Non-Photochemical Quenching. A Response to Excess Light Energy[1]

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Plants and algae have a love/hate relationship with light. As oxygenic photoautotrophic organisms, they require light for life; however, too much light can lead to increased production of damaging reactive oxygen species as byproducts of photosynthesis. In extreme cases, photooxidative damage can cause pigment bleaching and death, a phenomenon all too familiar to anyone who has tried to move a houseplant outdoors into full sunlight.

The quantity of the light in natural environments can vary over several orders of magnitude and on a time scale that ranges from seconds to seasons. Because light is such an important environmental parameter, plants have evolved numerous biochemical and developmental responses to light that help to optimize photosynthesis and growth. For example, plants rely on photoreceptors such as phytochrome for shade avoidance responses. Some plants are able to adjust their capacity for harvesting sunlight through leaf and chloroplast movements. During long-term acclimation to changes in light intensity many algae and plants regulate the size of their light-harvesting pigment antennae through changes in gene expression and/or proteolysis. Large antennae are necessary for efficient light capture in limiting light, but they can be a liability when light is abundant or excessive.

On a daily as well as seasonal basis most plants receive more sunlight than they can actually use for photosynthesis. Under these circumstances, regulation of light harvesting is necessary to balance the absorption and utilization of light energy, thereby minimizing the potential for photooxidative damage. Besides adjusting light absorption, algae and plants have ways of getting rid of excess light energy that has already been absorbed. This update will focus on protective non-photochemical mechanisms that quench singlet-excited chlorophylls (Chl) and harmlessly dissipate excess excitation energy as heat. These non-photochemical quenching (NPQ) processes occur in almost all photosynthetic eukaryotes, and they help to regulate and protect photosynthesis in environments in which light energy absorption exceeds the capacity for light utilization. We will summarize progress in understanding NPQ that has been made since the last *Update* article on NPQ appeared in this journal (Horton et al., 1994).

NPQ AFFECTS CHL FLUORESCENCE

Absorption of sunlight for photosynthesis is accomplished by light-harvesting pigment-protein complexes (LHCs) that are associated with reaction centers. Light absorption results in singlet-state excitation of a Chl *a* molecule (^{1}Chl*), which can return to the ground state via one of several pathways (Fig. 1). Excitation energy can be re-emitted as Chl fluorescence, it can be transferred to reaction centers and used to drive photochemistry, it can be de-excited by thermal dissipation processes (NPQ), or it can decay via the triplet state (^{3}Chl*). Although the triplet pathway can be a significant valve for excess excitation (4%–25% of absorbed photons; Foyer and Harbinson, 1999), ^{3}Chl* can transfer energy to ground-state O_2 to generate singlet oxygen ($^1O_2$*), an extremely damaging reactive oxygen species. At room temperature, Chl fluorescence mainly originates from photosystem (PS) II, and the yield of fluorescence is generally low (0.6%–3%; Krause and Weis, 1991). The yields of ^{3}Chl* and fluorescence vary in proportion to the average lifetime of ^{1}Chl*, which in turn depends on the yields of the other pathways. For example, the high quantum efficiency of photochemistry in limiting light results in a decrease, or quenching, of fluorescence that is termed photochemical quenching (qP). Non-photochemical processes that dissipate excitation energy also quench Chl fluorescence and are collectively called NPQ (or qN). A summary of Chl fluorescence quenching processes is given in Table I.

In practice Chl fluorescence quenching is usually measured with a commercial fluorometer that can measure fluorescence yield in the presence of varying background white light (Fig. 2). Over a wide range of light intensities, plants are able to maintain a low steady-state fluorescence yield and ^{3}Chl* yield due to a combination of qP and NPQ. Thus, qP and NPQ help to minimize production of $^1O_2$* in the PSII antenna. The quenching due solely to NPQ can be determined periodically by measuring the fluorescence during a brief (≤1 s) pulse of light that satu-

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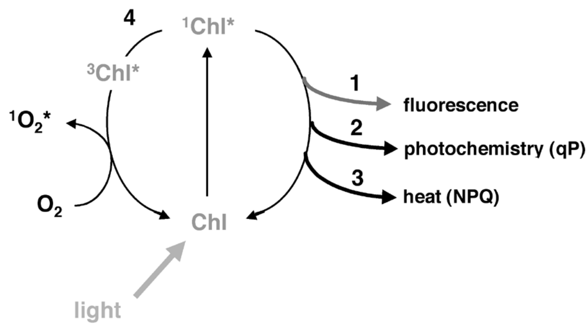

Figure 1. Possible fates of excited Chl. When Chl absorbs light it is excited from its ground state to its singlet excited state, ^{1}Chl*. From there it has several ways to relax back to the ground state. It can relax by emitting light, seen as fluorescence (1). Its excitation can be used to fuel photosynthetic reactions (2), or it can de-excite by dissipating heat (3); both of these mechanisms reduce the amount of fluorescence. They are therefore referred to as qP and NPQ of Chl fluorescence. Last, ^{1}Chl* can, by intersystem crossing, produce ^{3}Chl* (4), which in turn is able to produce $^1O_2$*, a very reactive oxygen species.

rates photochemistry so that there is no quenching anymore due to qP (Fig. 2).

NPQ HAS MULTIPLE COMPONENTS

NPQ can be divided into at least three different components according to their relaxation kinetics in darkness following a period of illumination, as well as their response to different inhibitors (Fig. 2; Horton and Hague, 1988). The major and most rapid component in most algae and plants is the pH- or energy-dependent component, qE. A second component, qT, relaxes within minutes and is more important in algae, but rather negligible in most plants during exposure to excess light. This component is due to the phenomenon of state transition, the uncoupling of LHCs from PSII. qT will not be considered further here because it does not seem to be important for photoprotection (Niyogi, 1999). The third component of NPQ shows the slowest relaxation and is the least defined. It is related to photoinhibition of photosynthesis and is therefore called qI.

RAPIDLY INDUCIBLE AND REVERSIBLE qE QUENCHING IS pH-DEPENDENT

Absorption of sunlight that exceeds a plant's capacity for CO_2 fixation results in a buildup of the thylakoid ΔpH that is generated by photosynthetic electron transport. The decrease in pH within the thylakoid lumen is an immediate signal of excessive light that triggers the feedback regulation of light harvesting by qE. The control by lumen pH allows induction or reversal of qE within seconds of a change in light intensity (see Fig. 2), which is fast enough to cope with natural fluctuations in light intensity that are due to, for example, passing clouds on a partly sunny day.

The requirement for low lumen pH is evidenced by the inhibition of qE by uncouplers of the ΔpH such as nigericin. Screening for mutants with lower qE levels has uncovered several mutants that are defective in generation of the ΔpH due to defects in photosynthetic electron transport. In these mutants qP is also affected (Shikanai et al., 1999). However, low lumen pH that induces qE does not have to be generated by light-dependent reactions. Using isolated thylakoids it is possible to induce qE in darkness by simply lowering the pH of the buffer or by generating a ΔpH via ATP hydrolysis and reverse proton pumping by the ATP synthase (Gilmore and Yamamoto, 1992; Krieger et al., 1992).

Intensive research during the past several years has led to a concept of the role of the ΔpH in qE. A decrease in lumen pH induces qE through protonation of PSII proteins and activation of xanthophyll synthesis via a xanthophyll cycle. Together, binding of protons and xanthophylls to specific sites in the PSII antenna causes a conformational change that switches a PSII unit into a quenched state with a short ^{1}Chl* lifetime and a low fluorescence yield (Gilmore, 1997). In the following sections we will describe further the mechanistic details of this pH-dependent switch and the physiological significance of qE.

Table I. *Summary of Chl fluorescence quenching processes*

Abbreviation	Definition
qP	Photochemical quenching; fluorescence yield is lowered because of use of excitation energy for photochemical reactions
NPQ	Non-photochemical quenching; all mechanisms that lower the fluorescence yield apart from photochemistry, divided into qE, qT, and qI according to their relaxation kinetics
qE	Energy-dependent quenching; requires the build-up of a proton gradient; relaxes within seconds to minutes
qT	State-transition quenching; the major light-harvesting complex separates from PSII, thereby reducing the amount of excitation energy in PSII that can de-excite to fluorescence; relaxes within tens of minutes
qI	Photoinhibitory quenching; this quenching is caused by photoinhibition and shows very slow relaxation kinetics in the range of hours

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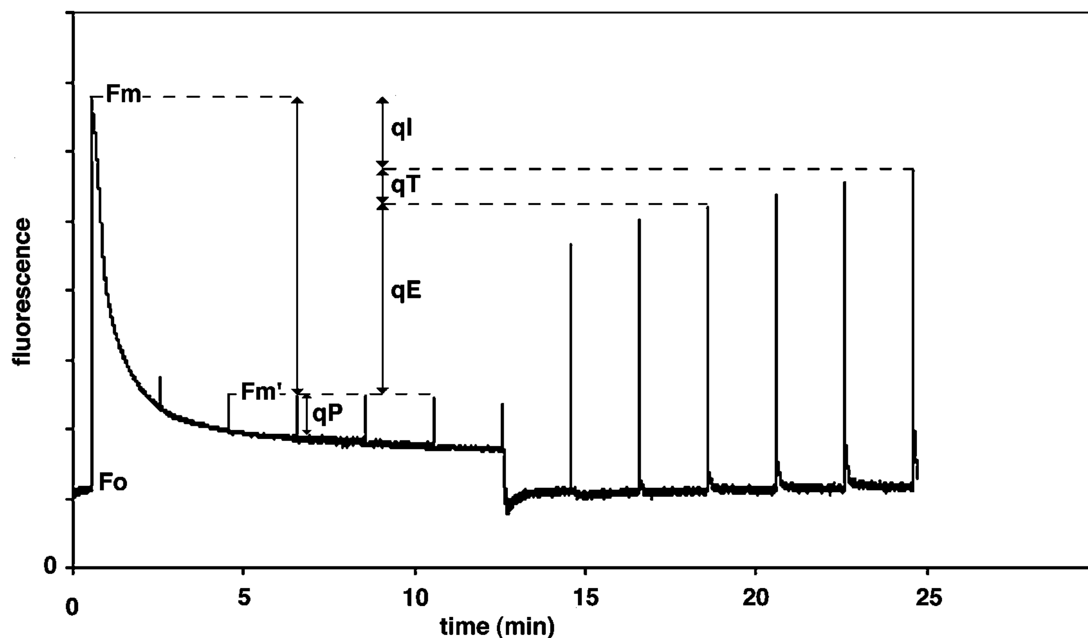

Figure 2. Chl fluorescence measurement from an Arabidopsis leaf. In the presence of only weak measuring light the minimal fluorescence (F_o) is seen. When a saturating light pulse is given, the photosynthetic light reactions are saturated and fluorescence reaches a maximum level (F_m'). Upon continuous illumination with moderately excess light (750 μmol photons m^{-2} sec^1; growth light was 130 μmol photons m^{-2} sec^{-1}), a combination of qP and NPQ lowers the fluorescence yield. NPQ (qE + qT + qI) can be seen as the difference between F_m and the measured maximal fluorescence after a saturating light pulse during illumination (F_m'). After switching off the light, recovery of F_m' within a few minutes reflects relaxation of the qE component of NPQ.

LOW LUMEN pH ACTIVATES A XANTHOPHYLL CYCLE

The decrease in lumen pH in excessive light activates the interconversion of specific xanthophyll pigments (oxygenated carotenoids) that are mostly bound to LHC proteins. This interconversion occurs on a timescale of minutes as part of a xanthophyll cycle, as depicted in Figure 3. All organisms that exhibit qE have a xanthophyll cycle, of which there are two main types. The violaxanthin cycle in plants, green algae (Chlorophyta), and brown algae (Phaeophyceae) consists of the pH-dependent conversion from violaxanthin, a xanthophyll with two epoxide groups, first to antheraxanthin (one epoxide group) and then to zeaxanthin (no epoxide group). Diatoms and most other eukaryotic algae have a different xanthophyll cycle (the diadinoxanthin cycle) that involves a conversion from diadinoxanthin (one epoxide group) to diatoxanthin (no epoxide group). Under certain conditions these algae can be observed to operate both the violaxanthin cycle and the diadinoxanthin cycle (Lohr and Wilhelm, 1999).

In plants the de-epoxidation reaction is catalyzed by violaxanthin de-epoxidase (VDE). VDE is a 43-kD nucleus-encoded protein that is localized in the thylakoid lumen (Bugos and Yamamoto, 1996). The purified VDE enzyme is activated by low pH (Eskling et al., 1997), and cloning of the VDE gene revealed that the enzyme has a Glu-rich region that may be protonated at low pH (Bugos and Yamamoto, 1996). Upon acidification of the lumen, VDE associates with the thylakoid membrane (Hager and Holocher, 1994) where it can interact with its substrate violaxanthin. VDE uses ascorbic acid (vitamin C) to reduce the epoxide group, and it has a K_m for ascorbic acid that is strongly dependent on pH, probably because ascorbic acid rather than ascorbate is the actual cosubstrate (Bratt et al., 1995).

A different enzyme, zeaxanthin epoxidase (ZE), catalyzes the epoxidation reactions that complete the violaxanthin cycle. ZE is a flavin adenine dinucleotide-containing, O_2-dependent mono-oxygenase that uses reduced ferredoxin to epoxidize first zeaxanthin and then antheraxanthin (Bouvier et al., 1996). Because of its pH optimum of 8, ZE is thought to be located on the stromal side of the thylakoid membrane and to be constitutively active. The level of zeaxanthin is therefore determined by the activity of VDE compared with ZE, with rapid accumulation of zeaxanthin occurring upon activation of VDE in excessive light. ZE and VDE are the first known plant members of the lipocalin family, a diverse group of proteins that bind small lipophilic molecules and share a conserved tertiary structure of eight β-strands in a barrel configuration (Bugos et al., 1998).

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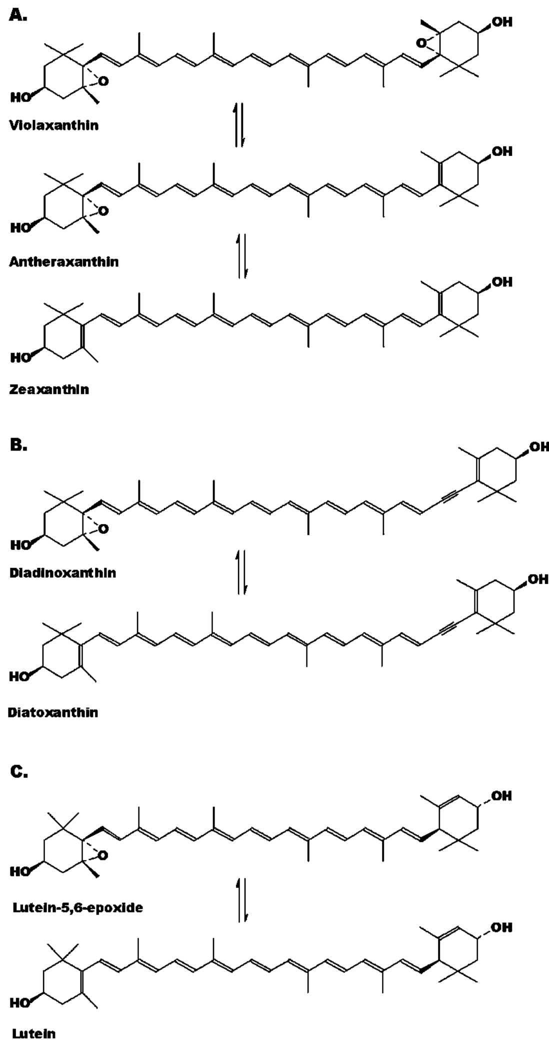

Figure 3. Xanthophyll cycles. A, The violaxanthin cycle consists of the de-epoxidation of violaxanthin in high light to first antheraxanthin and then zeaxanthin, catalyzed by VDE; ZE catalyzes the reverse reaction. B, The diadinoxanthin cycle consists of the conversion of diadinoxanthin to diatoxanthin by diadinoxanthin de-epoxidase in high light and the reverse reaction in low light. C, In the recently discovered lutein-5,6-epoxide cycle, conversion of lutein-5,6-epoxide to lutein might also be catalyzed by VDE.

XANTHOPHYLLS ARE NECESSARY, BUT NOT SUFFICIENT FOR qE IN VIVO

The amount of zeaxanthin synthesized via the violaxanthin cycle is highly correlated with the level of qE in a large number of plants under a variety of conditions (Demmig-Adams, 1990). In a similar manner, a correlation has been shown between qE and the conversion of diadinoxanthin to diatoxanthin in diatoms (Arsalane et al., 1994). Isolated thylakoids that are devoid of zeaxanthin have been observed to exhibit high levels of qE (Rees et al., 1992), but only at lumen pH values that are lower than those normally occurring in vivo.

The requirement for the xanthophylls in qE has been investigated in vivo by using inhibitors and mutants. Dithiothreitol has been used extensively as a remarkably specific inhibitor of VDE and diadinoxanthin de-epoxidase. Blocking zeaxanthin synthesis in leaves with dithiothreitol results in inhibition of qE, the extent of inhibition depending on the plant species examined (Horton et al., 1994). Mutants that are unable to convert violaxanthin to antheraxanthin and zeaxanthin have been isolated from Arabidopsis and *Chlamydomonas* (for review, see Niyogi, 1999). As in the inhibitor studies, lower levels of qE accompanied the lack of zeaxanthin in these *npq1* mutants. Recent studies using antisense VDE in tobacco have confirmed the results obtained from mutant analyses (Chang et al., 2000).

The alga *Mantoniella squamata* has an incomplete xanthophyll cycle, only leading to antheraxanthin in vivo, but still exhibits qE (Goss et al., 1998). This implies that the role of zeaxanthin in qE can be replaced by antheraxanthin in this alga. The involvement of antheraxanthin in plants had been proposed in earlier studies where zeaxanthin-independent qE could be explained by taking the amounts of antheraxanthin into account (Gilmore and Yamamoto, 1993). Therefore, it has become a common practice to calculate the level of de-epoxidation of a given organism as the amount of antheraxanthin and zeaxanthin in comparison with the total amount of antheraxanthin, zeaxanthin, and violaxanthin.

In addition to antheraxanthin and zeaxanthin, a third xanthophyll, lutein, has also been implicated in qE. These suggestions were supported by studies on the *Chlamydomonas lor1* mutant, which only lacks lutein and loroxanthin, but shows lower qE than the wild type (Niyogi et al., 1997). A similar mutant in Arabidopsis, *lut2*, which is defective in the lycopene ϵ-cyclase and therefore lacks lutein, also has less qE (Pogson et al., 1998). Double mutants of *Chlamydomonas* or Arabidopsis that lack lutein and zeaxanthin are totally devoid of any qE and are very sensitive to high light (Niyogi et al., 1997, 2001). Furthermore, plants that overexpress the lycopene ϵ-cyclase have an increased lutein content and show a slight, but significant increase in the rate of qE induction, even though their xanthophyll cycle pool size is reduced in comparison with the wild type (Pogson and Rissler, 2000).

It is interesting that a third kind of xanthophyll cycle involving lutein-5,6-epoxide has been found in a parasitic plant, *Cuscuta reflexa* (Fig. 3). In this plant neoxanthin is missing and is replaced by lutein-5,6-epoxide. In high light, lutein-5,6-epoxide is de-epoxidized to lutein, presumably by VDE, which was previously shown to use lutein-5,6-epoxide as substrate (Bungard et al., 1999). The existence of this new cycle is consistent with the idea that lutein has a photoprotective function that the epoxide lacks.

Although zeaxanthin is generally necessary for maximal qE in vivo, it is not sufficient. In mutants that accumulate zeaxanthin constitutively, qE must

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still be induced by a low pH (Niyogi, 1999). This demonstrates that the low pH has an additional role in qE, besides activation of the xanthophyll cycle.

A CONFORMATIONAL CHANGE IS INVOLVED IN qE

Lowering the pH in the thylakoid lumen not only activates the de-epoxidation of violaxanthin to zeaxanthin, but it is also necessary for a conformational change in the thylakoid membrane that can be monitored by absorbance changes. Two high light-induced absorbance changes in leaves or isolated thylakoids are associated with qE. One absorbance change occurs at 505 nm and is due to the conversion of violaxanthin to zeaxanthin. The second one at 535 nm (ΔA_{535}) depends on both zeaxanthin and low pH and is thought to be due to a conformational change in the thylakoid membrane (Krause, 1973; Bilger and Björkman, 1994). qE is always accompanied by the ΔA_{535}.

Conformational changes have also been inferred from measurements of Chl fluorescence lifetime distributions, which depend on the molecular environment of the excited Chl (Gilmore, 1997). In these experiments the presence of a ΔpH alone causes a lifetime shift from approximately 2 to 1.6 ns. This shift likely reflects a protonation-dependent conformational change that is independent of zeaxanthin. When both ΔpH and zeaxanthin are present, a fluorescence lifetime component at 0.4 ns appears at the expense of the 1.6 ns component. The amount of the 0.4 ns component is proportional to qE. Together, the absorbance and Chl fluorescence lifetime results suggest that a conformational change due to binding of protons and xanthophylls (maybe zeaxanthin) is necessary for qE.

THE PsbS PROTEIN IS ESSENTIAL FOR qE

Several LHC proteins associated with PSII have been implicated in qE. In particular the minor LHC proteins CP29 and CP26 were suggested to be involved in qE based on the relative enrichment of associated xanthophyll cycle pigments (Bassi et al., 1997) and binding of *N*,*N′*-dicyclohexylcarbodiimide (Walters et al., 1994), an inhibitor of qE that reacts with proton active residues. However, these proteins have not been found in some organisms that exhibit qE such as *Mantoniella* and diatoms.

To identify proteins involved in qE Arabidopsis mutants have been isolated that are defective in qE, but have normal xanthophyll levels (Li et al., 2000; Peterson and Havir, 2000). Characterization of one of these mutants, *npq4-1*, revealed that a PSII protein, PsbS, is essential for qE (Li et al., 2000). PsbS belongs to the LHC protein superfamily, but it has four transmembrane helices instead of three and different pigment-binding characteristics (Funk et al., 1995). Despite the absence of the PsbS protein, light harvesting is not impaired in the *npq4-1* mutant and the levels of the other LHC proteins are normal. However, in addition to lacking qE, *npq4-1* also lacks the conformational change monitored by ΔA_{535} (Li et al., 2000).

WHAT IS THE BIOPHYSICAL MECHANISM OF ^{1}CHL* DE-EXCITATION?

The pH- and xanthophyll-dependent conformational change and the PsbS protein are necessary for qE, but the actual biophysical mechanism of ^{1}Chl* de-excitation is still unknown. The central and long-standing question is whether the involvement of xanthophylls is direct or indirect. The xanthophylls may act indirectly as allosteric regulators of the LHCs that cause a switch from light harvesting to energy dissipation (qE; Horton et al., 2000). In this case the conformational change must somehow facilitate ^{1}Chl* de-excitation, which may occur by internal conversion of Chl itself to the ground state, releasing excitation energy as heat. Isolated, detergent-solubilized LHCs can exhibit pH-dependent Chl fluorescence quenching, which is inhibited by violaxanthin and promoted by zeaxanthin (Ruban et al., 1997).

On the other hand, xanthophylls may directly de-excite ^{1}Chl*. This is theoretically possible according to recent spectroscopic experiments, which showed that isolated xanthophyll cycle pigments possess a lowest singlet excited state that is below that of ^{1}Chl* (Polívka et al., 1999; Frank et al., 2000). However, because both violaxanthin and zeaxanthin could potentially accept energy from ^{1}Chl*, these results do not explain why the xanthophyll cycle is necessary for qE. In vivo, other factors such as different binding sites for zeaxanthin and violaxanthin, as well as distance and orientation of these xanthophylls relative to Chl may be important in determining if energy transfer occurs. Therefore, structural rather than energetic differences between zeaxanthin and violaxanthin are likely to be critical. For example, the xanthophylls that are important for qE (zeaxanthin, antheraxanthin, lutein, and diatoxanthin) all have a de-epoxidized 3-hydroxy β-ring endgroup (Fig. 3), in contrast to violaxanthin, diadinoxanthin, and other xanthophylls.

A WORKING MODEL FOR qE

Figure 4 shows a summary model for how qE might function. Under limiting light conditions, qE is not induced and the PSII antenna is characterized by efficient transfer of excitation energy to the reaction center (Fig. 4A). Upon high light exposure, proteins in the antenna become protonated, causing a switch in the Chl fluorescence lifetime from 2 to 1.6 ns (Fig. 4B). We propose that one of these proteins is PsbS. At the same time, VDE is activated, but the conversion

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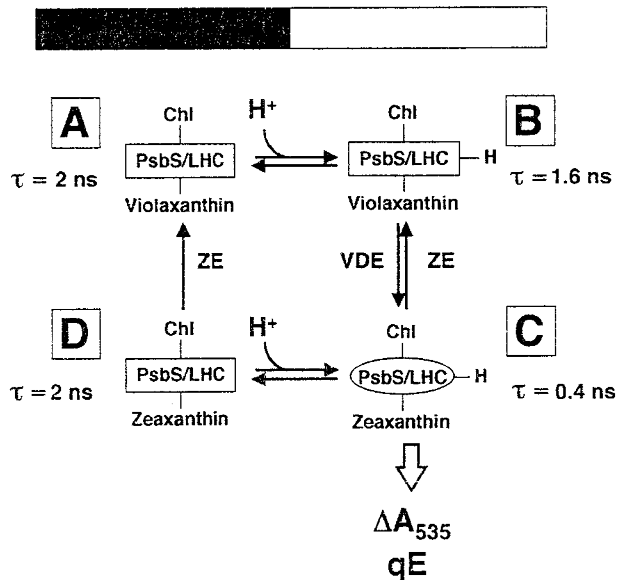

Figure 4. A summary model for qE. A, In limiting light or darkness (black bar above) no quenching occurs. B, In high light (white bar) a proton, likely several protons, bind to PsbS and LHC proteins, causing a shift in Chl fluorescence lifetime to 1.6 ns. C, A quenching complex with a different conformation (measurable as ΔA_{535}) is formed when zeaxanthin and protons are bound, reducing the Chl fluorescence lifetime to 0.4 ns. Conversion of violaxanthin to zeaxanthin occurs more slowly than protonation. Zeaxanthin might bind to the same site as violaxanthin or a different one. D, When the light stress has ended, the PSII proteins are de-protonated rapidly, whereas the epoxidation of zeaxanthin to violaxanthin is slower. In the dark, formation of complex D from complex A is not possible in vivo, but can artificially be induced by decreasing the pH in the thylakoid lumen. The above model is based in part on previous models by Horton (Horton et al., 1991).

of violaxanthin to zeaxanthin is slower than protonation. Binding of zeaxanthin to a protonated protein, possibly PsbS itself, causes the formation of a quenching complex, evident in a further decrease in the Chl fluorescence lifetime to 0.4 ns (Fig. 4C). To form the quenching complex, a conformational change must occur within the PSII antenna, perhaps originating in a conformational shift of PsbS induced by the binding of zeaxanthin and protons. The conformational change in the antenna can be followed by measuring ΔA_{535}. Formation of the quenching complex could involve changes within the dimeric PSII or changes in the interaction of several supercomplexes with each other. Upon a decrease in light intensity, a decrease in ΔpH should result in the relatively rapid de-protonation of the antenna protein(s) and the disassembly of the quenching complex, changing the fluorescence lifetime back to 2 ns (Fig. 4D). Zeaxanthin conversion to violaxanthin occurs more slowly. Therefore, a plant exposed to fluctuating light such as sunflecks is able to reach maximum qE faster after previous exposure to high light by directly switching from state D shown in Figure 4 to state C (Demmig-Adams et al., 1999).

qE AND XANTHOPHYLLS ARE IMPORTANT FOR PHOTOPROTECTION

The *npq* mutants have been useful for studying the photoprotective function of qE during high light stress. The Arabidopsis mutants *npq1* and *npq4* are more sensitive to photoinhibition than the wild type in a short-term high-light treatment (time scale of hours; Niyogi et al., 1998; Havaux and Niyogi, 1999), suggesting that qE normally functions to protect PSII. The photoprotective effect of qE may be due to decreased production of $^1O_2^*$ and other reactive oxygen species. qE may also prevent the over-reduction of the electron transport chain and the over-acidification of the lumen, which are known to sensitize PSII to photodamage.

After several days in high light, the *npq1* mutant showed more photooxidative bleaching and lipid peroxidation than *npq4* (Havaux and Niyogi, 1999). Similar to *npq1*, antisense tobacco plants that lack VDE showed a significant increase in photoinhibition and a decrease in pigment content when subjected to high light or a combination of moderate light and water stress in a growth chamber (Verhoeven et al., 2001). When transferring *npq1 lut2*, an Arabidopsis double mutant missing zeaxanthin and lutein, into high light even more photooxidative bleaching and premature senescence was visible (Niyogi et al., 2001). These results indicate that xanthophylls have a function not only in qE, but also in the protection of the thylakoid membrane against photooxidative damage.

Zeaxanthin may directly protect the thylakoid membrane against photooxidation. Thylakoid membranes are enriched in polyunsaturated fatty acids that are particularly susceptible to $^1O_2^*$-initiated lipid peroxidation reactions. Generation of $^1O_2^*$ within leaves infiltrated with the photosensitizing chemical eosin caused severe lipid peroxidation in mature leaves of *npq1*, but not in wild-type leaves, suggesting that the photoprotective role of zeaxanthin is not restricted to the LHCs (Havaux et al., 2000). Zeaxanthin may be an important antioxidant in the thylakoid membrane bilayer itself, where it could scavenge reactive oxygen species and/or terminate lipid peroxidation chain reactions. Zeaxanthin and lutein slowed down the lipid peroxidation in artificial membranes made from egg yolk lecithin in response to a peroxyl radical generator (Sujak et al., 1999). Investigating the antioxidant roles of zeaxanthin and lutein has important implications not only for thylakoids, but also for retinal membranes of the primate macula lutea where these xanthophylls are found specifically. Zeaxanthin could also have a structural function in the lipid bilayer itself. This xanthophyll has been shown to decrease the fluidity of the membrane (Tardy and Havaux, 1997), and a decrease in fluidity could be important by lowering the penetration of reactive oxygen species inside the thylakoid.

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In summary, qE protects PSII against short-term high light and fluctuations in light intensities, whereas xanthophylls have an additional photoprotective role in longer-term high light. Although the light sensitivity of the *npq* mutants of Arabidopsis and the VDE antisense plants of tobacco has been demonstrated clearly, these mutants are remarkably tolerant of strong light. In particular, young leaves of the *npq* mutants are quite resistant to high light or oxidative stress (Havaux et al., 2000; Niyogi et al., 2001), suggesting that other important photoprotective mechanisms like tocopherols or other antioxidants can compensate at least partially for the lack of qE and/or xanthophylls.

qI QUENCHING IS INVOLVED IN LONG-TERM DOWN-REGULATION OF PSII

Under more prolonged, severe light stress qE is replaced by a sustained, slowly reversible component of NPQ, called qI. In contrast to qE, qI is much less characterized and might be due to a mix of photoprotection and photodamage. Chl fluorescence measurements can help to distinguish between photoprotective mechanisms and photoinhibition. The minimum fluorescence level in the dark-adapted state, F_o (see Fig. 2), is decreased in direct proportion to the maximal fluorescence, F_m, by the photoprotective quenching like qE, whereas photoinhibition normally increases the F_o level while decreasing the F_m level (Gilmore et al., 1996).

Overwintering plants show an acclimation to the cold by increasing the xanthophyll pool size, as well as by having an increased retention of zeaxanthin and antheraxanthin that is associated with qI (Demmig-Adams et al., 1999). Overwintering snow gum trees appear to form special Chl-quenching complexes that dissipate excess excitation energy (Gilmore and Ball, 2000). Measurements of Chl fluorescence lifetime distributions of this qI state have revealed lifetime changes that are similar to those observed during qE, but that are reversed only gradually at room temperature (Gilmore and Ball, 2000). Induction and reversal of qI in these overwintering leaves may involve major reorganization of pigment-protein complexes in the thylakoid membrane.

Some part of the persistent qI induced by low temperature is actually better described as sustained qE because it is pH-dependent. By adding nigericin, an uncoupler, this kind of qI can be relaxed quickly (Gilmore and Björkman, 1995). It may be due to the maintenance of the ΔpH by the reverse proton pumping catalyzed by the ATP-synthase (Gilmore, 1997). Therefore, in contrast to normal qE it relaxes only slowly in darkness.

CONCLUSIONS AND FUTURE DIRECTIONS

Much has happened since the last *Update* on this topic was published in this journal (Horton et al., 1994). Based on physiological studies, the main function for qE seems to be the protection of PSII from photoinhibition. However, when qE is impaired, other mechanisms are able to compensate for long-term acclimation, at least in the absence of additional stresses. Further studies using qE mutants are necessary to test the ecological importance of qE and to uncover other important photoprotective mechanisms that complement qE.

The PsbS protein is an essential component of the mechanism of qE. PsbS itself may be the site of qE in the antenna, or it may function in concert with other LHC proteins. To understand how PsbS actually functions in qE, its location within the antenna, as well as possible proton- and pigment-binding sites within PsbS have to be determined. Characterization of additional mutants or the use of reverse genetics may provide insights into the involvement of other proteins.

Another new development in the field has been the finding that both zeaxanthin and violaxanthin are potential acceptors of excitation energy from ^{1}Chl*. In the future it will be important to design experiments that enable measurement of the energy levels of xanthophylls in their native protein environment and ultimately to determine if there is a direct energy transfer from Chl to a xanthophyll. The application of new and diverse techniques, from chemistry to genetics to ecology, will be necessary to understand qE, a nearly ubiquitous response of photosynthetic eukaryotes to excess light energy.

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