

## Mini Review

# Plants in light

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**Abbreviations:** PFD, photon flux density; PS, photosystem; LHCII, light harvesting II complex; ATP, adenosine triphosphate; DCCD, dicyclohexylcarbodiimide; NADPH, nicotinamide adenine dinucleotide phosphate; NPQ, nonphotochemical chlorophyll fluorescence quenching

**Key words:** photosynthesis, light adaptations, acclimation, photoinhibition, photosystem, light harvesting complex

In nature, plants have to face frequent fluctuations of intensity and spectral quality of their primary source of life—light, whose energy is needed to drive the processes of photosynthesis. A multilevel network of adaptations exists to help the plant to track and cope with fluctuations in the light environment. At the molecular level, the light harvesting antenna complex of photosystem II (LHCII), which collects the most significant part of the light energy, was found to play a central regulatory role by finely controlling the amount of energy delivered to the reaction centers. This is achieved by several mechanisms, which are summarized in this review. The fundamental features of the design of the photosynthetic antenna make photosynthetic light harvesting efficient, physiologically competent and flexible at the same time, ensuring high levels of plant survival and productivity within a wide range of light environments on our planet.

## Introduction

The success of oxygenic photosynthesis in our biosphere is a great biological phenomenon that relies first of all upon the efficient design and adaptability to the changing environmental conditions of the molecular machinery of the photosynthetic apparatus. The intensity and spectral quality of light exhibits large temporal and spatial variation. For example, the daily photon flux available for plants grown in the deep shade of the tropical forest is more than 100 times less than that available for plants exposed to direct sunlight. The spectral quality of light, available for plants in the shade environment, is often different from that of the full sun light due to filtering by the canopy.<sup>1</sup> Plants are also frequently exposed to rapid and irregular changes in light intensity, which could be caused by clouds and sudden shading by other plants, occurring in natural biocenoses. And finally, diurnal changes in light quality and quantity inevitably modulate photosynthetic processes in plants on a day-to-day basis.

The dependency of the photosynthetic efficiency upon the light environment originates from the existence of a number of limiting factors, restricting the rate of light energy consumption. As a result of evolution under frequent exposure to low light fluxes, the photosynthetic machinery has evolved in such a way that only a small fraction of chlorophyll molecules are actually photosynthetically competent. They are the reaction center chlorophylls. The rest of the chlorophyll has a purely light-collecting function and is called antenna chlorophyll. This chlorophyll is specifically assembled along with carotenoids and lipids on proteins to form various light-harvesting antenna complexes, LHC's. The latter deliver excitation energy to the reaction center.

Under *low light intensities* (light starvation) the photosynthetic yield will depend upon the efficiency of light energy capture by antenna pigments and its delivery to the reaction centers. It also relies on the captured light energy redistribution between photosystem I and II, which operate in series to form the electron transfer chain from water to NADP. The major fundamental limitation under *elevated light intensities* (light saturation) arises from differences in the rates of energy absorption and transfer to the reaction centers of photosystems and subsequent electron transport. Being much slower than energy transfer, electron transport rates fulfill the fundamental thermodynamic requirement—to minimize the uphill reactions and therefore stabilize energy, which is to be used in the chain of electron/proton transfer reactions leading to NADPH and ATP synthesis. With an increase in light intensity, the reaction centers will be progressively saturated with energy (closed) leading to the reduction of the fraction of energy utilized in photosynthesis and therefore to the build up of the “unused” potentially harmful excitation energy in the photosynthetic membrane.

## The Multilevel Strategies of Plant Adaptation to the Light Environment

During the evolution plants have developed an entire network of adaptation mechanisms to cope with fluctuations in the light environment. These can be divided into two major groups: A—adaptations to control light absorption capacity and B—adaptations to deal with the light energy, which has been captured.

To optimize light absorption, plants respond on different levels of organization, i.e., systemically. On the level of the whole organism the adaptation involves adjustment of the leaf orientation.<sup>2</sup> This

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adaptation helps many land plants to cope with excess irradiation particularly during the midday. Leaf movements can be of developmental (rather slow and irreversible), passive (drought related) and active nature (reversible). The latter employs a blue-light absorbing pigment system of unknown nature as a sensor and the pulvinar motor tissue to drive leaf orientation movements.<sup>3</sup> This adaptive system is very effective in some shade plants with very low photosynthetic capacity, which are occasionally exposed to light bursts. Some desert plants have also developed a number of adaptations to increase leaf reflectance and therefore reduce the amount of absorbed light. The adaptations may include building up inorganic deposits on the leaf surface (for example, salt crystals) or developing air-filled hairs. As a rule, the efficiency of these protective methods is good but as with the developmental leaf movements, these adaptations occur on rather slow time scale.

On the cellular level, light absorption can be regulated by chloroplast movements.<sup>4</sup> These are relatively fast adaptations, occurring within minutes, but are only able to reduce light absorption by 10–20% in the environment of excess light.<sup>5</sup> The similarity of the action spectra for chloroplast and leaf movements suggests that they possess common photoreceptors.<sup>5</sup> Factors limiting chloroplast movements could be the presence of obstacles such as large vacuoles and other cellular organelles. Generally, adaptations on the cell level or at the level of the whole plant have limited capacity. In the natural environment light tends to be scattered in all directions by clouds, fog or simply high air humidity. External scattering of the incident light may substantially reduce the various leaf orientation adaptations. The absorbed light energy is also often highly scattered within the leaf by starch and various leaf tissue structures making chloroplast movements even less effective.

A most profound type of plant adaptation to light occurs at the molecular level. The type A adaptations, the regulation of light absorption, occur by long-term control of chlorophyll content in leaves. Other responses involve short-term adaptations of the photosynthetic membrane consisting of dynamic changes in light harvesting antenna size and efficiency.

### Long-Term Light Adaptations: Acclimation

Acclimation is predominantly of developmental nature, and often is a result of regulation of the complex light-dependent gene expression, occurring on transcriptional, translational and post-translational levels.<sup>6</sup> This process takes days and weeks and on the level of the thylakoid membrane involves significant compositional and structural alterations. One of these is modulation of PSI and PSII antenna size by light intensity. This is a well documented and highly conserved phenomenon.<sup>7</sup> In plants, grown under high light intensity the antenna is always smaller than in those grown in shade. It was found that only the outer-LHCII complex (peripheral part of light harvesting antenna complex of photosystem II) is involved in acclimative modulation of the PSII antenna size. It was discovered that the reduction in the amount of outer-LHCII is caused by the proteolysis of its apoprotein.<sup>8</sup> The protease is suggested to be of serine and/or cysteine type of ATP-dependent enzyme, located extrinsically on the stroma side of the thylakoid membrane. It takes up to two days for the enzyme expression/post-translational activation after exposing plants to the high light environment. Once activated, it takes less than a day to complete proteolysis and reduce the outer-LHCII by a half, which is approximately

30% of the total LHCII content. “Wasting” this amount of LHCII will release about 160 kDa of the protein mass and 80 chlorophyll molecules per PSII unit—a significant metabolic event.

The other long-term acclimative responses of the thylakoid membrane to the change in the light environment (quantity and quality) include an alteration of the ratio between a number of photosystem I and II units and inactivation of a subpopulation of PSII reaction centers.<sup>9,10</sup> For example, shade plants grown under light filtered on the forest canopy (more far-red light) will possess a higher PSII/PSI ratio to compensate for the reduction in the amount of red light, which is required to excite PSII.<sup>1</sup> Various aspects of the signal reception pathway, *factor—receptor—transducer—gene* are still not well understood. Redox state of the plastoquinone pool is suggested to play an important role in the governing multiple stress signaling and transducing mechanisms.<sup>7</sup> It has been proposed that protein phosphorylation (see below), including LHCII, plays a key part in the signal transduction path in the light acclimation processes.<sup>8</sup>

### Major Determinants of the Efficiency of the Photosynthetic Light Harvesting

Before introducing the short-term molecular light adaptation strategies it is important to define what determines the efficiency of the light energy utilization in the photosynthetic membrane. Two fundamental properties of the photosynthetic light harvesting antenna determine its functioning: (1) *absorption cross-section*—the size and spectral breadth and (2) *excitation energy lifetime*—the time of energy storage in the antenna before it reaches reaction centers. Cross-section depends on the type and amount of pigments, spectral redistribution of extinction coefficients and the oscillator strength. The first two factors are adjustable and determine the difference between PSI and PSII antennae. Usually PSII antenna has more pigments than that of PSI. Spectrally, PSI and PSII are also distinct. The reason for this is different absorption of their reaction center chlorophyll. Whilst the PSI reaction center absorbs at 700 nm (called P700), the PSII reaction center absorbs at 680 nm (called P680). Therefore, to match the energy of reaction center, PSI antenna absorbs longer wavelength light than PSII. This is due to enrichment in chlorophyll *b* in PSII, on one hand, and the presence of the long-wavelength chlorophyll *a* in PSI on the other. Spectral differences between PSI and PSII make the linear electron transfer efficiency potentially vulnerable to changes in the spectral quality of light and require the existence of suitable compensatory mechanisms.

The time during which antenna remains excited is the other important feature. It is generally proportional to the probability of energy delivery to the reaction center. The excitation lifetime largely depends on the chlorophyll environment. The latter could influence chlorophyll conformation, directly interfere with the optical  $\pi$ -electron configuration or remove excitation via energy transfer. Carotenoids, amino acids and lipids are potential modulators of the antenna chlorophyll lifetime. The excitation lifetime can be monitored by the fluorescence lifetime or yield measurements, which reveal their remarkable dynamics, reflecting the existence of control over the light harvesting process in plants.

### Short-Term Molecular Adaptations to Low Light

Normally, during the short-term adaptations no changes in gene expression are involved. They occur within seconds and minutes and

serve to counteract faster changes in the light environment, such as diurnal fluctuations in the light quantity and quality, sun flecks, light filtering by the canopy.<sup>1</sup> The most documented adaptation to the light quality resulting in the imbalance of the energy absorbed by photosystems is known as the State Transitions.<sup>11</sup> This process occurs within minutes and is effective at low/moderate light intensity, for example when light intensity is not limiting photosynthesis.<sup>12</sup>

The molecular mechanism involved is designed to restore this energy imbalance, utilizing the redox state of the plastoquinone pool<sup>13,14</sup> or/and cytochrome *b*<sup>15</sup> as a sensor/transducer, which are involved in activation of a protein kinase.<sup>16,17</sup> When the energy imbalance is in favor of PSII, this kinase phosphorylates some polypeptides of LHCII complex, targeting *Thr* or *Ser* residues of the stroma-exposed N-terminus.<sup>18</sup> A so-called “mobile” LHCII (originating exclusively from the *outer*-LHCII pool) becomes dissociated from the PSII under the influence of the negative charge, introduced by phosphate.<sup>19</sup> As a consequence the PSII antenna becomes reduced. Phosphorylation leads to a partial unstacking of the grana and the detached *phospho*-LHCII can migrate into those regions of the thylakoid membrane, which are enriched in PSI (grana margins and stroma lamellae), and interact with the PSI complex. According to the low temperature fluorescence experiments *phospho*-LHCII brings to PSI 20–35% of additional excitation energy.<sup>20</sup> Assuming approximately 30% of PSII antenna decrease and about the same increase in PSI antenna, the total energy balance change between photosystems will reach 85%. This is large enough to be considered as significant for the adaptation to the changes in the light environment, particularly in shade growing plants, which have a larger pool of the peripheral LHCII.

### Photosynthesis under High Light: Photoinhibition

In high light the build-up of the excess excitation energy in antenna systems will inevitably lead to photoinhibition, a sustained decline in the photosynthetic efficiency and productivity, associated with the damage of photosynthetic reaction centers.<sup>21</sup> The reaction center of the PSII is more susceptible to the damage, because of a very strong oxidation potential of the P680 ( $-1,17$  V) needed to oxidize water (see above). Under some conditions, when electron donation to P680 is less efficient than oxidation, an increase in the P680<sup>+</sup> lifetime will occur. The powerful oxidant P680<sup>+</sup> will inevitably oxidize the nearest pigments and amino acids, causing their degradation and the subsequent D1 degradation will follow.<sup>22</sup> In other circumstances, when the acceptor side is less efficient, a radical pair is formed. The recombination of this pair will lead to the P680 triplet formation. In this state P680 can interact with the atmospheric triplet oxygen, causing formation of singlet oxygen, which in turn will bleach P680.<sup>23</sup> Therefore, the number of active PSII units will be decreased and because of the slow D1 repair<sup>24</sup> the decline in electron transfer will remain even when excess light is no longer present.

### Regulation of the Photosystem II Efficiency

A great deal of evidence exists that PSII efficiency can be regulated.<sup>25,26</sup> It was shown that it depends reversibly upon the light intensity in a clearly non-linear manner. This contradicted the view that this dependency was associated entirely with the proportion of open reaction centers, which assumes a linear relationship between their number and the PSII quantum yield. The most feasible explanation of the observed non-linearity is opening of excess energy

dissipation channel in PSII antenna. The latter became possible to detect by the chlorophyll fluorescence measurements in so-called quenching analysis using pulse-amplitude modulation technique.<sup>27</sup> One of the parameters of this analysis is called non-photochemical fluorescence quenching (NPQ). It measures the extent of the excess energy dissipation in PSII, when reaction centers are closed, i.e., not receiving excitation energy from antenna. Quantum efficiency of PSII was found to be in reciprocal linear relationship with NPQ.<sup>26</sup> This gave reasons to believe that PSII efficiency is under control of nonradiative energy dissipation, an NPQ-underlying process, which is triggered by the establishment of the proton gradient across the thylakoid membrane ( $\Delta$ pH).<sup>28</sup>

### The Site of NPQ

For a number of years several lines of evidence suggesting that the site of NPQ is localized in LHCII antenna and not in the PSII reaction center have been acquired.<sup>29</sup> Quenching is associated with a significant decrease in the antenna fluorescence when all reaction centers are open (Fo).<sup>30</sup> The process persists if samples are frozen to 77 K and is associated with quenched fluorescence bands originating from LHCII.<sup>31</sup> Time resolved fluorescence data recorded for leaves are consistent with quenching in the antenna.<sup>32</sup> Direct measurement of heat emission in NPQ state shows it to occur within 1.4  $\mu$ sec, much faster than estimates for rates of the recombination reactions in PSII.<sup>33</sup> Cross-linkers block NPQ and also transition of isolated LHCII complexes into a dissipative state.<sup>34</sup> NPQ and LHCII respond in the same way to a number of factors: antimycin A, tertiary amines and magnesium.<sup>35,36</sup> NPQ is almost entirely dependent upon the presence of exclusively LHCII-bound xanthophylls, lutein and zeaxanthin.<sup>36,37</sup>

### The Mechanism of Photoprotective Energy Dissipation

In order to understand the molecular mechanism leading to NPQ it is necessary to view it as a sequence of following events: *trigger—site—change—quencher*. The identities of the trigger,  $\Delta$ pH, and the site, LHCII, have been introduced above. It is important to establish where exactly protons bind to the antenna and what kind of *change* occurs as a result of the protonation. Finally, it is necessary to identify a molecule, which act as an excitation energy trap or *quencher*.

Reagent DCCD, which reacts with aspartate or glutamate residues of some proteins, was found to bind to LHCII proteins and inhibit NPQ.<sup>38</sup> Two DCCD-binding amino acids have been identified on one of the LHCII complexes.<sup>39</sup> It is tempting to conclude that the proton binding directly results in the induction of quenching. DCCD binding can either inhibit the *change* leading to NPQ, or block the proton pathway leading to the site of quenching. Thus, the LHCII proteins have a function not just in light harvesting but also in proton translocation, and the latter function is involved in some way in NPQ.

The knowledge of the event triggering energy dissipation has been enriched by the discovery of an involvement of the protein PsbS in NPQ.<sup>40</sup> Initially thought to be a carrier of the quencher,<sup>40</sup> it is believed now to be not a pigment-binding protein.<sup>41</sup> Two lumen-exposed glutamates of PsbS have been found to bind protons.<sup>42</sup> It was also shown that  $\Delta$ pH induces monomerization of the dimeric PsbS and its lateral migration out of PSII domains of the thylakoid membrane.<sup>43,44</sup> Plants lacking this protein lost the ability to fast-track light intensity changes<sup>45</sup> but only marginally lost the

photoprotection capacity under constant high light.<sup>46</sup> The new evidence is emerging that this protein plays a role of LHCII mobility or dynamics enhancer (Goral and Mullineaux, personal communications). Indeed being a highly hydrophobic protein PsbS could affect the fluidity of the crowded photosynthetic membrane<sup>47</sup> and therefore influence the *change* LHCII undergoes in order to switch into NPQ state.

The *change* in antenna underlying NPQ is currently in the focus of intense studies. It was proposed that protonation causes aggregation of LHCII in the membrane.<sup>48</sup> Isolated aggregated LHCII was found to be in highly dissipative state; therefore the “aggregation model” for NPQ has become common for explaining the *change*. According to the model, the extent of aggregation and therefore NPQ can be controlled by peripherally-bound xanthophylls, violaxanthin and zeaxanthin.<sup>29</sup> Violaxanthin, a highly polar xanthophyll, was suggested to inhibit LHCII aggregation, whereas hydrophobic zeaxanthin—to promote the process. Elevated light intensities cause conversion of violaxanthin into zeaxanthin by the enzyme de-epoxidase. In the dark another enzyme, epoxidase, converts zeaxanthin back into violaxanthin by attaching back two epoxy oxygens to the molecule. The interconversion process of these xanthophylls was called xanthophyll cycle.<sup>49</sup> De-epoxidase is activated by  $\Delta\text{pH}$ , whilst epoxidase is active when the gradient is absent. It has been proposed that the action of the xanthophylls cycle carotenoids is to allosterically control NPQ by modulating the transition of LHCII antenna in to a dissipative photoprotective state.<sup>50</sup> LHCII aggregation was a “first approximation” of this conformational change and further investigations have revealed that the dissipative state in LHCII is not a direct result of protein-protein interactions during the aggregation process but rather a consequence of intrinsic conformational change within the monomeric unit of the complex induced by protonation and/or environmental changes, such as protein aggregation.<sup>34</sup>

The nature of the NPQ *quencher* has been addressed in several studies. The formation of chlorophyll-chlorophyll associates possessing properties of energy traps has been suggested.<sup>51</sup> However, calculations and ultrafast absorption techniques have revealed that at least the configuration of steady-state chlorophyll dimers is unlikely to be of a quenched nature<sup>52</sup> and instead, intrinsically-bound xanthophyll, lutein, has been proposed to play a role of *quencher*.<sup>53</sup> Indeed, carotenoids are efficient energy sinks, provided they interact very closely and specifically with chlorophyll. The atomic structure of LHCII shows that lutein molecules are indeed in a very close contact with some chlorophylls, having high dipole-dipole interaction strength.<sup>54</sup> The latter is important for the energy transfer from chlorophyll to carotenoid. In other studies, it has been found that xanthophylls cycle carotenoid, zeaxanthin can form a radical pair with chlorophyll of some minor LHCII complexes.<sup>55,56</sup> This radical state was suggested to play a role in the formation of another NPQ *quencher*. The debates on the relative impact of lutein and zeaxanthin on NPQ are under way.<sup>56,57</sup> One point is clear, though, since the mutants lacking both of these xanthophylls show no NPQ, that both of them are important pigments for the photoprotective energy dissipation in LHCII antenna.<sup>37,57</sup>

## Concluding Remarks

Plants adapt to light in a number of different ways and on different levels of organization: whole plant, cellular and molecular

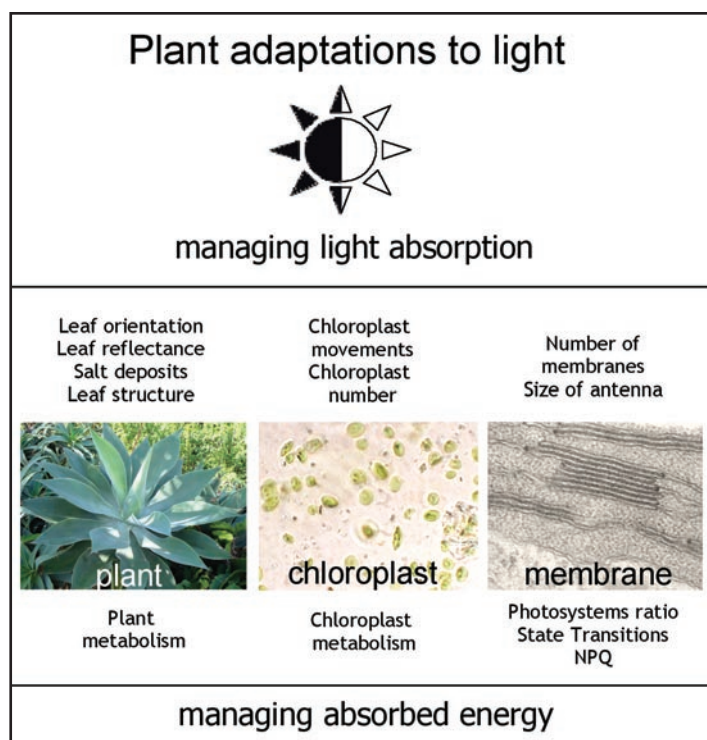


Figure 1. Multilevel strategies of plant adaptations to the light environment.

(Fig. 1). The systemic character of adaptations simply broadens the adaptability range. Whilst the low light molecular adaptations balance the energy capture rate and distribution into two photosystems, the high light adaptations act to avoid absorption and to safely deal with the captured energy. The long-term adaptations modulate the short-term strategies. For example, there is coherence between the long-term light adaptation and the short-term adaptation to the light quality, since under limiting light the highly abundant mobile part of LHCII antenna will ensure maximum alteration in the cross-section of photosystem I and II. High light growth environment frequently leads to saturation of photosynthesis, which makes light-quality adaptation obsolete. At this point the excess light dissipation becomes a dominant adaptation. The maximum extent of NPQ at light saturation is also dependent on the plant growth conditions, mainly the type of light and temperature environment and the type of plant species. It is clear that the published values for NPQ in crop plants are relatively low.<sup>58</sup> Conversely, in plants adapted for growth in adverse environments, the extent of NPQ is three-fold higher than in the plants that have been studied most extensively in the laboratory.<sup>59</sup> Therefore it is likely that there is genetic variability in the capacity for NPQ in a manner that is related to the natural habitat of the plant. This idea has been confirmed by the screening a large number of ecologically contrasting species.<sup>58</sup>

The biochemical basis of NPQ variability is either related to the composition of LHCII, including PsbS, xanthophyll cycle and  $\Delta\text{pH}$ . It is known that, when plants are grown in high light, the amount of LHCII decreases, whilst PsbS increases. However, the situation is not as simple as this—mutants deficient in LHCII show reduced levels of quenching,<sup>60</sup> suggesting that it is not the total content of LHCII that is important rather the actual composition of LHCII proteins,

both, trimeric and minor, which in turn shape the macrostructure of antenna system.

The knowledge, obtained in recent studies of plant responses to light is crucial for understanding the possible consequences of global warming and the associated effects on the world plant communities. The results and methodologies developing in these studies can be applied to the investigation of problems associated with biodiversity as a function of energy input into the ecosystem. Space exploration research utilizing plants on space stations and to investigate plant invasion strategies with the perspective of inhabiting other planets, should benefit unequivocally from using the knowledge of the photosynthetic mechanisms of plant adaptation to light regimes. This work is also valuable for the crop production, as it offers some important clues where and how genetic engineering could enhance plant resistance and productivity. It also raises the question of tighter links between fundamental research in plant physiology and genetic manipulation. Finally, the results of a fundamental nature and dynamics of the properties of photosynthetic antenna membrane proteins and their co-factors, chlorophylls and carotenoids are likely to be useful for the developing of technologies utilizing the energy of light.

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