

# Regulation of Light Harvesting in Green Plants<sup>1</sup>

## Indication by Nonphotochemical Quenching of Chlorophyll Fluorescence

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Chl fluorescence has become one of the most powerful methods for assessing photosynthetic performance in plant physiological experiments (Horton and Bowyer, 1990; Krause and Weis, 1991). This has resulted almost entirely from the development of methods to distinguish photochemical and nonphotochemical quenching of fluorescence. Moreover, it is now clear that the process of nonphotochemical quenching itself indicates important regulatory adjustments in the photosynthetic membrane in response to altered external and internal conditions (Demmig-Adams and Adams, 1992; Horton and Ruban, 1992). In particular, the dissipation of excess absorbed excitation that is monitored by the main component of nonphotochemical quenching is a process that is necessary if plants are to avoid photoinhibition and photodestruction under conditions of light stress.

When light is absorbed by the Chl molecules in the thylakoid membrane, the excited state has several alternative and competing fates: a small proportion is emitted as fluorescence, but, under light-limiting conditions, the major pathway of de-excitation is through photosynthetic electron transfer. The effect of photochemical utilization of energy is to quench fluorescence, and it is well known that when photosynthetic electron flow is saturated the yield of fluorescence rises. This photochemical quenching has been termed  $qP$  and, using the light-doubling principle as applied with modulated fluorimetry, it is easy to calculate it in leaves, chloroplasts, and cells (Schreiber et al., 1986; Horton and Bowyer, 1990; van Kooten and Snel, 1990). However,  $qP$  does not account for all of the quenching observed. Indeed, in light saturating for electron transport,  $qP$  tends to zero, yet there can be large amounts of quenching. Such quenching is therefore called nonphotochemical quenching and refers to the difference between the initial, dark-adapted maximum level of fluorescence and that recorded after a period of illumination. This quenching can be calculated in a number of ways, leading to it being termed variously as  $qN$  (Schreiber et al., 1986; van Kooten and Snel, 1990), NPQ (Bilger and Björkman, 1994), or  $SV_n$  (Gilmore and Björkman, 1994); these all refer to the

same biological process and in this article we will use the term  $qN$ .

$qN$  results from the nonphotochemical de-excitation of the singlet excited state of Chl associated with PSII. A number of processes occurring in the thylakoid membrane contribute to  $qN$  in vivo, but the major proportion arises from an increase in nonradiative decay as heat, the quenching processes being referred to as  $qE$  and  $qI$  (Horton and Bowyer, 1990; Krause and Weis, 1991). Both these are induced under conditions of light stress (i.e. at intensities above those that can be used completely in photosynthetic electron transport). They were distinguished initially in aqueous phase measurement (chloroplasts and protoplasts) by virtue of their sensitivity to DCMU and uncouplers. Thus,  $qE$  is dependent on the energization of the thylakoid membrane or, more specifically, the thylakoid  $\Delta pH$  formed upon illumination. On the other hand,  $qI$  remains after dissipation of  $\Delta pH$  and it relaxes only slowly; it has been attributed to a range of processes including both PSII damage or inactivation and quenching in the antenna, possibly as a result of zeaxanthin formation. The kinetics of relaxation of quenching after darkening of an illuminated leaf can be complex, and long-lived forms of  $qE$  can apparently exist that interfere with estimation of  $qI$  (Walters and Horton, 1991; Gilmore and Björkman, 1994). Except under extreme stress,  $qE$  is by far the dominant nonphotochemical quenching process, and it is this that will form the basis of this article. The essential feature of  $qE$  is that it is a feedback control mechanism induced under conditions in which absorbed light intensity is greater than what can be used by photosynthetic electron transport; it is the level of  $\Delta pH$  that "senses" that light is in excess, and the increased heat emission that is being monitored is a means of protecting against photoinhibition of photosynthesis.

There are two very different ideas concerning the mechanism of  $qE$  that relate in the first instance to where energy dissipation occurs (Horton and Ruban, 1992). PSII comprises

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Abbreviations: DCCD, dicyclohexylcarbodiimide;  $\Delta pH$ , transthylakoid pH gradient; LHClI, light-harvesting complexes of PSII;  $Q_A$ , bound quinone in the PSII reaction center;  $qE$ , nonphotochemical quenching of Chl fluorescence that occurs due to the presence of a  $\Delta pH$ ;  $qI$ , nonphotochemical quenching of Chl fluorescence that relaxes slowly or is irreversible;  $qN$ , nonphotochemical quenching of Chl fluorescence;  $qP$ , photochemical quenching of Chl fluorescence.

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a reaction center and a light-harvesting system or antenna. Therefore, quenching could occur while excitation is in the antenna or after it has been trapped by the reaction center. Different sites imply different mechanisms and different ways in which the  $\Delta\text{pH}$  can induce quenching. Thus, the first step in the elucidation of the molecular mechanism of qE is to find out where it occurs.

### QUENCHING IN THE PSII REACTION CENTER

It is well known that inhibition of the donation of electrons to PSII can cause quenching. This forms the basis of a model put forward by Weis and co-workers in which the primary effect of low pH in the thylakoid lumen is to cause release of bound  $\text{Ca}^{2+}$ ; this not only inhibits the donor side but also raises the redox potential of the acceptor side, promoting charge recombination in the reaction center between  $\text{P680}^+$  and  $\text{Q}_\text{A}^-$ . Excitation energy trapped by PSII would then be dissipated as heat (Krieger et al., 1992). Evidence in support of this model comes principally from the effects of  $\text{Ca}^{2+}$  channel inhibitors and from the reported absence of quenching when PSII centers are open (the quenching center would behave exactly as a permanently open reaction center). In addition, quenching was found to be inhibited if an artificial electron donor was provided to reduce  $\text{P680}^+$ , thus preventing its recombination with  $\text{Q}_\text{A}^-$ . There is little doubt that this scheme describes events that can occur in the PSII reaction center. However, the question remains as to whether it provides the mechanism of qE or whether it represents another form of inactivation or even damage to PSII under stress conditions.

### QUENCHING IN THE PSII ANTENNA

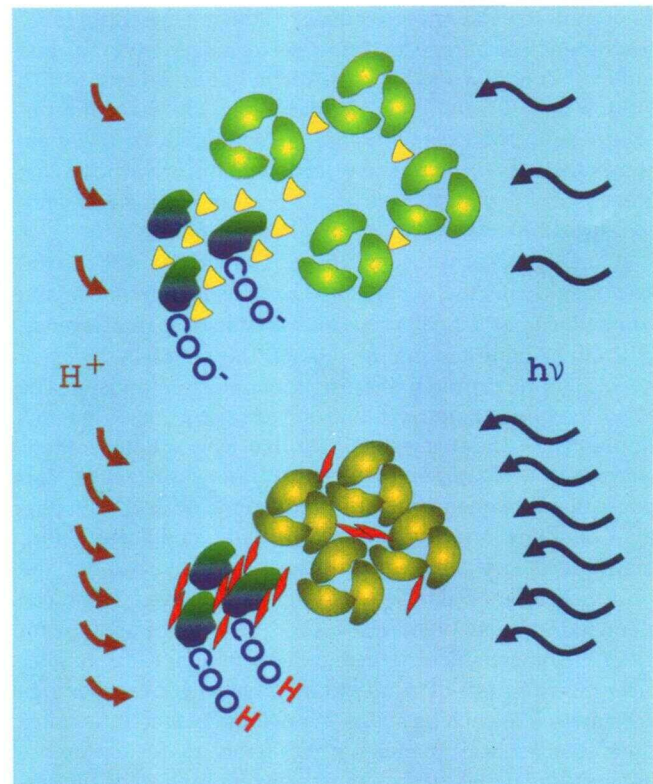
A number of lines of evidence suggest that the site of quenching *in vivo* is not in the PSII reaction center but in the antenna (reviewed by Horton and Ruban, 1992, 1994). (a) Quenching has repeatedly been found to be associated with a decrease in the level of fluorescence recorded when all PSII centers are open. In leaves this can be as high as 50%. Quenching of the level of fluorescence recorded when all PSII centers are open is best interpreted in terms of energy dissipation prior to trapping in the reaction center; however, more recent ideas concerning the energetics of PSII (the shallow trap model) indicate that this is not a conclusive argument in favor of antenna quenching. (b) Quenching persists if samples are frozen to 77 K, which is not expected since stable  $\text{Q}_\text{A}$  reduction at this temperature does not require an intact donor side. (c) Time-resolved fluorescence data recorded for leaves are consistent with quenching in the antenna, not the reaction center. (d) Measurements of the decrease in quantum yield of PSII show a decrease in absorption cross-section of PSII rather than an inactivation of reaction centers. (e) Modeling of the quantitative relationships between fluorescence parameters is not consistent with quenching in the reaction center. (f) Direct measurement of the increase in heat emission upon qE formation shows it to occur within 1.4  $\mu\text{s}$ , much faster than estimates for rates of the charge recombination reactions in PSII (Mullineaux et al., 1994).

The remainder of this article, which follows earlier reviews (Horton and Ruban, 1992, 1994), will focus on recent ideas to explain quenching in the PSII antenna, in particular the proposed role of the LHCII as depicted in Figure 1.

## THE ROLE OF THE LHCII IN QUENCHING

### Antenna Proteins of PSII

The antenna system of green plants is complex and consists of at least six different Chl protein complexes. CP47 and CP43 form part of the PSII core, whereas the Chl *a/b*-binding LHCII proteins form the light-harvesting system. The proteins for LHCII are coded for by six genes of the *Lhc* family (Jansson, 1994). LHCII comprises four different complexes referred to as LHCIIa, LHCIIb, LHCIIc, and LHCII d (also known as CP29, LHCII, CP26, and CP24, respectively) (Peter and Thornber, 1991). LHCIIb binds 60% of PSII Chl and is the most widely studied pigment protein in green plants. Its structure has been determined to 3.4 Å by electron crystallography of two-dimensional crystals (Kühlbrandt et al.,



**Figure 1.** Model to describe the proposed changes in LHCII associated with qE. Change from low light (top) to high light (bottom) results in increased proton concentration (red arrows) in the thylakoid lumen. Protonation of the carboxyl residues on the minor LHCII (blue/green) and de-epoxidation of violaxanthin (yellow) to zeaxanthin (red) results. Quenching, an increase in nonradiative dissipation of energy (shown as a decrease in brightness of the LHCII), is associated with a change in organization of LHCII, including LHCIIb (green), and results from increased proximity of either Chls or xanthophyll and Chl.

1994). It is organized into trimers that are mixtures of three polypeptides of molecular mass approximately 28, 27, and 25 kD coded for by *Lhcb1*, *Lhcb2*, and *Lhcb3*, respectively. The other three, "minor" complexes each bind about 5 to 10% of PSII Chl.

A principle feature of LHCII is that it is associated with a class of hydroxy carotenoids known as xanthophylls. The xanthophyll composition of purified LHCII components shows significant variability (Peter and Thornber, 1991; Bassi et al., 1993; Ruban et al., 1994). In particular, violaxanthin comprises only 5% of carotenoid in LHCIIb but 25 and 40% for LHCIIa and c.

### Xanthophyll Cycle

Violaxanthin is an important carotenoid with respect to qE because conditions that induce qE also bring about its reversible de-epoxidation to zeaxanthin (Demmig-Adams, 1990). There are strong correlations between zeaxanthin formation and the extent of quenching, but there appears not to be an obligatory requirement for zeaxanthin, since high levels of quenching have been observed in its absence. Instead, it has been proposed that it has a facilitating or activating role (Horton et al., 1991; Horton and Ruban, 1992; Ruban et al., 1993). Hence, the value for the lumen  $[H^+]$  required for quenching is reduced in the presence of zeaxanthin. This view is still controversial given the fact that zeaxanthin-independent quenching has been explained by the presence of the semi-epoxidated xanthophyll, antheraxanthin (Gilmore and Yamamoto, 1992). At present there is no proven mechanism to explain the role of zeaxanthin in quenching, but there are two suggestions: first, that zeaxanthin can directly accept, and quench, the singlet excited state of Chl (Owens et al., 1992), and second, that xanthophylls are important factors in controlling the structure of LHCII (Horton et al., 1991).

The basis for the idea of direct quenching by zeaxanthin is the estimation of the energy level of a hidden excited state of carotenoid known as the  $2A_1^g$  state; this analysis predicts that energy transfer from Chl to zeaxanthin could occur if the molecules were close enough together. However, because the energy level of this state is inversely proportional to the length of the conjugate chain, energy transfer from Chl to violaxanthin could not occur. Because thermal de-excitation from this excited carotenoid state is so efficient, energy transfer to it would be sufficient to explain quenching of Chl fluorescence. In this model the xanthophyll cycle is a process for creating a quencher. The  $\Delta pH$  may then be required to cause the change in LHCII organization needed to bring Chl and zeaxanthin close enough together for energy transfer.

The alternative mode of action of the xanthophyll cycle rests on the different chemical properties of violaxanthin and zeaxanthin (Horton and Ruban, 1994). The removal of the epoxide group from violaxanthin will not only affect the excited state but also the polarity of the molecule; zeaxanthin is much more hydrophobic than violaxanthin. This is evident from their chromatographic behavior and has been quantified by their solubility in ethanol/water mixtures. In fact, xanthophylls in general resemble detergents to some extent, and this amphipathic property is probably strongest for violaxan-

thin but weakened upon its conversion to zeaxanthin. Hence, an alternative explanation of the xanthophyll cycle is that it exerts control over thylakoid membrane structure, in particular the organization of LHCII via changes in the detergent-like properties of bound LHCII xanthophylls. This would provide a ready explanation of the facilitating role of zeaxanthin in qE, with  $\Delta pH$  being the only obligatory requirement for quenching; protonation of LHCII resulting in formation of a quenched state of the complex would be the sole requirement for qE.

At present it is not possible to distinguish between these two ideas, and both can readily be accommodated by the LHCII model for qE (Fig. 1). However, the notion that carotenoids are involved in determining the structure of LHCII proteins is a novel concept that finds support from the xanthophyll requirement for LHCII assembly *in vitro* (Plumley and Schmidt, 1987). In fact, there is also evidence that zeaxanthin decreases the fluidity of the lipids in the thylakoid membrane (Gruszecki and Strzalka, 1991). The message is that, just because carotenoids are colored molecules, it does not mean that they necessarily have a direct role photophysically.

There is relatively little knowledge of the biochemistry of the enzymes of the xanthophyll cycle. Violaxanthin de-epoxidase has been associated with an extrinsic lumen protein and is activated by lumen acidification, which induces binding of the enzyme to the membrane surface (Hager and Holocher, 1994). The epoxidase has been reported to be a constituent of LHCII itself (Gruszecki and Krupa, 1993), and it may be that the xanthophyll cycle is completely contained within LHCII, possibly in the minor complexes.

### The Nature of qN

The above rationalization of the role of the xanthophyll cycle immediately prompts a radical approach to understanding quenching: perhaps quenching is a "natural" property of the Chl in LHCII. In fact, quenching is not an abnormal process; to the contrary, solutions of Chl readily show energy quenching at high concentration or if forced to aggregate in polar solvents (Beddard and Porter, 1976). The exact mechanism of this "concentration" quenching has not been described but could provide a beautifully simple explanation of qE. But is there evidence for Chl-mediated quenching in a Chl protein? The observation of Arntzen and co-workers 15 years ago provided the first demonstration: although detergent-solubilized LHCII is highly fluorescent, upon aggregation fluorescence is quenched (Burke et al., 1978). It was this phenomenon that first prompted us to propose that similar changes in LHCII aggregation could underlie qE (Horton et al., 1991). In fact, absorption changes occur upon LHCII aggregation that resemble those observed for Chl and xanthophyll aggregation (Horton and Ruban, 1994).

At present it is not possible to interpret these changes; however, there is now a detailed structure for LHCII aggregates following the electron crystallographic studies of Kühlbrandt et al. (1994), and the observed positions of the Chl molecules offer numerous opportunities for Chl-Chl interactions. The fundamental question is whether the "quencher" is a particular minor Chl species formed upon aggregation or

whether it is a property of excitonic interactions shared across the whole aggregate. The answer will surely require application of advanced spectroscopic methods to LHCII. Although these experiments will undoubtedly increase our knowledge about LHCII, it is obviously crucial to determine whether LHCII aggregation provides the explanation of quenching *in vivo*, as shown in Figure 1.

Having obtained a "signature" for LHCII quenching *in vitro*, it has been possible to compare this to a spectral analysis of qE in both leaves and isolated chloroplasts. A first approach was to use low-temperature fluorescence-emission spectroscopy to determine the site of quenching; thus, if quenching was occurring in LHCII, at 77 K it should be possible to observe selective quenching of excitation in this complex, relative to the reaction center. A comparison of quenching due to photochemistry with that due to qE showed a clear difference in site; moreover, the qE spectrum resembled that of aggregated LHCII (Ruban et al., 1993; Ruban and Horton, 1994). Excitation spectra for the "lost" fluorescence also resembled an absorption spectrum of LHCII.

Next, an absorption spectrum for qE was obtained. This spectrum was remarkably similar to that observed for LHCII aggregation, showing a negative band in the Soret region indicative of Chl association (see Horton and Ruban, 1994). Similarly, shifts in the red band indicated an altered Chl environment; whether this is due to Chl-Chl association or changes in Chl-protein interaction cannot be ascertained, of course, but it is clear that LHCII is undergoing a structural change during induction of qE. Evidence was also obtained for changes in xanthophylls, with a negative band near 485 nm being found in the qE spectrum.

A change in  $A_{535}$  has been known for many years to be associated with the formation of the thylakoid  $\Delta$ pH. Referred to as "light-scattering," it was formerly thought to result from macroscopic changes in chloroplast structure consequent upon  $\Delta$ pH formation. However, it is now clear that it accompanies qE rather than  $\Delta$ pH, and in fact a perfect linear relationship exists between quenching and  $A_{535}$  in both leaves (Ruban et al., 1993; Bilger and Björkman, 1994) and chloroplasts (Noctor et al., 1993). Although the origin of this change in  $A_{535}$  is not proven, a band in this wavelength region is observed when xanthophyll aggregates are formed and when LHCII is aggregated (reviewed by Horton and Ruban, 1994). It is important to point out that this spectroscopic evidence suggests that the mechanism of energy quenching for qE is the same as for LHCII aggregation; however, this does not necessarily mean that large-scale LHCII aggregation occurs for qE, since rather minor pH-dependent changes in LHCII conformation may be sufficient to induce the quenching pigment configuration.

As discussed earlier, LHCII is a heterogeneous assembly of six different gene products. Is quenching occurring in all these complexes or is it confined to a specific complex? Based on the enrichment of xanthophyll cycle carotenoids in the minor LHCII, it has been suggested that these complexes are the sites of quenching (Bassi et al., 1993; Ruban et al., 1994). This idea has some attraction; these complexes are generally viewed as providing the link between the bulk LHCIIb and the PSII core complexes, and control at these points is easy to imagine. However, studies of energy transfer from LHCII

to PSII have not established such a role for the minor LHCII, and there is no direct proof that these are the quenching sites. Nevertheless, the minor LHCII appear to have a crucial role in qE, as described in the next section.

### Proton Binding Sites in LHCII

The simple view that protonation of lumen-exposed residues on LHCII (or any other PSII complex) is responsible for induction of quenching is not consistent with the effect of various inhibitors; in particular, the reagent dibucaine eliminates  $\Delta$ pH yet qE remains, its formation even being accelerated (Noctor et al., 1993). This suggests that protonation within LHCII or another localized domain may be involved. The reagent DCCD was found to block qE formation: this reagent binds to carboxy amino acid residues (glutamate or aspartate) in hydrophobic domains of membrane proteins (see Horton and Ruban, 1994). For example, it is an inhibitor of the  $CF_0$  proton channel of the ATP synthase, and it blocks proton channel activity in a variety of membrane proteins.

In isolated chloroplasts, DCCD inhibits the transport of protons into the thylakoid lumen, and this effect is correlated with DCCD binding to LHCII and LHCI proteins (Jahns and Junge, 1990). The inhibition of qE was also found to be correlated with its binding to these proteins, and these binding sites have been localized on the minor LHCII species LHCIIa and LHCIIc (see Horton and Ruban, 1994); examination of the sequences of these proteins reveals the presence of a motif on the B helix that shows homology to DCCD-binding sites on other membrane proteins, and recent data indicate that this is indeed a site of DCCD binding (R.G. Walters, unpublished data).

It is tempting to conclude that it is the binding of protons to these sites on the minor LHCII that directly results in the induction of quenching (Fig. 1). However, some caution must be applied at this time, since an alternative explanation is that DCCD binding merely blocks the proton pathway leading to the site of quenching. There is evidence that thylakoids contain a rather large proton domain that could include a major part of the LHCII system (Renganathan and Dille, 1994). This domain may not always be in equilibrium with the lumen pH. The lack of understanding of the relationships between proton domains, qE, LHCII, and control of electron transport and photophosphorylation represents an important knowledge gap in thylakoid membrane bioenergetics. What is abundantly clear, however, is that the LHCII (and perhaps also the light-harvesting complex of PSI) proteins have a function not just in light harvesting but in proton translocation, and that the latter function is involved in some way in qE. Changes in LHCII organization may therefore be tightly linked to proton channel activity and with control of the xanthophyll cycle.

An extrapolation of these ideas also leads to the suggestion that this putative channel on the minor LHCII may also have a role in PSII electron transport. It is known that proton release from PSII is blocked by DCCD and that proton-binding sites on LHCII are influenced by accumulation of charge in the oxygen-evolving system (Jahns and Junge, 1993). Therefore, it is possible that protonation of the minor LHCII is also responsible for an inhibition of electron trans-

port on the donor side of PSII, perhaps including a role for  $\text{Ca}^{2+}$  bound to the LHCIIa complex. Such a pivotal role for these complexes would mean that regulation of the PSII antenna and reaction center were closely integrated to provide effective photoprotection. The balance of "importance" of control in the two sites could therefore vary according to quite subtle differences in PSII organization.

#### FACTORS AFFECTING THE CAPACITY OF $q_N$

The maximum extent of  $q_E$  at light saturation is dependent on the conditions of illumination, the type of environment used for the growth of plants, and the species selected. In terms of assessing the practical importance of understanding this form of photoprotection, the question of what determines the maximum extent of  $q_E$  is of the utmost significance. In other words, could crop plants be made more resistant to environmental stress by increasing their capacity of  $q_E$ ? At present we do not know the answer to this question, although it is clear that the published values for this parameter are relatively low for crop species (Johnson et al., 1993). Conversely, in plants adapted for growth in adverse environments (e.g. *Guzmania monostachia*), the extent of  $q_E$  is 3-fold higher than in the plant species most commonly used in laboratory experiments (Ruban et al., 1993). Thus, there is reason to believe that there is genetic variability in the capacity for  $q_E$  in a manner that is related to the natural habitat of the plant. This notion has been confirmed by the screening of a large number of ecologically contrasting species (Johnson et al., 1993).

The biochemical basis of this variability is unclear, but must be related either to the composition of LHCII and the xanthophyll cycle or to features of  $\Delta\text{pH}$  generation. In the case of LHCII, it is known that the content of LHCIIb decreases when plants are grown in high light, and there may be an antagonistic relationship between a large antenna size and high levels of  $q_E$ . In this regard, it is significant that the level of the outer pool of LHCIIb, enriched in the 28- and 27-kD polypeptides but missing the 25-kD species, has been shown to be selectively reduced in high light (Melis, 1991). Possibly this peripheral pool of LHCII impedes the organizational changes necessary for strong  $q_E$ . The induction of higher levels of quenching in plants grown under high light is also associated with an increased pool size of xanthophyll cycle carotenoids and an increased capacity for de-epoxidation of violaxanthin (Demmig-Adams and Adams, 1992). Although it is clear that different LHCII components have different contents of xanthophyll cycle carotenoid, there have been no studies to establish whether the determined binding stoichiometries are fixed; thus, it cannot be stated whether the increase in content of the xanthophyll cycle requires an alteration in LHCII composition. However, the picture is not a simple one; for example, mutants deficient in LHCIIb show reduced levels of quenching, despite having an increased content of xanthophyll cycle carotenoids and increased extent of convertibility to zeaxanthin (Leverenz et al., 1992).

It is interesting that an increase in quenching capacity and xanthophyll cycle pool size can be induced at relatively low light if photosynthetic capacity has been reduced by expres-

sion of an antisense gene for Fru bisphosphatase (W. Bilger, personal communication), showing that the maximum extent of  $q_E$  is closely associated with the balance between the light environment and photosynthetic capacity. In the same way, reductions in photosynthesis upon water stress or nutrient deficiency also result in increases in quenching and xanthophyll cycle pool size (reviewed by Demmig-Adams and Adams, 1992). Why the same maximum capacity for  $q_E$  is not expressed in all conditions is not understood; inevitably there is a conflict between having maximum light harvesting in low light and minimum photoinhibition in high light (Horton, 1987), but what this actually means in molecular terms is unknown. There are also insufficient studies to be able to conclude whether all species are capable of achieving the very high levels of  $q_E$  found in plants such as *Guzmania* if growth conditions are manipulated. These unanswered questions reveal severe deficiencies in our understanding not only of the LHCII system itself, but of how it is assembled during growth and development and what factors are important in determining the pigments and proteins found in it.

It is also possible that the capacity for  $q_E$  is determined by the features of thylakoid proton accumulation. In plants with high photosynthetic capacity it seems unlikely that the lumen pH will fall much below 6.0 in saturating light because this would inevitably inhibit electron flow at the level of the Cyt *bf* complex. In these situations it would seem that the formation of zeaxanthin would be essential to induce maximum  $q_E$  in order to reduce the requirement for a high proton concentration. Alternatively,  $q_E$  may be driven by a localized proton domain, which in effect would uncouple it from the effect of  $\Delta\text{pH}$  on electron transport rate. In plants growing under extreme conditions it is likely that the rate of photosynthetic carbon assimilation is already suppressed (e.g. by water or nutrient deficit), and here one could imagine a large  $\Delta\text{pH}$  being generated that would exert a strong feedback control over PSII at the level of light harvesting and perhaps also electron transport. In fact, the adenylate energy charge has been shown to be high under such conditions (Gilmore and Björkman, 1994) and, in this context, the involvement of Mehler reactions and PSI cyclic electron transport should be mentioned.

Although electron transport through to  $\text{O}_2$  has frequently been considered as a way of safely relieving electron pressure, recent work has pointed out the possible role of these reactions in providing the means of raising  $\Delta\text{pH}$  under stress conditions (Schreiber and Neubauer, 1990; Neubauer and Yamamoto, 1992). The pathway involves a number of enzymes, with the ascorbate peroxidase playing a key role. Because ascorbate is also an essential substrate for violaxanthin de-epoxidation, linkage arises among three apparently vital aspects of stress tolerance: oxygen metabolism, energy dissipation, and the xanthophyll cycle. In the same way, cyclic electron transport around PSI may be important as a means of maintaining or enhancing chloroplast energization under stress conditions. One would predict that in plants tolerant to light-dependent stress each of these would be expressed to a high level. Therefore,  $q_N$  and the changes in function of the light-harvesting system are inextricably linked to the metabolic processes in the leaf, clearly illustrating the interplay that must occur between environmental and meta-

bolic factors in the regulation of photosynthetic electron transport (Horton, 1987).

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