Nonphotochemical Chlorophyll Fluorescence Quenching: Mechanism and Effectiveness in Protecting Plants from Photodamage[1]

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We review the mechanism underlying nonphotochemical chlorophyll fluorescence quenching (NPQ) and its role in protecting plants against photoinhibition. This review includes an introduction to this phenomenon, a brief history of major milestones in our understanding of NPQ, definitions, and a discussion of quantitative measurements of NPQ. We discuss the current knowledge and unknown aspects in the NPQ scenario, including the following: ΔpH, the proton gradient (*trigger*); light-harvesting complex II (LHCII), PSII light harvesting antenna (*site*); and changes in the antenna induced by ΔpH (*change*), which lead to the creation of the *quencher*. We conclude that the minimum requirements for NPQ in vivo are ΔpH, LHCII complexes, and the PsbS protein. We highlight the most important unknown in the NPQ scenario, the mechanism by which PsbS acts upon the LHCII antenna. Finally, we describe a novel, emerging technology for assessing the photoprotective "power" of NPQ and the important findings obtained through this technology.

"Real knowledge is to know the extent of one's ignorance."
Confucius

Nonphotochemical chlorophyll fluorescence quenching (NPQ) is a process in which excess absorbed light energy is dissipated into heat. This process takes place in the photosynthetic membranes of plants, algae, and cyanobacteria (Demmig-Adams et al., 2014). Early photosynthetic organisms have dealt with the problem of surviving in shady environments by evolving the light-harvesting antenna, which collects dilute light energy for photosynthetic reaction centers (Clayton, 1980; Blankenship, 2002). However, high light exposure causes rapid saturation of the photosynthetic reaction centers and their eventual closure, leading to a reduction in the fraction of energy utilized in photosynthesis and the subsequent build-up of harmful excess excitation energy in the photosynthetic membrane (Björkman and Demmig-Adams, 1995). This energy can damage the most delicate part of the photosynthetic apparatus, the PSII reaction center (RCII), which drives water splitting and oxygen evolution (Powles, 1984; Barber, 1995; Ohad et al., 1984). A RCII repair mechanism exists, but this repair process occurs on the order of hours (Barber and Andersson, 1992; Aro et al., 1993; Nixon et al., 2010; Nath et al., 2013). In addition, excess light can potentially harm the antenna pigments (Fleming et al., 2012), which can lead to a sustained decline in photosynthetic efficiency and, under extreme conditions, death of the photosynthetic cell, tissue, or organism.

Evolution has supplied a range of solutions to the problem of high light exposure that vary in efficiency, level of action, and promptness of response (Gall et al., 2011; Niyogi and Truong, 2013; Ruban, 2015; Demmig-Adams et al., 2014; Goss and Lepetit, 2015). There are adaptations to control light absorption capacity as well as adaptations that deal with the light energy that has already been captured (Chow et al., 1988; Koller, 1990; Ruban, 2009; Cazzaniga et al., 2013; Xu et al., 2015a). At the molecular level, there is both long-term (acclimation) and short-term (regulatory mechanisms) control of the input of light energy into reaction centers. The first type of mechanism is predominantly developmental in nature and is the result of light-dependent regulation of complex gene expression occurring at the transcriptional, translational, and posttranslational levels (Anderson et al., 1988). However, the long response time of acclimation limits its photoprotective efficiency while at the same time allowing energy and resources to be consumed. On its own, acclimation is insufficient for photoprotection, since profound damage to RCII can occur within minutes of excess light exposure (Tyystjärvi and Aro, 1996).

NPQ is a molecular adaptation that represents the fastest response of the photosynthetic membrane to excess light (Demmig-Adams et al., 2014). The NPQ process is directly or indirectly related to the processes of light harvesting by the photosynthetic antenna complexes, their structure, captured energy transfer to

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reaction centers, electron transport, proton translocation across the membrane, ATPase activity, and carbon assimilation (Walker, 1987; Ruban, 2013; Demmig-Adams et al., 2014). At various times, NPQ research has led to the development of new methods to define and quantify this protective process (Papageorgiu and Govindjee, 1968; Murata and Sugahara, 1969; Schreiber, 1986; Oxborough and Horton, 1988; Weis and Berry, 1987), the structure of the photosynthetic antenna complexes (Nield and Barber, 2006; Liu et al., 2004) and their organization in the membrane (Dekker and Boekema, 2005; Ruban and Johnson, 2015), the dynamics of the antenna complexes (Garab et al., 1988; Ruban et al., 1994; Miloslavina et al., 2008; Krüger et al., 2012, Liguori et al., 2015), pigment compositions (Rees et al., 1989; Demmig-Adams, 1990) and dynamics in the membrane (Demmig-Adams and Adams III, 1992; Matsubara et al., 2001; Jahns et al., 2009), and excitation energy transfer and dissipation (Van Amerongen et al., 2000; Polívka and Sundström, 2004; Renger and Holzwarth, 2008; Cheng and Fleming, 2009; Scholes et al., 2011). A long and often convoluted pathway has led to the current understanding of the molecular mechanism underlying NPQ. Indeed, it took some time to define and separate NPQ processes, learn how to measure and quantify it, obtain molecular insights into antenna structure, reveal its dynamic nature, and understand its role in photoprotection. Recently, numerous review articles about various aspects of NPQ have emerged, a recent collection of which was published in the fortieth volume of the series Advances in Photosynthesis and Respiration, 2014 (Demmig-Adams et al., 2014). Hence, the aim of this review is to provide complementary information highlighting the most current known and unknown aspects of the most highly investigated *mechanism* of NPQ that takes place in plants. This article also discusses emerging work on quantitative approaches to assessing the *effectiveness* of NPQ in protecting plants against photoinhibition.

DEFINITION OF NPQ

NPQ was introduced as a reflection of the processes that arise in the photosynthetic membrane that are not photochemical in origin. Indeed, the activity of RCII causes a significant reduction, or quenching, of chlorophyll fluorescence, since it consumes light energy that otherwise could be released through fluorescence, interconversion, or intersystem crossing (Duysens and Sweers, 1963; Govindjee, 1971; Myers, 1974). However, fluorescence can also be quenched when all RCIIs are closed, hence not consuming any absorbed light energy (Papageorgiu, 1968; Murata and Sugahara, 1969; Wraight and Crofts, 1970). This closure was first achieved by treating chloroplasts that were constantly illuminated with actinic light with the PSII acceptor site inhibitor DCMU. The inhibitor caused the closure of RCIIs within the first second of illumination, quickly reversing the photochemically quenched fluorescence, while the remaining quenched fluorescence was reversed on a much slower time scale (Papageorgiu, 1968). This slowly relaxing quenching is called nonphotochemical quenching, or energy-dependent quenching (qE; Wraight and Crofts, 1970). The term qE remains popular and is considered to be the major component of NPQ (Fig. 1A).

In the 1980s, the introduction of the pulse amplitude modulated (PAM) fluorescence technique opened up new opportunities for detailed study of NPQ (Schreiber, 1986; Oxborough and Horton, 1988). Figure 1A depicts a typical PAM induction measurement assessing the state of PSII in the dark, the F_o fluorescence level, when all RCIIs are open, and the F_m level, when all RCIIs are closed in response to a high-intensity pulse (normally 0.5-1.0 s in duration). From this simple process, one can calculate the quantum efficiency of PSII as $\Phi_{PSII} = (F_m - F_o)/F_m$. In fact, this value is actually the relative amount of fluorescence that is photochemically quenched due to the activity of the reaction centers. Interestingly, the fluorescence does not immediately return to the initial F_o level, because the acceptor site of PSII remains reduced for some time. This process can be accelerated by the use of far red light, which preferentially excites PSI, causing faster oxidation of the Cyt*b*/*f* complex and producing a pool of oxidized mobile electron carriers, plastoquinones, which remove electrons from PSII (Hill and Bendall, 1960; Blankenship, 2002). Actinic light illumination is then applied for approximately 5 min. During this time, saturating light pulses are used every minute to determine the level of F_m. This level is progressively quenched and stabilizes at the end of the illumination period. The quenched F_m is termed F_m'. Hence, the level of NPQ can be calculated as $(F_m - F_m')/F_m'$. Another parameter, qN, is used to calculate nonphotochemical quenching: $qN = (F_m - F_m')/F_m$. This parameter describes the percentage of quenching in a similar manner to Φ_{PSII}. The NPQ calculation reflects the ratio of the rate constant of NPQ to the sum of the remaining constants reflecting all other dissipation pathways in the membrane, such as fluorescence, internal, and interconversion (Krause and Weis, 1991). qE is defined in the context of this analysis as the rapidly reversing component of qN or NPQ (Fig. 1A). Normally, this component is considered to recover within 5 min of switching off the actinic light. Notably, the trigger of qE, ΔpH, usually collapses within 10 to 20 s (Ruban, 2013). Hence, it was proposed in the early days of NPQ research that the NPQ process involved some conformational changes within the photosynthetic membrane that respond to ΔpH. As shown in the figure, qE appears to be the major component of NPQ. The remainder was previously termed qI, i.e. the irreversible NPQ component related to photoinhibition/damage to RCII (Krause and Weis, 1991). It was later discovered that the formation of zeaxanthin is closely related to the NPQ mechanism (Demmig-Adams et al., 1989, 1990; Demmig-Adams and Adams III, 1992; for review see Demmig-Adams et al., 2014) and as such, a portion of qI is often termed qZ to reflect the long-term quenching

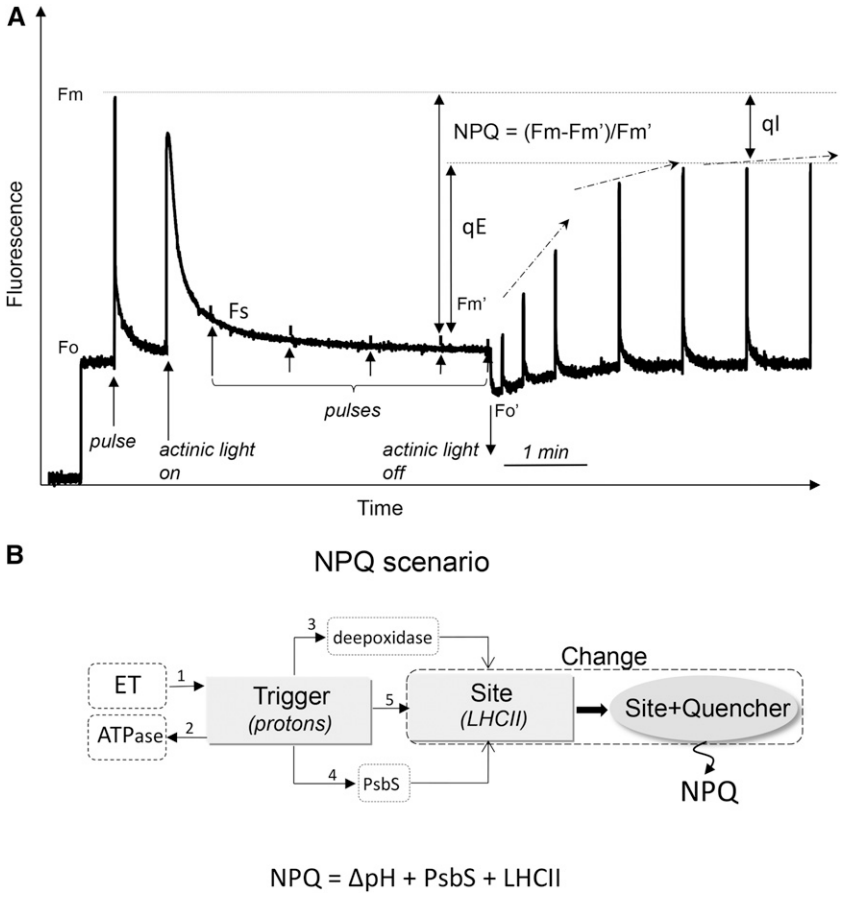

Figure 1. A, Typical PAM fluorescence trace of an Arabidopsis leaf showing induction and relaxation of NPQ. Fm and F_o are the maximum and minimum fluorescence levels in the dark before actinic light illumination (1000 μmol $m^{-2}s^{-1}$), respectively. Fs is the steady-state fluorescence level. F_m' is maximum fluorescence during actinic light illumination. Pulses of light (10,000 μmol $m^{-2}s^{-1}$) were applied to close all RCIIs and were used to estimate F_m and F_m'. qE and qI are quickly and slowly reversible components of NPQ, respectively. B, Model of NPQ development (NPQ scenario) showing key factors triggering and regulating the process (for more details, see the text). The formula for the minimum component requirement for NPQ is shown below the diagram.

effect that is correlated with the presence of this pigment (Nilkens et al., 2010). In addition, other sustained components of NPQ are triggered by low temperature acclimation (Verhoeven, 2013), prolonged illumination in the presence of zeaxanthin (Ruban and Horton, 1995), slow proton equilibration between different membrane compartments (Ruban and Horton, 1995; Joliot and Finazzi, 2010), or simply the formation of high levels of NPQ in some types of photosynthetic materials (Ruban et al., 1993, 2004; Ware et al., 2015b). Hence, qI appears to be a highly complex component of NPQ that remains difficult to interpret, and the temporal criterion for quantification of qE is rather ambiguous. Therefore, we will use the term protective NPQ (or simply NPQ) instead of qE; the former includes all moderately or slowly reversible components that are not related to photoinhibition (for details, see "Protective Effectiveness of NPQ").

MECHANISM OF NPQ

NPQ resides in the antenna (Bassi and Caffarri, 2000; Fleming et al., 2012; Ruban et al., 2012; Wilk et al., 2013; *site*), which undergoes a *change* triggered by ΔpH (*trigger*; Horton et al., 1996; Strand and Kramer, 2014). As a result of this change, the *quencher* pigment(s) begins receiving the energy harvested by the light-harvesting complex (LHCII) antenna and dissipating it as heat. Hence, ΔpH provides feedback control over light harvesting efficiency in the photosynthetic membrane (Ruban et al., 2012; Strand and Kramer, 2014).

Trigger: Protons

NPQ is triggered by ΔpH either directly, by protonation of antenna components, or indirectly, by the activity of the xanthophyll cycle(s) (Ruban et al., 2012). It also makes sense to refer to the proton gradient as the *trigger*, since in some organisms such as diatom algae, high levels of NPQ can be induced and sustained in the dark or upon addition of uncouplers in the absence of ΔpH (Ruban et al., 2004; Lepetit et al., 2012). In addition, acidification of the incubation buffer can induce a type of fluorescence quenching that possesses features similar to NPQ (Rees et al., 1992). This finding provides justification for the use of acidification techniques to study fluorescence quenching in isolated antenna complexes (Ruban et al., 1994; Bassi and Caffarri, 2000). Importantly, since ΔpH buildup is generated as a result of electron transport, a variety of pathways contribute to its amplitude (for a recent, comprehensive review, see Strand and Kramer, 2014). In addition, by consuming protons, ATPase exerts a modulatory effect

upon ΔpH. Also, a recent report showed that not only ATPase, but also a specialized proton/potassium antiporter, can influence the rate of NPQ relaxation under low light by accelerating the collapse of ΔpH (Armbruster et al., 2014). In fact, the *trigger* is kept under control as well (Fig. 1B, regulatory points 1 and 2). It appears that cyclic electron transport around PSI is the major contributor to the component of ΔpH that triggers the largest portion of NPQ (Munekage et al., 2004). Recent work by Sato et al. (2014) revealed that cyclic electron transport-generated ΔpH contributes 60% to 80% to NPQ formation. Therefore, the ratio of PSII to PSI defined, for example, over the course of acclimation is likely to affect the *trigger*, and therefore the amplitude, of NPQ (Brestic et al., 2015). Remarkably, chloroplasts from plants grown on lincomycin, which had therefore lost almost all of PSII and 80% of PSI, had ΔpH values close to those of the control, as well as very high levels of NPQ (Belgio et al., 2012, 2015). Recently, modulating ΔpH with artificial proton shuttles such as diaminodurene has successfully been used to uncover vital mechanistic clues about the sensitivity of responses of antenna components to lumen acidification during the induction of NPQ (see below in "*Site*: LHCII Antenna and PsbS"). Lumen protons target three key components involved in NPQ: violaxanthin de-epoxidase (Fig. 1B, target point 3; Jahns et al., 2009), the PsbS protein (Fig. 1B, target point 4; Li et al., 2004), and the LHCII antenna (Fig. 1B, target point 5; Ruban et al., 1994, 1996; Walters et al., 1994; Liu et al., 2008; Belgio et al., 2013). The pK of the lumen-exposed side of the thylakoid membrane is as low as 4.1 (Åkerlund et al., 1979). In vivo lumen acidification resulting from ΔpH formation is estimated to lead to a pH of 5.5 (Noctor et al., 1991; Kramer et al., 1999). The pK for NPQ in chloroplasts devoid of zeaxanthin is 4.7, and the pK of quenching in the isolated major LHCII complex without zeaxanthin is approximately 4.5 (Wentworth et al., 2001) but is 1 to 2 pH units higher in the presence of zeaxanthin or the monomeric LHCII protein CP26 (Ruban and Horton, 1999; Wentworth et al., 2001). The pK for PsbS, according to Dominici et al. (2002), should be approximately 6.0 to 6.5. A similar pK for violaxanthin de-epoxidation was reported by Jahns et al. (2009). Hence, it appears that the most lumen pH-sensitive components of the thylakoid membrane are PsbS, violaxanthin de-epoxidase, monomeric antenna complexes, and LHCII that carries zeaxanthin produced by de-epoxidase (Ruban at al., 2012). Therefore, for the LHCII antenna to respond to lumen pH (Fig. 1B, target point 5) and become quenched, it is important to achieve activation of de-epoxidase (target point 3) to produce zeaxanthin and activation of PsbS (target point 4). Both LHCII and PsbS contain a number of lumen-exposed residues that can receive protons. Two of these residues in monomeric LHCII and two in PsbS have been identified using N,N′-dicyclohexylcarbodiimide (DCCD) labeling and site-directed mutagenesis (Walters et al., 1996; Li et al., 2004). However, tritium labeling of LHCII in vivo suggested that each monomer can sequester up to 17 protons (Zolotareva et al., 1999). It may well be possible that since monomeric antenna receive protons at lower levels of ΔpH, they are the primary sites for the quenching that eventually spreads to the bulk of LHCII trimers. The idea that the minor antenna is the site for NPQ is currently the most supported idea that has emerged from the work of Fleming and Bassi (Ahn et al., 2008; Avenson et al., 2009).

There has never been an easy way to measure the proton gradient. The use of 9-aminoacridine is the most common way to assess this gradient in thylakoids and chloroplasts (Ruban, 2013); however, this technique is difficult to perform in leaves. This task was previously accomplished through indirect measurements based on the light-induced change in absorption at 518 nm, which is believed to reflect the electrochromic shift of carotenoids (Kramer et al., 1999). However, this method was recently subjected to a critical reassessment, which claimed that the observed steady-state component of the 518-nm absorption change that was used as a measure of the proton gradient (Kramer et al., 1999) was due to interference from the NPQ-associated absorption at 535 nm (for a more detailed discussion, see Johnson and Ruban, 2014). This work also casts doubt that the electric field gradient $\Delta\psi$ makes a noticeable contribution to the proton motive force in photosynthesis. The 535-nm change is closely related to NPQ and, since the latter is triggered by ΔpH, measurements of absorption at 518 nm would, to a certain extent, reflect the amplitude of NPQ and therefore, indirectly, ΔpH. Therefore, developing accurate, direct, nondestructive ways to measure ΔpH in vivo would be a crucial step toward monitoring the dynamics of this important parameter during the course of light and metabolic alterations in order to identify the causes of altered NPQ levels.

Site: LHCII Antenna and PsbS

Some 25 years ago, a model of the relationship between NPQ and the PSII yield pointed toward the involvement of the PSII antenna in NPQ (Genty et al., 1989). Indeed, the NPQ quencher was found to reduce not only F_m, but also F_o fluorescence (Fig. 1A; Horton and Ruban, 1993). The quencher persists at 77 K and preferentially quenches major LHCII complex bands at 680 and 700 nm (Ruban et al., 1991). Results from early fluorescence lifetime analysis were consistent with quenching taking place in the PSII antenna (Genty et al., 1992). Later, this type of spectroscopy revealed similarities between decay-associated spectral changes upon the transition into the quenching state in both isolated LHCII complexes and intact chloroplasts (Johnson and Ruban, 2009). Plants lacking a majority of LHCII antenna complexes display strongly reduced NPQ (Jahns and Krause, 1994; Havaux et al., 2007). The remaining quenching in the chlorina mutants or plants grown under intermittent light was attributed to the presence of some minor LHCII antenna complexes

(Jahns and Krause, 1994; Havaux et al., 2007), as was previously proposed (Andrews et al., 1995). NPQ is modulated by cross-linkers, tertiary amines, antimycin A, DCCD, and magnesium in the same way as quenching in isolated LHCII antenna complexes (Ruban et al., 1992, 1994, 1996; Johnson and Ruban, 2009). The latter is induced at detergent concentrations below critical micelle concentration and leads to the aggregation of the complex. Hence, a hypothesis has been put forward that the in vivo aggregation of the LHCII antenna is a mechanism underlying NPQ (Horton et al., 1991; for further discussion, see "*Change*: LHCII Aggregation and Other"). Moreover, the discovery that xanthophyll cycle carotenoids are localized exclusively to LHCII antenna complexes (Thayer and Björkman, 1992; Bassi et al., 1993) and the subsequent discovery that NPQ is entirely dependent on the xanthophylls zeaxanthin and lutein (Pogson et al., 1998; Niyogi et al., 2001) leave little doubt that the NPQ site is the LHCII antenna (for more details, see Ruban et al., 2012).

The evolving knowledge of PSII antenna composition, structure, and organization in the photosynthetic membrane reveals its structural and functional heterogeneity (Boekema et al., 1995; Jansson, 1999; Dekker and Boekema, 2005; Caffarri et al., 2009; Kouřil et al., 2011, 2012). The current model suggests that the LHCII antenna comprises three monomeric LHCII antenna complexes, CP24, CP26, and CP29, collectively known as the minor LHCII antenna, as well as several trimeric LHCIIs known as the major LHCII antenna. The minor LHCII antenna comprises a structural and apparently functional (Dall'Osto et al., 2014) bridge between the major trimeric LHCII complexes and the core antenna in the PSII supercomplex dimer (Fig. 2). Three types of LHCII trimers are distinguished based on their binding strength to the PSII supercomplex: S, M, and L, i.e. strongly, moderately, and loosely bound, respectively.

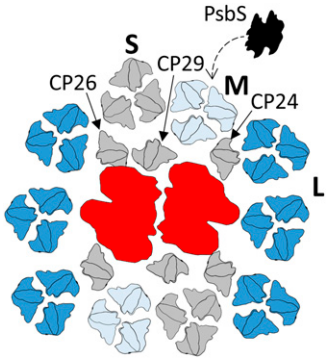

Figure 2. The structure of PSII antenna components. S, M, and L are the major LHCIIs that are strongly, moderately, and loosely bound to the RCII core trimers, respectively. CP24, 26, and 29 are the minor monomeric antenna complexes. PSII core dimer is shown in red. PsbS dimer is shown with a dashed line pointing to the putative preferential interaction site in the dark.

Only the localizations of S and M trimers have been identified. Loosely bound trimers are thought to diffuse relatively freely in the membrane, and therefore it is difficult to predict their localization. There can be two to four (and sometimes more) loosely bound trimeric LHCII complexes per PSII monomer (Melis and Anderson, 1983; Kouřil et al., 2012; Wientjes et al., 2013). Studies of DCCD binding, in vitro quenching, and carotenoid binding on the monomeric LHCII complexes CP26 and CP29 have shown that both of these complexes can accept protons, can attain high levels of quenching, and are enriched in xanthophyll cycle carotenoids (Walters et al., 1994, 1996; Ruban et al., 1996, 1998; Bassi and Caffarri, 2000). These findings prompted researchers to propose that the site of NPQ is localized to the monomeric LHCII complexes (Bassi and Caffarri, 2000; Ahn et al., 2008; Avenson et al., 2009). This proposal was weakened by the observation that antisense and knockout mutants of Arabidopsis (*Arabidopsis thaliana*) lacking one or even two of the three monomeric LHCIIs (CP24/29 double mutant) possess significant levels of NPQ (Andersson et al., 2001; de Bianchi et al., 2008). In addition, the efficiency of violaxanthin de-epoxidation located in the L2 site (Pan et al., 2011) is very low in the minor antenna complexes, particularly in CP29 due to strong binding at the site (Duffy and Ruban, 2012), implying that they cannot bind significant amounts of the postulated quencher zeaxanthin at this site. However, it may well be that quenching in the monomeric LHCII antenna complexes proceeds by the same mechanism (Mozzo et al., 2008) suggested for the major trimeric LHCII (Ruban et al., 2007). Further clarification of the role of monomeric LHCII complexes in NPQ is expected to come from investigations of the triple minor antenna knockout mutant (no-minor-antenna mutant, NOM; Dall'Osto et al., 2014).

Another component that plays a crucial role in enabling the rapidly reversible component of NPQ, qE, is PsbS (Li et al., 2000). Structural work on the localization of this protein in the photosynthetic membrane suggested that it is not a part of the PSII supercomplex (Nield et al., 2000). Biochemical work convincingly showed that PsbS does not specifically bind pigments (Bonente et al., 2008). The atomic structure of PsbS has recently been solved (Fan et al., 2015). This protein is a dimer that is more stable at low pH. Acidification was suggested to cause a conformational change associated with alteration in lumenal intermolecular interactions. Hence, it appears that PsbS acts like a *switch* that is triggered by ΔpH and not like a quenching site. Therefore, this switch must be localized closer to the LHCII antenna to prompt it into the NPQ state or make it sensitive to protonation (Ruban et al., 2012). It is appropriate to use the term "sensitive" here, since qE can actually form without PsbS, provided ΔpH is high enough (Johnson and Ruban, 2011). Hence, in the model shown in Figure 1B, a straight line was drawn from the *trigger* to the *site* (LHCII antenna; action point 5), bypassing PsbS and zeaxanthin, which are presented

as components of modulation. These components are actually important for physiological adjustment of NPQ (see "*Change*: LHCII Aggregation and Other"). Since PsbS was not detected in the structure of PSII supercomplex, it must be localized somewhere in the domains of the LHCII antenna (Fig. 2). In a recent study in which the site of PsbS binding in PSII in the moss *Physcomitrella patens* was probed biochemically, it was suggested that in the dark PsbS binds to several Lhcb proteins, with preferential binding to the periphery of the LHCII M trimer of the PSII supercomplex (Gerotto et al., 2015). The most recent report by Correa-Galvis et al. (2016) revealed that in higher plants in the dark, PsbS is localized around PSII supercomplexes, while in the NPQ state, PsbS begins to interact with various LHCII antenna components, with preferential binding to the major trimeric LHCII complex. Hence, the likely NPQ site could be trimeric rather than monomeric LHCII complexes. Interestingly, plants that grew on lincomycin (mentioned above) and possessed very few RCII (retaining trimeric and some reduced amounts of monomeric LHCII complexes) also contained PsbS (see above; Belgio et al., 2012, 2015). NPQ in these plants was modulated by PsbS (Ware et al., 2015b), suggesting that the site of NPQ is the LHCII antenna and PsbS together. However, this work did not prove that the monomeric LHCII is not involved in this process, but it provided a simpler model system for NPQ studies. It appears that only ΔpH, the LHCII antenna, and PsbS are required for NPQ in vivo. It is likely that PsbS is needed to make the LHCII antenna more rapidly responsive to natural levels of ΔpH. The structural arrangement of the LHCII antenna and PsbS around PSII does not appear to be required for the quenching to be observed, provided they are present in the membrane. However, the core complex may play a role in tuning NPQ kinetically by initiating the reassembly of the antenna around it in the dark (Dong et al., 2015; Ware et al., 2015b). The notion that the RCII core complex is not essential for quenching is consistent with the results of a recent work involving reconstitution of PsbS and the major LHCII complex into liposomes (Wilk et al., 2013). Interestingly, the liposomal system did not contain any minor antenna complexes, suggesting that LHCII trimers are sufficient partners for PsbS interaction and for the formation of the *quencher*.

Change: LHCII Rearrangements/Aggregation and the Formation of the NPQ *quencher*

The requirement for the ΔpH-triggered change in the LHCII antenna was first proposed by Horton's group (Horton et al., 1991). They hypothesized that the proton gradient triggers LHCII antenna aggregation, which is required to establish the NPQ state. Indeed, isolated major LHCII complex aggregates under low detergent concentrations, which is greatly enhanced by acidification of the incubation buffer. This process is followed by fluorescence quenching that is strong enough to explain any levels of NPQ observed in nature (Ruban et al., 1994). Another attractive physiological implication of this hypothesis is that LHCII antenna aggregation is modulated by xanthophyll cycle carotenoids, which explains the occurrence of NPQ with or without zeaxanthin, as well as the concept of "plant illumination memory" and the effect of hysteresis (Horton et al., 1996; Ruban et al., 2012). Xanthophyll cycle carotenoids are localized to peripheral binding site V1 of the major LHCII complex (Ruban et al., 1999; Liu et al., 2004), although they also bind peripherally to the minor antenna complexes (Ruban et al., 1999; Xu et al., 2015b). This peripheral localization and the ability to regulate LHCII antenna aggregation have been explained by the differential hydrophobicity/polarity of violaxanthin and zeaxanthin (Ruban and Johnson, 2010; Ruban et al., 2012). The presence of zeaxanthin is thought to slow the reversibility of NPQ and promote the sustained component qZ due to the tuning of the aggregation of the LHCII antenna, a process that is slowly reversible (Noctor et al., 1991; Ruban and Horton, 1999). In addition, violaxanthin de-epoxidation alters the LHCII antenna aggregation state in vivo as well as energy transfer pathways within the LHCII antenna, bringing minor LHCII antenna complexes such as CP29 in closer contact with LHCII trimers (Ilioaia et al., 2013).

Although the LHCII antenna aggregation hypothesis for NPQ has prompted much research around LHCII complexes and many attempts to link it to NPQ using indirect biochemical and spectroscopic methods (for a recent review, see Ruban et al., 2012), there was a lack of crucial, direct proof of in vivo aggregation or rearrangements of the LHCII antenna triggered by ΔpH and of an explanation for the role of PsbS in the proposed rearrangements (Ruban et al., 2012). Several groups have undertaken a number of approaches to address these important points (Miloslavina et al., 2008; Holzwarth et al., 2009; Betterle et al., 2009; Johnson et al., 2011; Ware et al., 2015b). Indirect but novel spectroscopic in vivo evidence has emerged suggesting that upon formation of NPQ, a portion of the major LHCII complexes, undergoes both separation from the PSII supercomplex and aggregation (Miloslavina et al., 2008; Holzwarth et al., 2009). Furthermore, biochemical and structural evidence has been obtained suggesting that during NPQ, PsbS controls the dissociation of the portion of the PSII–LHCII supercomplex containing LHCII, CP24, and CP29 and that the average distances between PSII core complexes become shorter (Betterle et al., 2009). Subsequently, freeze-fracture electron microscopy studies revealed similar alterations in PSII distances and most importantly, clustering of LHCII antenna particles on the protoplasmic fracture face of the stacked thylakoid membrane (Johnson et al., 2011; Ruban et al., 2012). This clustering was found to be promoted by the presence of zeaxanthin and PsbS (Johnson et al., 2011; Goral et al., 2012). Furthermore, overexpression of PsbS caused massive LHCII antenna aggregation, even in the absence of RCII complexes (Ware et al., 2015b). It was also shown that the antenna

composition has a strong effect on NPQ and the dynamics of the related rearrangements triggered by ΔpH (Goral et al., 2012). These advances provide the first direct experimental confirmation of the LHCII antenna aggregation hypothesis of NPQ. Moreover, the data reveal the common nature of qE and zeaxanthin-dependent qZ NPQ components as manifestations of the same LHCII aggregation phenomenon. Crucially, the observed structural alterations induced by illumination occurred on a timescale consistent with the formation and relaxation of qE (Johnson et al., 2011).

Despite all of this progress, many details of the *change* that leads to the establishment of the quenched state remain to be confirmed. Although there is no denial that the LHCII antenna undergoes reorganization into the NPQ state, recent data suggest that it does not uncouple energetically from RCII (Johnson and Ruban, 2009; Belgio et al., 2014), as was previously proposed (Holzwarth et al., 2009), which is in total agreement with the earlier established and experimentally confirmed relationship between the yield of PSII and NPQ (Genty et al., 1989). Moreover, it was shown that NPQ protects closed, not open, RCII, which makes this protective strategy *economical*, as it does not allow much competition between NPQ and RCII traps for energy under low or moderate light intensity (Belgio et al., 2014). Figure 3A shows a model of the fragment of the grana membrane showing the arrangement of PSII core and LHCII complexes. The part of the diagram showing the arrangement of cores and C2S2M2 supercomplexes (orientation and distances) containing core dimer, all monomeric LHCII, S, and M trimers, was reprinted from Kouřil et al. (2011). The L trimers were added randomly (positions and orientations) to match the LHCII trimer/RCII ratio of 5. Figure 3B shows a schematic diagram of the clustering of PSII and LHCII complexes in the NPQ state (adapted from Johnson et al., 2011). Note that the major assumption here is that the structure of the C2S2 supercomplex is preserved. However, this remains to be verified (Dong et al., 2015), as does the localization of PsbS. This protein changes its conformation (Fan et al., 2015; Correa-Galvis et al., 2016), which can alter, for example, its binding affinity within the LHCII antenna, a process that could trigger the observed rearrangement. However, several important questions remain: What is the mechanism underlying this PsbS effect, its interaction with the LHCII antenna, and its specificity? Is the interaction promoted by altered hydrophobicity or potentiated by the promotion of N-terminal interactions? If the scheme in Figure 1B is correct, why does PsbS make the LHCII antenna more sensitive to lumen pH? Is it because it somehow enhances hydrophobicity of the environment of proton-receiving amino acids, which would certainly make their pK values higher (Mehler et al., 2002; Thurlkill et al., 2006)? Also, while both PsbS and zeaxanthin promote rapid formation of NPQ (Li et al., 2000; Demmig-Adams et al., 1989), why has the former an acceleratory and the latter an inhibitory effect on its recovery, as well as opposite effects on chlorophyll excited state relaxation dynamics (Sylak-Glassman et al., 2014)?

Another important issue is whether LHCII antenna clustering is a primary cause of the quenching or simply a thermodynamic consequence of the inner conformational change within each trimer or monomer that actually creates the *quencher*. Preliminary evidence that isolated LHCII complexes can be quenched without significant aggregation has been obtained using high hydrostatic pressure treatment of these complexes or by polymerizing them into a polyacrylamide gel and gradually removing the detergent (van Oort et al., 2007; Ilioaia et al., 2008). The features of this quenching were similar to those of the aggregated low-pH-quenched LHCII. It has begun to emerge that the LHCII monomer/trimer undergoes some type of conformational change into the quenching state that involves specific changes in some of the xanthophyll (neoxanthin and lutein) and chlorophyll pigments, as was previously observed for LHCII aggregates (Robert et al., 2004; Ilioaia et al., 2011). However, to date, only the structure of the quenched conformation of trimeric LHCII has been solved (Liu et al., 2004; Pascal et al., 2005). Recently, a few attempts have been made to understand the scale and possible specificity of the conformational transition into the quenched state. Exciton annihilation experiments along with high hydrostatic pressure work have revealed very small changes in the volume of quenched trimeric LHCII (van Oort et al., 2007; Rutkauskas et al., 2012). NMR studies and accompanying theoretical analysis revealed subtle alterations in some chlorophyll *a* pigments and their interactions with neoxanthin and lutein 1 and 2 (Pandit at al., 2013: Duffy et al., 2014). These observations are consistent with the discovered role of the lumenal loop of trimeric LHCII, which is localized near the neoxanthin domain, in modulating quenching in vitro (Belgio et al., 2013). This notion was recently confirmed by the first molecular dynamics study revealing significant flexibility of trimeric LHCII, primarily in the neoxanthin and lutein 1 (terminal emitter) domains (Liguori et al., 2015).

In parallel with the structural work on the LHCII antenna, novel single molecule fluorescence spectroscopy on all types of LHCIIs (both trimeric and monomeric) has been intensely performed in recent years (Krüger et al., 2012; 2013, 2014). The rapidly fluctuating levels of LHCII fluorescence, known as fluorescence intermittency or *blinking*, were found to be modulated by both the xanthophyll cycle composition and low pH treatments and are therefore closely related to NPQ. The blinking reflects local conformational fluctuations within the complex, which thermally access distinct conformational states that have strong quenching (lutein 1 and 2 domains) or red-shifted fluorescence properties (around 700 nm; Krüger et al., 2014).

All of these studies on the intrinsic dynamics of the LHCII complexes were absolutely essential in the search for the possible NPQ quencher(s). The quencher is simply "born" out of the change in conformation

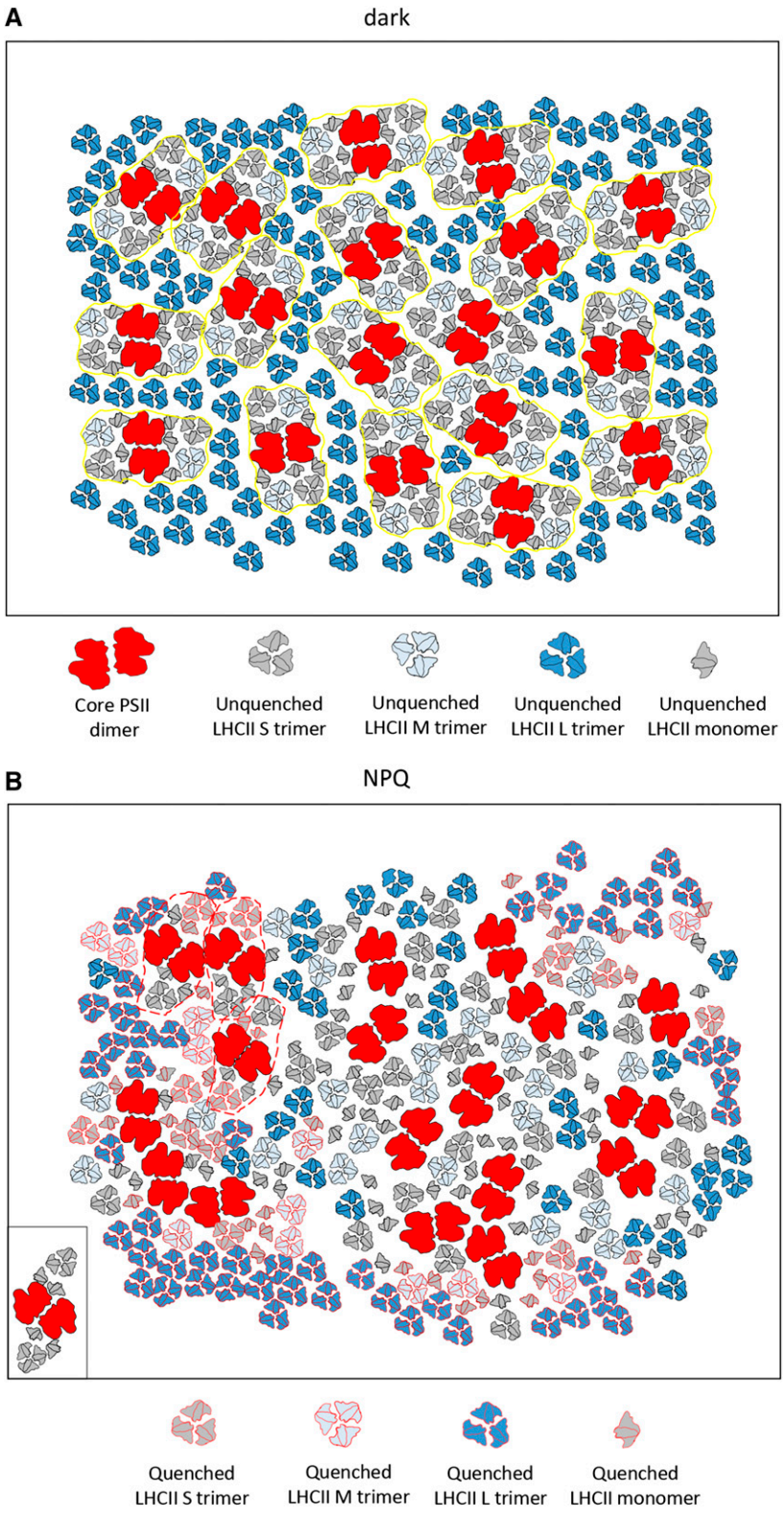

Figure 3. Schematic representation of putative PSII arrangements in the grana membrane in the dark (A) and NPQ (B) states. A, 18 PSII C2S2M2 complexes (outlined by yellow lines) with peripheral LHCII trimers (L trimers; after Kouřil et al., 2011). The total LHCII trimer-to-RCII monomer ratio is approximately 5. B, 18 PSII core dimers rearranged/clustered into the NPQ state (following Johnson et al., 2011). C2S2 structure is shown (outlined with a dashed red line; see the inset) preserved in the three supercomplexes shown in the far left corner. A mix of unquenched (black contour) and quenched (red contour) S, M, and L trimers and monomers of the minor antenna (not specified here) is shown.

triggered by protonation (Formaggio et al., 2001). Currently, there are several theories describing the possible identity and physical mechanism of the quenching process. Since this falls out of the scope of this review, the reader is referred to the most recent account of the state of our knowledge on the physics of the NPQ quencher (Duffy and Ruban, 2015). In brief, the pigments zeaxanthin, lutein, and chlorophyll *a* have

been proposed as possible NPQ quenchers. The suggestion that zeaxanthin is a quencher was proposed some time ago (Frank et al., 1996; for review, see Demmig-Adams, 1990) and has recently received strong, insightful support from Fleming, Niyogi, and Bassi, who proposed that the quencher is localized within the minor LHCII antenna complex CP29 (Holt et al., 2005; Ahn et al., 2008). Several groups have proposed that lutein bound to the major and minor LHCII serves as a quencher (Ruban et al., 2007; Avenson et al., 2009). While there is currently only a single theory about the role of zeaxanthin in quenching, i.e. radical cation formation with chlorophyll (Holt et al., 2005), there are several theories explaining how lutein (and other xanthophylls) can quench excess energy, which include coherent and incoherent energy transfer pathways from chlorophyll to xanthophyll (Duffy and Ruban, 2015). While there is some evidence showing how zeaxanthin becomes activated as a quencher (Holt et al., 2005; Ahn et al., 2008), there are numerous reports attempting to explain the changes in protein and lutein that point to this pigment as a quencher, as well as modeling work assessing the effectiveness of this quencher in taking excess excitation energy from chlorophyll *a* (Ilioaia et al., 2013; Duffy et al., 2013a, 2013b, 2014; Chmeliov et al., 2015). The formation of quenching chlorophyll-chlorophyll dimers has also been recently advocated (Müller et al., 2010). Notably, the multiplicity of the possible identity and physics of the NPQ quencher(s) may well reflect the complex nature of the process involving the formation of a variety of pigment-pigment interactions. Therefore, the existence of multiple types of quenchers, which include xanthophylls as well as chlorophylls, was recently contemplated (Holzwarth et al., 2009; Liguori et al., 2015).

PROTECTIVE EFFECTIVENESS OF NPQ

The attention to the details of the mechanism underlying NPQ has been and remains enormous. By contrast, little is actually known about how (quantitatively) efficient NPQ is in protecting the photosynthetic membrane against photodamage and how to separate its protective components. In addition, some reports claim that NPQ plays little or no role in photoprotection of PSII against photodamage (Santabarbara et al., 2001). However, the majority of in vivo studies have clearly established a crucial role for NPQ in protection against photoinhibition, leading to early senescence and reduced plant growth and fitness (Niyogi et al., 1998; Havaux et al., 2000; Verhoeven et al., 2001; Külheim et al., 2002; Niyogi and Truong, 2013). Understanding the quantitative aspects of the protective effectiveness of NPQ and determining the light intensity plants can tolerate without showing signs of photoinhibition require the development of new approaches. As mentioned in the beginning of this review, qE is a rather inaccurate parameter, since there are some less readily reversible (but also protective) aspects of NPQ different from qI that also reflect photoinhibition. Existing and commonly used measures of photoinhibition include the dark-adapted F_v/F_m ratio or the yield of PSII, O_2 evolution, and D1 protein degradation. While these measures have been effective for assessing the threshold for damage, they have drawbacks when used for physiological analyses, especially where laboratory-based biochemical analysis is required (D1 turnover). In addition, these methods require disruption of the light treatment, either by destructive sampling or by imposing a sustained dark period. The length of the dark period used for F_v/F_m measurements itself can be ambiguous. Recently, we developed a novel principle of NPQ analysis that enables a better understanding and quantification of the effectiveness of the protective action of NPQ. In this approach, the extent of photochemical quenching (qP) measured in the dark is used to monitor the state of active RCIIs, enabling detection of the early signs of photoinhibition (Ruban and Murchie, 2012; Ruban and Belgio, 2014). Importantly, both NPQ/qE and photodamage to RCIIs diminish the quantum yield of PSII, which is illustrated by the following formula derived by Ruban and Murchie (2012):

$$\Phi_{PSII} = qP \times (F_v/F_m)/[1 + (1 - F_v/F_m) \times NPQ] \quad (1)$$

, where qP is photochemical quenching and F_v/F_m is the yield of PSII before illumination. qP is defined as $(F_m'\text{-}F_{o\,act.}')/(F_m'\text{-}F_{o\,calc.}')$, where $F_{o\,act.}'$ is the measured dark fluorescence level and $F_{o\,calc.}'$ is the dark fluorescence level calculated using Fm′ (Oxborough and Baker, 1997). When Equation (1) was applied to leaves that had been exposed to gradually increasing light intensity, like that used in light saturation curves but for longer periods of illumination with short periods of darkness in order to assess qP levels (Fig. 4A), the formula perfectly matched the experimental data (Fig. 4B) up to a certain high actinic light intensity, above which the experimentally determined yield started to decrease more steeply with NPQ than the theoretical value (Fig. 4B). This discrepancy between the measured and calculated yield came from the fact that qP started to show values <1 (Fig. 4B), which occurred because the measured values of F_o started to become higher than the values of F_o predicted using F_m' amplitude (Oxborough and Baker, 1997; Fig. 4A). This discrepancy comes from the observation that when RCIIs become closed due to photoinhibition, they stay closed in the dark. Hence, they cannot photochemically quench fluorescence, causing an increase in F_o' in a similar way to the increase in F_o' that would be caused by the addition of DCMU or illumination, making this level effectively F_s. Therefore, under this condition, F_o' becomes appreciably less quenched in relation to F_m', which is manifested in the observed deviation of the experimental F_o' levels from their predicted values and hence brings the qP level down from 1. This qP was designated qP_d to indicate that it is always measured in the dark under the regime of

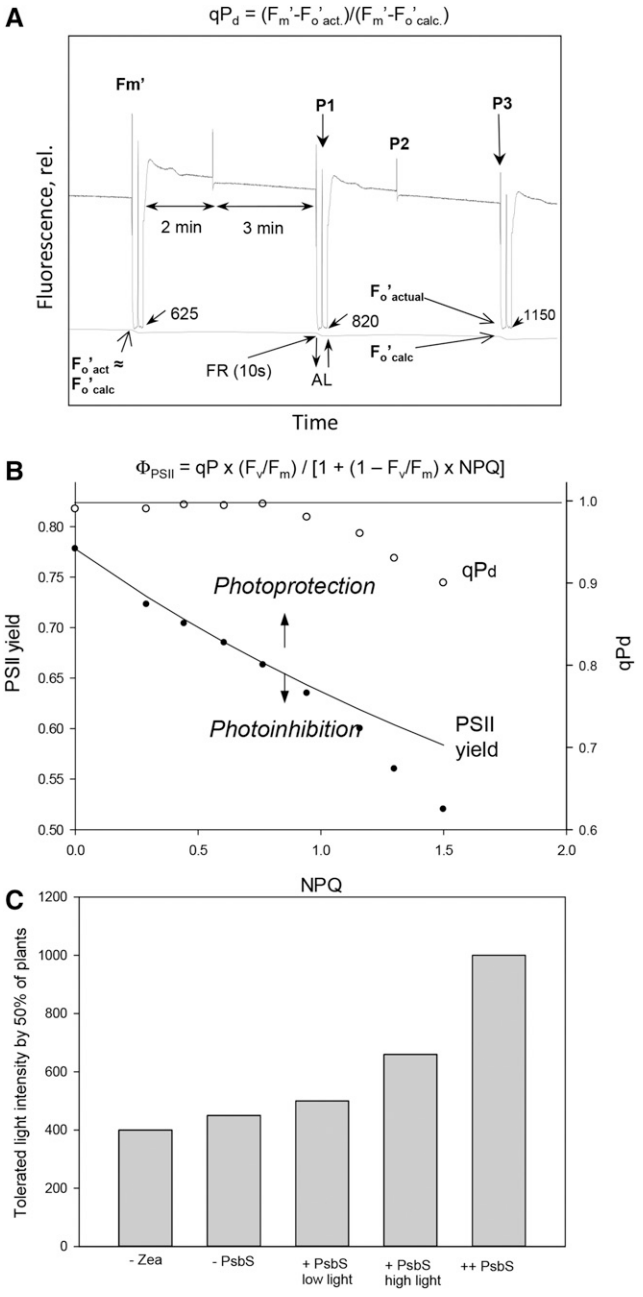

Figure 4. A, Part of the gradually increasing illumination procedure used in PAM measurements of Arabidopsis leaves. The formula at the top shows how qP_d is calculated. $F_o'_{act.}$ and $F_o'_{calc.}$ are the measured and calculated (Oxborough and Baker, 1997) dark fluorescence levels, respectively. P1, 2, and 3 are saturating pulses, AL and FR are actinic and far red light, respectively, and 625 and 820 are the intensities of actinic light in μmol $m^{-2}s^{-1}$. B, The relationships between the PSII yield, qP_d, and NPQ in the dark over the course of the gradually increasing actinic light intensity procedure (Ruban and Belgio, 2014). The formula shows the relationship between PSII yield, qP, and NPQ. C, Light intensity (in μmol $m^{-2}s^{-1}$) tolerated by 50% of the Arabidopsis mutant plants examined: –*Zea* (npq1); –PsbS (npq4); +PsbS (wt), and ++PsbS (PsbS overexpressor, L17).

gradually increasing actinic light intensity (Ruban and Murchie, 2012; Ruban and Belgio, 2014). Critical work has been undertaken to ensure that this novel method is free from artifacts from the contribution of PSI to the novel PAM fluorescence measurements (Giovagnetti et al., 2015) and that the fluorescence parameter qP_d is in good correlation with the electron transport rates measured by oxygen evolution techniques (Giovagnetti and Ruban, 2015).

The application of this approach enabled a number of important parameters to be obtained without the use of the dark relaxation step: (1) the amplitude of all protective components of NPQ, pNPQ; (2) the maximum tolerated light intensity at which all RCIIs remain functional; (3) the minimum pNPQ sufficient to protect against a unit of light intensity; (4) the amount of potentially wasteful pNPQ; and (5) the light tolerance curves for a particular type of plant (Ruban and Belgio, 2014; Ware et al., 2014). As a result of this development, the highest light intensity tolerated by 50% of various tested plants has been identified (Fig. 4C). One important conclusion of this work is that regardless of the type of mutation, the light tolerance was solely determined by the amplitude of pNPQ (Ruban and Belgio, 2014; Ware et al., 2014). Hence, pNPQ of approximately 1 in Arabidopsis could protect plants exposed to roughly 400 μmol $m^{-2}s^{-1}$ PAR (Photosynthetically Active Radiation). This relationship is nearly linear, meaning that to tolerate 1600 μmol $m^{-2}s^{-1}$ PAR of light intensity, almost the highest attainable level on the planet (total light intensity of approximately 3200 μmol $m^{-2}s^{-1}$), plants must develop pNPQ of approximately 4, which is probably the top value for this species. As expected, plants acclimated to low light exhibited lower light tolerance (Ware et al., 2015a). The formation of a larger antenna causes higher excitation pressure, hence changing the steepness in the relationship between NPQ and tolerated light intensity. Also, different plant species differ in their sensitivity to light, and therefore the requirement for pNPQ may vary significantly (Ruban, 2015). In addition, in low light-acclimated plants, part of the large LHCII antenna is uncoupled from RCII. Interestingly, this uncoupling is associated with increased levels of F_o quenching. However, this additional quenching does not contribute to light tolerance, implying that if uncoupled LHCII indeed participates in the NPQ process, as previously suggested (Holzwarth et al., 2009), it would contribute little to protection, a fact rendering the existence of two uncoupled sites for NPQ totally unnecessary. In addition, an interesting trend in light tolerance was observed during ontogenetic development (Carvalho et al., 2015): 1-week-old seedlings are almost 20 times less tolerant to light than established 8-week-old plants. This finding indicates that the most significant high-light damage occurs in young plants or developing leaves. Therefore, the major focus of plant physiologists, ecologists, and breeders should be directed toward monitoring and improving light tolerance, specifically at early stages of plant development.

The novel method of NPQ assessment described in this review should be very useful for evaluating the true effectiveness of NPQ in photoprotection in cyanobacteria, diatoms, and other classes of photosynthetic organisms. Knowing of the existence of NPQ is not enough. Modern times require that we obtain a complete understanding of its value in protecting plants by analyzing NPQ amplitude and the efficiency of photochemistry in parallel.

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LITERATURE CITED

Ahn TK, Avenson TJ, Ballottari M, Cheng YC, Niyogi KK, Bassi R, Fleming GR (2008) Architecture of a charge-transfer state regulating light harvesting in a plant antenna protein. Science **320:** 794–797

Åkerlund HE, Andersson B, Persson A, Albertsson PA (1979) Isoelectric points of spinach thylakoid membrane surfaces as determined by cross partition. Biochim Biophys Acta **552:** 238–246

Anderson JM, Chow WS, Goodchild DJ (1988) Thylakoid membrane organisation in sun/shade acclimation. Aust J Plant Physiol **15:** 11–26

Andersson J, Walters RG, Horton P, Jansson S (2001) Antisense inhibition of the photosynthetic antenna proteins CP29 and CP26: implications for the mechanism of protective energy dissipation. Plant Cell **13:** 1193–1204

Andrews JR, Fryer MJ, Baker NR (1995) Consequences of LHCII deficiency for photosynthetic regulation in chlorina mutants of barley. Photosynth Res **44:** 81–91

Armbruster U, Carrillo LR, Venema K, Pavlovic L, Schmidtmann E, Kornfeld A, Jahns P, Berry JA, Kramer DM, Jonikas MC (2014) Ion antiport accelerates photosynthetic acclimation in fluctuating light environments. Nat Commun **5:** 5439

Aro EM, Virgin I, Andersson B (1993) Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. Biochim Biophys Acta **1143:** 113–134

Avenson TJ, Ahn TK, Niyogi KK, Ballottari M, Bassi R, Fleming GR (2009) Lutein can act as a switchable charge transfer quencher in the CP26 light-harvesting complex. J Biol Chem **284:** 2830–2835

Barber J (1995) Molecular-basis of the vulnerability of photosystem-II to damage by light. Aust J Plant Physiol **22:** 201–208

Barber J, Andersson B (1992) Too much of a good thing: light can be bad for photosynthesis. Trends Biochem Sci **17:** 61–66

Bassi R, Caffarri S (2000) Lhc proteins and the regulation of photosynthetic light harvesting function by xanthophylls. Photosynth Res **64:** 243–256

Bassi R, Pineau B, Dainese P, Marquardt J (1993) Carotenoid-binding proteins of photosystem II. Eur J Biochem **212:** 297–303

Belgio E, Duffy CDP, Ruban AV (2013) Switching light harvesting complex II into photoprotective state involves the lumen-facing apoprotein loop. Phys Chem Chem Phys **15:** 12253–12261

Belgio E, Johnson MP, Jurić S, Ruban AV (2012) Higher plant photosystem II light-harvesting antenna, not the reaction center, determines the excited-state lifetime-both the maximum and the nonphotochemically quenched. Biophys J **102:** 2761–2771

Belgio E, Kapitonova E, Chmeliov J, Duffy CDP, Ungerer P, Valkunas L, Ruban AV (2014) Economic photoprotection in photosystem II that retains a complete light-harvesting system with slow energy traps. Nat Commun **5:** 4433

Belgio E, Ungerer P, Ruban AV (2015) Light-harvesting superstructures of green plant chloroplasts lacking photosystems. Plant Cell Environ **38:** 2035–2047

Betterle N, Ballottari M, Zorzan S, de Bianchi S, Cazzaniga S, Dall'osto L, Morosinotto T, Bassi R (2009) Light-induced dissociation of an antenna hetero-oligomer is needed for non-photochemical quenching induction. J Biol Chem **284:** 15255–15266

Björkman O, Demmig-Adams B (1995) Regulation of photosynthetic light energy capture, conversion and dissipation in leaves of higher plants. *In:* ED Schulze and MM Caldwell, eds, Ecophysiology of Photosynthesis: Ecological Studies. Springer-Verlag, Berlin

Blankenship R (2002) Molecular Mechanisms of Photosynthesis. Blackwell Science, London

Boekema EJ, Hankamer B, Bald D, Kruip J, Nield J, Boonstra AF, Barber J, Rögner M (1995) Supramolecular structure of the photosystem II complex from green plants and cyanobacteria. Proc Natl Acad Sci USA **92:** 175–179

Bonente G, Howes BD, Caffarri S, Smulevich G, Bassi R (2008) Interactions between the photosystem II subunit PsbS and xanthophylls studied *in vivo* and *in vitro.* J Biol Chem **283:** 8434–8445

Brestic M, Zivcak M, Kunderlikova K, Sytar O, Shao H, Kalaji HM, Allakhverdiev SI (2015) Low PSI content limits the photoprotection of PSI and PSII in early growth stages of chlorophyll *b*-deficient wheat mutant lines. Photosynth Res **125:** 151–166

Caffarri S, Kouril R, Kereïche S, Boekema EJ, Croce R (2009) Functional architecture of higher plant photosystem II supercomplexes. EMBO J **28:** 3052–3063

Carvalho FEL, Ware MA, Ruban AV (2015) Quantifying the dynamics of light tolerance in Arabidopsis plants during ontogenesis. Plant Cell Environ **38:** 2603–2617

Cazzaniga S, Dall' Osto L, Kong SG, Wada M, Bassi R (2013) Interaction between avoidance of photon absorption, excess energy dissipation and zeaxanthin synthesis against photooxidative stress in Arabidopsis. Plant J **76:** 568–579

Cheng Y-C, Fleming GR (2009) Dynamics of light harvesting in photosynthesis. Annu Rev Phys Chem **60:** 241–262

Chmeliov J, Bricker WP, Lo C, Jouin E, Valkunas L, Ruban AV, Duffy CDP (2015) An 'all pigment' model of excitation quenching in LHCII. Phys Chem Chem Phys **17:** 15857–15867

Chow WS, Anderson JM, Hope AB (1988) Variable stoichiometries of photosystem II to photosystem I reaction centres. Photosynth Res **17:** 277–281

Clayton RK (1980) Photosynthesis. Physical mechanisms and chemical patterns. Cambridge, Cambridge University Press

Correa-Galvis W, Poschmann G, Melzer M, Stuhler K, Jahns P (2016**)** PsbS interactions involved in the activation of energy dissipation in Arabidopsis. Nature Plants, doi: 10.1038/NPLANTS.2015.225

Dall'Osto L, Ünlü C, Cazzaniga S, van Amerongen H (2014) Disturbed excitation energy transfer in Arabidopsis thaliana mutants lacking minor antenna complexes of photosystem II. Biochim Biophys Acta **1837:** 1981–1988

de Bianchi S, Dall'Osto L, Tognon G, Morosinotto T, Bassi R (2008) 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:** 1012–1028

Dekker JP, Boekema EJ (2005) Supramolecular organization of thylakoid membrane proteins in green plants. Biochim Biophys Acta **1706:** 12–39

Demmig-Adams B (1990) Carotenoids and photoprotection: a role for the xanthophyll zeaxanthin. Biochim Biophys Acta **1020:** 1–24

Demmig-Adams B, Adams WW III (1992) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol **43:** 599–626

Demmig-Adams B, Garab G, William Adams III, Govindgee (2014) Nonphotochemical quenching and energy dissipation in plants, algae and cyanobacteria, Advances in Photosynthesis and Respiration **40**. Springer Science+Business Media Dordrecht

Demmig-Adams B, Winter K, Krüger A, Czygan F-C (1989) Light response of CO_2 assimilation, dissipation of excess excitation energy, and zeaxanthin content of sun and shade leaves. Plant Physiol **90:** 881–886

Dominici P, Caffarri S, Armenante F, Ceoldo S, Crimi M, Bassi R (2002) Biochemical properties of the PsbS subunit of photosystem II either purified from chloroplast or recombinant. J Biol Chem **277:** 22750–22758

Dong L, Tu W, Liu K, Sun R, Liu C, Wang K, Yang C (2015) The PsbS protein plays important roles in photosystem II supercomplex remodeling under elevated light conditions. J Plant Physiol **172:** 33–41

Duffy CDP, Ruban AV (2012) A theoretical investigation of xanthophyll-protein hydrogen bonding in the photosystem II antenna. J Phys Chem B **116:** 4310–4318

Duffy CDP, Chmeliov J, Macernis M, Sulskus J, Valkunas L, Ruban AV (2013a) Modeling of fluorescence quenching by lutein in the plant light-harvesting complex LHCII. J Phys Chem B **117:** 10974–10986

Duffy CDP, Valkunas L, Ruban AV (2013b) Quantum mechanical calculations of xanthophyll-chlorophyll electronic coupling in the light-harvesting antenna of photosystem II of higher plants. J Phys Chem B **117:** 7605–7614

Duffy CDP, Pandit A, Ruban AV (2014) Modeling the NMR signatures associated with the functional conformational switch in the major light-harvesting antenna of photosystem II in higher plants. Phys Chem Chem Phys **16:** 5571–5580

Duffy CDP, Ruban AV (2015) Dissipative pathways in the photosystem-II antenna in plants. J Photochem Photobiol B **152**(Pt B): 215–226

Duysens LNM, Sweers HE (1963) Mechanism of two photochemical reactions in algae as studied by means of fluorescence. In: Japanese society of plant physiologists (ed) Studies on microalgae and photosynthetic bacteria. University of Tokyo press, Tokyo, pp 353-371

Fan M, Li M, Liu Z, Cao P, Pan X, Zhang H, Zhao X, Zhang J, Chang W (2015) Crystal structures of the PsbS protein essential for photoprotection in plants. Nat Struct Mol Biol **22:** 729–735

Fleming GR, Schlau-Cohen GS, Amarnath K, Zaks J (2012) Design principles of photosynthetic light-harvesting. Faraday Discuss **155:** 27–41, discussion 103–114

Formaggio E, Cinque G, Bassi R (2001) Functional architecture of the major light-harvesting complex from higher plants. J Mol Biol **314:** 1157–1166

Frank HA, Cogdell RJ (1996) Carotenoids in photosynthesis. Photochem Photobiol **63:** 257–264

Gall A, Berera R, Alexandre MTA, Pascal AA, Bordes L, Mendes-Pinto MM, Andrianambinintsoa S, Stoitchkova KV, Marin A, Valkunas L, Horton P, Kennis JTM, et al (2011) Molecular adaptation of photoprotection: triplet states in light-harvesting proteins. Biophys J **101:** 934–942

Garab G, Leegood RC, Walker DA, Sutherland JC, Hind G (1988) Reversible changes in macroorganization of the light-harvesting chlorophyll *a/b* pigment-protein complex detected by circular dichroism. Biochemistry **27:** 2430–2434

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta **990:** 87–92

Genty B, Goulas Y, Dimon B, Peltier G, Briantais J-M, Moya I (1992) Modulation of efficiency of primary conversion in leaves. Photosynth Res **34:** 106

Gerotto C, Franchin C, Arrigoni G, Morosinotto T (2015) *In vivo* identification of photosystem II light harvesting complexes interacting with photosystem II subunit S. Plant Physiol **168:** 1747–1761

Giovagnetti V, Ruban AV (2015) Discerning the effects of photoinhibition and photoprotection on the rate of oxygen evolution in Arabidopsis leaves. J Photochem Photobiol B **152:** 272–278.

Giovagnetti V, Ware MA, Ruban AV (2015) Assessment of the impact of photosystem I chlorophyll fluorescence on the pulse-amplitude modulated quenching analysis in leaves of Arabidopsis thaliana. Photosynth Res **125:** 179–189

Goral TK, Johnson MP, Duffy CDP, Brain APR, Ruban AV, Mullineaux CW (2012) Light-harvesting antenna composition controls the macromolecular organization and dynamics of thylakoid membranes in Arabidopsis. Plant J **69:** 289–301

Goss R, Lepetit B (2015) Biodiversity of NPQ. J Plant Physiol **172:** 13–32

Govindjee PG (1971) Chlorophyll fluorescence and photosynthesis: fluorescence transients. In: Giese AC (ed) Photophysiology, **v.6** Academic, New York, pp 1-46

Havaux M, Bonfils J-P, Lütz C, Niyogi KK (2000) Photodamage of the photosynthetic apparatus and its dependence on the leaf developmental stage in the npq1 Arabidopsis mutant deficient in the xanthophyll cycle enzyme violaxanthin de-epoxidase. Plant Physiol **124:** 273–284

Havaux M, Dall'osto L, Bassi R (2007) Zeaxanthin has enhanced antioxidant capacity with respect to all other xanthophylls in Arabidopsis leaves and functions independent of binding to PSII antennae. Plant Physiol **145:** 1506–1520

Hill R, Bendall F (1960) Function of the two cytochrome components in chloroplasts: a working hypothesis. Nature **186:** 136–137

Holt NE, Zigmantas D, Valkunas L, Li X-P, Niyogi KK, Fleming GR (2005) Carotenoid cation formation and the regulation of photosynthetic light harvesting. Science **307:** 433–436

Holzwarth AR, Miloslavina Y, Nilkens M, Jahns P (2009) Identification of two quenching sites active in the regulation of photosynthetic light-harvesting studied by time-resolved fluorescence. Chem Phys Lett **483:** 262–267

Horton AV, Ruban AV (1993) Delta-pH-dependent quenching of the Fo-level of chlorophyll orescence in spinach leaves. Biochim Biophys Acta **1142:** 203–206

Horton P, Ruban AV, Rees D, Pascal A, Noctor GD, Young A (1991) Control of the light harvesting function of chloroplast membranes: The LHCII-aggregation model for non-photochemical quenching. FEBS Lett **292:** 1–4

Horton P, Ruban AV, Walters RG (1996) Regulation of light harvesting in green plants. Annu Rev Plant Physiol Plant Mol Biol **47:** 655–684

Ilioaia C, Duffy CDP, Johnson MP, Ruban AV (2013) Changes in the energy transfer pathways within photosystem II antenna induced by xanthophyll cycle activity. J Phys Chem B **117:** 5841–5847

Ilioaia C, Johnson MP, Horton P, Ruban AV (2008) Induction of efficient energy dissipation in the isolated light-harvesting complex of Photosystem II in the absence of protein aggregation. J Biol Chem **283:** 29505–29512

Ilioaia C, Johnson MP, Liao P-N, Pascal AA, van Grondelle R, Walla PJ, Ruban AV, Robert B (2011) Photoprotection in plants involves a change in lutein 1 binding domain in the major light-harvesting complex of photosystem II. J Biol Chem **286:** 27247–27254

Jahns P, Latowski D, Strzalka K (2009) Mechanism and regulation of the violaxanthin cycle: the role of antenna proteins and membrane lipids. Biochim Biophys Acta **1787:** 3–14

Jahns P, Krause GH (1994) Xanthophyll cycle and energy-dependent fluorescence quenching in leaves from pea plants grown under intermittent light. Planta 176–182

Jansson S (1999) A guide to the Lhc genes and their relatives in Arabidopsis/IT>. Trends Plant Sci **4:** 236–240

Johnson MP, Goral TK, Duffy CDP, Brain APR, Mullineaux CW, Ruban AV (2011) Photoprotective energy dissipation involves the reorganization of photosystem II light-harvesting complexes in the grana membranes of spinach chloroplasts. Plant Cell **23:** 1468–1479

Johnson MP, Ruban AV (2009) Photoprotective energy dissipation in higher plants involves alteration of the excited state energy of the emitting chlorophyll in LHCII. J Biol Chem **284:** 23592–23601

Johnson MP, Ruban AV (2011) Restoration of rapidly reversible photoprotective energy dissipation in the absence of PsbS protein by enhanced pH. J Biol Chem **286:** 19973–19981

Johnson MP, Ruban AV (2014) Rethinking the existence of a steady-state $\Delta\psi$ component of the proton motive force across plant thylakoid membranes. Photosynth Res **119:** 233–242

Joliot PA, Finazzi G (2010) Proton equilibration in the chloroplast modulates multiphasic kinetics of nonphotochemical quenching of fluorescence in plants. Proc Natl Acad Sci USA **107:** 12728–12733

Koller D (1990) Light-driven leaf movements. Plant Cell Environ **13:** 615–632

Kouřil R, Dekker JP, Boekema EJ (2012) Supramolecular organization of photosystem II in green plants. Biochim Biophys Acta **1817:** 2–12

Kouřil R, Oostergetel GT, Boekema EJ (2011) Fine structure of granal thylakoid membrane organization using cryo electron tomography. Biochim Biophys Acta **1807:** 368–374

Kramer DM, Sacksteder CA, Cruz JA (1999) How acidic is the lumen? Photosynth Res **60:** 151–163

Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol **42:** 313–349

Krüger TPJ, Ilioaia C, Johnson MP, Belgio E, Ruban AV, van Grondelle R (2013) The specificity of controlled protein disorder in the photoprotection of plants. Biophys J **105:** 1018–1026

Krüger TPJ, Ilioaia C, Johnson MP, Ruban AV, Papagiannakis E, Horton P, van Grondelle R (2012) Controlled disorder in plant light-harvesting complex II explains its photoprotective role. Biophys J **102:** 2669–2676

Krüger TPJ, Ilioaia C, Johnson MP, Ruban AV, van Grondelle R (2014) Disentangling the low-energy states of the major light-harvesting complex of plants and their role in photoprotection. Biochim Biophys Acta **1837:** 1027–1038

Külheim C, Ågren J, Jansson S (2002) Rapid regulation of light harvesting and plant fitness in the field. Science **297:** 91–93

Lepetit B, Goss R, Jakob T, Wilhelm C (2012) Molecular dynamics of the diatom thylakoid membrane under different light conditions. Photosynth Res **111:** 245–257

Li XP, Björkman O, Shih C, Grossman AR, Rosenquist M, Jansson S, Niyogi KK (2000) A pigment-binding protein essential for regulation of photosynthetic light harvesting. Nature **403:** 391–395

Li XP, Gilmore AM, Caffarri S, Bassi R, Golan T, Kramer D, Niyogi KK (2004) Regulation of photosynthetic light harvesting involves intrathylakoid lumen pH sensing by the PsbS protein. J Biol Chem **279:** 22866–22874

Liguori N, Periole X, Marrink SJ, Croce R (2015) From light-harvesting to photoprotection: structural basis of the dynamic switch of the major antenna complex of plants (LHCII). Sci Rep **5:** 15661

Liu C, Zhang Y, Cao D, He Y, Kuang T, Yang C (2008) Structural and functional analysis of the antiparallel strands in the lumenal loop of the major light-harvesting chlorophyll *a/b* complex of photosystem II (LHCIIb) by site-directed mutagenesis. J Biol Chem **283:** 487–495

Liu Z, Yan H, Wang K, Kuang T, Zhang J, Gui L, An X, Chang W (2004) Crystal structure of spinach major light-harvesting complex at 2.72 A resolution. Nature **428:** 287–292

Matsubara S, Gilmore AM, Osmond CB (2001) Diurnal and acclimatory responses of violaxanthin and lutein epoxide in the Australian mistletoe *Amyema miquelii*. Aust J Plant Physiol **28:** 793–800

Mehler EL, Fuxreiter M, Simon I, Garcia-Moreno EB (2002) The role of hydrophobic microenvironments in modulating pKa shifts in proteins. Proteins **48:** 283–292

Melis A, Anderson JM (1983) Changes in composition and function of thylakoid membranes as a result of photosynthetic adaptation of chloroplasts from pea-plants grown under different light conditions. Biochim Biophys Acta **723:** 392–399

Miloslavina Y, Wehner A, Lambrev PH, Wientjes E, Reus M, Garab G, Croce R, Holzwarth AR (2008) Far-red fluorescence: a direct spectroscopic marker for LHCII oligomer formation in non-photochemical quenching. FEBS Lett **582:** 3625–3631

Mozzo M, Passarini F, Bassi R, van Amerongen H, Croce R (2008) Photoprotection in higher plants: the putative quenching site is conserved in all outer light-harvesting complexes of Photosystem II. Biochim Biophys Acta **1777:** 1263–1267

Müller MG, Lambrev P, Reus M, Wientjes E, Croce R, Holzwarth AR (2010) Singlet energy dissipation in the photosystem II light-harvesting complex does not involve energy transfer to carotenoids. ChemPhysChem **11:** 1289–1296

Munekage Y, Hashimoto M, Miyake C, Tomizawa K, Endo T, Tasaka M, Shikanai T (2004) Cyclic electron flow around photosystem I is essential for photosynthesis. Nature **429:** 579–582

Murata N, Sugahara K (1969) Control of excitation transfer in photosynthesis. 3. Light-induced decrease of chlorophyll a fluorescence related to photophosphorylation system in spinach chloroplasts. Biochim Biophys Acta **189:** 182–192

Myers J (1974) Conceptual developments in photosynthesis, 1924-1974. Plant Physiol **54:** 420–426

Nath K, Jajoo A, Poudyal RS, Timilsina R, Park YS, Aro EM, Nam HG, Lee CH (2013) Towards a critical understanding of the photosystem II repair mechanism and its regulation during stress conditions. FEBS Lett **587:** 3372–3381

Nield J, Barber J (2006) Refinement of the structural model for the Photosystem II supercomplex of higher plants. Biochim Biophys Acta **1757:** 353–361

Nield J, Funk C, Barber J (2000) Supermolecular structure of photosystem II and location of the PsbS protein. Philos Trans R Soc Lond B Biol Sci **355:** 1337–1344

Nilkens M, Kress E, Lambrev P, Miloslavina Y, Müller M, Holzwarth AR, Jahns P (2010) 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:** 466–475

Nixon PJ, Michoux F, Yu J, Boehm M, Komenda J (2010) Recent advances in understanding the assembly and repair of photosystem II. Ann Bot (Lond) **106:** 1–16

Niyogi KK, Grossman AR, Björkman O (1998) Arabidopsis mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. Plant Cell **10:** 1121–1134

Niyogi KK, Shih C, Soon Chow W, Pogson BJ, Dellapenna D, Björkman O (2001) Photoprotection in a zeaxanthin- and lutein-deficient double mutant of *Arabidopsis*. Photosynth Res **67:** 139–145

Niyogi KK, Truong TB (2013) Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. Curr Opin Plant Biol **16:** 307–314

Noctor G, Rees D, Young A, Horton P (1991) The relationship between zeaxanthin, energy-dependent quenching of chlorophyll fluorescence and the transthylakoid pH-gradient in isolated chloroplasts. Biochim Biophys Acta **1057:** 320–330

Ohad I, Kyle DJ, Arntzen CJ (1984) Membrane protein damage and repair: removal and replacement of inactivated 32-kilodalton polypeptides in chloroplast membranes. J Cell Biol **99:** 481–485

Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence of photosynthetic efficiency into photochemical components — calculation of qP and Fv′/Fm′ without measuring Fo′. Photosynth Res **54:** 135–142

Oxborough K, Horton P (1988) A study of the regulation and function of energy-dependent quenching in pea-chloroplasts. Biochim Biophys Acta **934:** 135–143

Pan X, Li M, Wan T, Wang L, Jia C, Hou Z, Zhao X, Zhang J, Chang W (2011) Structural insights into energy regulation of light-harvesting complex CP29 from spinach. Nat Struct Mol Biol **18:** 309–315

Pandit A, Reus M, Morosinotto T, Bassi R, Holzwarth AR, de Groot HJM (2013) An NMR comparison of the light-harvesting complex II (LHCII) in active and photoprotective states reveals subtle changes in the chlorophyll a ground-state electronic structures. Biochim Biophys Acta **1827:** 738–744

Papageorgiu C, Govindjee (1968) Light-induced changes in the fluorescence yield of chlorophyll *a in vivo* II. *Chlorella pyrenoidosa*. Biophys J **8:** 1299–1315

Pascal AA, Liu Z, Broess K, van Oort B, van Amerongen H, Wang C, Horton P, Robert B, Chang W, Ruban A (2005) Molecular basis of photoprotection and control of photosynthetic light-harvesting. Nature **436:** 134–137

Pogson BJ, Niyogi KK, Björkman O, DellaPenna D (1998) Altered xanthophyll compositions adversely affect chlorophyll accumulation and nonphotochemical quenching in *Arabidopsis* mutants. Proc Natl Acad Sci USA **95:** 13324–13329

Polívka T, Sundström V (2004) Ultrafast dynamics of carotenoid excited States-from solution to natural and artificial systems. Chem Rev **104:** 2021–2071

Powles SB (1984) Photoinhibition of photosynthesis induced by visible-light. Annu Rev Plant Physiol Plant Mol Biol **35:** 15–44

Rees D, Noctor G, Ruban AV, Crofts J, Young A, Horton P (1992) pH dependent chlorophyll fluorescence quenching in spinach thylakoids from light treated or dark adapted leaves. Photosynth Res **31:** 11–19

Rees D, Young A, Noctor G, Britton G, Horton P (1989) Enhancement of the ΔpH-dependent dissipation of excitation energy in spinach chloroplasts by light-activation; correlation with the synthesis of zeaxanthin. FEBS Lett **256:** 85–90

Renger T, Holzwarth A (2008) Theory of excitation energy transfer and optical spectra of photosynthetic systems. Advances in photosynthesis and respiration **26**: 421-443

Robert B, Horton P, Pascal AA, Ruban AV (2004) Insights into the molecular dynamics of plant light-harvesting proteins *in vivo*. review. Trends Plant Sci **9:** 385–390

Ruban A (2013) The Photosynthetic Membrane: Molecular Mechanisms and Biophysics of light harvesting. Wiley-Blackwell, Chichester

Ruban AV (2009) Plants in light. Commun Integr Biol **2:** 50–55

Ruban AV (2015) Evolution under the sun: optimizing light harvesting in photosynthesis. J Exp Bot **66:** 7–23

Ruban AV, Belgio E (2014) The relationship between maximum tolerated light intensity and non-photochemial chlorophyll fluorescence quenching: chloroplast gains and losses. Phil Trans Royal Society of London B, **369:** 20130222.

Ruban AV, Berera R, Ilioaia C, van Stokkum IHM, Kennis JTM, Pascal AA, van Amerongen H, Robert B, Horton P, van Grondelle R (2007) Identification of a mechanism of photoprotective energy dissipation in higher plants. Nature **450:** 575–578

Ruban AV, Horton P (1995) An investigation of the sustained component of nonphotochemical quenching of chlorophyll fluorescence in isolated chloroplasts and leaves of spinach. Plant Physiol **108:** 721–726

Ruban AV, Horton P (1999) The xanthophyll cycle modulates the kinetics of nonphotochemical energy dissipation in isolated light-harvesting complexes, intact chloroplasts, and leaves of spinach. Plant Physiol **119:** 531–542

Ruban AV, Johnson MP (2010) Xanthophylls as modulators of membrane protein function. Arch Biochem Biophys **504:** 78–85

Ruban AV, Johnson MP (2015) Visualizing the dynamic structure of the plant photosynthetic membrane. Nature Plants **1:** 15161

Ruban AV, Johnson MP, Duffy CD (2012) The photoprotective molecular switch in the photosystem II antenna. Biochim Biophys Acta **1817:** 167–181

Ruban A, Lavaud J, Rousseau B, Guglielmi G, Horton P, Etienne A-L (2004) The super-excess energy dissipation in diatom algae: comparative analysis with higher plants. Photosynth Res **82:** 165–175

Ruban AV, Lee PJ, Wentworth M, Young AJ, Horton P (1999) Determination of the stoichiometry and strength of binding of xanthophylls to the photosystem II light harvesting complexes. J Biol Chem **274:** 10458–10465

Ruban AV, Murchie EH (2012) Assessing the photoprotective effectiveness of non-photochemical chlorophyll fluorescence quenching: a new approach. Biochim Biophys Acta **1817:** 977–982

Ruban AV, Pesaresi P, Wacker U, Irrgang K-DJ, Bassi R, Horton P (1998) The relationship between the binding of dicyclohexylcarbodiimide and quenching of chlorophyll fluorescence in the light-harvesting proteins of photosystem II. Biochemistry **37:** 11586–11591

Ruban AV, Rees D, Noctor GD, Young A, Horton P (1991) Long-wavelength chlorophyll species are associated with amplification of high-energy state excitation quenching in higher-plants. Biochim Biophys Acta **1059:** 355–360

Ruban AV, Walters RG, Horton P (1992) The molecular mechanism of the control of excitation energy dissipation in chloroplast membranes. Inhibition of delta pH-dependent quenching of chlorophyll fluorescence by dicyclohexylcarbodiimide. FEBS Lett **309:** 175–179

Ruban AV, Young AJ, Horton P (1993) 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:** 741–750

Ruban AV, Young A, Horton P (1994) Modulation of chlorophyll fluorescence quenching in isolated light harvesting complex of photosystem II. Biochim Biophys Acta **1196:** 123–127

Ruban AV, Young AJ, Horton P (1996) 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:** 674–678

Rutkauskas D, Chmeliov E, Johnson M, Ruban AV, Valkunas L (2012) Exciton annihilation as a probe of the light-harvesting antenna transition into the photoprotective mode. Chem Phys **404:** 123–128

Santabarbara S, Barbato R, Zucchelli G, Garlaschi FM, Jennings RC (2001) The quenching of photosystem II fluorescence does not protect the D1 protein against light induced degradation in thylakoids. FEBS Lett **505:** 159–162

Sato R, Ohta H, Masuda S (2014) Prediction of respective contribution of linear electron flow and PGR5-dependent cyclic electron flow to non-photochemical quenching induction. Plant Physiol Biochem **81:** 190–196

Scholes GD, Fleming GR, Olaya-Castro A, van Grondelle R (2011) Lessons from nature about solar light harvesting. Nat Chem **3:** 763–774

Schreiber U (1986) Detection of rapid induction kinetics with a new type of high-frequency modulated chlorophyll fluorometer. Photosynth Res **9:** 261–272

Strand DD, Kramer DM (2014) Control of non-photochemical exciton quenching by the proton circuit of photosynthesis. In: Non-photochemical quenching and energy dissipation in plants, algae and cyanobacteria, Advances in Photosynthesis and Respiration **40**. Springer Science+Business Media Dordrecht

Sylak-Glassman E, Malnoë A, De Re E, Brooks MD, Fisher AL, Niyogi KK, Fleming GR (2014) Proc Natl Acad Sci USA **111:** 2213–2218

Thayer SS, Björkman O (1992) Carotenoid distribution and deepoxidation in thylakoid pigment-protein complexes from cotton leaves and bundle-sheath cells of maize. Photosynth Res **33:** 213–225

Thurlkill RL, Grimsley GR, Scholtz JM, Pace CN (2006) Hydrogen bonding markedly reduces the pK of buried carboxyl groups in proteins. J Mol Biol **362:** 594–604

Tyystjärvi E, Aro EM (1996) The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity. Proc Natl Acad Sci USA **93:** 2213–2218

Van Amerongen H, van Grondelle R, Valkunas L (2000) Photosynthetic excitons. World Scientific Pub Co Inc.

van Oort B, van Hoek A, Ruban AV, van Amerongen H (2007) Equilibrium between quenched and nonquenched conformations of the major plant light-harvesting complex studied with high-pressure time-resolved fluorescence. J Phys Chem B **111:** 7631–7637

Verhoeven A (2013) Sustained energy dissipation in winter evergreens. New Phytol **201:** 57–65

Verhoeven AS, Bugos RC, Yamamoto HY (2001) Transgenic tobacco with suppressed zeaxanthin formation is susceptible to stress-induced photoinhibition. Photosynth Res **67:** 27–39

Walker D (1987) The use of the oxygen electrode and fluorescence probes in simple measurements of photosynthesis. Oxygraphics Ltd., Sheffield

Walters RG, Ruban AV, Horton P (1994) Light-harvesting complexes bound by dicyclohexylcarbodiimide during inhibition of protective energy dissipation. Eur J Biochem **226:** 1063–1069

Walters RG, Ruban AV, Horton P (1996) Identification of proton-active residues in a higher plant light-harvesting complex. Proc Natl Acad Sci USA **93:** 14204–14209

Ware MA, Belgio E, Ruban AV (2014) Comparison of the protective effectiveness of NPQ in *Arabidopsis* plants deficient in PsbS protein and zeaxanthin. J Exp Bot **66:** 1259–1270

Ware MA, Belgio E, Ruban AV (2015a) Photoprotective capacity of non-photochemical quenching in plants acclimated to different light intensities. Photosynth Res **126:** 261–274

Ware MA, Giovagnetti V, Belgio E, Ruban AV (2015b) PsbS protein modulates non-photochemical chlorophyll fluorescence quenching in membranes depleted of photosystems. J Photochem Photobiol B **152**(Pt B): 301–307

Weis E, Berry JA (1987) Quantum efficiency of Photosystem-II in relation to energy-dependent quenching of chlorophyll fluorescence. Biochim Biophys Acta **894:** 198–208

Wentworth M, Ruban AV, Horton P (2001) Kinetic analysis of non-photochemical quenching of chlorophyll fluorescence. II. Isolated light harvesting complexes. Biochemistry **40:** 9902–9908

Wientjes E, van Amerongen H, Croce R (2013) Quantum yield of charge separation in photosystem II: functional effect of changes in the antenna size upon light acclimation. J Phys Chem B **117:** 11200–11208

Wilk L, Grunwald M, Liao P-N, Walla PJ, Kühlbrandt W (2013) Direct interaction of the major light-harvesting complex II and PsbS in non-photochemical quenching. Proc Natl Acad Sci USA **110:** 5452–5456

Wraight CA, Crofts AR (1970) Energy-dependent quenching of chlorophyll alpha fluorescence in isolated chloroplasts. Eur J Biochem **17:** 319–327

Xu DQ, Chen Y, Chen GY (2015a) Light-harvesting regulation from leaf to molecule with the emphasis on rapid changes in antenna size. Photosynth Res **124:** 137–158

Xu P, Tian L, Kloz M, Croce R (2015b) Molecular insights into Zeaxanthin-dependent quenching in higher plants. Sci Rep **5:** 13679

Zolotareva EK, Podorvanov VV, Tereshchenko AF, Ruban AV, Horton P (1999) Energy-dependent tritium incorporation into LHCII proteins of chloroplasts. Dokl Acad Sci Ukr SSR B **11:** 152–156