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Review

The photoprotective molecular switch in the photosystem II antenna ☆

Alexander V. Ruban *, Matthew P. Johnson, Christopher D.P. Duffy

Queen Mary University of London, School of Biological & Chemical Sciences, Mile End Road, London E1 4TN, UK

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ABSTRACT

We have reviewed the current state of multidisciplinary knowledge of the photoprotective mechanism in the photosystem II antenna underlying non-photochemical chlorophyll fluorescence quenching (NPQ). The physiological need for photoprotection of photosystem II and the concept of feed-back control of excess light energy are described. The outline of the major component of nonphotochemical quenching, qE, is suggested to comprise four key elements: *trigger* (ΔpH), *site* (antenna), *mechanics* (antenna dynamics) and *quencher(s)*. The current understanding of the identity and role of these qE components is presented. Existing opinions on the involvement of protons, different LHCII antenna complexes, the PsbS protein and different xanthophylls are reviewed. The evidence for LHCII aggregation and macrostructural reorganization of photosystem II and their role in qE are also discussed. The models describing the qE locus in LHCII complexes, the pigments involved and the evidence for structural dynamics within single monomeric antenna complexes are reviewed. We suggest how PsbS and xanthophylls may exert control over qE by controlling the affinity of LHCII complexes for protons with reference to the concepts of hydrophobicity, allostery and hysteresis. Finally, the physics of the proposed chlorophyll–chlorophyll and chlorophyll–xanthophyll mechanisms of energy quenching is explained and discussed. This article is part of a Special Issue entitled: Photosystem II.

1. Introduction

1.1. The need for photoprotection of photosystem II

Light varies in intensity both temporally (as a result of the diurnal cycle) and spatially (as a result of shading by clouds and other organisms and objects) throughout the day. The resulting frequent exposure to low light exerted a strong selection pressure during the evolution of photosynthetic organisms for molecular apparatus that could increase the spatial and spectral cross-section of the chlorophyll pigments responsible for the trapping of photon energy by charge separation in the photosynthetic reaction center. Evolution's solution to this problem was to build light harvesting systems or antennae of many interconnected pigments capable of efficiently absorbing and delivering photon energy to the photosynthetic reaction center pigments. Hence, photosynthetic antennae function to increase the power input into the energy transforming machinery. However, while the photosynthetic antenna can advantage the organism in low light it can also produce negative effects when the light intensity is elevated.

The major fundamental problem of exposure to elevated light intensities 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 processes leading to NADPH and ATP synthesis. Under increasing light intensity, the photosynthetic reaction centers become progressively saturated (closed), resulting in a reduction in the fraction of energy utilized in photosynthesis and the subsequent build-up of "unused", potentially harmful, excitation energy in the photosynthetic membrane (Fig. 1). Build up of this energy can cause various detrimental effects on the organism, particularly on the delicate photosynthetic machinery. The most sensitive part of it is photosystem II (PSII) — the photosystem that splits water and evolves oxygen possesses one of the strongest oxidizers in nature, the special-pair chlorophylls known as P680 which undergo charge separation. Excess light leads to photoinhibition, a sustained decline in photosynthetic efficiency, associated with damage to P680 [1]. When electron donation to P680 is less efficient than oxidation, an increase in the $P680^+$ lifetime will take place. $P680^+$ will oxidize the nearest pigments and amino acids eventually leading to degradation of the PSII reaction center protein D1. Conversely, when electron donation from P680 to oxidized plastoquinone is inhibited by the build-up of reduced plastoquinone charge recombination can occur triggering P680 triplet formation [2,3]. In the triplet state P680 will interact with atmospheric triplet oxygen, causing formation of singlet

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* Corresponding author at: School of Biological and Chemical Sciences, Queen Mary University of London, Fogg Building, Mile End Road, London E1 4NS, UK. Tel.: +44 2078826314; fax: +44 2089830973.
E-mail address: a.ruban@qmul.ac.uk (A.V. Ruban).
URL: http://queenmaryphotosynthesis.org/~ruban/ (A.V. Ruban).

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oxygen, which in turn will bleach P680. Photoinhibition inevitably leads to a reduction in the number of active PSII units and because of the slow repair of damaged D1 proteins the decline in electron transfer normally persists for several hours, even in the dark or at low light intensity [4]. The need to avoid photoinhibition has therefore created a strong selective pressure for mechanisms that reduce excess energy accumulation in high light environments [5].

1.2. The concept of photoprotective feed-back control of excess light energy in photosystem II. Nonphotochemical chlorophyll fluorescence quenching (NPQ)

During the course of evolution plants have developed an entire network of adaptive mechanisms to cope with high light exposure. There are adaptations to control light absorption capacity as well as adaptations that deal with the light energy that has already been captured. Plants also respond on different levels of organization, for instance at the whole organism level via leaf movements and leaf deposits, at the cellular level via chloroplast movements and control of chloroplast number and at the molecular level by control of the number of pigments within the antenna, etc. [6–8]. The molecular level of adaptations to high light is the most fundamental and there has been a great deal of research performed in the last 40 years aimed at understanding its basis. The molecular adaptations can be divided into two distinct groups, long-term (acclimation) and short-term (regulatory mechanisms). The first type is predominantly developmental in nature, and is the result of light-dependent regulation of complex gene expression, occurring on transcriptional, translational and post-translational levels [9–12]. However, since the response time of acclimation is long it limits the photoprotective efficiency while at the same time consuming energy and resources.

The short-term adaptation to light is therefore a necessity for control over the sequence of energy transformation events in the photosynthetic membrane (Fig. 2A). The fundamental principle of this type of adaptation is the utilization of feed-back control mechanisms. In the light phase of photosynthesis, overproduction of ATP (NADPH) will cause the accumulation of protons in the inter-thylakoid membrane space (lumen), which in turn will lead to inhibition of a number of key electron transport enzymes (Cyt *b*/*f* and the oxygen evolving complex of PSII) causing reduction in electron transport rates. Indeed, as with respiratory control in mitochondria, the photosynthetic membrane possesses photosynthetic control as a feed-back mechanism for balancing ATP (NADPH) production with

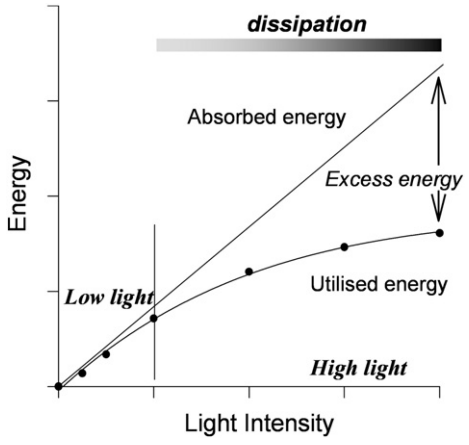

Fig. 1. The need for photoprotection in photosystem II. In low light the amount of light energy absorbed and the amount utilized in photosynthesis are well matched. Absorption continues unabated as the light intensity increases but photosynthesis becomes saturated with light. The difference between the amount of light energy absorbed and that utilized in photosynthesis is the 'excess energy' which if left unchecked has the potential to cause photo-oxidative damage to the photosynthetic membrane.

electron transport. However, no matter how efficient photosynthetic control is, the path of photon to electron energy conversion requires an additional control loop. Such feedback control exists in the form of the proton effect upon the PSII energy conversion events (Fig. 2A, photon–exciton–electron) [13–15]. This process can be monitored in the form of the PSII antenna chlorophyll fluorescence yield decline under conditions of excess excitation energy accumulation in the photosynthetic membrane [14–17] and is called nonphotochemical chlorophyll fluorescence quenching or NPQ.

It can be shown that NPQ is a very efficient process that protects PSII reaction centers from photoinhibition. Fig. 2B displays the time course of development of NPQ in leaves induced by high light when the proton gradient is present or absent (infiltration with the uncoupler nigericin). The state of PSII was monitored in parallel by calculation of so-called photochemical chlorophyll fluorescence quenching that reflects the number of closed or indeed damaged PSII reaction centers [17,18]. The rapid onset of NPQ triggered by ΔpH in control leaves caused more than half of all PSII to re-open as a result of excess energy dissipation and the consequential relief of the excitation pressure. After 1 h the illumination was switched off and the proportion of active (open) PSII reaction centers was assessed. In the presence of ΔpH the NPQ was fully photoprotective since all PSII reaction centers are in the open state when probed by a light pulse in the dark following illumination (qP = 1). On the contrary, leaves which were not able to form ΔpH demonstrated much higher excitation pressure in PSII evident from the very low qP levels (<0.1) during illumination. The NPQ generated in the absence of ΔpH was not photoprotective since nearly half of all reaction centers remain in the closed state when probed by a light pulse in the dark following illumination (qP = 0.5). Thus, NPQ generated in the presence of ΔpH is a very effective short-term regulatory mechanism capable of protecting PSII under conditions of excess light. Since the NPQ generated in the absence of ΔpH was not photoprotective it is described as photoinhibitory quenching and is believed to arise from permanently damaged reaction centers acting as weak energy traps.

2. The qE scenario

NPQ is a heterogeneous process, both kinetically and mechanistically. NPQ can be divided on the basis of these kinetic differences into several different components. The most slowly forming and relaxing NPQ component is qI, sustained quenching that was originally ascribed to the photoinhibitory damage of PSII reaction centers, which can persist for several hours in the dark following illumination. The second component, qT or state transitions, forms and relaxes on a timescale of tens of minutes, is predominantly observed in low light and is related to the balancing of excitation energy between PSII and photosystem I (PSI) [19–21]. The major part of NPQ in high light develops and relaxes within seconds to minutes and is called qE or energy dependent quenching [19,22,23]. qE was shown to be triggered by ΔpH but followed somewhat slower kinetics of formation and, in particular, relaxation than the proton gradient [14,22,24,25]. This lack of a tight kinetic relationship between the proton gradient and qE led to the proposal that the thylakoid membrane (or specifically a part of PSII) should undergo some kind of conformational change in order to attain the photoprotective state [19,24]. This was the first indication that protons (the *trigger*) must act upon the *site* where qE is formed to bring about some kind of change (the *mechanics*) leading to formation of a *quencher* responsible for the fluorescence decrease in PSII. We can call this chain of events the NPQ outline or to be more focused on the major and effective photoprotective component — the *qE scenario* (Fig. 3).

In the 1980s and the beginning of 1990s a strong debate took place about the site of qE. Two opposing proposals were put forward. According to the one of them, the site of qE was the PSII reaction center itself. It was suggested that quenching arises from

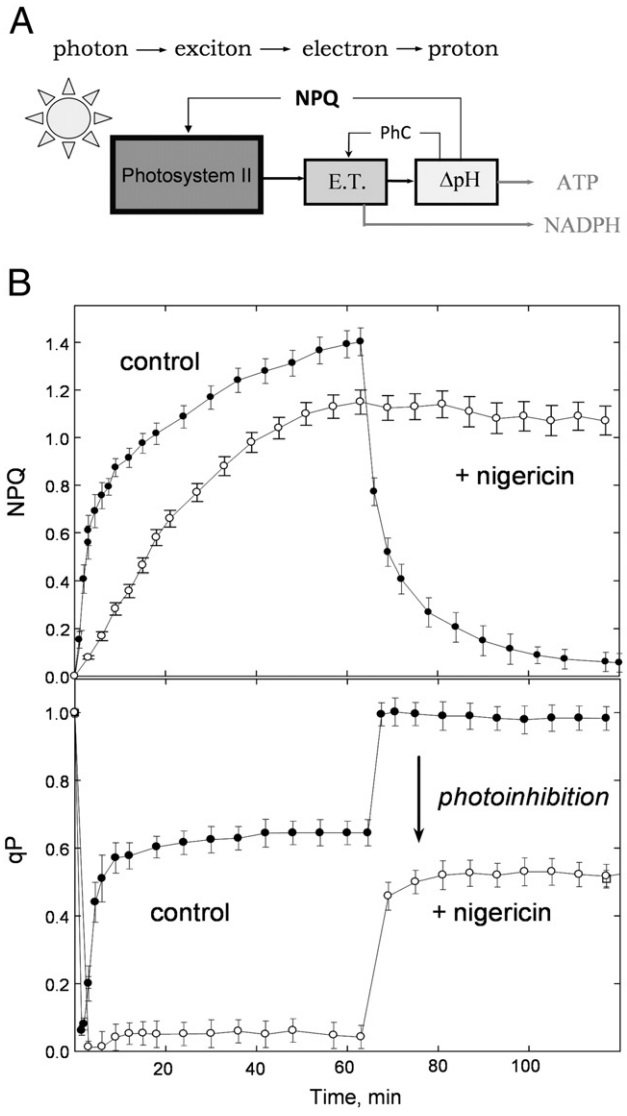

Fig. 2. (A) Scheme depicting photosynthetic and feedback control of photosynthesis in higher plants. PSII absorbs light and produces electrons (E.T.) which are transported through the thylakoid membrane to produce NADPH and drive formation of ΔpH for ATP synthesis. Build up ΔpH exerts control over photosynthesis via inhibition of electron transport (photosynthetic control, PhC) and via regulation of the excitation pressure in PSII (NPQ). (B) The photoprotective effect of NPQ. In control leaves NPQ forms rapidly (upper panel) and causes the relief of PSII excitation pressure (rise in qP — lower panel). When the light (400 μmol photons m^{-2} s^{-1}) is switched off after 60 min the NPQ relaxes and qP returns to 1 (all PSII reaction centers are open). However, if ΔpH is inhibited with the uncoupler nigericin, NPQ forms much more slowly (upper panel) and is unable to relive PSII excitation pressure (lower panel). Thus, when the light is switched off qP is only ~0.5, thus ~50% of PSII reaction centers have been permanently damaged by light and remain closed. The NPQ in this case is photoinhibitory quenching and doesn't relax in the dark (upper panel, for experimental details see ref. [119]).

recombination between $P680^+$ and the acceptor side of PSII [26,27]. The other proposal suggested that the PSII antenna (major and minor LHCII complexes, for the detailed classification see Section 3), was the site of quenching. Numerous lines of evidence emerged at the beginning of 1990s supporting the latter view that qE is localized in LHCII and not in the reaction center [23]. qE was found to be associated with a significant decrease in LHCII fluorescence when all PSII reaction centers are open (F_o quenching) [28]. qE was discovered to persist if samples were frozen to 77 K and was associated with quenched fluorescence bands originating from LHCII [29]. Indeed, spectral analysis of qE and qP showed that different emitters are quenched: qP preferentially quenches the PSII core and qE quenches emitters near 680 and 700 nm, which suggested that excitation in LHCII is selectively quenched [29,30]. The time resolved fluorescence data recorded for leaves were consistent with quenching taking place in the antenna [31]. Direct measurement of heat emission in the qE state showed it to occur within 1.4 μs, much faster than estimates for rates of the recombination reactions in PSII reaction centers [32]. Cross-linkers blocked qE and also transition of isolated trimeric LHCII complexes into a dissipative state [33]. qE and trimeric LHCII responded in the same way to a number of factors: antimycin A, tertiary amines and magnesium [34,35]. Other approaches studying chlorophyll *b*-less plants lacking all LHCII complexes [36–38], the effect of dicyclohexylcarbodiimide (DCCD) [39,40] and the similarities between quenching induced in all isolated LHCII complexes and qE [41–45] provided further evidence that the site of quenching was located within LHCII antenna. Additionally, qE was found to be almost entirely dependent upon the presence of exclusively LHCII antenna-bound xanthophylls, lutein and zeaxanthin [35,46].

Hence, nowadays it is widely accepted that the *site* in the photoprotective qE scenario is in the LHCII antenna. Still this definition is vague, since there is no common agreement in the current literature on which part or parts of LHCII antenna (major or minor or both) carry the qE quencher(s). In the following pages of this review we will also discuss what is known until now about the *mechanics* involved in the establishment of qE, regulation of the process, as well as the physical identity and photo-physical mechanism of the qE *quencher*.

3. qE site and change

3.1. Discovery of the involvement of zeaxanthin in NPQ: "qE or not qE ?"

In the late 1980s and the beginning of the 1990s Demmig-Adams and coworkers made a major breakthrough towards understanding the mechanism of qE that is reviewed in [47,48]. They provided the first evidence of a connection between the xanthophyll cycle and NPQ. The xanthophyll cycle was discovered by Sapozhnikov's group in 1957 [49] and its properties and enzymatics were initially characterized by Yamomoto and Hager's groups [50,51]. The cycle involves two enzymes, the de-epoxidase and the epoxidase which reversibly interconvert violaxanthin and zeaxanthin (for recent reviews see [52,53]). The work of Demmig-Adams and coworkers clearly demonstrated that the conversion of violaxanthin into zeaxanthin induced by the formation of ΔpH in high light strongly enhances NPQ [25,54,55]. The group suggested that zeaxanthin may be the pigment responsible for quenching [47]. Not only qE correlated with the amount of zeaxanthin but so did a part of qI, the slowly-reversible NPQ component associated with a sustained reduction in the yield of PSII. At first the qI component was attributed solely to photoinhibitory damage to the PSII reaction centers [56]. Later however, it was recognized that the major part of qI was in fact due to a sustained quenching in LHCII antenna related to the photoprotective down-regulation of PSII [56,57]. Further investigation of zeaxanthin-dependent qI revealed that it consists of two components, one is

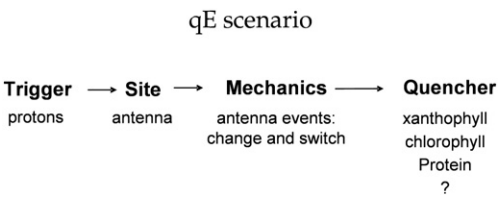

Fig. 3. Scheme depicting the qE scenario. The trigger (protons) acts upon the site (antenna) to bring about the mechanics (change and switch) that activates the pigment(s) that dissipate the excess energy as heat (quencher).

sensitive to uncouplers and the other is not [58]. Simultaneous measurements of ΔpH and NPQ revealed that the uncoupler-sensitive component of zeaxanthin-dependent qI persists for relatively long periods in darkness and is not associated with the bulk ΔpH [58]. Therefore, a new aspect of photoprotective quenching in the antenna was revealed — namely that the longevity of the photoprotective state (conformational state) in the darkness could vary depending upon zeaxanthin concentration and pre-illumination history [59–61]. The modulation of qI by zeaxanthin led to the possibility that it may originate from the same site and the same process that underlies qE. It was found, for example, that conditions promoting the zeaxanthin-dependent qI component caused a concomitant decrease in qE [57,58], as if qE was in fact becoming less and less reversible. An extreme of such behavior of qE when it became gradually totally irreversible even after addition of an uncoupler was discovered in diatom algae [62] and also in plants exposed to high light and low temperature conditions [63,64].

3.2. qE without zeaxanthin: the LHCII aggregation model

The discovery of zeaxanthin involvement in qE led to a detailed study on the relationship between qE and ΔpH at different levels of zeaxanthin in the thylakoid membrane. Horton and coworkers undertook a series of detailed titration studies on isolated intact chloroplasts and thylakoids where they found that it is possible to induce high levels of qE without zeaxanthin provided the lumen pH was lower than 4.5–5.0 [65,66]. Zeaxanthin was found to simply shift the relationship between qE and ΔpH so that the quenching could be activated at much lower ΔpH, i.e. higher lumen pH. This shift was found to be about 1.3–1.7 pH units, which enabled significant quenching to be formed at a lumen pH of 5.7–6.2. A very similar effect of zeaxanthin on qE was also observed in the experiments on uncoupled thylakoids where the acidification of the buffer led to a pH-induced quenching in the dark [67]. It was therefore proposed that zeaxanthin may act as an allosteric modulator of qE by controlling the affinity of the LHCII antenna for protons rather than as a direct energy quencher [67,68]. Furthermore, experiments on leaves confirmed the existence of significant quenching in the absence of zeaxanthin and highlighted not only the fact that zeaxanthin controlled the amplitude of qE but also its kinetics, accelerating qE formation and decelerating qE relaxation [22,57,69].

The spectroscopic similarities between qE with and without zeaxanthin and quenching induced by aggregation of isolated major LHCII complex under low detergent conditions *in vitro*[35,41–45] led Horton and co-workers to propose a hypothesis for the mechanics of qE known as the “LHCII aggregation model” [70]. It was proposed that since the 680 nm low temperature (77 K) fluorescence band was preferably quenched by qE the quencher must reside somewhere in the LHCII antenna [29,70]. Zeaxanthin was suggested to promote LHCII trimer aggregation *in vivo* since it caused the emergence and quenching of a low temperature fluorescence band emitting at 700 nm [29,71], typical of LHCII aggregates *in vitro*[35,41,42,69,70]. In addition, the similar sensitivity of qE and LHCII aggregation to magnesium cations, tertiary amines, low pH [35] and the inhibitory effect antimycin [42,70] all provided evidence that the two processes shared a common origin. According to the Horton model there are four different structural/functional states in the LHCII antenna, I, II, III and IV (an updated model shown in Fig. 4A). I corresponds to dark-adapted, violaxanthin-containing unquenched state. Illumination causes violaxanthin de-epoxidation and protonation of the LHCII antenna, both driving the system into the deeply quenched state IV by promoting LHCII aggregation. Violaxanthin inhibits LHCII aggregation. If zeaxanthin is not formed LHCII will be only partially aggregated and quenched (III). After qE relaxation the antenna will still contain zeaxanthin and therefore remains partially aggregated and quenched (II), since it takes much longer for the epoxidation of zeaxanthin back into violaxanthin than for the relaxation of ΔpH. All four states therefore have different degrees of heat dissipation proportional to the degree of aggregation.

The aggregation model of qE was not only consistent with many physiological, spectroscopic and biochemical observations but also explained the origins of zeaxanthin-dependent qI components, the kinetic behavior of qE, and the role of zeaxanthin as an allosteric modulator of qE. The control of the sensitivity of qE to ΔpH by the xanthophyll cycle is a process of great significance since in high light it allows qE formation at sub-saturating levels of ΔpH, which simultaneously allow high electron transfer rates, while in low light qE is switched-off at levels of ΔpH which are still sufficient for ATP synthesis. Indirect estimates of ΔpH *in vivo* suggest that the steady state levels of ΔpH are relatively low [72,73], explaining the low levels of qE observed in the absence of zeaxanthin *in vivo*. Another important aspect of the LHCII aggregation model is that it remains the only model that explains large variations in NPQ levels observed among various plants and algae, since the fluorescence of LHCII during the aggregation process, can be quenched up to 20 times [41,43–45]. Therefore, the LHCII antenna was proposed to possess an inherent property for the control of excitation energy density and for the fine regulation of qE sensitivity to the feedback signal, ΔpH [57,74].

The evidence for LHCII antenna aggregation in the photosynthetic membrane went beyond the qE mechanism. Formation of large aggregates of LHCII has been documented in overwintering evergreen plants [63,64]. The process was followed by the appearance of a long wavelength fluorescence characteristic of aggregated LHCII around 700–715 nm registered at 77 K [75]. LHCII aggregation has also been observed in plants grown under a CO_2 starvation regime [76,77] and with delayed senescence [78]. The phenomenon of antenna protein aggregation in the photosynthetic membrane seems to be of a universal regulatory significance and its primary studies laid the foundation for various structural and spectroscopic studies determined to underpin the dynamics of the photosynthetic membrane landscape and its role in the regulation of the light phase of photosynthesis.

3.3. Minor vs major LHCII: proton- and zeaxanthin-binding at the qE site

Although the aggregation model of qE described many physiological phenomena of photoprotection in plants in algae and suggested the modulating role of the xanthophyll cycle it lacked key structural details. At the time the model was proposed, knowledge of the PSII antenna composition and architecture was poor as was the understanding of how and where protons acted in order to trigger qE. At the beginning of 1990s Jahns and Junge discovered an interesting phenomenon related to proton translocation in PSII [79]. They found that DCCD, which covalently binds to glutamate and aspartate residues buried in hydrophobic environments forming zero cross-links, could interact with some LHCII polypeptides. The binding caused a proton short-circuit within the PSII water-splitting complex [80,81]. The authors therefore concluded that some LHCII complexes play an important role in providing part of the proton release pathway from the oxygen-evolving complex into the lumen [80,81]. Horton's group found that DCCD could effectively inhibit qE before it caused a total uncoupling in the isolated thylakoid membranes [39]. At the same time the work of Bassi's group made progress in the separation and preparative isolation of a number of LHCII antenna complexes, which were called the major LHCII (LHCIIb), CP24 (LHCIId), CP26 (LHCIIc) and CP29 (LHCIIa). While the first complex was dominating (~80% of chlorophyll in the PSII antenna[82]) and existed in the form of a trimer [83], the last three were called minor antenna and were all monomeric [84,85]. Work on ^{14}C-labeled DCCD demonstrated that the two minor antenna complexes, CP26 and CP29 were the most effectively labeled [40]. In CP26 two of the amino acid residues bound by DCCD were identified as glutamates on the lumen-facing domain [86]. It is possible that protonation of these residues may be the

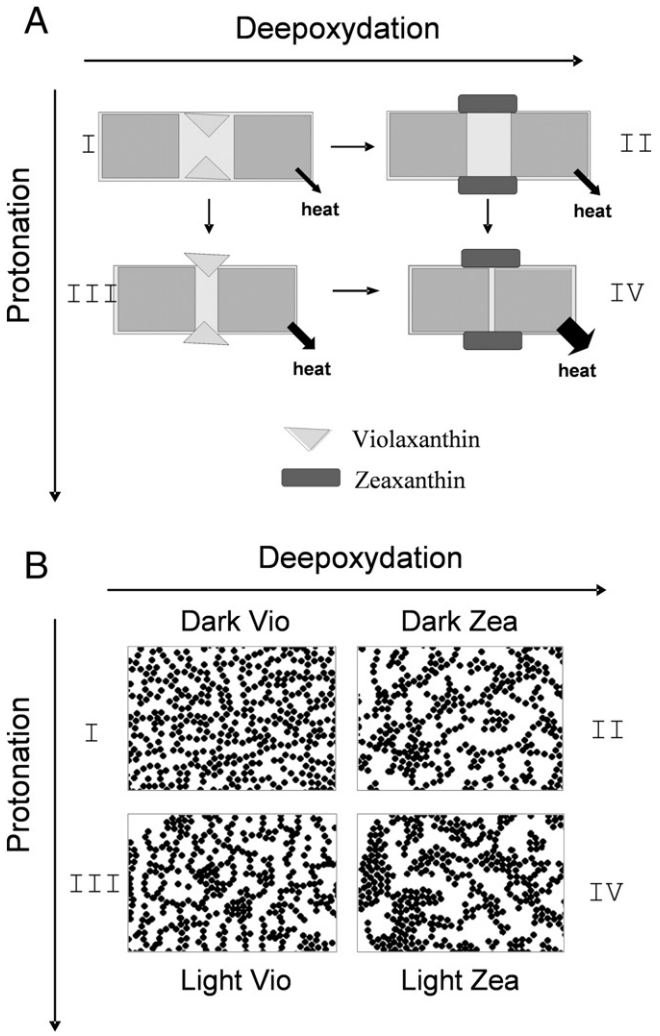

Fig. 4. (A) The LHCII aggregation model for NPQ. According to the Horton model there are four different structural/functional states in the LHCII antenna, I, II, III and IV. I corresponds to dark-adapted, violaxanthin-containing unquenched state. Illumination causes violaxanthin de-epoxidation and protonation of LHCII, both driving the system into the deeply quenched state IV by promoting LHCII aggregation. Violaxanthin inhibits LHCII aggregation. If zeaxanthin is not formed LHCII will be only partially aggregated and quenched (III). After qE relaxation the antenna will still contain zeaxanthin and therefore remains partially aggregated and quenched (II), since it takes much longer for the epoxidation of zeaxanthin back into violaxanthin than for the relaxation of ΔpH. All four states therefore have different degrees of heat dissipation proportional to the degree of aggregation. (B) Patterning of LHCII particles in intact spinach chloroplasts determined by freeze-fracture electron microscopy (each LHCII trimer was fitted with a 50 nm^2 circle using image recognition software and their positions are presented in the figure) [130], the four states of organization were observed in dark-adapted chloroplasts (Dark Vio, analogous to State I in the LHCII aggregation model), chloroplasts frozen immediately after 5 min illumination at 350 μmol photons $m^{-2}\,s^{-1}$ in the absence of zeaxanthin (Light Vio, analogous to State III), chloroplasts frozen immediately after 5 min illumination at 350 μmol photons $m^{-2}\,s^{-1}$ in the presence of zeaxanthin (Light Zea, analogous to State IV) and chloroplasts frozen following 5 min illumination at 350 μmol photons $m^{-2}\,s^{-1}$ in the presence of zeaxanthin and a further 5 min dark adaptation (Dark Zea, analogous to State II).

primary trigger for qE, inducing conformational changes in the lumen-facing domain of the complex leading to the formation of the quenching state. However, it is unlikely that these two residues can form the whole proton domain and their direct role in qE is not proven by these observations. An alternative idea would be that that they may constitute a proton channel into the qE locus, which is elsewhere within the PSII antenna. The major LHCII bound the DCCD label less effectively than the minor complexes [40] however tritium labeling demonstrated that the major LHCII could sequester up to 17 additional protons in the qE state and upon aggregation *in vitro* [87,88].

The debate on the preferable site for qE was continued with the work that analyzed xanthophyll cycle carotenoid binding in the four LHCII antenna complexes [82,84,85,89,90]. The group of Bassi claimed that only the minor antenna complexes bound violaxanthin in large amounts [85]. Later it was found that the major LHCII could also bind violaxanthin as well as zeaxanthin in stoichiometric amounts (one per LHCII monomer) with somewhat lower affinity of binding than the minor complexes [90]. This finding was later confirmed by the crystal structure of the major LHCII complex [91]. The efficiency of violaxanthin de-epoxidation was found to be the highest in the major LHCII [89,90]. This was explained by the need for violaxanthin to be easily accessible (removable from its binding site) for the action of the de-epoxidase [90]. On the other hand, violaxanthin found in the CP29 complex was found to be almost inaccessible to the de-epoxidase and very strongly bound to the complex [89,90]. Therefore, it was concluded that this complex may be an unlikely candidate for the qE site [89,90]. Nevertheless, the interest to the quenching properties *in vitro* of the minor antenna continued. The groups of Horton and Bassi found that these complexes could form stronger quenching states than the major LHCII and that DCCD could reverse this quenching more effectively in the minor complexes than in the major LHCII [43,69,92]. However, all four LHCII complexes were found to possess similar spectral quenching fingerprints like 77 K 700 nm fluorescence and red-shifted chlorophyll absorption [43,93], suggesting that any of them could potentially be the site qE quenching.

Work on PSII antenna genetic manipulation, pioneered in Jansson's group and later continued in the laboratory of Bassi, enabled the construction of *Arabidopsis* mutant plants where various LHCII components were selectively removed (by antisense or knock-out techniques) to study the consequences for qE [94–97]. Unexpectedly, given its affinity for DCCD the absence of CP26 had no effect on qE [94]. However, the absence of CP29 complexes decreased qE by ~30% [94], while deletion of the CP24 complex (which did not bind DCCD) led to an almost 50% reduction in qE [95]. Surprisingly, however simultaneous deletion of CP24 and CP26 complexes and a concomitant 50% reduction in CP29 levels did not cause any decrease in qE [96]. On the other hand, the absence of the two major polypeptides of LHCIIb, Lhcb1 and 2, caused approximately a 35% decrease in qE [97]. However, CP26 trimers have been found in these plants, suggesting that this complex could have taken the function (including qE) of the major LHCII [98]. In addition, the early work by Jahns and Krause on plants containing only the CP26 complex showed significant but not complete reduction in qE levels [37]. It is therefore likely that no individual LHCII complex acts as the sole site of qE and that, in principle, the quenching could occur in any of the four types of LHCII. It is worth to note, however, that the theoretical calculations based on the course-grained model for energy transfer in the PSII antenna and reaction center landscapes of the thylakoid membrane suggest that the major LHCII would act as the most effective site for qE [99]. Section 5.1 will specifically discuss the spectroscopic evidence arguing further the question of the qE site.

3.4. Discovery of PsbS involvement in qE: localization, proton and pigment binding

In the mid-1990s a novel PSII-related protein, PsbS, was discovered [100,101]. Funk and coworkers concluded that while the protein was related to the antenna family, since it was stable in the absence of pigments it was unlikely to directly participate in light harvesting [101]. A few years later the group of Niyogi isolated *Arabidopsis* mutants that lacked this protein and discovered that qE was almost totally absent in them [102,103]. *In vitro* work indicated that PsbS could bind zeaxanthin and cause a strong alteration in its spectral

properties (red-shift) [104]. In addition, PsbS was shown to bind DCCD via two lumen-exposed glutamate residues [105]. When these two glutamate residues on PsbS were mutated in *Arabidopsis* plants DCCD binding and the ability to form qE were lost [106]. It was therefore suggested that binding of protons by PsbS triggered zeaxanthin binding and its activation as a quencher of chlorophyll excited states in the PSII antenna via interaction with a connected LHCII complex [106]. According to this model the actions of PsbS and zeaxanthin are interconnected in one site. In order to check this proposal the group of Horton used plants with normal and over-expressed levels of PsbS [107] where zeaxanthin formation was inhibited by dithiothreitol (DTT) [108]. They found that increased levels of PsbS enhanced qE proportionally in both the presence and the absence of zeaxanthin. Moreover, while zeaxanthin decelerated the qE recovery kinetics, PsbS seemed to have an acceleration effect upon qE reversal [109,110], suggesting that zeaxanthin and PsbS may have quite different roles in qE. Furthermore, the group of Bassi provided new evidence arguing that PsbS was in fact unable to specifically and selectively bind zeaxanthin [111]. Therefore, an alternative explanation of PsbS function in qE started to emerge. It was proposed that the protein acted as a catalyst of the conformational change in LHCII [68,105,111,112]. At the same time structural evidence began to appear that suggested PsbS affected the rigidity of the grana membrane, its resistance to detergent solubilization and could accelerate the grana stacking process induced by magnesium cations — suggesting that it may play a role as an enhancer of thylakoid membrane dynamics [113]. Electron microscopy revealed an increase in the percentage of PSII units assembled into semi-crystalline arrays in grana membranes lacking PsbS that in principle should make the membrane more rigid [114]. These findings were consistent with the fact that PsbS was found not to be an intrinsic structural component of the PSII–LHCII supercomplex [115,116]. Giacometti's group reported that PsbS existed in dimeric state associated with PSII in the dark and undergoes monomerization upon illumination and migration towards LHCII [117]. Later the same group showed using crosslinking studies that PsbS was rather loosely associated with a wide variety of thylakoid membrane complexes including trimeric LHCII, CP29, PSI and ATP synthase [118]. In addition, they showed that upon qE formation PsbS migrated from the stacked grana thylakoids to the grana margins and stromal lamellae [118]. These results implied that PsbS has a highly dynamic nature in terms of its oligomerization state as well as its lateral mobility in the thylakoid membrane. Recently we have found that it is possible to reach the same level of NPQ as in the wild type in plants lacking PsbS [119]. Most importantly, the quenching is photoprotective and possesses many of the same characteristics as qE. This work appeared to confirm the notion that PsbS is a catalyst kinetically controlling NPQ but is not the site of quenching and is not obligatory for the photoprotective process that occurs in antenna.

3.5. Change: global structural reorganization of PSII in the qE state

It has been known for a long time that the organization and lateral redistribution of photosynthetic complexes in the thylakoid membrane depend upon cations [120–123]. Magnesium is particularly crucial for stabilization of grana stacking and the lateral segregation of PSI from PSII, as well as the assembly of PSII–LHCII supercomplexes. Upon illumination formation of ΔpH leads to neutralization of point charges on the lumen exposed surface of the thylakoid membrane, resulting in displacement of bound magnesium cations and there diffusion into the stromal space [124]. These changes in charge distribution were found to cause significant conformational alterations — the thylakoid membrane became thinner, dehydrated and more hydrophobic [125,126]. Barber suggested that the alteration in charge distribution brought about by ΔpH formation could cause alteration in the lateral interactions and aggregation state of thylakoid membrane proteins [123]. Recently several new lines of evidence have emerged that provide further insight into the *change* in PSII and LHCII organization brought about by ΔpH formation. Firstly, Holzwarth's group provided indirect spectroscopic evidence suggesting that upon formation of qE part of the major LHCII undergoes separation from the PSII supercomplex [127,128]. This conclusion was based on the appearance of a new red-shifted emitting band in decay-associated fluorescence spectra in the qE state that was suggested to arise from LHCII aggregates [127,128]. Secondly, the group of Bassi obtained biochemical evidence suggesting that PsbS controlled the dissociation of a part of the PSII–LHCII supercomplex containing LHCII, CP24 and CP29 occurred under NPQ conditions [129]. Bassi's group also found structural evidence that the distance between PSII core complexes decreased under NPQ conditions in detergent solubilized grana membranes, providing further evidence that NPQ involved a reorganization of the PSII antenna [129]. Recently, our group obtained further structural evidence based upon freeze-fracture electron microscopy of intact chloroplasts displaying clustering of PSII core units as well as LHCII antenna aggregation upon qE formation [130]. Importantly our findings support the original LHCII aggregation model by demonstrating that ΔpH and de-epoxydation of violaxanthin to zeaxanthin cooperatively drive LHCII aggregation (Fig. 4B). Moreover the data confirm the common nature of qE and zeaxanthin-dependent qI as manifestations of the same LHCII aggregation phenomenon (Fig. 4B) [130]. Crucially the observed structural alterations induced by illumination occur on a timescale consistent with the formation and relaxation of qE [130]. This data therefore provide the first direct link between the structural change in PSII antenna and qE in intact, unsolubilized thylakoid membranes.

The model presented in Fig. 5 summarizes recent structural evidence regarding the *change* in PSII–LHCII macro-organization underlying qE. In the qE state part of the PSII–LHCII supercomplex containing the LHCII m-trimer, CP24 and CP29 is dissociated, a phenomenon that relies upon the presence of PsbS [129], this reorganization leads to the aggregation and partially segregation of LHCII from PSII [130]. Unfortunately the current resolution does not permit the exact location of each specific LHCII complex or indeed PsbS to be known with certainty. It is also not known whether all complexes, only one, or few in the locus are in the quenched state; indeed, Holzwarth has suggested that the aggregated LHCII and the PSII core and the remaining LHCII complexes form two different quenching sites [128].

4. qE control

4.1. qE without PsbS: control of the antenna sensitivity to ΔpH

Further exploring the hypothesis of catalytic control of qE by PsbS we utilized the proteolithic agents diaminodurene (DAD) and phenazine metasulfate (PMS) to enhance ΔpH in intact chloroplasts. Fig. 6 shows typical PAM fluorescence quenching traces measured in parallel with the ΔpH indicator 9-aminoacridine. DAD enhanced ΔpH and led to formation of rapid NPQ in *npq4* mutant chloroplasts. This quenching was rapidly reversible, quenched F_o, was enhanced by zeaxanthin, and was associated with the typical qE-related absorption change at 535 nm (the details of the origins of this absorption will be described in the next paragraph) and was photoprotective. Therefore, these data support the idea that PsbS enhances the sensitivity of LHCII to protons by shifting the pK of qE towards higher values. The fact that qE can be obtained without PsbS suggests that the quenching process itself is independent of this protein. Altered PSII–LHCII organization present in plants lacking PsbS (discovered in the studies described in the previous paragraph) somehow shifts the ΔpH versus qE titration curve to a lower pK, increasing the co-operativity of the process. Thus in the absence of PsbS a larger ΔpH driving force (i.e. a more acidic lumen) is required to trigger the reorganization of the PSII–LHCII

macro-structure associated with qE (as discussed in the previous paragraph). qE cannot be observed in *npq4* plants simply because of the relatively low levels of ΔpH that occur in natural conditions [72,73]. Our data therefore provide direct experimental support for the catalyst hypothesis for the role of PsbS in qE.

4.2. Regulation of qE by xanthophyll hydrophobicity: memory of light exposure and hysteresis

The proposed role of the xanthophyll cycle in the allosteric control of qE was primarily based on the ΔpH titration curves described in previous paragraphs. Horton's group suggested that the differential effect of zeaxanthin (stimulatory) and violaxanthin (inhibitory) on qE arose from the different shape (i.e. the cyclic group orientation relative to the polyene chain) and hydrophobicity of the two molecules [131,132]. The hydrophobicity of each xanthophyll was determined using a simple empirical measure of the percentage of ethanol in water/ethanol mixtures needed to solubilize aggregates of each xanthophyll [133]. Zeaxanthin was the most hydrophobic, followed by lutein and violaxanthin, while neoxanthin was the most polar. These structural differences were found to affect the tertiary and quaternary structures of LHC proteins [134,135]. The more hydrophobic xanthophylls such as zeaxanthin were found to favor condensed states of LHCs (aggregates) [35,41–43,69–71], while the more polar xanthophylls, such as violaxanthin favored fluorescent LHCII conformations [35,69,70,136]. The character of xanthophyll cycle tuning of qE became apparent as a result of the analysis of the kinetics of NPQ formation and relaxation [35,43,57,60,69,137]. It was shown that the rate of transition into the quenched state in leaves, chloroplasts and isolated LHCII containing violaxanthin (less hydrophobic) is slower than that containing zeaxanthin (more hydrophobic), while the relaxation rate demonstrated the opposite character. This is a typical behavior of transitions with memory, like those occurring in ferromagnetic or semi-elastic materials. Therefore, since the formation and relaxation of the photoprotective mode in plants do not follow the same route, qE is revealed to possess a *hysteretic* character. More hydrophobic xanthophylls cause faster development of NPQ but drastically decrease its relaxation. Therefore LHCII antenna *xanthophyll hydrophobicity* is an important property enabling the memory of the photoprotective state [138,139]. Violaxanthin conversion into zeaxanthin in high light provides a light exposure memory or counter for plants living in the frequently changing environments. More light exposure, continuous or, indeed, intermittent will cause an increase in hydrophobicity of antenna xanthophylls and make NPQ more sensitive/responsive to illumination while concomitantly slowing its relaxation. This light conditioning of antenna function is a remarkable achievement of the molecular evolution of the natural light harvesting process.

4.3. The mechanism of qE control

But what is the mechanism of the effect of xanthophyll hydrophobicity and PsbS upon the affinity of the LHCII structure for protons? The answer may lie in evidence that shows that the apparent pKa of amino acids strongly depends upon their environment [140–142]. Hydrogen bonding, steric hindrance and the di-electric constant of the environment can all affect the pKa of amino acids [140–142]. For example the pKa of the carboxyl group on aspartate can be as low as 2.4, in water environment due to hydrogen bonding, while in hydrophobic environment the pKa can be as high as 6.4 [143]. The shift of the qE vs ΔpH titration curve in the presence of hydrophobic xanthophylls such as zeaxanthin and hydrophobic proteins such as PsbS may be explained in this way. The proximity of numerous acidic amino acid residues on the lumenal side of the published LHCII structures to the xanthophyll binding domains provides a possible explanation of the ability of xanthophylls to influence their pKa [91]. Indeed, zeaxanthin binding was shown to shift the pI point of CP26 to higher values [143]. The careful regulation of the qE vs ΔpH titration curve by xanthophyll de-epoxidation and PsbS is essential to maximize the efficiency of photosynthesis. Since, the experimentally determined pKa of the lumenal side of the thylakoid membrane is as low as 4.1 [144], factors such as PsbS and zeaxanthin which raise the pKa will allow efficient qE at lower values of ΔpH than would otherwise be required but impossible to attain *in vivo*[72,73]. The scheme presented in Fig. 7 aims to explain the role of zeaxanthin and PsbS in qE by regulation of the sensitivity of the LHCII system to protons. Without zeaxanthin and PsbS the pK for LHCII protonation is very low (~4.0) and therefore the complex(es) remains unprotonated and qE is absent. The pI point of PsbS protonation is much higher at ~6.0 [105] thus it senses even low levels of ΔpH. Protonation of PsbS is known to promote the observed reorganization of the PSII–LHCII macro-structure in excess light [129] leading to LHCII aggregation [130] and this in turn may increase the pK for LHCII protonation. The pK for the activation of the violaxanthin de-

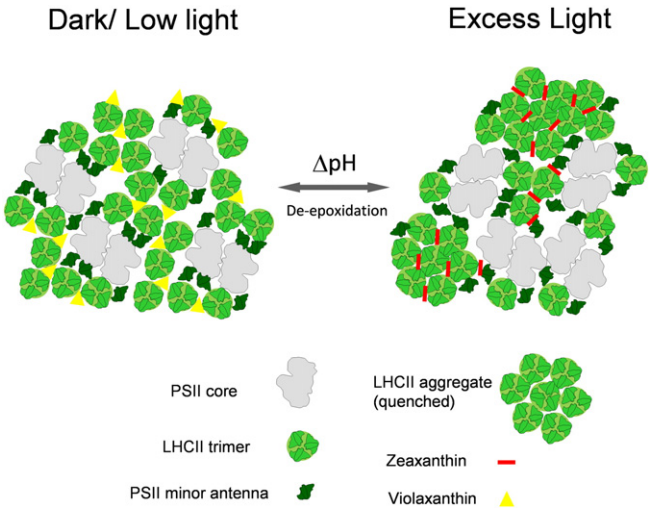

Fig. 5. Scheme depicting the current knowledge regarding the structural reorganization of the PSII–LHCII macrostructure occurring in the NPQ state. Dissociation of the CP24–CP29–LHCII-M part of the PSII–LHCII supercomplex [129] leads to the reorganization and aggregation [130] of antenna components.

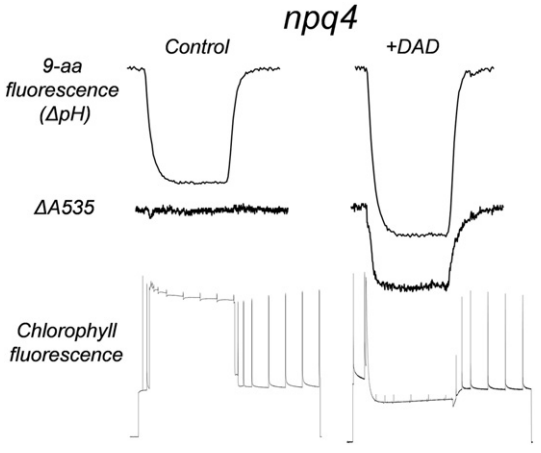

Fig. 6. The effect of DAD on chlorophyll fluorescence quenching in intact *Arabidopsis* chloroplasts (36 μM [Chl]) lacking PsbS (*npq4*). In Control *npq4* chloroplasts no qE or the qE-related ΔA535 absorption change (measured using the wavelength pair 565–535 nm as previously described [137]) is observed during 5 min of illumination at 350 μmol photons m^{-2} s^{-1} despite the presence of ΔpH (shown by the simultaneous quenching of fluorescence from 1 μM 9-aminoacridine). 400 μM DAD increases the amount of ΔpH and this allows qE and ΔA535 to occur in the absence of PsbS.

epoxidase is ~6.0 and the formation of zeaxanthin then further promotes LHCII aggregation such that the pK is further shifted up to ~5.7–6.2 allowing protonation at moderate levels of ΔpH thus amplifying qE. Hence, hydrophobicity and aggregation (reorganization) of the PSII antenna are mutually enhancing processes favoring the establishment of the quenching conformation of the individual LHCII complexes. We propose that the cooperative nature of qE in the absence of zeaxanthin and/or PsbS arises from the tendency of the protonated LHCII conformation to aggregate. As LHCII begins to aggregate so the hydrophobicity of the structure and thus affinity for protons increases, thus proton binding is cooperative. By promoting aggregation zeaxanthin and PsbS thus reduce the cooperativity of the proton binding equilibrium.

Tuning the photoprotective response is ecologically important, since different light environments require different light-tracking strategies within a light harvesting system. LHCII with more hydrophilic xanthophylls and faster NPQ recovery would be more desirable at very frequent but low amplitude fluctuations of light intensity. On the contrary, LHCII carrying hydrophobic xanthophylls with the tendency for NPQ with slow recovery would be better suited to slower but stronger fluctuations in light environment. The xanthophyll cycle fulfills the LHCII tuning role in wild-type plants to a certain extent. However, already available *Arabidopsis* xanthophyll mutants cover a much wider dynamic range of NPQ and hence offer a wider light adaptation capacity [138,139] that could become useful for crop design in the future.

5. The qE *switch* and the *quencher(s)*: atomic structure and dynamics of the quenching site

We have discussed so far the *site* and *change* in the PSII membranes associated with the establishment of ΔpH and qE state. The change is apparently important to study in order to understand, first of all, the principles of qE regulation as a basis for the light management by plants on the molecular level. Another important aspect to be reviewed is: what is the state of the knowledge of the events in the LHCII antenna leading to *quencher* formation on the atomic scale? In other words, to address the question of what is the qE *quencher* and how it is "born" in the antenna as a result of protonation and the macrostructural reorganization of PSII.

5.1. Spectral signatures of the qE state

First of all, we will review the knowledge that started to emerge at the beginning of 1990s on the various spectroscopic features of the qE state *in vivo* and quenching state of LHCII *in vitro*. Horton's group characterized qE and isolated aggregated major and later minor LHCII complexes with steady-state low temperature absorption and fluorescence spectroscopy as was already mentioned above [29,30,41–43]. It was found and later confirmed that the quenched state *in vivo* and *in vitro* was characterized by the appearance of the red-shifted Chl *a* absorption at ~685 nm, decline in the Chl *a* absorption at ~435 nm, Chl *b* at 472 nm and xanthophyll absorption at ~488 and ~495 nm, belonging to neoxanthin and lutein 1 respectively [41–43,45,137,145]. Zeaxanthin was found to promote the formation of the 77 K 700 nm fluorescence band *in vivo*, that is characteristic of quenched aggregated LHCII *in vitro* [29,41,45]. Later, linear dichroism spectroscopy revealed specific strong enhancement in the Chl *b* red region which is interpreted as an excitonic feature [45]. In addition, circular dichroism spectroscopy of aggregated LHCII also revealed a conservative signal in this region in support of the excitonic nature of the change in the LD spectra [45]. The CD spectrum of aggregates also displayed a strong negative Chl *a* band at 438 nm that appeared in parallel with the decrease in the 677 nm transition [45]. The amplitude of this decrease correlated well with the extent of quenching [45]. Resonance Raman studies on the state of chlorophyll in aggregated LHCII revealed specific alterations in the hydrogen bonding pattern to a subpopulation of Chl *a* and *b* [146]. Recently, time-resolved fluorescence

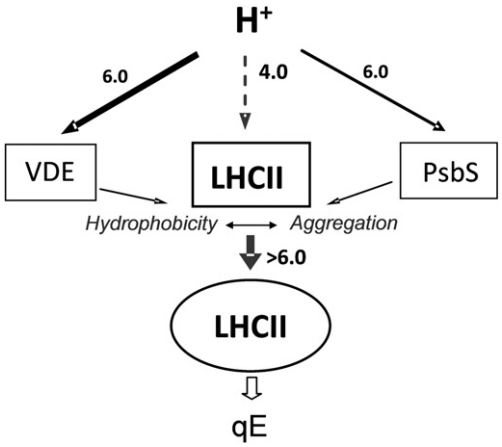

Fig. 7. Model explaining the effect of PsbS and xanthophyll cycle on the pKa of qE-active residues in LHCII in chloroplasts. The pKa of LHCII is ~4.0 [82,144], too low for qE activation by physiological lumen pH values ~5.8 [73], however PsbS and VDE have a pKa of ~6.0 [53,105] and thus bind protons, together they trigger the aggregation of LHCII increasing the hydrophobicity of the environment of the qE-active residues and shifting the pKa to ~6.0 — thus activating qE at physiological lumen pH values.

spectroscopy revealed that qE and quenched state of LHCII are both characterized by a red-shifted room temperature fluorescence component [127,145]. Based on the spectral changes described it was proposed that the qE quencher could be a consequence of pigment–pigment interactions within the LHCII antenna [23,41,112]. Homo- or heterodimers of chlorophyll have been proposed to be involved in the quenching [23,112,127,128,147]. Indeed, chlorophyll associates, permanent or transient, were known to possess very low fluorescence yield and similar spectroscopic features to those observed in aggregates of LHCII [148,149]. Hence, Crofts and Yerkes in addition/as opposed to LHCII aggregation model proposed formation of Chl–Chl dimers in the minor LHCII antenna triggered by the protonation of the lumen-exposed glutamates [150]. However, it must be pointed out that the maximum fluorescence red shift in quenched LHCII was discovered to be only about 4 nm (~80 cm^{-1}) [33,145]. Although in some configurations chlorophyll aggregates can be only slightly red-shifted, the alternative explanation for the observed spectral alterations in the Chl *a* region and their relationship to the *quencher* could be the influence of the environment: protein, lipid or xanthophyll [41,112]. Nothing is known so far about the role of lipids and specific amino-acids in the process of *quencher* formation. However, the changes in xanthophyll absorption in the quenched state described above were paralleled by distinct alterations in neoxanthin configuration *in vitro* as well as *in vivo* [151–154]. Since qE was present in plants lacking neoxanthin it is unlikely that changes in this pigment directly caused quenching [155]. In addition, the well-documented absorption change at 535 nm associated with qE was found to originate from a sub-population of red-shifted xanthophyll cycle carotenoids, zeaxanthin and violaxanthin [156–158]. This absorption was only observed *in vitro* when LHCII contained significant amounts of these xanthophylls and was absent without them [33,45,157]. The red-shifted xanthophyll cycle carotenoids were concluded to have rather indirect role in qE as indicators of LHCII aggregation [153,157,158]. The conclusion is based, first of all, upon the lack of their coupling to chlorophylls revealed by fluorescence excitation spectroscopy [145] and secondly since they were not required for quenching in aggregated LHCII [41]. Finally, in the *Arabidopsis lut2npq2* mutant possessing zeaxanthin as the only xanthophyll the correlation between 535 nm absorption and qE is broken [137].

5.2. Quenching in single LHCII complexes

In spite of the abundant biochemical and spectroscopic evidence suggesting that LHCII aggregation is involved in qE the model has

been subjected to various criticism. The possibility that single trimeric LHCII complexes could undergo a conformational change or switch into a photoprotective state was opposed notably by Kühlbrandt's group [159,160]. Indeed, in the past quenching in aggregates of antenna complexes was proposed to be a result of existence of a small number of permanently dissipating complexes [161]. Aggregation was proposed to enhance energetic interactions between many LHC complexes enabling permanently quenched complexes to quench the fluorescence of connected unquenched LHCs. Therefore, the *change* and *switch* into the quenched state were a matter of connectivity in antenna. Furthermore, it was proposed that the quenching in the major LHCII aggregates as well as the aggregation process itself could be totally artificial processes: the former, due to the constant presence of nonspecific quenching admixtures in the preparation media; and the latter due to the upside-down orientation of LHCs in the large aggregates [159,160]. However, Pascal and coworkers discovered using FLIM, 77 K fluorescence and resonance Raman spectroscopy that fluorescence of the major LHCII crystals (structure of which was solved at 2.72 Å resolution) was quenched and the state of neoxanthin corresponded to the state found in quenched aggregated LHCII [147]. The authors concluded that the available atomic structure corresponds to a quenched rather than light harvesting state of LHCII. The group of Kühlbrandt continued to argue, producing evidence that the 77 K fluorescence spectra could have been distorted due to artifacts [162]. Nevertheless, the fluorescence lifetime they obtained for their crystals was ~1 ns — very similar to the lifetime published by Pascal and coworkers [147]. However, the first group of authors argued that the structure was unquenched while the second was quenched, but not as much as aggregated LHCII [163]. It is interesting to note that the other spectroscopic signatures of the crystals, such as the neoxanthin and Chl *b* Raman fingerprints, were very typical for the quenched LHCII state [146,151–153]. It seems that the crystals containing violaxanthin as the only xanthophyll cycle carotenoid were simply dissipating energy not as much as LHCII with zeaxanthin but with similar efficiency to chloroplasts or LHCII containing violaxanthin [127,136,139,145]. Hence, the crystals are quenched to the anticipated extent consistent with their xanthophyll composition.

In addition to the crystal spectroscopy several lines of evidence emerged that apparently convinced the group of Kühlbrandt to accept the LHCII aggregation system as a viable model for qE and join the search for the *quencher* in aggregates [164]. Firstly, the support for the conformational change within the monomeric LHCII unit came from experiments using binary reagents — cross-linkers. Glutaraldehyde was found to inhibit formation as well as relaxation of both, quenching in LHCII upon aggregation and qE [145]. Secondly, high hydrostatic pressure induced quenching in the absence of aggregation [165]. The quenching revealed spectroscopic and thermodynamic features typical for the aggregated LHCII [41,45,166]. Thirdly, strong quenching was obtained in LHCII polymerized into polyacrylamide or gelatin gels displaying all features of aggregated quenched LHCII, including the reaction to cross-linkers and fluorescence lifetime spectra [33]. Fourthly, the group of van Grondelle discovered recently, using single molecule fluorescence spectroscopy, that individual LHCII trimers possess an intrinsic capacity to reversibly switch between quenched and unquenched states [167]. The listed work provides strong support for the view that for qE a conformational transition between unquenched and quenched LHCII unit underlies the molecular switch that produces the act of quenching or generates the *quencher*[68,112,168].

Currently there are two models proposed to explain the molecular features of the *switch* that activates quenching in LHCII complexes. Fig. 8A shows the model of Bassi and coworkers, that suggests zeaxanthin binds into the L2 site in the minor antenna complexes, CP29, CP26 and CP24 and forms a quenching association with the two chlorophylls, A_5 (a603) and B_5 (b609) [168,169]. The second model of the qE switch is based upon the major LHCII (but does not principally exclude the minor antenna) (Fig. 8B). The model proposes small conformational or configurational alteration within the monomeric unit of the complex that causes twist in the neoxanthin molecule (shown by the arrow) and simultaneous (co-operative) movement of lutein bound to L1 site (shown by three arrows). Since this lutein is very closely located to the terminal emitter locus containing Chl a610–612 (A1, A2 and B2) it is proposed that protonation that causes the change in the protein structure brings lutein close enough to these pigments (almost at the van-der-Waals contact) to act as the terminal emitter chlorophyll excited state quencher [154].

6. Physical mechanism(s) of the qE *quencher(s)*

6.1. Excited states and quenching

Chlorophyll excitation energy is dissipated via three main channels: 1) energy transfer to another pigment, 2) fluorescence or 3) internal conversion to the ground state (loss as heat). Energy may also be lost via intersystem crossing to a low-lying triplet state followed by phosphorescence. However, these processes are several orders of magnitude slower than the three main processes mentioned above. NPQ relies upon either an increase in the efficiency of internal conversion via modification of the environment and/or molecular configuration of the fluorescent species (thus itself becoming a quencher) or the connection of a species with an intrinsically high internal conversion rate (quencher) to the fluorescent species. Typically in ethanol the excited state lifetime of chlorophyll *a* is long, ~5 ns. Within the isolated LHCII trimer this is reduced to ~4 ns and when this complex is incorporated into the membrane the lifetime is further reduced to ~2 ns (in the Fm state when all PSII reaction centers are closed by light) [145,170]. Thus, even without the presence of ΔpH there is already significant (60–70%) quenching of chlorophyll incorporated into the complexes of the thylakoid membrane. Yet, at the concentration present in the thylakoid membrane (>0.6 M), chlorophyll would be nearly completely quenched in organic solvent by concentration quenching effects [148]. Thus, it is actually a remarkable achievement of nature that chlorophyll is relatively unquenched in the membrane. This can in part be attributed to the very specific microenvironment provided by the ligating protein to each pigment it binds, we refer to as the 'programmed solvent'. Upon formation of qE in the presence of ΔpH the chlorophyll excited state lifetime in the PSII antenna is further reduced to ~0.6–0.4 ns [127,128,145,170]. Thus, the key question for qE researchers is what is the physical cause of this further reduction in lifetime when qE is formed and does it have the same or a different nature to the 'pre-quenching' seen at Fm?

6.2. Xanthophylls — natural born quenchers?

Xanthophylls possess two low-lying singlet excited states which are known as S_1 (2Ag) and S_2 (1Bu), according to their spatial symmetry. The S_1 state has the same spatial symmetry as the ground (S_0 or $1A_g$) and, in accordance with the selection rules for electronic transitions, is dipole-forbidden. The S_2 state possesses a different spatial symmetry to the ground state and is therefore dipole-allowed, meaning it is connected to the ground state via an electric dipole transition. Upon excitation of the S_2 state there is rapid (~300 fs) internal conversion to the S_1 state. The latter possesses an extremely short lifetime (~10 ps) likely due to the crossing of its potential energy surface with that of the ground state (at a so called conical intersection). The short lifetime and close proximity of the S_1 state energy to that of the lowest excited state of chlorophyll in the Q_y band arguably makes xanthophylls 'natural born quenchers'.

6.2.1. The molecular gearshift

The discovery by Demmig-Adams and co-workers of the zeaxanthin effect upon qE led them to the suggestion that this pigment could

act as the qE *quencher*[47]. This idea was elaborated by Thomas Owens, Harry Frank and co-workers who calculated, using the 'energy gap-law', that the energy of the S_1 state of zeaxanthin should lie below that of chlorophyll *a*, allowing it to act as an energy acceptor [171,172]. In contrast, the S_1 energy of violaxanthin was predicted to lie above that of chlorophyll *a* and was thus predicted to be an energy donor. The xanthophyll cycle was thus proposed to act as a 'molecular gearshift' activating qE when zeaxanthin was formed. However, it was later shown experimentally using transient absorption (TA) and fluorescence spectroscopy that the S_1 energy of both violaxanthin and zeaxanthin lie below that of chlorophyll *a*, first in organic solution and then in LHCII itself [173–175]. These data indicated that the relative positioning of the energy levels of the various xanthophylls alone could not explain quenching. However, both of these methods are likely to have measured the energy of the relaxed S_1 state i.e. the energy after the nuclear geometry of the molecule has relaxed to accommodate the excitation. Energy transfer however predominantly occurs into the vertical state i.e. the excited state prior to relaxation. For instance Dreuw has calculated that the relaxation energy of the excited states of violaxanthin and zeaxanthin is rather high (0.2 eV) and so conceivably the vertical energies may be correctly positioned for the gearshift to work [176].

6.2.2. Excitonic coupling between chlorophylls and xanthophylls

The first evidence for direct carotenoid involvement in qE was provided by Fleming and co-workers with TA studies on thylakoid membranes [177]. They showed that upon chlorophyll excitation in the NPQ state an instantaneous population of a xanthophyll S_1 state was observed, which was attributed to zeaxanthin based on comparisons of various xanthophyll mutants [177]. Recently, a similar instantaneous rise of a xanthophyll S_1 state was observed in quenched LHCII aggregates devoid of zeaxanthin by Walla and co-workers [164]. This observation and those made by the same group using two-photon excitation in LHCII aggregates and on leaves in the qE state have led to the suggestion that excitonic interactions between chlorophylls and xanthophylls are involved in quenching [164,178,179]. Excitonic coupling is realized between two pigments when the exciton transfer integral coupling the two molecules (a measure of the energy interaction between the electronic transitions of the two molecules) is much greater than the de-phasing energy (a measure of the interaction between a molecule and its environment) [180]. Two pigment molecules are said to be excitonically coupled if excitation energy can be coherently transferred between them. The molecular dimer then behaves as a single quantum mechanical entity and the excitation is delocalized across both molecules. Thus, excitonic coupling could explain the instantaneous appearance of the xanthophyll S_1 signal upon chlorophyll excitation. In principle, such excitonic interactions could result in low-lying states that possess more carotenoid than chlorophyll character showing enhanced coupling to the ground state, thus making them efficient quenchers. It is possible however that a distortion of the xanthophyll brought about during quenching could allow direct excitation of S_1 by removing the symmetry restriction that makes the state forbidden and arguably this could explain the instantaneous rise. Indeed, Raman spectroscopy has found that neoxanthin is distorted upon qE formation [146,151–153].

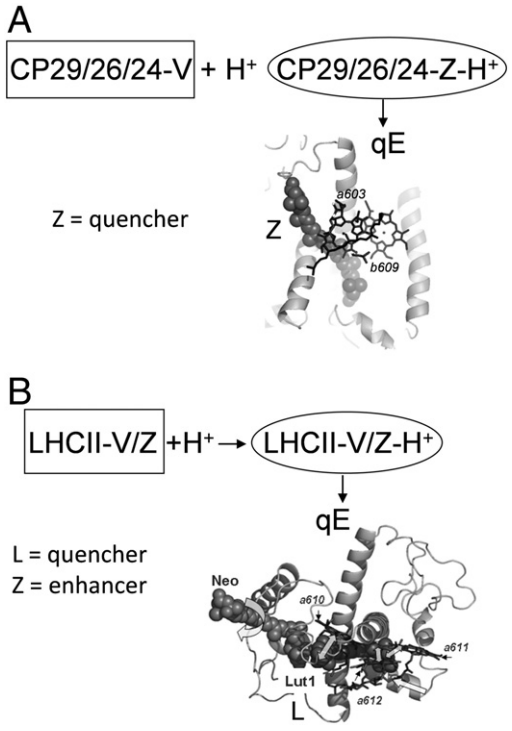

Fig. 8. Atomic structure level models of possible quenching sites. (A) Bassi and co-workers [183,184] propose that protonation of the PSII minor antenna complexes leads to a conformational change leading to formation of a xanthophyll–chlorophyll quenching interaction between zeaxanthin bound at the L2 site and chlorophylls A_5 and B_5. (B) van Grondelle and co-workers [154] propose that protonation of LHCII leads to a conformational change causing a quenching interaction between the lutein bound at the L1 site and chlorophylls *a*612–*a*611–*a*610. Neoxanthin distortion is a spectroscopic signature of this conformational change.

6.2.3. Involvement of xanthophyll–chlorophyll charge transfer states in quenching

When two molecules are coherently coupled the lowest lying excited states mix resulting in two delocalized excitonic states (as mentioned above) and additionally two charge transfer states which correspond to the hole (the positive part of the exciton) and the electron being localized to individual molecules within the dimer [180]. For a homodimer i.e. one for which the individual molecular excited states have very similar excitation energies these charge transfer states lie above the excitonic states. Conversely a heterodimer composed of molecules with very different excitation energies the charge transfer states generally lie below the excitonic states. Under certain circumstances it is energetically favorable for the charge transfer state to dissociate via charge separation into an anion and a cation. The hole and electron that make up the exciton are no longer bound together and the exciton is destroyed, charge recombination between the anion and cation follows and the energy is dissipated as heat. Dreuw and co-workers thus recognized that in the event of xanthophyll involvement in quenching the charge transfer states may play a role in the mechanism and the cation could provide a spectroscopic signature to search for in the near infra-red region (~900–1000 nm) [181]. A xanthophyll radical cation signal with a rise time of ~11 ps, attributed to zeaxanthin, was detected in thylakoids in the qE state upon chlorophyll excitation by Fleming and co-workers using TA spectroscopy [182]. As with the xanthophyll S_1 signal detected in the visible region the extent of the cation signal scaled with the amount of quenching [183]. The same zeaxanthin cation signal was observed in isolated PSII minor antenna complexes and was shown to be dependent in the case of CP29 on the presence of chlorophylls A_5 and B_5 in close proximity to the xanthophyll L2 site (Fig. 8A) [183,184]. It was thus suggested that the charge transfer state forms by delocalization of the electron across the two chlorophylls. Fleming and co-workers were unable to detect the zeaxanthin cation in LHCII trimers although it was later observed by Wachtveitl and co-workers who created it directly via two-photon ionization spectroscopy [185,186]. However, whether in LHCII or the PSII minor antenna, the presence of the zeaxanthin radical cation has not yet been found to be associated with any significant quenching of chlorophyll fluorescence compared to violaxanthin containing complexes [184–186]. Whether the zeaxanthin radical cation is able to

better act as a quencher *in vivo* via interaction with ΔpH, PsbS or some other factors as suggested by Fleming and co-workers remains to be established [184]. Recently, lutein cations have also been detected in the PSII minor antenna proteins and these have been suggested to play a role in quenching, particularly under circumstances when zeaxanthin is absent [187,188].

6.2.4. Quenching via incoherent interactions between xanthophylls and chlorophylls

Transient absorption studies on LHCII aggregates performed by van Grondelle and co-workers, also revealed the transient population of a xanthophyll S_1 state upon chlorophyll excitation [154]. The position of the S_0–S_2 bleach in the TA kinetics indicated that the species involved was lutein 1 rather than zeaxanthin which was absent from the prepared complexes. In contrast to the work of van Grondelle and coworkers, Fleming found no evidence to indicate that a carotenoid radical cation was involved in the quenching [154]. Also unlike the work by groups of Walla and Fleming the rise time of the xanthophyll S_1 state was not instantaneous but rather only peaked after ~20–40 ps. van Grondelle and co-workers proposed a model in which conformational change in LHCII brought about by aggregation opens a channel for energy transfer from chlorophyll to the lutein S_1 state (Fig. 8B[154]). Such a mechanism would invoke incoherent coupling between the states for which the coupling between molecules is much weaker than the coupling of each molecule to its local environment (the opposite limit to excitonic (coherent) interactions). Transfer of energy between them is thus said to occur 'incoherently', hopping from one to another while at any time being localized on a single molecule, the short lifetime of the xanthophyll S_1 state in this case making it an efficient quencher. Förster classically described incoherent energy transfer between dipole-allowed states, via interactions between the transition dipole moments of each molecule [189]. By this logic Förster transfer to the forbidden xanthophyll S_1 state cannot occur. Generalized Förster theory however, which takes into account the size of the molecule does permit transfer to and from forbidden states [190]. Alternatively there is the exchange interaction-mediated Dexter mechanism that describes the incoherent transfer of energy between two molecules whose electronic orbitals closely overlap, allowing electron exchange [191].

6.3. Chlorophylls as quenchers — back to the future

The consensus that was emerging around the involvement of carotenoids in qE has recently been challenged however. Using TA spectroscopy on LHCII aggregates Holzwarth and co-workers failed to find any evidence of carotenoid involvement in quenching [192]. However, it is worth noting that pure TA kinetics in both the study of Ruban et al.[154] and Muller et al.[192] are nearly identical suggesting that it is the modeling approaches used by each group that have resulted in the radically different interpretations of the data. Holzwarth and co-workers have suggested that the red-emitting states formed during NPQ *in vivo* and in LHCII aggregates are related to the formation of coherent chlorophyll–chlorophyll interactions with charge transfer character [127,192]. Chlorophyll–chlorophyll charge transfer states are characterized by enhanced coupling to the ground state and can thus potentially act as efficient quenchers. Normally the charge transfer states lay above the excitonic states for two interacting chlorophylls, however in principle an anisotropic environment could invert this order creating low-lying charge transfer states. These ideas are similar to earlier proposals by Ruban et al.[41] who linked quenching to appearance of red-shifted chlorophyll *a* absorption (683–687 nm) and 77 K fluorescence bands (F700) [29,41,57]. Such red-shifted spectra are typical of excitonically coupled chlorophylls. These red-shifted fluorescence emission bands were found to be temperature dependent being nearly absent at room temperature while growing rapidly in amplitude as the temperature is lowered toward 77 K and below, changes that coincided with a gradual cancellation of quenching [41,193]. Cancellation of quenching at low temperature can be the result of suppression of molecular vibrations. Calculations of the mean vibronic (phonon) energy and electron–phonon coupling for the F700 emission bands indicated that both quenching and the temperature dependency could in principle be explained by coupling of the electronic transition of this state to low frequency vibrations within LHCII aggregates [41]. However, time-resolved fluorescence experiments showed that the amplitude of the lifetime of the F700 band was much longer than that of the main F680 band in LHCII aggregates, suggesting that it may not be the quenching species [194].

Discussions about the exact photo-physical origins of the quencher thus remain the subject of great debate in the NPQ field. New approaches in quantum mechanical modeling of the excited states of carotenoids and chlorophylls should provide new insights in the future into the complex interactions of these molecules. While, new experimental techniques such as femtosecond stimulated Raman spectroscopy may reveal new details of the fates of excitation energy in quenched systems.

7. Conclusions

We have reviewed the current state of mechanistic knowledge about the photoprotective molecular mechanism of qE active in plants and some algae. Four key elements of qE, *trigger*, *site*, *mechanics* and *quencher (s)* were defined and reviewed in their connection with dynamics and regulation of this process. It must be concluded that there exist several important points in qE research to be addressed in order to build a complete picture of the mechanism of this outstanding, intriguing and long investigated process. Among them are the following: Is there a specific complex responsible for qE or can the process take place in all LHCII complexes, major and minor? Could genetic manipulation approaches help to solve this question? Is there only one qE quencher or several? What is (are) the precise photo-physical mechanism(s) of quenching? How effective is the qE process in the photoprotection of photosystem II and for the crops in general? Is the work on mutations in antenna pigments and proteins feasible for the use in agriculture?

In our view the answers to these questions may not be as clear cut as we desire. For instance, while we take the view that mechanistically qE is a single process that is regulated in a rather complex way, we cannot exclude that the quenching process itself may originate from a nonspecific environmental effect that is felt by all pigments. The fact that the quenching can exist in both, minor and major antenna complexes and the evidence implicating different sets of pigments in quenching is consistent with such a view. One point is clear, that the development of new mechanistic approaches and further integration of the disciplines of biology, chemistry and physics could shed light upon these and other important questions related to the one of the most significant mechanisms of photoprotection and regulation of the light phase of photosynthesis.

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References

[1] S.B. Powles, Photoinhibition of photosynthesis induced by visible-light, Ann. Rev. Plant Physiol. Plant Mol. Biol. 35 (1984) 15–44.
[2] J. Barber, Molecular-basis of the vulnerability of photosystem-II to damage by light, Aust. J. Plant Physiol. 22 (1995) 201–208.
[3] A. Telfer, W.Z., J. Barber, Spectral resolution of more than one chlorophyll electron donor in the isolated photosystem-II reaction center complex, Biochim. Biophys. Acta 1017 (1990) 143–151.
[4] I. Ohad, D.J. Kyle, C.J. Arntzen, Membrane-protein damage and repair — removal and replacement of inactivated 32-kilodalton polypeptides in chloroplast membranes, J. Cell Biol. 99 (1984) 481–485.
[5] O. Björkman, B. Demmig-Adams, Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants, in: E.D. Schulze,

M.M. Caldwell (Eds.), Ecophysiology of Photosynthesis: Ecological Studies, Springer-Verlag, Berlin, 1995, pp. 14–47.
[6] O. Björkman, S.B. Powles, Leaf movement in the shade species *Oxalis oregana* L I. Response to light level and light quality, Year B Carnegie Inst. Wash. 80 (1987) 59–62.
[7] D. Koller, Light-driven leaf movements, Plant Cell Environ. 13 (1990) 615–632.
[8] W.S. Chow, J.M. Anderson, A.B. Hope, Variable stoichiometries of photosystem-II to photosystem-I reaction centers, Photosynth. Res. 17 (1988) 277–281.
[9] K. Kloppstech, Light regulation of photosynthetic genes, Physiol. Plant. 100 (1997) 739–747.
[10] J.M. Anderson, W.S. Chow, Y.I. Park, The grand design of photosynthesis: acclimation of the photosynthetic apparatus to environmental cues, Photosynth. Res. 46 (1995) 129–139.
[11] B. Andersson, E.M. Aro, Proteolytic activities and proteases of plant chloroplasts, Physiol. Plant. 100 (1997) 780–793.
[12] J.M. Anderson, W.S. Chow, D.J. Goodchild, Thylakoid membrane organisation in sun/shade acclimation, Aust. J. Plant Physiol. 15 (1988) 11–26.
[13] E. Weis, J.A. Berry, Quantum efficiency of Photosystem-II in relation to energy-dependent quenching of chlorophyll fluorescence, Biochim. Biophys. Acta 894 (1987) 198–208.
[14] C.A. Wraight, A.R. Crofts, Energy-dependent quenching of chlorophyll *a* fluorescence in isolated chloroplasts, Eur. J. Biochem. 17 (1970) 319–327.
[15] J.-M. Briantais, C. Vemotte, M. Picaud, G.H. Krause, A quantitative study of the slow decline of chlorophyll *a* fluorescence in isolated chloroplasts, Biochim. Biophys. Acta 548 (1979) 128–138.
[16] B. Genty, J.-M. Briantais, N.R. Baker, The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence, Biochim. Biophys. Acta 990 (1989) 87–92.
[17] U. Schreiber, Detection of rapid induction kinetics with a new type of high-frequency modulated chlorophyll fluorometer, Photosynth. Res. 9 (1986) 261–272.
[18] K. Oxborough, P. Horton, A study of the regulation and function of energy-dependent quenching in pea-chloroplasts, Biochim. Biophys. Acta 934 (1988) 135–143.
[19] G.H. Krause, E. Weis, Chlorophyll fluorescence and photosynthesis: the basics, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 313–349.
[20] A.V. Ruban, M.P. Johnson, Dynamics of the photosystems cross-section associated with the state transitions in higher plants, Photosynth. Res. 99 (2009) 173–183.
[21] J.F. Allen, J. Forsberg, Molecular recognition in thylakoid structure and function, Trends Plant Sci. 6 (2001) 317–326.
[22] P. Horton, A.V. Ruban, Regulation of photosystem II, Photosynth. Res. 34 (1992) 375–385.
[23] P. Horton, A.V. Ruban, R.G. Walters, Regulation of light harvesting in green plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 655–684.
[24] G.H. Krause, Changes in chlorophyll fluorescence in relation to light-dependent cation transfer across thylakoid membranes, Biochim. Biophys. Acta 333 (1974) 301–313.
[25] B. Demmig, K. Winter, A. Kruger, F.C. Czygan, Photoinhibition and zeaxanthin formation in intact leaves: a possible role of the xanthophyll cycle in the dissipation of excess light, Plant Physiol. 84 (1987) 218–224.
[26] A. Krieger, I. Moya, E. Weis, Energy-dependent quenching of chlorophyll a fluorescence: effect of pH on stationary fluorescence and picosecond-relaxation kinetics in thylakoid membranes and photosystem II preparations, Biochim. Biophys. Acta 1102 (1992) 167–176.
[27] A. Krieger, E. Weis, The role of calcium in the pH-dependent control of photosystem II, Photosynth. Res. 37 (1993) 117–130.
[28] P. Horton, A.V. Ruban, Delta-pH-dependent quenching of the Fo-level of chlorophyll fluorescence in spinach leaves, Biochim. Biophys. Acta 1142 (1993) 203–206.
[29] A.V. Ruban, D. Rees, G.D. Noctor, A. Young, P. Horton, Long-wavelength chlorophyll species are associated with amplification of high-energy state excitation quenching in higher-plants, Biochim. Biophys. Acta 1059 (1991) 355–360.
[30] A.V. Ruban, P. Horton, Spectroscopy of non-photochemical and photochemical quenching of chlorophyll fluorescence in leaves; evidence for a role of the light harvesting complex of photosystem II in the regulation of energy dissipation, Photosynth. Res. 40 (1994) 181–190.
[31] B. Genty, Y. Goulas, B. Dimon, G. Peltier, J.-M. Briantais, I. Moya, Modulation of efficiency of primary conversion in leaves, Photosynth. Res. 34 (1992) 106.
[32] C.W. Mullineaux, A.V. Ruban, P. Horton, Prompt heat release associated with delta-pH dependent quenching in spinach thylakoid membranes, Biochim. Biophys. Acta 1185 (1994) 119–123.
[33] C. Ilioaia, M. Johnson, P. Horton, A.V. Ruban, Induction of efficient energy dissipation in the isolated light harvesting complex of photosystem II in the absence of protein aggregation, J. Biol. Chem. 283 (2008) 29505–29512.
[34] G. Noctor, A.V. Ruban, P. Horton, Modulation of ΔpH-dependent nonphotochemical quenching of chlorophyll fluorescence in spinach-chloroplasts, Biochim. Biophys. Acta 1183 (1993) 339–344.
[35] A.V. Ruban, A. Young, P. Horton, Modulation of chlorophyll fluorescence quenching in isolated light-harvesting complex of photosystem-II, Biochim. Biophys. Acta 1186 (1994) 123–127.
[36] J.-M. Briantais, Light harvesting chlorophyll a–b complex requirement for regulation of photosystem II photochemistry by nonphotochemical quenching, Photosynth. Res. 40 (1994) 287–294.
[37] P. Jahns, G.H. Krause, Xanthophyll cycle and energy-dependent fluorescence quenching in leaves from pea plants grown under intermittent light, Planta 192 (1994) 176–182.
[38] H. Lokstein, H. Härtel, P. Hoffmann, P. Woitke, G. Renger, The role of light-harvesting complex II in excess excitation energy dissipation: an *in vivo* fluorescence study on the origin of high energy quenching, J. Photochem. Photobiol. B 26 (1994) 174–185.
[39] A.V. Ruban, R.G. Walters, P. Horton, The molecular mechanism of the control of excitation energy dissipation in chloroplast membranes: inhibition of ΔpH-dependent quenching of chlorophyll fluorescence by dicyclohexylcarbodiimide, FEBS Lett. 309 (1992) 175–179.
[40] R.G. Walters, A.V. Ruban, P. Horton, Light-harvesting complexes bound by dicyclohexylcarbodiimide during inhibition of protective energy dissipation, Eur. J. Biochem. 226 (1994) 1063–1069.
[41] A.V. Ruban, P. Horton, Mechanism of ΔpH-dependent dissipation of absorbed excitation energy by photosynthetic membranes I: spectroscopic analysis of isolated light-harvesting complexes, Biochim. Biophys. Acta 1102 (1992) 30–38.
[42] A.V. Ruban, D. Rees, A.A. Pascal, P. Horton, Mechanism of ΔpH-dependent dissipation of absorbed excitation energy by photosynthetic membranes II: the relationships between LHCII aggregation in vitro and qE in isolated thylakoids, Biochim. Biophys. Acta 1102 (1992) 39–44.
[43] A.V. Ruban, A.J. Young, P. Horton, Dynamic properties of the minor chlorophyll a/b binding proteins of photosystem II — an *in vitro* model for photoprotective energy dissipation in the photosynthetic membrane of green plants, Biochemistry 35 (1996) 674–678.
[44] D. Phillip, A.V. Ruban, P. Horton, A. Asato, A.J. Young, Quenching of chlorophyll fluorescence in the major light-harvesting complex of photosystem II: a systematic study of the effect of carotenoid structure, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 1492–1497.
[45] A.V. Ruban, F. Calkoen, S.L.S. Kwa, R. van Grondelle, P. Horton, J.P. Dekker, Characterisation of the aggregated state of the light harvesting complex of photosystem II by linear and circular dichroism spectroscopy, Biochim. Biophys. Acta 1321 (1997) 61–70.
[46] K.K. Niyogi, C. Shih, W.S. Chow, B.J. Pogson, D. DellaPenna, O. Björkman, Photoprotection in a zeaxanthin and lutein deficient double mutant of *Arabidopsis*, Photosynth. Res. 67 (2001) 139–145.
[47] B. Demmig-Adams, Carotenoids and photoprotection: a role for the xanthophyll zeaxanthin, Biochim. Biophys. Acta 1020 (1990) 1–24.
[48] B. Demmig-Adams, W.W. Adams III, Photoprotection and other responses of plants to high light stress, Annu. Rev. Plant Physiol. Plant Mol. Biol. 43 (1992) 599–626.
[49] D.I. Sapozhnikov, T.A. Kransovskaya, A.N. Maevskaya, Change in the interrelationship of the basic carotenoids of the plastids of green leaves under the action of light, Dokl. Acad. Nauk USSR 113 (1957) 465–467.
[50] H.Y. Yamamoto, T.O.M. Nakayama, C.O. Chichester, Studies on the light and dark interconversions of leaf xanthophylls, Arch. Biochem. Biophys. 97 (1962) 168–173.
[51] A. Hager, Die Zusammenhange zwischen Lichtinduzierten Xanthophyll-Umwandlungen und Hill-Reaktion, Ber. Dtsch. Bot. Ges. 79 (1966) 94–107.
[52] M. Eskling, The xanthophyll cycle, its regulation and components, Physiol. Plant. 100 (1997) 806–816.
[53] P. Jahns, D. Latowski, K. Strzalka, Mechanism and regulation of the violaxanthin cycle: the role of antenna proteins and membrane lipids, Biochim. Biophys. Acta 1787 (2009) 3–14.
[54] B. Demmig-Adams, K. Winter, A. Krüger, F.-C. Czygan, Light response of CO_2 assimilation, dissipation of excess excitation energy, and zeaxanthin content of sun and shade leaves, Plant Physiol. 90 (1989) 881–886.
[55] B. Demmig-Adams, K. Winter, A. Kruger, F.-Z. Czygan, Zeaxanthin synthesis, energy dissipation, and photoprotection of photosystem II at chilling temperatures, Plant Physiol. 90 (1989) 894–898.
[56] N.R. Baker, P. Horton, Physiological factors associated with fluorescence quenching during photoinhibition, in: C.J. Arntzen, D.J. Kyle, C.B. Osmond (Eds.), Topics in Photosynthesis, Photoinhibition, vol. 9, Elsevier, Amsterdam, 1987, pp. 145–168.
[57] A.V. Ruban, A.J. Young, P. Horton, Induction of nonphotochemical energy dissipation and absorbance changes in leaves. Evidence for changes in the state of the light-harvesting system of photosystem II *in vivo*, Plant Physiol. 102 (1993) 741–750.
[58] A.V. Ruban, P. Horton, An investigation of the sustained component of nonphotochemical quenching of chlorophyll fluorescence in isolated chloroplasts and leaves of spinach, Plant Physiol. 108 (1995) 721–726.
[59] P. Horton, A. Ruban, The role of LHCII in energy quenching, in: N.R. Baker, J.R. Bowyer (Eds.), Photoinhibition of Photosynthesis — From Molecular Mechanisms to the Field, Bios Scientific Publishers, Oxford, 1994, pp. 111–128.
[60] P. Horton, A.V. Ruban, R.G. Walters, Regulation of light harvesting in green plants. Indication by nonphotochemical quenching of chlorophyll fluorescence, Plant Physiol. 106 (1994) 415–420.
[61] M. Nilkens, E. Kress, P. Lambrev, Y. Miloslavina, M. Müller, A.R. Holzwarth, P. Jahns, Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated under steady-state conditions in *Arabidopsis*, Biochim. Biophys. Acta 1797 (2010) 466–475.
[62] A.V. Ruban, J. Lavaud, B. Rousseau, G. Guglielmi, P. Horton, A.-L. Etienne, The super-excess energy dissipation in diatom algae: comparative analysis with higher plants, Photosynth. Res. 82 (2004) 165–175.
[63] G. Oquist, W.S. Chow, J.M. Anderson, Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II, Planta 186 (1992) 450–460.
[64] G. Oquist, N.P.A. Huner, Photosynthesis of overwintering evergreen plants, Annu. Rev. Plant Biol. 54 (2003) 329–355.

[65] D. Rees, A. Young, G. Noctor, G. Britton, P. Horton, Enhancement of the ΔpH-dependent dissipation of excitation energy in spinach chloroplasts by light-activation; correlation with the synthesis of zeaxanthin, FEBS Lett. 256 (1989) 85–90.
[66] G. Noctor, D. Rees, A. Young, P. Horton, The relationship between zeaxanthin, energy-dependent quenching of chlorophyll fluorescence and the transthylakoid pH-gradient in isolated chloroplasts, Biochim. Biophys. Acta 1057 (1991) 320–330.
[67] D. Rees, G. Noctor, A.V. Ruban, J. Crofts, A. Young, P. Horton, pH dependent chlorophyll fluorescence quenching in spinach thylakoids from light-treated or dark-adapted leaves, Photosynth. Res. 31 (1992) 11–19.
[68] P. Horton, A.V. Ruban, M. Wentworth, Allosteric regulation of the light harvesting system of photosystem II, Philos. Trans. R. Soc. Lond. B 355 (2000) 1361–1370.
[69] A.V. Ruban, P. Horton, The xanthophyll cycle modulates the kinetics of nonphotochemical energy dissipation in isolated light-harvesting complexes, intact chloroplasts, and leaves of spinach, Plant Physiol. 119 (1999) 531–542.
[70] P. Horton, A.V. Ruban, D. Rees, A.A. Pascal, G. Noctor, A.J. Young, Control of the light-harvesting function of chloroplast membranes by aggregation of the LHCII chlorophyll–protein complex, FEBS Lett. 292 (1991) 1–4.
[71] A.V. Ruban, D. Phillip, A.J. Young, P. Horton, Carotenoid-dependent oligomerization of the major chlorophyll a/b light harvesting complex of photosystem II of plants, Biochemistry 36 (1997) 7855–7859.
[72] J. Harbinson, C. Hedley, The kinetics of P700 reduction in leaves: a novel in situ probe of thylakoid functioning, Plant Cell Environ. 12 (1989) 357–369.
[73] D.M. Kramer, C.A. Sacksteder, J.A. Cruz, How acidic is the lumen? Photosynth. Res. 60 (1999) 151–163.
[74] A.V. Ruban, P. Horton, Regulation of nonphotochemical quenching of chlorophyll fluorescence in plants, Aust. J. Plant Physiol. 22 (1995) 221–230.
[75] A.M. Gilmore, S. Matsubara, M.C. Ball, D.H. Barker, S. Itoh, Excitation energy flow at 77 K in the photosynthetic apparatus of overwintering evergreens, Plant Cell Environ. 26 (2003) 1021–1034.
[76] P. Siffel, F. Vacha, Aggregation of the light-harvesting complex in intact leaves of tobacco plants stressed by CO_2 deficit, Photochem. Photobiol. 67 (1998) 304–311.
[77] P. Siffel, Z. Braunova, Release and aggregation of the light-harvesting complex in intact leaves subjected to strong CO_2 deficit, Photosynth. Res. 61 (1999) 217–226.
[78] M.-H. Oh, Y.-H. Moon, C.-H. Lee, Increased stability of LHCII by aggregate formation during dark-induced leaf senescence in the *Arabidopsis* mutant, *ore10*, Plant Cell Physiol. 44 (2003) 1368–1377.
[79] P. Jahns, W. Junge, Dicyclohexylcarbodiimide-binding proteins related to the short-circuit of the proton-pumping activity of photosystem II, J. Biochem. 193 (1990) 731–736.
[80] P. Jahns, W. Junge, Another role of chlorophyll alb binding proteins of higher plants: they modulate proteolytic reactions associated with photosystem II, Photochem. Photobiol. 57 (1993) 120–124.
[81] P. Jahns, A. Polle, W. Junge, The photosynthetic water oxidase: its proton pumping activity is short-circuited within the protein by DCCD, EMBO J. 7 (1988) 589–594.
[82] A.V. Ruban, A. Young, A. Pascal, P. Horton, The effects of illumination on the xanthophyll composition of the photosystem II light harvesting complexes of spinach thylakoid membranes, Plant Physiol. 104 (1994) 227–234.
[83] P.J.G. Butler, W. Kühlbrandt, Determination of the aggregate size in detergent solution of the light-harvesting chlorophyll a/b-protein complex from chloroplast membranes, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 3797–3801.
[84] R. Bassi, P. Dainese, A supramolecular light-harvesting complex from chloroplast photosystem II membranes, Eur. J. Biochem. 204 (1992) 317–326.
[85] R. Bassi, B. Pineau, P. Dainese, J. Marquardt, Carotenoid binding proteins of photosystem II, Eur. J. Biochem. 212 (1993) 297–303.
[86] R.G. Walters, A.V. Ruban, P. Horton, Identification of proton-active residues in a higher plant light-harvesting complex, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 14204–14209.
[87] A.A. Pascal, Spinach LHCII: spectral and biochemical changes associated with aggregation and with violaxanthin de-epoxidation, PhD Thesis. University of Sheffield, 1995.
[88] E.K. Zolotareva, V.V. Podorvanov, A.F. Tereshchenko, A.V. Ruban, P. Horton, Energy-dependent tritium incorporation into LHCII proteins of chloroplasts, Dokl. Acad. Sci. Ukr. SSR B 11 (1999) 152–156.
[89] A. Färber, A.J. Young, A.V. Ruban, P. Horton, P. Jahns, Dynamics of xanthophyll cycle activity in different antenna subcomplexes in the photosynthetic membranes of higher plants, Plant Physiol. 115 (1997) 1609–1618.
[90] A.V. Ruban, P.J. Lee, M. Wentworth, A.J. Young, P. Horton, Determination of the stoichiometry and strength of binding of different xanthophylls to the photosystem II light harvesting complexes, J. Biol. Chem. 274 (1999) 10458–10465.
[91] Z.F. Liu, H.C. Yan, K.B. Wang, T.Y. Kuang, J.P. Zhang, L.L. Gui, X.M. An, W.R. Chang, Crystal structure of spinach major-light harvesting complex at 2.72 Å resolution, Nature 428 (2004) 287–292.
[92] A.V. Ruban, P. Pesaresi, U. Wacker, K.-D.J. Irrgang, R. Bassi, P. Horton, The relationship between the binding of dicyclohexylcarbodiimide and pH-dependent quenching of chlorophyll fluorescence in the light harvesting proteins of photosystem II, Biochemistry 37 (1998) 11586–11591.
[93] M. Ballottari, J. Girardon, N. Betterle, T. Morosinotto, R. Bassi, Identification of the chromophores involved in aggregation-dependent energy quenching of the monomeric photosystem II antenna protein Lhcb5, J. Biol. Chem. 285 (2009) 28309–28321.
[94] J. Andersson, R.G. Walters, P. Horton, S. Jansson, Antisense inhibition of the photosynthetic antenna proteins CP29 and CP26: implications for the mechanism of protective energy dissipation, Plant Cell 13 (2001) 1193–1204.
[95] L. Kovacs, J. Damkjær, S. Kereïche, C. Ilioaia, A.V. Ruban, E.J. Boekema, S. Jansson, P. Horton, Lack of the light harvesting complex CP24 affects the structure and function of the grana membranes of higher plant chloroplasts, Plant Cell 18 (2006) 3106–3120.
[96] S. de Bianchi, L. Dall'Osto, G. Tognon, T. Morosinotto, R. Bassia, Minor antenna proteins CP24 and CP26 affect the interactions between photosystem II subunits and the electron transport rate in grana membranes of *Arabidopsis*, Plant Cell 20 (2008) 1012–1028.
[97] J. Andersson, M. Wentworth, R.G. Walters, C.A. Howard, A.V. Ruban, P. Horton, S. Jansson, Absence of the Lhcb1 and Lhcb2 proteins of the light-harvesting complex of photosystem II — effects on photosynthesis, grana stacking and fitness, Plant J. 35 (2003) 350–361.
[98] A.V. Ruban, M. Wentworth, A.E. Yakushevska, W. Keegstra, P.J. Lee, J.P. Dekker, S. Jansson, E. Boekema, P. Horton, Plants lacking the main light harvesting complex retain photosystem II macro-organisation, Nature 421 (2003) 648–652.
[99] L. Valkunas, G. Trinkunas, J. Chmeliov, A.V. Ruban, Modeling of exciton quenching in photosystem II, Phys. Chem. Chem. Phys. 11 (2009) 7576–7584.
[100] C. Funk, W.P. Schroder, A. Napiwotzki, S.E. Tjus, G. Renger, B. Andersson, The PSII-S protein of higher-plants — a new-type of pigment-binding protein, Biochemistry 34 (1995) 11133–11141.
[101] C. Funk, I. Adamska, B.R. Green, B. Andersson, G. Renger, The nuclear-encoded chlorophyll-binding Photosystem-II-S protein is stable in the absence of pigments, J. Biol. Chem. 270 (1995) 30141–30147.
[102] X.P. XP, O. Björkman, C. Shih, A.R. Grossman, M. Rosenquist, S. Jansson, K.K. Niyogi, A pigment-binding protein essential for regulation of photosynthetic light harvesting, Nature 403 (2000) 391–395.
[103] X.P. Li, P. Muller-Moule, A.M. Gilmore, K.K. Niyogi, PsbS-dependent enhancement of feedback de-excitation protects photosystem II from photoinhibition, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 15222–15227.
[104] M. Aspinall-O'Dea, M. Wentworth, A. Pascal, B. Robert, A.V. Ruban, P. Horton, *In vitro* reconstitution of the activated zeaxanthin state associated with energy dissipation in plants, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 16331–16335.
[105] P. Dominici, S. Caffarri, F. Armenante, S. Ceoldo, M. Crimi, R. Bassi, Biochemical properties of the PsbS subunit of photosystem II either purified from chloroplast or recombinant, J. Biol. Chem. 277 (2002) 22750–22758.
[106] X.P. Li, A.M. Gilmore, S. Caffarri, R. Bassi, T. Golan, D. Kramer, K.K. Niyogi, Regulation of photosynthetic light harvesting involves intrathylakoid lumen pH sensing by the PsbS protein, J. Biol. Chem. 279 (2004) 22866–22874.
[107] X.P. Li, A.M. Gilmore, K.K. Niyogi, Molecular and global time-resolved analysis of a psbS gene dosage effect on pH- and xanthophyll cycle-dependent nonphotochemical quenching in photosystem II, J. Biol. Chem. 277 (2002) 33590–33597.
[108] S. Crouchman, A.V. Ruban, P. Horton, PsbS enhances nonphotochemical fluorescence quenching in the absence of zeaxanthin, FEBS Lett. 580 (2006) 2053–2058.
[109] A Zia, Study of factors controlling the photoprotection capacity of the thylakoid membrane, PhD Thesis. Queen Mary University of London, 2010.
[110] A. Zia, M.P. Johnson, A.V. Ruban, Acclimation- and mutation-induced enhancement of PsbS levels increases photoprotective energy dissipation in the absence of antheraxanthin and zeaxanthin. Planta. doi:10.1007/s00425-011-1380-5.
[111] G. Bonente, B.D. Howes, S. Caffarri, G. Smulevich, R. Bassi, Interactions between the photosystem II subunit PsbS and xanthophylls studied *in vivo* and *in vitro*, J. Biol. Chem. 283 (2008) 8434–8445.
[112] P. Horton, M. Wentworth, A.V. Ruban, Control of the light harvesting function of chloroplast membranes: the LHCII-aggregation model for non-photochemical quenching, FEBS Lett. 579 (2005) 4201–4206.
[113] A.Z. Kiss, A.V. Ruban, P. Horton, The PsbS protein controls the organisation of the photosystem II antenna in higher plant thylakoid membranes, J. Biol. Chem. 283 (2008) 3972–3978.
[114] S. Kereïche, A.Z. Kiss, R. Kouril, E.J. Boekema, P. Horton, The PsbS protein controls the macro-organisation of photosystem II complexes in the grana membranes of higher plant chloroplasts, FEBS Lett. 584 (2010) 754–764.
[115] J. Nield, C. Funk, J. Barber, Supermolecular structure of photosystem II and location of the PsbS protein, Philos. Trans. R. Soc. Lond. B 355 (2000) 1337–1343.
[116] J.P. Dekker, E.J. Boekema, Supramolecular organization of thylakoid membrane proteins in green plants, Biochim. Biophys. Acta 1706 (2005) 12–39.
[117] E. Bergantino, A. Segalia, A. Brunetta, E. Teardo, F. Rogoni, G.M. Giacometti, I. Szabo, Light- and pH-dependent structural changes in the PsbS protein of photosystem II, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15265–15270.
[118] E. Teardo, P.P. de Laureto, E. Bergantino, F. Dalla Vecchia, F. Rigoni, D. Szabo, G.M. Giacometti, Evidences for interaction of PsbS with photosynthetic complexes in maize thylakoids, Biochim. Biophys. Acta 1767 (2007) 703–711.
[119] M.P. Johnson, A.V. Ruban, *Arabidopsis* plants lacking PsbS protein possess photoprotective energy dissipation, Plant J. 61 (2010) 283–289.
[120] J.M. Anderson, The molecular organisation of chloroplast thylakoids, Biochim. Biophys. Acta 416 (1975) 191–235.
[121] J. Barber, W.S. Chow, A mechanism for controlling the stacking and unstacking of chloroplast thylakoid membranes, FEBS Lett. 105 (1979) 5–10.
[122] B. Andersson, J.M. Anderson, Lateral heterogeneity in the distribution of chlorophyll–protein complexes of the thylakoid membranes of spinach chloroplasts, Biochim. Biophys. Acta 593 (1980) 427–440.
[123] J. Barber, Influence of surface charges on thylakoid structure and function, Ann. Rev. Plant Physiol. 33 (1982) 261–295.
[124] J. Barber, J. Mills, J. Nicolson, Studies with cation specific ionophores show that within the intact chloroplast Mg^{2+} acts as the main exchange cation for H^+ pumping, FEBS Lett. 49 (1974) 106–110.
[125] S. Murakami, L. Packer, Light-induced changes in the conformation and configuration of the thylakoid membrane of *Ulva* and *Porphyra* chloroplasts in vivo, Plant Physiol. 45 (1970) 289–299.

[126] S. Murakami, L. Packer, Protonation and chloroplast membrane structure, J. Cell Biol. 47 (1970) 332–351.
[127] Y. Miloslavina, A. Wehner, P.H. Lambrev, E. Wientjes, M. Reus, G. Garab, R. Croce, A.R. Holzwarth, Far-red fluorescence: a direct spectroscopic marker for LHCII oligomer formation in non-photochemical quenching, FEBS Lett. 582 (2008) 3625–3631.
[128] A.R. Holzwarth, Y. Miloslavina, M. Nilkens, P. Jahns, Identification of two quenching sites active in the regulation of photosynthetic light-harvesting studied by time-resolved fluorescence, Chem. Phys. Lett. 483 (2009) 262–267.
[129] N. Betterle, M. Ballottari, S. Zorzan, S. de Bianchi, S. Cazzaniga, L. Dall'Osto, T. Morosinotto, R. Bassi, Light-induced dissociation of an antenna hetero-oligomer is needed for non-photochemical quenching induction, J. Biol. Chem. 284 (2009) 15255–15266.
[130] M.P. Johnson, T.K. Goral, C.D.P. Duffy, A.P.R. Brain, C.W. Mullineaux, A.V. Ruban, Photoprotective energy dissipation involves the reorganization of photosystem II light harvesting complexes in the grana membranes of higher plant chloroplasts. Plant Cell (in press) doi:10.1105/tpc.110.081646.
[131] A.J. Young, D. Phillip, A.V. Ruban, P. Horton, H.A. Frank, The xanthophyll cycle and carotenoid-mediated dissipation of excess excitation energy in photosynthesis, Pure Appl. Chem. 69 (1997) 2125–2130.
[132] A.V. Ruban, D. Phillip, A.J. Young, P. Horton, Excited-state energy level does not determine the differential effect of violaxanthin and zeaxanthin on chlorophyll fluorescence quenching in the isolated light-harvesting complex of photosystem II, Photochem. Photobiol. 68 (1998) 829–834.
[133] A.V. Ruban, P. Horton, A.J. Young, Aggregation of higher plant xanthophylls: differences in absorption spectra and in the dependency on solvent polarity, J. Photochem. Photobiol. B 21 (1993) 229–234.
[134] H. Paulsen, in: H.A. Frank, A.J. Young, G. Britton, R. Cogdell (Eds.), The Photochemistry of Carotenoids, Kluwer Academic Publishers, Dordrecht, 1999, pp. 123–135, First.
[135] H. Lokstein, L. Tian, J.E. Polle, D. DellaPenna, Xanthophyll biosynthetic mutants of *Arabidopsis thaliana*: altered nonphotochemical quenching of chlorophyll fluorescence is due to changes in photosystem II antenna size and stability, Biochim. Biophys. Acta 1553 (2002) 309–319.
[136] M.P. Johnson, A. Zia, P. Horton, A.V. Ruban, Effect of xanthophyll composition on the chlorophyll excited state lifetime in plant leaves and isolated LHCII, Chem. Phys. 373 (2010) 23–32.
[137] M.P. Johnson, M.L. Perez-Bueno, A. Zia, P. Horton, A.V. Ruban, The zeaxanthin-independent and zeaxanthin-dependent qE components of nonphotochemical quenching involve common conformational changes within the photosystem II antenna in *Arabidopsis*, Plant Physiol. 149 (2009) 1061–1075.
[138] M.L. Pérez-Bueno, M.P. Johnson, A. Zia, A.V. Ruban, P. Horton, The Lhcb protein and xanthophyll composition of the light harvesting antenna controls the ΔpH-dependency of nonphotochemical quenching in *Arabidopsis thaliana*, FEBS Lett. 582 (2008) 1477–1482.
[139] A.V. Ruban, M.P. Johnson, Xanthophylls as modulators of membrane protein function, Arch. Biochem. Biophys. 504 (2010) 78–85.
[140] R.L. Thurlkill, G.R. Grimsley, M. Scholtz, C.N. Pace, Hydrogen bonding markedly reduces the pK of buried carboxyl groups in proteins, J. Mol. Biol. 362 (2006) 594–604.
[141] E.L. Mehler, M. Fuxreiter, I. Simon, E.B. Garcia-Moreno, The role of hydrophobic microenvironments in modulating pKa shifts in proteins, Proteins Struc. Funct. Genet. 48 (2002) 283–292.
[142] H. Li, A.D. Robertson, J.H. Jensen, The determinants of carboxyl pK(a) values in Turkey ovomucoid third domain, Proteins 55 (2004) 689–704.
[143] L. Dall'Osto, S. Caffarri, R. Bassi, A mechanism of nonphotochemical energy dissipation, independent from PsbS, revealed by a conformational change in the antenna protein CP26, Plant Cell 17 (2005) 1217–1232.
[144] H.E. Åkerlund, B. Andersson, A. Persson, P.A. Albertsson, Isoelectric points of spinach thylakoid membrane surfaces as determined by cross partition, Biochim. Biophys. Acta 552 (1979) 238–246.
[145] M.P. Johnson, A.V. Ruban, Photoprotective energy dissipation in higher plants involves alteration of the excited state energy of the emitting chlorophyll in LHCII, J. Biol. Chem. 284 (2009) 23592–23601.
[146] A.V. Ruban, B. Robert, P. Horton, Resonance Raman spectroscopy of photosystem II light-harvesting complex of green plants. A comparison of trimeric and aggregated states, Biochemistry 34 (1995) 2333–2337.
[147] A.A. Pascal, Z. Liu, K. Broess, B. van Oort, H. van Amerongen, C. Wang, P. Horton, B. Robert, W. Chang, A. Ruban, Molecular basis of photoprotection and control of photosynthetic light-harvesting, Nature 436 (2005) 134–137.
[148] G.S. Beddard, G. Porter, Concentration quenching in chlorophyll, Nature 260 (1976) 366–367.
[149] A. Agostiano, M. Della Monica, G. Palazzo, M. Trotta, Chlorophyll-a autoaggregation in water-rich region, Biophys. Chem. 47 (1993) 193–202.
[150] A.R. Crofts, C.T. Yerkes, A molecular mechanism for qE-quenching, FEBS Lett. 352 (1994) 265–270.
[151] A.V. Ruban, A. Pascal, B. Robert, Xanthophylls of the major photosynthetic light-harvesting complex of plants: identification, conformation and dynamics, FEBS Lett. 477 (2000) 181–185.
[152] B. Robert, P. Horton, A. Pascal, A.V. Ruban, Insights into the molecular dynamics of plant light-harvesting proteins *in vivo*, Trends Plant Sci. 9 (2004) 385–390.
[153] A.V. Ruban, Identification of carotenoids in photosynthetic proteins: xanthophylls of the light harvesting antenna, in: J.T. Landrum (Ed.), Carotenoids: Physical, Chemical and Biological Functions and Properties, CRC Press, Florida, 2009, pp. 113–136.
[154] A.V. Ruban, R. Berera, C. Ilioaia, I.H. van Stokkum, J.T. Kennis, A.A. Pascal, H. van Amerongen, B. Robert, P. Horton, R. van Grondelle, Identification of a mechanism of photoprotective energy dissipation in higher plants, Nature 450 (2007) 575–578.
[155] K.K. Niyogi, A.R. Grossman, O. Björkman, *Arabidopsis* mutants define a central role for the xanthophyll cycle in regulation of photosynthetic energy conversion, Plant Cell 10 (1998) 1121–1134.
[156] A.V. Ruban, A.A. Pascal, B. Robert, P. Horton, Activation of zeaxanthin is an obligatory event in the regulation of photosynthetic light harvesting, J. Biol. Chem. 277 (2002) 7785–7789.
[157] C. Ilioaia, M.P. Johnson, C.D.P. Duffy, A.A. Pascal, R. van Grondelle, B. Robert, A.V. Ruban, The origin of the absorption changes associated with the photoprotective energy dissipation in the absence of zeaxanthin, J. Biol. Chem. 284 (2011) 91–98.
[158] C.D.P. Duffy, M.P. Johnson, M. Macernis, L. Valkunas, W. Barford, A.V. Ruban, A theoretical investigation of the photo-physical consequences of major plant light-harvesting complex aggregation within the photosynthetic membrane, J. Phys. Chem. B 114 (2010) 15244–15253.
[159] J. Standfuss, A.C. Terwisscha van Scheltinga, M. Lamborghini, W. Kühlbrandt, Mechanisms of photoprotection and nonphotochemical quenching in pea light harvesting complex at 2.5 Å resolution, EMBO J. 24 (2005) 919–928.
[160] T. Barros, W. Kühlbrandt, Crystallisation, structure and function of plant light-harvesting Complex II, Biochim. Biophys. Acta 1787 (2009) 747–752.
[161] T. Gillbro, Å. Sandström, M. Spangfort, V. Sundström, R. van Grondelle, Excitation energy annihilation in aggregates of chlorophyll a/b complexes, Biochim. Biophys. Acta 934 (1988) 369–374.
[162] T. Barros, A. Royant, J. Standfuss, A. Dreuw, W. Kuhlbrandt, Crystal structure of plant light-harvesting complex shows the active, energy transmitting state, EMBO J. 28 (2009) 298–306.
[163] B. van Oort, A. van Hoek, A.V. Ruban, H. van Amerongen, Aggregation of light-harvesting complex II leads to formation of efficient excitation energy traps in monomeric and trimeric complexes, FEBS Lett. 581 (2007) 3528–3532.
[164] P.-N. Liao, C.-P. Holleboom, L. Wilk, W. Kühlbrandt, P.J. Walla, Correlation of Car S1 → Chl with Chl → Car S1 energy transfer supports the excitonic model in quenched light harvesting complex II, J. Phys. Chem. B 114 (2010) 15650–15655.
[165] B. van Oort, A. van Hoek, A.V. Ruban, H. van Amerongen, The equilibrium between quenched and non-quenched conformations of the major plant light-harvesting complex studied with high-pressure time-resolved fluorescence, J. Phys. Chem. B 111 (2007) 7631–7637.
[166] S. Santabarbara, P. Horton, A.V. Ruban, Comparison of the thermodynamic landscapes of unfolding and formation of the energy dissipative state in the isolated light harvesting complex II, Biophys. J. 97 (2009) 1188–1197.
[167] T.P.J. Krüger, V.I. Novoderezhkin, C. Ilioaia, R. van Grondelle, Fluorescence spectral dynamics of single LHCII trimers, Biophys. J. 98 (2010) 3093–3101.
[168] R. Bassi, S. Caffarri, Lhc proteins and the regulation of photosynthetic light harvesting function by xanthophylls, Photosynth. Res. 64 (2000) 243–256.
[169] T. Morosinotto, R. Baronio, R. Bassi, Dynamics of chromophore binding to Lhc proteins in vivo and in vitro during operation of the xanthophyll cycle, J. Biol. Chem. 277 (2002) 36913–36920.
[170] A.M. Gilmore, T.L. Hazlett, Govindjee, Xanthophyll cycle-dependent quenching of photosystem-II chlorophyll-a fluorescence — formation of a quenching complex with a short fluorescence lifetime, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 2273–2277.
[171] T.G. Owens, Excitation energy transfer between chlorophylls and carotenoids. A proposed molecular mechanism for non-photochemical quenching, in: N.R. Baker, J.R. Bowyer (Eds.), Photoinhibition of Photosynthesis from Molecular Mechanisms to the Field, Bios Scientific Publishers, London, 1994, pp. 95–107.
[172] H.A. Frank, A. Cua, T.V. Chynwa, A.J. Young, D. Gosztola, M.R. Wasielewski, Photophysics of the carotenoids associated with the xanthophyll cycle in photosynthesis, Photosynth. Res. 41 (1994) 389–395.
[173] H.A. Frank, J.A. Bautista, J.S. Josue, A.J. Young, Mechanism of nonphotochemical quenching in green plants. Energies of the lowest excited singlet state of zeaxanthin and violaxanthin, Biochemistry 39 (2000) 2831–2837.
[174] T. Polivka, J.H. Herek, D. Zigmantas, H.E. Akerlund, V. Sundstrom, Direct observation of the (forbidden) S1 state in carotenoids, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4914–4917.
[175] T. Polivka, D. Zigmantas, V. Sundstrom, E. Formaggio, G. Cinque, R. Bassi, Carotenoid S1 state in a recombinant light-harvesting complex of photosystem II, Biochemistry 41 (2002) 439–450.
[176] A. Dreuw, Influence of geometry relaxation on the energies of the S1 and S2 states of violaxanthin, zeaxanthin, and lutein, J. Phys. Chem. A 110 (2006) 4592–4599.
[177] Y.-Z. Ma, N.E. Holt, X.-P. Li, K.K. Niyogi, G.R. Fleming, Evidence for direct carotenoid involvement in the regulation of photosynthetic light harvesting, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 4377–4382.
[178] S. Bode, C.C. Quentmeier, P.N. Liao, N. Hafi, T. Barros, L. Wilk, F. Bittner, P.J. Walla, On the regulation of photosynthesis by excitonic interactions between carotenoids and chlorophylls, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 12311–12316.
[179] P.N. Liao, S. Bode, L. Wilk, N. Hafi, P.J. Walla, Correlation of electronic carotenoid–chlorophyll interactions and fluorescence quenching with the aggregation of native LHC II and chlorophyll deficient mutants, Chem. Phys. 373 (2010) 50–55.
[180] A.S. Davydov, The theory of molecular excitons, Sov. Phys. Usp., 1964.
[181] A. Dreuw, G. Fleming, M. Head-Gordon, Role of electron-transfer quenching of chlorophyll fluorescence by carotenoids in non-photochemical quenching of green plants, Biochem. Soc. Trans. 33 (2005) 858–862.
[182] N.E. Holt, D. Zigmantas, L. Valkunas, X.-P. Li, K.K. Niyogi, G.R. Fleming, Carotenoid cation formation and the regulation of photosynthetic light harvesting, Science 307 (2005) 433–436.

[183] T.J. Avenson, T.K. Ahn, D. Zigmantas, K.K. Niyogi, Z. Li, M. Ballotari, R. Bassi, G.R. Fleming, Zeaxanthin radical cation formation in minor light harvesting complexes of higher plant antenna, J. Biol. Chem. 283 (2008) 3550–3558.
[184] T.K. Ahn, T.J. Avenson, M. Ballottari, Y.C. Cheng, K.K. Niyogi, R. Bassi, G.R. Fleming, Architecture of a charge-transfer state regulating light harvesting in a plant antenna protein, Science 320 (2008) 794–797.
[185] S. Amarie, J. Standfuss, T. Barros, W. Kühlbrandt, A. Dreuw, J. Wachtveitl, Carotenoid radical cations as a probe for the molecular mechanism of nonphotochemical quenching in oxygenic photosynthesis, J. Phys. Chem. B 111 (2007) 3481–3487.
[186] S. Amarie, L. Wilk, T. Barros, W. Kühlbrandt, A. Dreuw, J. Wachtveitl, Properties of zeaxanthin and its radical cation bound to the minor light-harvesting complexes CP24, CP26 and CP29, Biochim. Biophys. Acta 1787 (2009) 747–752.
[187] T.J. Avenson, T.K. Ahn, K.K. Niyogi, M. Ballotarri, R. Bassi, G. Fleming, Lutein can act as a switchable charge transfer quencher in the CP26 light-harvesting complex, J. Biol. Chem. 284 (2009) 2830–2835.
[188] Z. Li, T.K. Ahn, T.J. Avenson, M. Ballotarri, J.A. Cruz, D.M. Kramer, R. Bassi, G. Fleming, J.D. Keasling, K.K. Niyogi, Lutein accumulation in the absence of zeaxanthin restores nonphotochemical quenching in the *Arabidopsis thaliana* npq1 mutant, Plant Cell 21 (2009) 1798–1812.
[189] T. Förster, Zwischenmolekulare Energiewanderung und Fluoreszenz, Ann. Phys. 437 (1948) 55–75.
[190] B.P. Krueger, G.D. Scholes, G.R. Flemming, Calculation of couplings and energy-transfer pathways between the pigments of LH2 by the *ab initio* transition density cube method, J. Phys. Chem. B 102 (1998) 5378–5386.
[191] D.L. Dexter, A theory of sensitized luminescence in solids, J. Chem. Phys. 21 (1953) 836–850.
[192] M.G. Muller, P. Lambrev, M. Reus, E. Wientjes, R. Croce, A.R. Holzwarth, Singlet energy dissipation in the photosystem II light-harvesting complex does not involve energy transfer to carotenoids, Chem. Phys. Chem. 11 (2010) 1289–1296.
[193] A.V. Ruban, J.P. Dekker, P. Horton, R. van Grondelle, Temperature dependence of chlorophyll fluorescence from the light harvesting complex II of higher plants, Photochem. Photobiol. 61 (1995) 216–221.
[194] C.W. Mullineaux, A.A. Pascal, P. Horton, A.R. Holzwarth, Excitation-energy quenching in aggregates of the LHCII chlorophyll–protein complex — a time-resolved fluorescence study, Biochim. Biophys. Acta 1141 (1993) 23–28.