light. Clearly this survey did not include any algae dependent on CAB light-harvesting systems. However, the large number of studies of *Chlamydomonas reinhardtii* over the last two decades has provided a solid base for understanding this system, with some recent revaluation of the finer details of the mechanisms.

### 6.3 Non-photochemical Quenching – Sensu Lato

Non-photochemical quenching is a set of processes, whereby some light energy is deactivated as heat before the rest is channeled to the reaction centres; it is a protective mechanism that is activated under high light, protecting the reaction centres from damaging levels of excitation, which can give rise to photoinhibition, through the degradation of key peptides and the activities of Reactive Oxygen Species (ROS). The oldest known process is the Xanthophyll Cycle, which is activated by key carotenoids and triggered though the polypeptide PSBS, in land plants, or

LHCSR (LHCX) in most algae (see Fig. 9). There is another process, which is simply triggered by low pH and involves a number of xanthophylls. Additionally, in some Cyanobacteria there is a specifi c mechanism brought about by the Orange Carotenoid Protein (OCP), which acts similarly to deactivate excitation energy as heat.

### 6.4 Spillover

Spillover is a term first used by Butler in the 1950s to denote a migration of excitation from one photosystem to the other (see above). In its earliest formulations it can probably be seen as a process that was confused with changes in absorption cross-section, brought about by reassignment of antenna unit or subunits. Nevertheless, a valid use of the term seems to have been that of Ley and Butler (1980) who studied the fluorescence changes in the unicellular red alga, *Porphyridium cruentum* . As recently reported by Kowalczyk et al. (2013) for an investigation of a similar situation in the high-light stressed intertidal red alga, *Chondrus crispus*, a real migration of energy from PSII to PSI can be detected, rather than a deactivation through an NPQ pathway or a redistribution of light harvesting antennae. Another situation, where spillover has been shown is in the lichen *Parmelia sulcata* , which harbors a unicellular Trebouxian green alga. It should be pointed out that few eukaryotic algae have thylakoids with grana and true appression, i.e. where PSII is physically segregated from PSI. It is therefore possible in algae, and in Cyanobacteria, for PSI and PSII to be physically close to one another, and therefore, theoretically, for energy to migrate from one photosystem to the other. How widespread this phenomenon is has yet to be shown.

# 7 Non-photochemical Quenching

## 7.1 The Xanthophyll Cycle

The Xanthophyll Cycle in its generally recognised formoccurs in most eukaryotic algae and in higher plants. The xanthophylls involved are violaxanthin, antheraxanthin and zeaxanthin or diatoxanthin and diadinoxanthin (Fig.8). While much work has been carried out on higher plants and green algae much less work has been carried out on algae, other than some green algae such as *Chlamydomonas*.

However, there is good evidence to believe that a similar cycle exists in many eukaryotic algae. In the Xanthophyll Cycle, in the light, violaxanthin or diatoxanthin are converted by de-epoxidation to zeaxanthin or diadinoxanthin (Fig. 8). The details of this de-epoxidation and subsequent epoxidation in the light have been well documented, and Demmig-Adams and Adams (1993). Thus one molecule of oxygen is liberated (de-epoxidated) or takenup (epoxidated) for a complete transition. In algae these changes were worked out in detail by Stransky and Hager (1970) and their general conclusions, shown in Fig. 8 were as follows:

- **Group 1** (Glaucophyta, Rhodophyceae, Cryptophyceae and Cyanobacteria), no epoxide cycle takes place althoughchanges in levels of zeaxanthin occur.
- **Group 2** (Bacillariophyceae, Chrysophyceae, Xanthophyceae, Chloromonadophyceae, Dinophyceae and Euglenophyceae), diadinoxanthin is the oxygenated carotenoid and diatoxanthin is the de-epoxidated carotenoid.
- **Group 3** (Phaeophyceae and Chlorophyceae and odd species of some other Classes), the Xanthophyll Cycle is present.

In micromonad algae only a part of the conventional Xanthophyll Cycle is present – that converting violaxanthin to antheraxanthin; furthermore, Lohr and Wilhelm (1999) have shown that some algae displaying the diadinoxanthin type of a Xanthophyll Cycle also display features of the violaxanthin-based cycle. In general, as light levels increase, so the level of violaxanthin/ diadinoxanthin decreases, reaching a steady level, and conversely, the level of zeaxanthin/diatoxanthin increases to an asymptote. The Xanthophyll Cycle involves, generally, three factors in the stimulation of a non-photochemical quenching of energy in the PSII/LHCII assemblage:

(i) an increased concentration of zeaxanthin (ordiatoxanthin),

(ii) the presence of PSBS, in land plants, or LHCSR ineukaryotic algae (and to a lesser extent in liverworts,mosses and other non-vascular plants, and, streptophyte green algae (see Fig. 9 ),

(iii) a ΔpH across the thylakoid membrane. With these three factors in operation, and in the presence of LHCII, excitation energy is transferred to the carotenoid and excitation energy is transduced to heat. There is still much to be learnt in eukaryotic algae, however. In Rhodophyta and Glaucophyta, which rely on a phycobilisome system the operation of LHCSR is not well established, nor has it been found in dinofl agellates (see Fig. 9). In a number of algae from these groups it is not clear whether a delta pH is necessary. A number of workers had earlier implicated LHCII in this process. However, more recently a specifi c role for the non-chlorophyll- binding, 22 kDa 4-α-helix membrane- spanning protein, PSBS, has been shown. This protein may lie in an intermediate position between LHCII and the inner antennae of RCII. The evidence suggests that energy- dependent quenching, Qe (which is defined as that component of the total non-photochemical quenching, qN, directly

attributable to the energisation of the thylakoid membrane, and therefore the rapid.

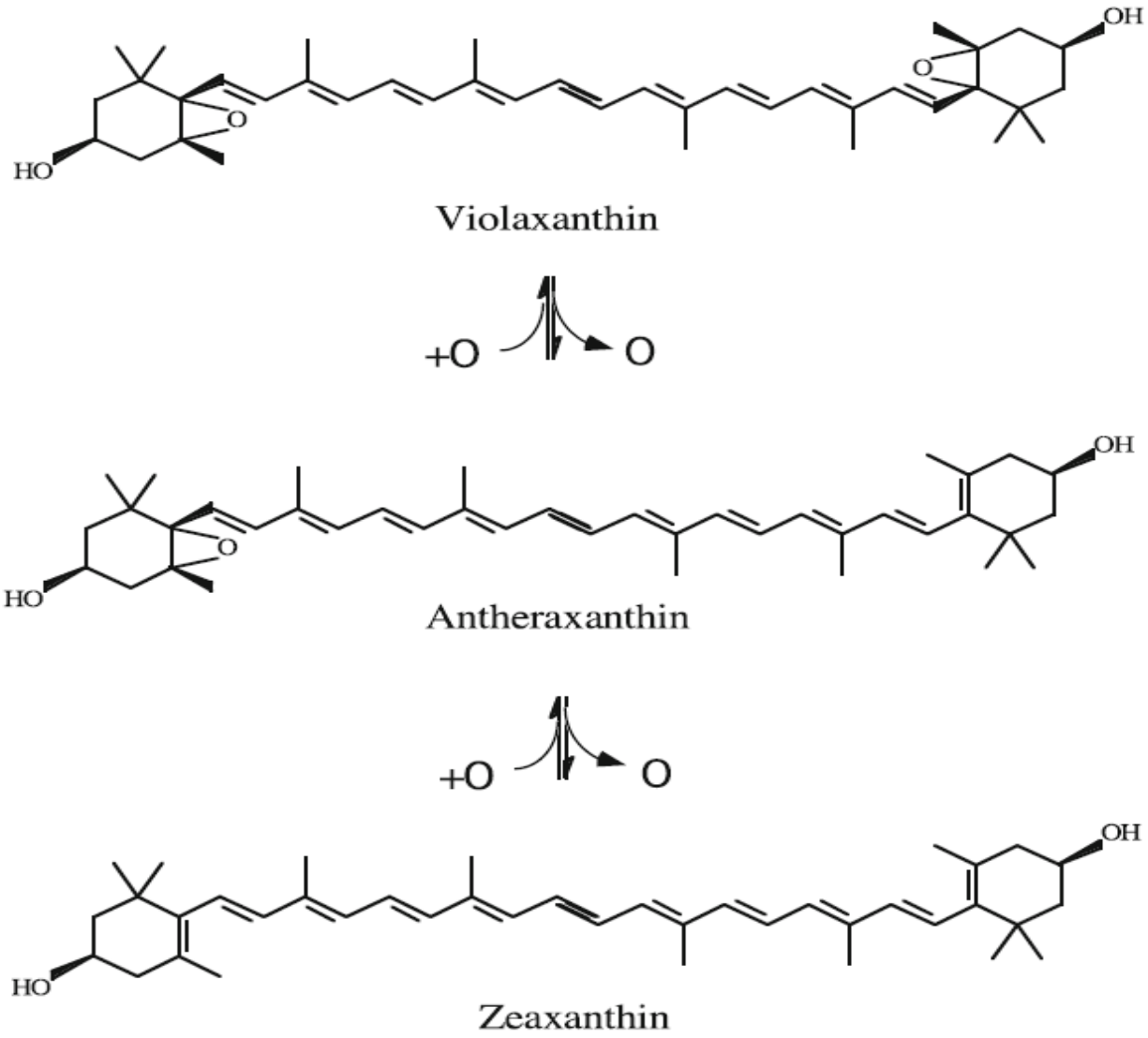


**Fig. 8** The xanthophyll cycle: the interconversion of violaxanthin and zeaxanthin, with light triggering the conversion of violaxanthin to zeaxanthin (and diadinothanxin to diatoxanthin in those algae which possess these xanthophylls). entrained component of qN) is directly dependent on PSBS.A number of specifi c details are known concerning thereactions involved in quenching by zeaxanthin (and diatoxanthin). For instance, dibucaine stimulates the quenching and antimycin A, dithiothreitol (DTT) and the protein carboxyl- modifying agent

dicyclohexylcarbodiimide (DCCD) inhibit the quenching. Horton *et al.* (1996) suggested that there is a pocket extending from the intrathylakoid lumen into the membrane by which low pH in the thylakoid lumen can infl uence a critical site in the thylakoid membrane. Since PSBS is essential for qE to occur it may be the protein, which senses the low pH and the binds zeaxanthin or it may play a crucial structural role in energy transfer/dissipation. The mechanistic details of energy quenching have yet to be fully worked out. Clearly if the mechanism is to work zeaxanthin has able to change its molecular excitation states, which would then allow it to dissipate excitation energy as heat when triggered by low pH. Recent work of the group of Frank suggest that the S1 state of carotenoids is important for this kind of down-regulation.

The situation in algae is more complex than in higher plants. Firstly chlororespiration may make up a larger component of electron fl ow than in higher plant plastids and chlororespiration may induce a pH gradient even in the dark (e.g. in *Euglena*). Secondly, qE seems to be independent of the xanthophyll cycle in *Euglena*. Thirdly, Cyanobacteria, admittedly oxygenic photosynthetic bacteria and not algae, but nevertheless with similar photosynthetic mechanisms, and sharing a common origin, do not have the conventional xanthophyll cycle yet carry out down- regulation of photosynthesis, both by conventional carotenoids and by the orange carotenoid protein.

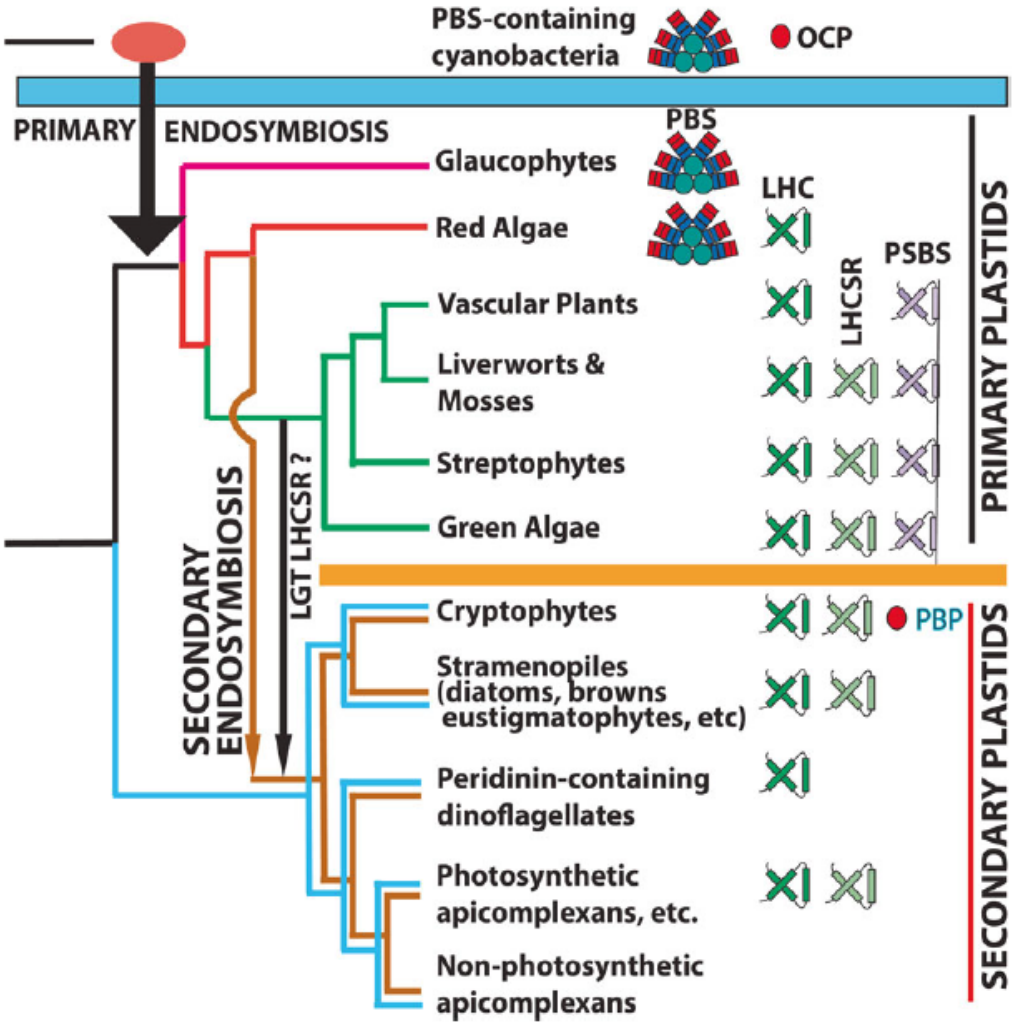


**Fig. 9** Scheme showing the likely evolutionary events in the formation of photosynthetic proteins involved in light-harvesting and photoprotection (Copyright: AWD Larkum: based on the scheme of Niyogi and Truong 2013)(LGT = lateral gene transfer)

## 7.2 pH Quenching

Many workers in the field have suggested a second mechanism by which eukaryotic algae and land plants can quench excitation energy. This is through the action of low pH on light-harvesting proteins in the vicinity of the reaction centres. However, much more work needs to be done before this is generally accepted.

## 7.3 Orange Carotenoid Protein

The orange carotenoid protein was discovered relatively recently. It is, as its name suggests, a caroteno-protein, which is produced under high light in cyanobacteria; initially it was thought that it occurred in only certain cyanobacteria but it has been found to exist quite widely in cyanobacteria. The OCP is located on the stalk of the phycobilisome and under high light channels excitation energy into the carotenoid where the energy is released as heat. However, the OCP has a second role in quenching singlet oxygen. When the light intensity is reduced to non-stressful levels another protein FRP decouples the OCP and excitation energy is once again passed on to the local reaction centre.