Optimization of light harvesting and photoprotection: molecular mechanisms and physiological consequences

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The distinctive lateral organization of the protein complexes in the thylakoid membrane investigated by Jan Anderson and co-workers is dependent on the balance of various attractive and repulsive forces. Modulation of these forces allows critical physiological regulation of photosynthesis that provides efficient light-harvesting in limiting light but dissipation of excess potentially damaging radiation in saturating light. The light-harvesting complexes (LHCII) are central to this regulation, which is achieved by phosphorylation of stromal residues, protonation on the lumen surface and de-epoxidation of bound violaxanthin. The functional flexibility of LHCII derives from a remarkable pigment composition and configuration that not only allow efficient absorption of light and efficient energy transfer either to photosystem II or photosystem I core complexes, but through subtle configurational changes can also exhibit highly efficient dissipative reactions involving chlorophyll–xanthophyll and/or chlorophyll–chlorophyll interactions. These changes in function are determined at a macroscopic level by alterations in protein–protein interactions in the thylakoid membrane. The capacity and dynamics of this regulation are tuned to different physiological scenarios by the exact protein and pigment content of the light-harvesting system. Here, the molecular mechanisms involved will be reviewed, and the optimization of the light-harvesting system in different environmental conditions described.

Keywords: light-harvesting complex; non-photochemical quenching; thylakoid; grana; photoprotection

1. INTRODUCTION

The collection of sunlight to provide energy for cellular processes by photosynthesis is one of the pinnacles of evolution, being also a driving force for profound changes to our planet and the life upon it. One of the most important constraints on photosynthesis is balancing the efficient absorption of sunlight to capture as much of the energy as possible against the risk of it causing damage [1]. The problem of balancing efficiency and risk is exacerbated by the intrinsic nature of photosynthesis and the properties of the environment. In plants, the two photosystems are served by light-harvesting complexes (LHCs), which bind numerous chlorophyll molecules to increase the absorption cross section, providing an antenna for the reaction centres, so that enough light is harvested to drive photosynthesis at a worthwhile rate. This poses two problems. Firstly, because the redox potentials of the donor/acceptor pairs of photosystem II (PSII) and photosystem I (PSI) are different, the absorption spectra of the antennas are correspondingly different, making the task of balancing electron flux through each system problematic. Secondly, the rate at which the LHCs of PSII and PSI deliver excitation energy to the reaction centres is proportional to the incident light intensity. Because the reaction centres interface with electron transfer complexes and enzyme-catalysed reactions that have finite maximum velocity, at some point, the intensity of sunlight must be saturating; then, the amount of light absorbed is in excess of that which can be used in photosynthesis. It is in this case of excess light that the risk of damage is highest, because a proportion of the absorbed energy can no longer be used and the probability increases that an absorbed photon will result in a deleterious reaction—photo-oxidation and generation of reactive oxygen species—that damages photosynthetic components. Achieving the dual goals of high efficiency and low risk is made more difficult by the continuous change in the environment: long-term seasonal and diurnal changes, accompanied by continuous fluctuations in cloud cover and wind-induced plant movement, cause massive shifts in the intensity and spectral quality of incident sunlight. In addition, alterations in temperature and the availability of CO₂, water and nutrients affect the capacity of photosynthesis, whereas internal metabolic and developmental features determine the demand for ATP and NADPH and the carbohydrate products of carbon fixation. Thus, excess light does not only arise when the intensity of sunlight is high.

While variation in composition of the chloroplast in response to the environmental conditions during...
Figure 1. Regulatory interactions between light harvesting, electron transport and carbon assimilation. Dotted lines describe the identified feedback and feed forward mechanisms that regulate photosynthetic energy flow, balancing light input and metabolic output in order to increase the efficiency of photosynthesis and reduce the production of reactive oxygen species (ROS). 1. NPQ; 2. pH control of electron transport; 3. state transition; 4. redox control over PSI cyclic electron transport; 5. redox control of ATP synthase; 6. redox control of Calvin cycle enzymes; 7. ΔpH control of Calvin cycle enzymes; 8. ATP control of Calvin cycle enzymes; 9. Pi control of ATP synthesis (modified and updated from Horton [3]).

development and to a lesser extent in mature plants (photosynthetic acclimation) works to balance the capacities of light harvesting and light utilisation [2], this is not enough. In contrast to the first descriptions of photosynthesis, in which the production and consumption of ATP and NADPH were seen as only passively connected, it became clear that active regulatory mechanisms controlled both the activity of the thylakoid processes producing ATP and NADPH and thestromal reactions of carbon assimilation that consume them [3] (figure 1). These feedback and feed forward mechanisms have the potential to balance the sub-processes of photosynthesis, enhancing efficiency and reducing risk in the face of short-term changes in the external and internal environment of plants. Given a fixed chloroplast composition and a fluctuating environment, regulatory mechanisms extend the range of conditions over which photosynthesis can remain in balance—they provide homeostasis of excitation energy level, redox state and ΔpH in the thylakoids, and ATP, NADPH and metabolite levels in the stroma.

Understanding how, in molecular terms, plants have a high efficiency of photosynthesis when light is limiting but the ability to reduce the risk of damage when it is in excess has been one of the major challenges in photosynthesis research. Equally, how the inevitable trade-offs between these two conflicting demands are ‘decided’ in different species in different environmental scenarios raises fundamental questions about the physiology and ecology of plants, the answers to which may have agricultural implication. In this review, it will be shown how the unique properties of the LHCs and their organization in the grana membranes of the chloroplast allow plants to achieve this remarkable balancing act.

2. THE LIGHT HARVESTING COMPLEXES OF PSII

The light-harvesting antenna of PSII consists of several proteins, which together bind around 200–300 chlorophyll molecules depending on growth conditions. Associated tightly with the D1/D2 reaction centre are the antenna complexes CP47 and CP43, forming the dimeric core (C2). The remainder of the antenna consists of the Lhcb proteins (Lhcb1–6). These bind chlorophyll a, chlorophyll b and xanthophylls to form several different complexes—LHCII (Lhcb1–3), CP29 (Lhcb4), CP26 (Lhcb5) and CP24 (Lhcb6). LHCII is the main complex and contains about 40 per cent of the PSII chlorophyll. Lhcb1, Lhcb2 and Lhcb3 associate in different combinations to form a population of heterotrimeric LHCII, termed LHCII-S, LHCII-M and LHCII-L, which show different strengths of binding to the PSII core complex. The resultant C2S2 and C2S2M2 LHCII–PSII supercomplexes also contain two monomers of CP26 and CP29, or of CP24, CP26 and CP29, respectively [4]. A single Lhcb monomeric unit is a relatively small protein (approx. 25 kDa), containing three transmembrane α-helical structures and binding up to 14 molecules of chlorophyll (up to eight chlorophyll a and six chlorophyll b) and up to four xanthophylls [5]. In the grana membrane, the supercomplexes form a network or macrodomain of connected antennas [6] and are sometimes seen as ordered semicrystalline arrays [4].

The high efficiency of light harvesting in PSII is determined by the number of LHCII subunits, the pigmentation order within them, the interaction between subunits and their closeness to the reaction centre complex. The high density of chlorophyll in LHCII is very significant. Not only does it contribute to the high concentration of chlorophyll in a leaf, but the high chlorophyll/protein ratio ensures efficient use of leaf N. The estimated concentration of 0.6 M led some to wonder how photosynthesis works at all, because in a solution of chlorophyll, at this concentration, nearly all absorbed light is rapidly dissipated as heat, ‘concentration quenching’ [7]. Obviously, the binding of chlorophyll to the protein scaffold prevents the interactions that would give such quenching. It is also noteworthy that peripheral location of some pigments in the symmetrical LHCII trimer allows energy transfer between complexes [8]. These attributes of LHCII provide the molecular basis for regulation of light harvesting and photoprotection. Two distinct strategies optimize light-harvesting function [1]. The first balances the input of light energy to PSI and PSII reaction centres in order to optimize electron transfer rate—this is called the state transition. The second regulates the amount of light energy dissipated in LHCII—this is called non-photochemical quenching (NPQ). Both state transitions and NPQ rely on the dynamic behaviour
of the LHCII system—its ability to sense the ‘state’ of the light–energy balance and to respond by structural change.

(a) LHCII phosphorylation
The Lhcbl and Lhcbl2 polypeptides of LHCII are reversibly phosphorylated by a redox-regulated thylakoid–associated protein kinase, STN7 [9]. Phosphorylations at threonine residues near the stromal facing N terminus of LHCII weaken the association between PSII and LHCII, allowing phospho-LHCII to associate with PSI. Thus, LHCII has the capacity to transfer energy efficiently to the cores of both PSII and PSI. Certain specific LHCII trimers seem to be preferentially dissociated from the PSII complex [10], and the kinetics of the state transition are correspondingly influenced by the exact composition of the Lhcb proteins, e.g. plants deficient in Lhcb3, not a phosphorylation substrate, show more rapid state transition kinetics than wild-type plants. LHCII phosphorylation is mediated by the thylakoid–associated protein kinase, STN7 [9]. Phosphorylations at threonine residues near the stromal facing N terminus of LHCII weaken the association between PSII and LHCII, allowing phospho-LHCII to associate with PSI. Thus, LHCII has the capacity to transfer energy efficiently to the cores of both PSII and PSI. Certain specific LHCII trimers seem to be preferentially dissociated from the PSII complex [10], and the kinetics of the state transition are correspondingly influenced by the exact composition of the Lhcb proteins, e.g. plants deficient in Lhcb3, not a phosphorylation substrate, show more rapid state transition kinetics than wild-type plants. LHCII phosphorylation is mediated by the thylakoid–associated protein kinase, STN7 [9]. Phosphorylations at threonine residues near the stromal facing N terminus of LHCII weaken the association between PSII and LHCII, allowing phospho-LHCII to associate with PSI. Thus, LHCII has the capacity to transfer energy efficiently to the cores of both PSII and PSI. Certain specific LHCII trimers seem to be preferentially dissociated from the PSII complex [10], and the kinetics of the state transition are correspondingly influenced by the exact composition of the Lhcb proteins, e.g. plants deficient in Lhcb3, not a phosphorylation substrate, show more rapid state transition kinetics than wild-type plants. LHCII phosphorylation is mediated by the thylakoid–associated protein kinase, STN7 [9].

(b) Energy dissipation in LHCII
Purified LHCII is readily transformed into a quenched state: lowering the detergent concentration, addition of Mg ions or decreasing the pH all induce a reduction in fluorescence yield. These conditions are associated with oligomerization and/or aggregation. A number of observations suggest that the quenching observed is the same as that causing photoprotective energy dissipation in NPQ [1], particularly the presence of similar or identical spectroscopic signatures in both cases and the common effects of reagents that stimulate or inhibit quenching. It seems that quenching arises because of changes in pigment configuration within the LHCII molecule and that aggregation per se is not required for quenching [14]. Rather, it may be proposed that the quenched state is protonated and then has a tendency to aggregate. Insights into this have come from single-molecule experiments: here, spontaneous switching of LHCII molecules between unquenched and quenched states is observed. Most significantly, conditions that promote LHCII aggregation (and NPQ) increase the dwell time in the quenched state [15]. Thus, it may be postulated that LHCII has an inherent disorder that allows it to exist in different energetic and spectral states. This property is exploited to regulate NPQ: in excess light, conditions arise which select and stabilize particular quenched states of LHCII (figure 2).

The physical process that gives rise to quenching is still not proved. Evidence for quenching by energy transfer from chlorophyll to the S1 state of lutein 1 has been obtained [17]. Equally, evidence consistent with a charge transfer quenching involving a carotenoid radical cation and a chlorophyll radical anion was found [18], probably involving zeaxanthin (see below). Finally, some data support the idea that quenching may arise from a chlorophyll–chlorophyll charge transfer [19]. These may not be mutually exclusive: there may be more than one quenching mechanism occurring in LHCII; or complexes may display different quenching reactions, because quenching is a feature also of the minor antenna complexes CP26 and CP29, which have different pigment compositions; or the dominant mechanism may vary according to particular conditions, for example, depending on the presence of zeaxanthin or other neighbouring proteins. However, in all cases, the high density of pigments in these complexes, an adaption for efficient light harvesting, also provides many opportunities for efficient energy dissipation by means of only very subtle configuration shifts [20].

In vivo, NPQ appears not to be a single process [21]. A rapidly relaxing NPQ dependent on the thylakoid ΔpH, called qE, is the major component under most conditions. A more sustained NPQ, broadly termed qI, but which is itself heterogeneous, becomes dominant under more extreme conditions, and is usually regarded as a form of photoinhibition (sustained decline in quantum efficiency of PSII) despite the fact that it is photoprotective (dissipation of excess energy) [22]. Because qI can include quenching arising from damaged reaction centres, the term qZ (see below) has been introduced to describe the photoprotective form of sustained NPQ [23]. Although not strictly a quenching process, the state transition also contributes to measurements of NPQ and has been referred to as qT. Its contribution in plants is small, except in low light. However, the idea that different kinetics defines a different molecular process is problematic: the rate of dark relaxation of qE is highly variable and gets slower as the illumination time is prolonged. Thus, although it appears as if qE is being replaced by something new [23], it is better to think that qE transforms into a more stable state, implying that the molecular mechanisms of the two may well be the same [24]. Similar transitions in qI occur, which with time is dominated by more slowly relaxing components. As discussed below, it is suggested that all of the heterogeneity of NPQ derives from different states of association between LHCII and its associated proteins, which merely control the pK of the protonation, an event that is obligatory for all quenching [16,24] (figure 2).

(c) The xanthophyll cycle
LHCII binds the carotenoids of the xanthophyll cycle. Studies that localized these carotenoids in LHCII-enriched fractions following solubilization of membranes [25] were confirmed by the identification of violaxanthin in the crystal structure of LHCII [5]. Its peripheral location suggested how it could easily be de-epoxidized to zeaxanthin by the violaxanthin de-epoxidase (VDE). The discovery that the accumulation of zeaxanthin
correlated with the extent of NPQ in vivo was a research landmark [26], and suggested a relatively simple mechanism for qE: VDE is activated by the low pH in the lumen occurring in excess light, and the zeaxanthin formed is the quencher of chlorophyll-excited states. However, not all NPQ can be explained in this way. The observation of qE in the absence of zeaxanthin and the different pH-dependencies of qE and VDE disproved such simple mechanisms. Instead, data suggest that the formation of zeaxanthin has an indirect effect, activating a quenching process that was intrinsic to LHCII and that was triggered by \( D_{pH} \). Direct support for this proposal is that the \( D_{pH} \) dependency of qE is controlled by the de-epoxidation state (DES) [27]: in the absence of zeaxanthin, the pK is around 4.5, shifting to 5.5 after light-induced de-epoxidation (typically a DES of around 0.6). In the complete absence of violaxanthin (DES 1.0) in Arabidopsis npq2 mutants, the pK is over 6 [28]. Therefore, qE is an allosteric process, under the control of the interacting effects of protonation and zeaxanthin binding, similar to many regulated enzymes [29]. This model for qE is consistent with the in vivo properties of qE: because the \( D_{pH} \) in vivo does not fall below 5.5, qE will depend almost completely on de-epoxidation of violaxanthin; and the kinetics of qE formation in which an initial rapid phase of quenching, reflecting the initial response to \( D_{pH} \), is followed by a slow phase as violaxanthin is de-epoxidized and qE activated [16].

Evidence for the indirect effect of the xanthophyll cycle on qE also comes from studies on isolated LHCII. Violaxanthin slows down, and zeaxanthin speeds up the rate with which LHCII is quenched following reduction in detergent concentration and/or lowering the pH [1]. The fluorescence yield of the quenched state is unaffected. This experimental protocol allowed the structure/function relationships of the effect of xanthophylls to be determined. Orientation of the xanthophyll head group is the determining feature [30], but how this affects LHCII remains to be proved. Other studies have investigated the effects of substituting the xanthophylls bound to the internal L2 site [31]. Here, exchange of violaxanthin bound to the L2 site in CP26 or CP29 with zeaxanthin increases the quenching in aggregated samples [32].

The involvement of zeaxanthin as a quencher in the more sustained forms of NPQ appears much more likely. Here, the correlations between qI (qZ) and DES are very strong (hence the term qZ) [33]. Nevertheless, the spectroscopic similarity between the more extreme qI (qZ) states and highly aggregated LHCII is clear [34], and it may be proposed again that the effect of zeaxanthin is an indirect one. Indeed, the intricate relationship between qE and qZ (activation of qE is associated with formation of qZ; the time-dependent increase in qZ is associated with diminution of qE) suggests they are acting by the same mechanism. Of course, it cannot be excluded that zeaxanthin is both

Figure 2. Multiple factors regulate the functional states of LHCII. The content of PSII and its organization, the amount and composition of Lhcb proteins, the appearance of stress-related proteins such as ELIPS, the content of specific lipids, the levels of epoxidized or de-epoxidized xanthophylls and the extent of grana stacking all influence the distribution between different functional states of the LHCII antenna. It is postulated that all of these factors affect the pK for NPQ. With length of time of exposure to factors promoting aggregation, it becomes deeper, more stable and less pH-dependent (adapted and updated from Horton et al. [16]).
a stimulator of LHCII aggregation and simultaneously also a quencher [35].

Studies of the role of the xanthophyll cycle are additionally complicated by the fact that zeaxanthin exhibits an anti-oxidant function in the thylakoid [36]. A putative role in preventing lipid peroxidation under stress was confirmed by the effects of increased xanthophyll cycle pool size in transgenic Arabidopsis [37]. Much of the extra xanthophyll in these plants is bound to sites on LHCII, which are not saturated in wild-type plants [38].

(d) Multiple states of LHCII

Lhcb-containing complexes can be reversibly phosphorylated, protonated and de-epoxidized, allowing them to fulfil their multiple functions [39]. Variable expression of these modifications in different complexes and variation in their relative concentrations gives scope for fine-tuning of light-harvesting regulation [24]. Multiple states of LHCII trimers can be defined: the light-harvesting state in which it strongly interacts with PSII cores, through gradually increasing LHCII—LHCII interaction in oligomers and aggregates (figure 2). This dynamic equilibrium is influenced by factors external to LHCII, and enables different physiological states of NPQ to be created—different amplitudes, kinetics of formation and kinetics of relaxation. Thus, the LHCII molecule has an intrinsic level of quenching determined by its structural dynamics [15], which are modulated by external factors to give rise to physiological regulatory mechanisms of appropriate amplitude and dynamic range. These factors are dependent on the organization of LHCII in the thylakoid membrane [16] and give rise to the changes to the pK observed in the ΔpH titration of qE [28].

3. THE THYLAKOID MEMBRANE

The thylakoid is a remarkable membrane. It is densely packed with the protein complexes that carry out photosynthetic electron transport and ATP synthesis. These are segregated laterally, the main effect of which is to separate domains rich in PSII from those containing PSI [40]. This lateral segregation arises from the interaction between the stromal side of the stroma and the differential surface charge of the protein complexes [41]. A consequence of this lateral segregation is the formation of the grana, whereby attraction between the stromal facing surfaces of the PSI domains causes membrane appression. The thylakoid membrane encloses an aqueous space, the lumen, the acidification of which as a result of light-driven proton release not only drives ATP synthesis but also results in protonation of surface residues, including those on LHCII and related complexes, and movements of Mg and Cl ions across the membrane. Accompanying the ΔpH, therefore, are changes in lumen volume and membrane structure [42]. These two attributes of the thylakoid membrane, the lateral organization controlled by stromal surface charge and the response to lumen pH change, provide the basis of the regulatory mechanisms that control light harvesting and photoprotection. In essence, they modulate protein–protein interactions in the grana membranes, and determine, for example, the ΔpH-dependency of NPQ.

As in state transitions, the regulation of NPQ depends on changes in the macro-organization of the LHCII—PSII supercomplexes [16]. There appears to be sufficient mobility of complexes in the plane of the membrane to allow a dynamic equilibrium between different associations of complexes. The rearrangements in the membrane accompanying qE create clusters of LHCII (and CP24) [43], resembling aggregates [44]—these are likely to contain the quenching sites (figure 3). The quenched LHCII aggregate is thought to be (functionally) detached from the reaction centre cores and the tightly bound LHCII, CP26 and CP29 complexes, where additional quenching centres appear to reside [23]. However, it cannot be excluded that the quenched aggregates, by remaining attached, also quench excitation energy from the core. Studies of plants missing one or more of these complexes all give rise to the same conclusion [47–50]: that NPQ is not a property of any one complex, rather all the Lhcb-containing complexes give rise to the formation of an efficient quenching domain [16,24]. Recently, the macro-organization of thylakoid membranes in these plants was determined [46], highlighting the role of CP24. Confirming previous results [49], in the absence of this complex, there is an increase in both uncoupled LHCII-M trimers and associations of C2S2 supercomplexes. Remarkably, large areas of semi-crystalline organization of the latter were observed in these membranes. As discussed further below, it seems that such membrane domains may be inactive in NPQ. This explains the low NPQ phenotype of these plants [49] and also the restoration of near wild type NPQ in plants which, in addition to lacking CP24, are deficient in other Lhcb proteins, affecting supercomplex stability [50]. Thus, in the grana membranes, two forces are seemingly in opposition—that driving LHCII aggregation (as depicted in figure 2) and that driving association of PSII supercomplexes into ordered semi-crystalline arrays (figure 3). The balance between these two tendencies determines physiological function by setting the pK of the vital protonation-dependent quenching.

(a) The role of PsbS

Rapidly relaxing NPQ is completely inhibited in the absence of PsbS [51], and is replaced by a 10 times slower qL-type of NPQ of similar magnitude [52]. In fact, in the presence of a large enough ΔpH, rapidly relaxing qE is completely restored [53]. These observations strongly suggest that PsbS, rather than being a site of quenching as first proposed [51], is a catalyst controlling the kinetics and pH-dependency of NPQ [54]. Three observations support such a view: PsbS controls the Mg dependency for the restoration of high fluorescence upon thylakoid restacking [55]; the amount of membrane in crystalline domains increases when PsbS is absent and decreases when PsbS is elevated [45]; and the lateral mobility of LHCII decreases when PsbS is absent [46]. PsbS appears to be an example of a protein that controls the interactions and associations between membrane protein complexes [16,45,54]. As pointed out recently [46], PsbS could be viewed as a lubricant that enables the rapid dynamic regulatory behaviour of the thylakoid to
work despite its very high protein density. Indeed, across a range of mutants, there is a correlation between NPQ and the mobility of complexes in the membrane [46]. Hence, the role of PsbS in qE is explained by it allowing the reorganization of LHCII/PSII to consolidate the quenching domain, a visible effect of which is the dissociation of the crystalline arrays [45](figure 3). Such arrays should then be viewed as areas closed to dynamic behaviour, and when forces promoting their formation are dominant the capacity and rapid kinetics of NPQ are held down, principally because the pK for protonation is much less than the lumen pH. It is interesting that in low-light-grown plants, there is an increase in the amount of LHCII/PSII in these domains [56], and such plants have reduced PsbS and lower NPQ. This illustrates the balance that has to be struck in the structure of the thylakoid—it seems that an increase in thylakoid protein density, such as occurs upon acclimation to low light, increases the likelihood of uncontrolled quenching by LHCII aggregation [57], and such plants have reduced PsbS and lower NPQ. This illustrates the balance that has to be struck in the structure of the thylakoid—it seems that an increase in thylakoid protein density, such as occurs upon acclimation to low light, increases the likelihood of uncontrolled quenching by LHCII aggregation [57], and such plants have reduced PsbS and lower NPQ.

4. PHYSIOLOGICAL REGULATION OF NPQ

The modulation of the kinetics (formation and relaxation), capacity and ∆pH-sensitivity of NPQ by the pigment, protein and lipid composition of the grana membranes gives the theoretical capability to fine tune the character of NPQ to the different kinds of environmental challenges presented in nature [24,40]. Is there any evidence to support this proposition?

(a) NPQ in high-intensity sunlight

In high light, the requirements are for a high electron transport rate, high rate of ATP synthesis and high levels of dissipation of excess light energy. Frequently, all three will simultaneously be at maximum rate or capacity. The allosteric model for qE [1,29,54] explains how the chloroplast could have a ∆pH high enough in limiting light to allow ATP synthesis without qE, yet in saturating light to have maximum electron transport rates and high qE simultaneously. Without such modulation by the xanthophyll cycle, either the
photosynthetic quantum yield would be compromised in limiting light because of the presence of qE or electron transport would be inhibited in high light because of inhibition of the activity cytochrome b6f complex by an excessive ΔpH. In *in vivo*, ΔpH is confined to a narrow range (lumen pH 5.8 and 6.5) [59] so therefore its function as a regulatory sensor in NPQ depends upon amplification via the xanthophyll cycle.

Prolonged illumination with high light sees important changes in the kinetics of NPQ [33]. NPQ becomes larger but more stable, it is quicker to form and slower to relax. With time, qE gradually transforms into a sustained qZ/qI type of NPQ. Here, it is suggested that LHCII becomes more and more aggregated when the DES is at a maximum and membrane reorganization is relatively slow (figure 2). Deep and stable quenching has adaptive value under prolonged continuous illumination.

It is interesting to consider the opposite situation, plants living in deep shade when risk of exposure to high light is very low, and therefore where a stable light harvesting mode could be most adaptive. Here, the adaption would be to a stable light-harvesting mode. Could these plants have predominantly semi-crystalline LHCII/PSII in their large grana stacks, as found in low-light-grown spinach [56], and be relatively inactive in NPQ? (figure 3).

(b) Low temperature

Relatively high light and low temperature are a frequent combination in nature, well documented, for example, in early morning in spring or in some mid-winter environments [33]. Here, the scenario is different—high energy dissipation has to be induced with only low or even zero electron (and proton) transport activity. In these conditions, very high DES is observed that makes the ΔpH requirement for NPQ very low. In addition, other proteins such as ELIPS as well as PsbS accumulate, which may stabilize LHCII aggregation (figure 2). Epoxidation is inhibited and high DES and even ΔpH appear to be maintained during night time. Phosphorylation of LHCII and PSII proteins also appears to persist. All these features promote formation of highly aggregated quenched LHCII [16] (figure 2), with increasingly little or no dependence on ΔpH, essentially ‘locking in’ [33] the dissipative photoprotected state. In more prolonged exposures, PSII reaction centres disappear, also further promoting LHCII aggregation (as implied by figure 3). Such quenching states are very deep, stable and require minimum input of cellular energy, exactly as required in over-wintering plants. The overnight retention of NPQ is clearly important; at low temperature, the low metabolic rate and high membrane rigidity would prevent rapid response to morning illumination.

(c) Sunflecks: on and off

The most challenging, and most common, condition in which NPQ is active is in fluctuating environments. Here, NPQ should be of the ‘right’ size, neither too large nor too small, it should form and relax at the ‘right’ speed, neither too fast nor too slow, and it should occur at the ‘right’ light intensity, so as not to limit photosynthesis but to provide photoprotection when necessary [16,24]. The initial formation of NPQ in response to a sudden increase in light intensity or a switch from darkness will depend on the magnitude of the ΔpH and the pK for the qE formation. As discussed earlier, for *Arabidopsis*, normally a small rapid NPQ is followed by a slower phase as violaxanthin is de-epoxidized and qE is activated. In repeated sunflecks, a steady-state level of zeaxanthin will be reached and the onset of NPQ thereby greatly accelerated. In essence, the thylakoid records and ‘remembers’ the intermittent light environment by the value of DES, so adapting it for when the next sunfleck comes. For reasons explained earlier, this response is different depending on the state of acclimation and adaption of the thylakoid membrane, e.g. shade plants will have a reduced capacity to rapidly induce NPQ.

The initial burst of qE may be transient if the light intensity is below saturation for steady-state photosynthesis; thus, as the induction period for photosynthesis proceeds and carbon assimilation accelerates, the rate of ATP consumption increases, the ΔpH decreases and qE diminishes [60]. In rice, this transient behaviour is very strong: high qE is observed within a minute of illumination, declining markedly as photosynthesis accelerates [61]. This phase of relaxation of NPQ is one where the level of quenching potentially limits photosynthetic electron transport. Observation of plants in which qE is altered by change in the concentration of PsbS sheds light on this limitation [61]: in PsbS-depleted plants, there is a higher rate of CO2 assimilation compared with wild-type plants, whereas the reverse happens in plants with higher PsbS. Thus, the presence of qE reduces the rate of photosynthesis during the induction period following the onset of illumination.

(d) Optimization of NPQ

These observations point to the trade-offs that occur in the extent and kinetics of NPQ. NPQ is necessary for photoprotection, and plants deficient in PsbS show elevated photoinhibition and a decreased photosynthetic capacity. Hence, the earlier-mentioned ‘advantage’ of qE deficiency when considering the rate of photosynthesis following an increase in light intensity is presumably offset by the lack of photoprotection. Equally, one might think more qE brought about by higher PsbS would be advantageous because of less photoinhibition. However, supra-optimal levels of qE compromise photosynthetic rate following transitions in light intensity. A similar compromise is found when considering the xanthophyll cycle [62]. A genetically engineered increase in pool size increases the level of zeaxanthin, which by way of its antioxidant effect increases resistance to low and high temperature stress [37,38]. Because this change relies on a single gene, one wonders why the wild-type plants do not have a larger xanthophyll cycle pool. The answer may lie in the effect of the elevated pool size on NPQ kinetics: because NPQ depends on the DES rather than the absolute level of zeaxanthin, the rate of formation of NPQ decreases in these plants, and this could increase the susceptibility to photodamage during bursts of high light.

Another example of trade-off is in the kinetics. A general observation is that in conditions which induce high steady-state NPQ (e.g. high DES), it forms rapidly, once ‘primed’, but will relax slowly.
So, during a sunfleck, which is better—rapidly forming NPQ for immediate photoprotection or rapid relaxation from NPQ so photosynthesis is not held back in a following period of low light?

Thus, the fine-tuning of NPQ to give maximum plant fitness is relatively complex, and still poorly understood. Genetic variability has been described, with some species showing exceptionally high capacities of NPQ [63]. Such plants experience environments in which high light intensity is accompanied by some type of severe environmental restriction on growth. Conversely, fast-growing plants adapted to largely favourable environments generally have poorly expressed NPQ [64].

(e) Integration of NPQ with regulation of electron transport

It has been pointed out that photoprotection by NPQ is just one of a range of responses of plants to excess light stress [65]. Leaves of Phaseolus in midday summer sun under drought conditions show only negligible NPQ because they turn away from the sun so that there is little light absorption [66]. Even within the chloroplast, the response to excess light is not limited to the formation of NPQ. The details of photosynthetic induction following the onset of illumination have been intensively studied. Initially, after a brief burst of photosynthesis, there is insufficient concentration of metabolites to sustain the Benson–Calvin cycle. ATP and NADPH accumulate, linear electron transport is inhibited and cyclic electron transport around PSI is stimulated. The result is a reduction of the PSII acceptors and a high ΔpH [3,60]. During this period, there is evidence for inactivation of PSII electron transport [67]. A low lumen pH can result in inactivation of the donor side reactions of oxygen evolution, and new pathways of electron donation to PSII are opened up [68]. Cytochrome b₅₉ₒ is affected [69], bound Ca is released and the redox potential of Qₐ increased [70]. Estimations of the intrinsic yield of PSII electron transport show a decline in efficiency separate from any effect of qE [71]. Some or all of these responses may be occurring during this period of high ΔpH. It is interesting that the transient inactivation of PSII is dependent on PsbS [67], suggesting that the influence of this protein on membrane organization (figure 3) affects not only the function of LHCCI but also that of the PSII core. However, the significance of all these responses remains to be determined—are they unavoidable responses to the overshoot of the ΔpH, with no physiological advantage, or are they temporary measures to provide photoprotection before qE can be engaged?

(f) Can NPQ be re-optimized?

An immediate question of practical significance arises—is there scope for genetic manipulation to adjust NPQ optimization to improve the performance of crop plants? It has been estimated that speeding up the relaxation of NPQ following a transition from high light to low light could increase crop yields by as much as 15 per cent [72]. But can this be done without compromising other aspects of light harvesting and its regulation? It has been argued that the favourable conditions of modern agriculture in which abiotic stress is minimized by agronomic practices mean that productivity could be boosted by eliminating or reducing mechanisms such as NPQ that protect against such stress [73]. Conversely, as we face the uncertain effects of global change on agriculture, can more resilient crops be created by elevating NPQ without negative effects on yield potential? Such questions give rise to research on light-harvesting regulation and the thylakoid membrane an extra urgency.

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