Chapter 11

Photoprotection of Photosystem II: Reaction Center Quenching Versus Antenna Quenching

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Summary

Understanding the role of the xanthophyll cycle and elucidating the mechanisms of antenna quenching through the non-photochemical dissipation of excess absorbed energy in the photoprotection of the photochemical apparatus continues to be a major focus of photosynthetic research. In addition to antenna quenching, there is evidence for the non-photochemical dissipation of excess energy through the PS II reaction center. Hence, this photoprotective mechanism is called reaction center quenching. One technique to assess reaction center quenching is photosynthetic thermoluminescence. This technique represents a simple but powerful probe of PS II photochemistry that measures the light emitted due to the reversal of PS II charge separation through the thermally-dependent recombination of the negative charges stabilized on Q_A^- and Q_B^- on the acceptor side of PS II with the positive charges accumulated in the S_2- and S_3-states of the oxygen evolving complex. Changes in the temperature maxima for photosynthetic thermoluminescence may reflect changes in redox potentials of recombining species within PS II reaction centers. Exposure of *Synechococcus* sp. PCC 7942, *Pinus sylvestris* L., *Arabidopsis thaliana*, and *Chlamydomonas reinhardtii* to either low temperatures or to high light induces a significant downshift in the temperature maxima for $S_2Q_B^-$

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and $S_3Q_B^-$ recombinations relative to $S_2Q_A^-$ and $S_3Q_A^-$ recombinations. These shifts in recombination temperatures are indicative of lower activation energy for the $S_2Q_B^-$ redox pair recombination and a narrowing of the free energy gap between Q_A and Q_B electron acceptors. This, in turn, is associated with a decrease in the overall thermoluminescence emission. We propose that environmental factors such as high light and low temperature result in an increased population of reduced Q_A (Q_A^-), that is, increased excitation pressure, facilitating non-radiative $P680^+Q_A^-$ radical pair recombination within the PS II reaction center. The underlying molecular mechanisms regulating reaction center quenching appear to be species dependent. We conclude that reaction center quenching and antenna quenching are complementary mechanisms that may function to photoprotect PS II to different extents in vivo depending on the species as well as the environmental conditions to which the organism is exposed.

I. Introduction

Changes in irradiance, temperature, nutrient, and water availability result in imbalances between the light energy absorbed through photochemistry and energy utilization through photosynthetic electron transport coupled to carbon, nitrogen, and sulphur reduction. This leads to photoinhibition of photosynthesis under controlled laboratory conditions as well as natural field conditions (Powles, 1984; Krause, 1988; Aro et al., 1993; Long et al., 1994; Keren and Ohad, 1998). Recovery from photoinhibition in plants, green algae, and cyanobacteria is thought to involve a PS II repair cycle in which photodamaged D1 is degraded and the resynthesized D1 is re-inserted to form a functional PS II reaction center (Aro et al., 1993; Keren and Ohad, 1998; Melis, 1999). It has been shown in some chilling-sensitive plant species, green algae, and cyanobacteria that protection against photoinhibition may be accounted for, in part, by the rate of repair relative to the rate of photodamage to D1 (Nishida and Murata, 1996; Keren and Ohad, 1998; Melis, 1999). Alternatively, certain cold tolerant plant species such as winter wheat (*Triticum aestivum* L), rye (*Secale cereale* L), and *Arabidopsis thaliana*, exhibit a minimal dependence on D1 repair but exhibit increased photosynthetic capacity and reprogramming of photosynthetic carbon metabolism in response to cold acclimation (Huner et al., 1993; Hurry et al., 1995; Strand et al., 1997; Demmig-Adams et al., 1999; Adams et al., 2001; Stitt and Hurry, 2002; A. Strand et al., 2003). Although the Mehler reaction appears to contribute to photoprotection in cold tolerant cereals, cold acclimation of Monopol wheat results in the repression of photorespiration (Savitch et al., 2000). This reprogramming of metabolism results in an increased capacity to keep Q_A oxidized and PS II reaction centers open under high excitation pressure induced by either excessive irradiance or low temperatures (Huner et al., 1998; Huner et al., 2003; Öquist and Huner, 2003). Thus, photoprotection in these species is accomplished, in part, through an increase in photochemical quenching (q_P) (Krause and Jahns, 2003).

In contrast to the D1 repair cycle and photochemical quenching, the concept of radiationless dissipation of excess energy through antenna quenching was originally developed on the basis of the Butler model for energy transfer and used to account for Chl fluorescence quenching (Butler, 1978). Non-photochemical quenching (NPQ) of excess excitation energy in the antenna pigment bed of PS II is considered to be the major PS II photoprotective mechanism (Demmig-Adams and Adams, 1992; Horton et al., 1999; Demmig-Adams et al., 1999; Gilmore, 2000; Gilmore and Ball, 2000; Ort, 2001; Demmig-Adams and Adams, 2002). Recently, the term, feedback de-excitation, has been

Abbreviations: A-band – thermoluminescence band between −15° and −10°C; A – antheraxanthin; B_1-band – thermoluminescence band between +20°C and +30°C in the absence of DCMU; B_2-band – thermoluminescence band between +35° and +40°C in the absence of DCMU; C-band – thermoluminescence band between +50° and +60°C; CHB – cold hard band; Cyt b_{559} – cytochrome b_{559}; D1 – photosystem II reaction center polypeptide; D2 – photosystem II reaction center polypeptide; ELIPs – early light inducible proteins; F_o – minimum yield of chlorophyll fluorescence at open PS II centers in dark-adapted leaves; F_m – maximum yield of fluorescence at closed PS II reaction centers in dark adapted leaves; F_v – variable yield of fluorescence in dark adapted leaves; F_v/F_m – maximum PS II photochemical efficiency in dark adapted leaves; LHCII – the major Chl a/b pigment-protein complex associated with PSII; NPQ – non-photochemical quenching; OEC – oxygen evolving complex; Pheo – pheophytin; PI – photoinhibition; PS I – photosystem I; PS II – photosystem II; PS IIα – photosystem IIα centers; PS IIβ – photosystem II β centers; PsbS – PS II subunit and gene product of the *PsbS* gene; PsbZ – PS II subunit and gene product of *ycf9*; PQ – plastoquinone; Q-band – thermoluminescence band between 0° and +10°C in the presence of DCMU; Q_A – primary electron-accepting quinone in PS II reaction centers; Q_B – secondary electron-accepting quinone in PS II reaction centers; q_E – ΔpH-dependent high energy quenching; q_N – non-photochemical quenching coefficient; q_O – quenching coefficient for basal fluorescence; q_P – photochemical quenching coefficient; Q_y – chlorophyll a absorption band; TL – thermoluminescence; T_M – temperature of maximum thermoluminescence emission; V – violaxanthin; Z – zeaxanthin; Zv – thermoluminescence band between −80° and −30°C

used to describe this protective mechanism (Kulheim et al., 2002). However, there is also evidence for the non-photochemical dissipation of excess energy through the PS II reaction center rather than through the antenna (Weis and Berry, 1987; Krause and Weis, 1991; Walters and Horton, 1993; Buhkov et al., 2001; Lee et al., 2001; Ivanov et al., 2001, 2002; Sane et al., 2002, 2003; Matsubara and Chow, 2004; Finazzi et al., 2004). Hence this photoprotective mechanism is called reaction center quenching.

We begin our discussion with a brief comparison of antenna quenching versus reaction center quenching and provide past and recent evidence that support a significant role for reaction center quenching in the photoprotection of PS II. This is followed by a discussion of thermoluminescence as a sensitive technique to detect reaction center quenching during photoinhibition of photosynthetic organisms as diverse as cyanobacteria, green algae, conifers, and herbaceous plants. We conclude that, as originally suggested by Krause and Weis (1991), it is probable that both reaction center and antenna quenching function in vivo to different extents depending on the environmental conditions to protect PS II from photodamage.

II. Antenna Quenching

Because antenna quenching is the focus of several other chapters in this volume, we will describe only its essential characteristics here. Although not always the case (Hurry et al., 1997), non-photochemical quenching (NPQ) of excess excitation energy is thought to occur through the interconversion of the light harvesting xanthophyll, violaxanthin (V), to the energy quenching xanthophylls, antheraxanthin (A) and zeaxanthin (Z), and is considered to be the major PS II photoprotective mechanism (Demmig-Adams and Adams, 1992; Horton et al., 1999; Demmig-Adams et al., 1999; Gilmore, 2000; Gilmore and Ball, 2000; Ort, 2001; Demmig-Adams and Adams, 2002). Two major mechanisms have been proposed to account for antenna quenching via the xanthophyll cycle. The direct mechanism proposes that the S_1 state of A and Z within LHCII is lower than that of Chl a within the antenna pigment bed. Thus, A and Z are not able to transfer energy to the S_1 state of antenna chlorophyll whereas V is able to transfer energy. Consequently, excited state A and Z decay to ground state with the release of heat (Frank et al., 1994). This light dependent, reversible interconversion of V to A and Z has been called a 'molecular gear shift' regulating energy transfer within LHCII (Frank et al., 1994). Although the data reported by Polivka et al. (1999; 2002) do not support the 'molecular gear shift' hypothesis, recent convincing evidence has been reported in support for the direct mechanism for non-photochemical quenching through zeaxanthin in the antenna (Ma et al., 2003; Dreuw et al., 2003a, 2003b; Holt et al., 2005). In contrast, the indirect mechanism proposes that the transthylakoid ΔpH gradient and the xanthophyll cycle pigments regulate the oligomerization state of LHCII that affects the rapidly relaxing energy-dependent component (q_E) of NPQ (Horton et al., 1999; Ruban et al., 2002; Aspinall-O'Dea et al., 2002; Wentworth et al., 2003). In support of the indirect mechanism, Elrad et al. (2002) reported that LHCII trimerization is required for antenna quenching in the *npq5* mutant of *Chlamydomonas reinhardtii*. Clearly, the underlying mechanism by which the xanthophyll cycle regulates antenna quenching remains controversial.

Regardless of the mechanism of antenna quenching, the persistent retention of Z and A in overwintering plants led to the development of the concept of sustained xanthophyll-dependent energy dissipation, which involved sustained thylakoid lumen acidification, even in the dark (Gilmore, 1997; Demmig-Adams et al., 1996; Adams et al., 2001). Sustained energy dissipation through the antenna has been suggested as an important protective mechanism enabling evergreen plants to maintain their leaves during the winter. Many mesophytic overwintering plants such as *Malva*, *Arabidopsis*, and winter cereals exhibit a 'cold-sustained', non-photochemical quenching which is rapidly reversible upon warming. In addition to this reversible form, schlerophytic evergreens also exhibit a sustained form of zeaxanthin-dependent non-photochemical quenching which predominates during the winter and is not rapidily reversible upon either warming or the presence of uncouplers. This persistent quenching appears to be associated with the reorganization of the LHCII into xanthophyll-containing aggregates induced by a combination of low temperature and high light (Gilmore and Ball, 2000; Öquist and Huner, 2003; Gilmore et al., 2003). However, this persistent type of sustained quenching has not been reported for mesophytic plant species (Adams et al., 2002; Adams et al., 2004). In addition to its role in non-photochemical quenching, Z also appears to act as an anti-oxidant to protect against photooxidative stress (Havaux and Niyogi, 1999; Baroli et al., 2003).

Recently, major insights into our understanding of the molecular mechanism(s) of NPQ have occurred as a consequence of the isolation of NPQ mutants of *Arabidopsis thaliana* and *Chlamydomonas reinhardtii*. The

PsbS deletion mutant, *npq4-1* (Li et al., 2000) and various *PsbS*-defective mutants of *Arabidopsis* (Niyogi, 1999; Havaux and Kloppstech, 2001; Peterson and Havir, 2001, 2003; Grasses et al., 2002) are impaired in the development of NPQ. However, despite the development of mutants specifically deficient in PsbS and NPQ, the precise function of the PsbS protein, and its specific role in NPQ remains equivocal. Based on the original observation that PsbS binds chlorophylls and xanthophylls (Funk, 2001), and its role in the development of q_E, Li et al. (2002) suggested that this protein is the site of ΔpH and xanthophyll-dependent NPQ. However, more detailed biochemical analyses suggest that the PsbS protein does not bind pigments (Dominici et al., 2002). This is in agreement with the fact that most of the highly conserved amino acids that form the ligands for chlorophyll in most of the LHC proteins (Kühlbrandt et al., 1994; Bassi et al., 1999) are not found in PsbS. In addition, the availability of the 3D map of the PS II supercomplex (Nield et al., 2000a) and the structure of the LHCII trimer (Kühlbrandt et al., 1994) indicate that there is not sufficient space to accommodate the PsbS protein within the LHCII-PSII supercomplex (Nield et al., 2000b). Through fluorescence analysis of the *npq4-1* mutant of *Arabidopsis thaliana* lacking PsbS, Peterson and Havir (2003) have suggested that the PsbS polypeptide may regulate exciton distribution within PS II. Wentworth et al. (2003) suggest that the role of PsbS is to regulate the oligomerization of antenna complexes involved in antenna quenching. However, Holt et al. (2005) reported that PsbS regulates NPQ through a chlorophyll-zeaxanthin heterodimer. A detailed summary of the structure and function of this intriguing protein is provided by Funk (2001) and by Niyogi et al. (2005).

Swiatek et al. (2001) have provided convincing evidence that the *ycf9* gene which encodes PsbZ, a core PS II subunit, plays a critical role in NPQ in tobacco and *Chlamydomonas reinhardtii*. PsbZ appears to stabilize the supramolecular organization of PS II core complexes with the peripheral antennae (Swiatek et al., 2001). Although NPQ was significantly inhibited in Δ*ycf9* tobacco plants (associated with a decrease in the level of PsbZ), PsbS accumulation was unaffected in this mutant. Furthermore, PsbZ is present in phycobilisome-containing eukaryotic and prokaryotic organisms that exhibit NPQ but no xanthophyll cycle. Swiatek et al. (2001) suggest that PsbZ is a critical component in the regulation of NPQ in these organisms.

Early light-inducible proteins (ELIPs) are a family of proteins related to the LHC gene family (Montane and Kloppstech, 2000). ELIPs have also been shown to accumulate under conditions of high light or low temperature stress in mature leaves under controlled as well as natural field conditions (Lindahl et al., 1997; Montane et al., 1997; Norén et al., 2003) and during chloroplast development (Meyer and Kloppstech, 1984; Krol et al., 1999). Although it is presumed that they are involved in the non-photochemical photoprotection of PS II, their precise role still remains to be elucidated.

III. Reaction Center Quenching

The concept of antenna quenching is based on Butler's model (Butler, 1978) and assumes that the rate constant for energy transfer to PS II reaction centers exceeds the rate constant for the back-transfer of energy from the reaction center to the antenna. This led to the concept of a PS II unit as an energy funnel. However, fluorescence lifetime measurements indicate that the equilibration of excitons between antennae and reaction centers is one order of magnitude faster than charge separation. Thus, PS II is trap limited and the reaction center appears to act not as a funnel but as a shallow trap (Schatz et al., 1988).

Although a major focus of recent research on photoprotection has been on the contribution of antenna quenching to NPQ, there is historical precedence for alternative mechanisms for the dissipation of excess light and photoprotection of PS II reaction centers (Krause, 1988; Krause and Weis, 1991; Walters and Horton, 1993). Considerable evidence for non-photochemical quenching through reaction center quenching has been provided in plants as well as cyanobacteria (Briantais et al., 1979; Weis and Berry, 1987; Krause, 1988; Vavilin and Vermaas, 2000; Bukhov et al., 2001; Sane et al., 2002). Exposure of spinach, wheat, and rye plants to photoinhibitory conditions is associated with quenching of F_v. This reflects the quenching of fluorescence within PS II reaction centers with minimal effects on antenna quenching as indicated by minimal changes in F_o fluorescence (Krause, 1988; Somersalo and Krause, 1990; Huner et al., 1993). It has been proposed that reaction center quenching is the result of the conversion of photochemically active, fluorescent, closed PS II reaction centers into photochemically inactive, non-fluorescent, PS II reaction centers (Krause, 1988; Krause and Weis, 1991). The photoinactivated PS II reaction centers act as PS II quenching centers and

$$(\text{PS II})_{\text{OPEN}} \overset{h\nu}{\leftrightarrow} (\text{PS II})_{\text{CLOSED}} \overset{[H^+]}{\leftrightarrow} (\text{PS II})_{\text{QUENCHER}} \quad (1)$$

ACTIVE INACTIVE

dissipate energy as heat, preventing further damage not only to the photoinactivated reaction centers themselves but also neighboring active PS II reaction centers through their role as sinks for excitation energy (Öquist et al., 1992; Lee et al., 2001; Matsubara and Chow, 2004). The relative proportion of active PS II centers versus inactive centers is dependent both on the intrathylakoid ΔpH as well as proportion of closed reaction centers measured as the relative reduction state of Q_A (Weis and Berry, 1987; Krause and Weis, 1991; Krause and Jahns, 2003). Furthermore, the proportion of PS II quenching centers has been shown to be sensitive to the level of photoinhibition to which plants are exposed (Lee et al., 2001). Based on a theoretical assessment of alternative mechanisms for NPQ in *Hordeum vulgare*, Walters and Horton (1993) concluded that reaction center quenching is operative only when reaction centers are closed, that is, when Q_A is in the reduced state.

The presence of functionally distinct populations of PS II, that is PS IIα and PS IIβ centers, was used to explain the biphasic Chl fluorescence induction kinetics induced upon illumination (Melis and Homan,1976). In the PS II repair cycle, functionally active PS IIα centers are thought to be damaged by photoinhibition (PI), transformed into inactive PS IIβ centers which are subsequently repaired by the de novo synthesis of D1 (Melis, 1999). Thus, recovery from photoinhibition was presumed to be dependent upon chloroplastic protein synthesis (Greer et al., 1986; Aro et al., 1993; Keren and Ohad, 1998; Melis, 1999). However, photoinhibition of PS II in spinach (Somersalo and Krause, 1990; van Wijk and van Hasselt, 1993), wheat as well as rye (Huner et al., 1993) indicated the presence of a form of PS II that quenched Chl fluorescence through the reaction center that exhibited a rapid, temperature-independent recovery, with a $t_{1/2}$ of 15–30 min, the formation of which was light-dependent but independent of chloroplastic protein synthesis. This fast recovery component of PS II was suggested to represent PS IIα quenching centers that are rapidly and reversibly interconverted to active PS IIα centers (Krause and Weis, 1991; Huner et al., 1993).

$$\text{PS II}\alpha\ \text{(active)} \underset{\text{fast}}{\overset{\text{PI}}{\leftrightarrow}} \text{PS II}\alpha\ \text{(quencher)} \qquad (2)$$

In a detailed flash-induced analysis of basal (F_o) and maximal (F_m) fluorescence, Delrieu (1998) demonstrated that fluorescence quenching might result from a conversion of PS IIα-centers (dimers) to PS IIβ–centers (monomers) in a low fluorescence state. The monomerization of PS II centers can be triggered by light (Kruse et al., 1997) that effectively decreases the absorption cross-section of PS II (Delrieu, 1998). Furthermore, it was shown that PS II quenching centers increase with the severity of photoinactivation (Lee et al., 2001) and dissipate excess excitation energy as heat (Krause, 1988). Krause and Weis (1991) suggested that both reaction center and antenna quenching play important roles in the photoprotection of PS II.

IV. Thermoluminescence

Photosynthetic thermoluminescence (TL) is the light emitted from a frozen (77°K), preilluminated leaf, algal, or cyanobacterial sample that is gradually heated in darkness. This technique represents a simple but powerful probe of PS II photochemistry (Inoue, 1996; Ke, 2001; Ducruet, 2003). Photosynthetic TL arises from the reversal of PS II charge separation through the thermally-dependent recombination of the negative charges stabilized on Q_A^- and Q_B^- on the acceptor side of PS II with the positive charges accumulated in the S_2 and S_3-states of the oxygen evolving complex (OEC) (Sane and Rutherford, 1986; Inoue, 1996; Ke, 2001; Ducruet, 2003). Light-induced PS II charge separation generates a singlet radical pair $^1[P680^+\ Pheo^-]$ within a few picoseconds that subsequently proceeds to stabilization of negative and positive equivalents at the acceptor (Q_A^-, Q_B^-) and donor sides (S_2^+, S_3^+-states) of PS II respectively and the regeneration of ground state P680. Although a major part of the light energy captured is stored as redox potential difference between the donor and acceptor sides of PS II, part of the captured energy is lost as 'stabilization energy' that results in the trapping of the separated donor-acceptor charge pair (Inoue, 1996; Ke, 2001; Ducruet, 2003). This free energy trap represents the activation energy barrier against charge recombination, and as a consequence, increases the probability of forward electron transfer relative to reverse electron transfer. However, when thermal activation energy is provided externally, charge recombination becomes possible with the re-excitation of P680 to P680* and the thermally-induced light emission from either P680* or core antenna Chl (Inoue 1996; Ducruet, 2003).

Thermally-induced PS II recombination events are distinguished by their characteristic emission peak temperatures (T_M). A deconvoluted TL glow curve of a photosynthetic sample pre-illuminated with continuous light (Fig. 1A) typically consists of six distinct emission peaks or bands (Vass and Govindjee, 1996; Inoue, 1996; Ke, 2001; Ivanov et al., 2002;

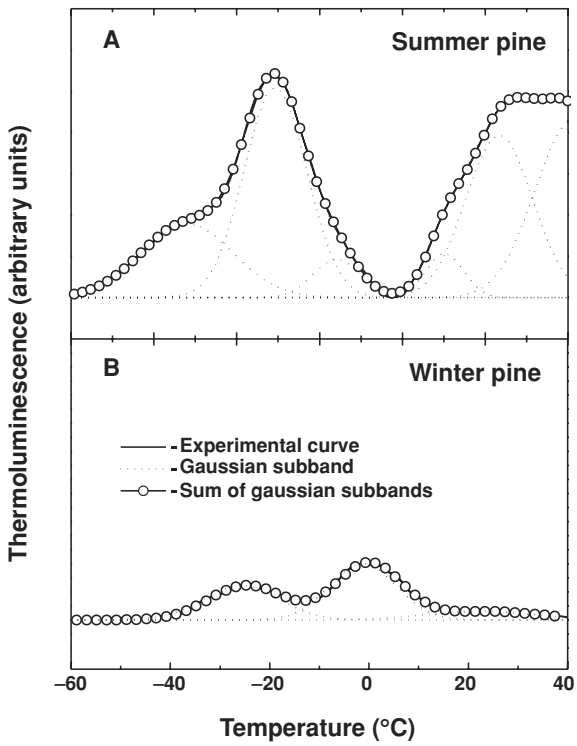

Fig. 1. Thermoluminescence glow curves and mathematical resolution of glow curves in sub-bands in Scots pine (*Pinus sylvestris* L.) needles collected during summer (A) and in winter (B). Experimental curves (-) represent averages of 3 to 5 scans. –o– Computer-generated sum of sub-bands; · · · – computer-fitted sub-band. (From Ivanov et al., 2002)

Ducruet, 2003). The Z_v-band occurs between −80° and −30°C and is thought to reflect $P680^+Q_A^-$ recombination whereas the A-band detected at about −10°C has been assigned to $S_3Q_A^-$ recombinations. The B_1-band assigned to $S_3Q_B^-$ recombinations and the B_2-band associated with $S_2Q_B^-$ recombinations are typically detected between +20° to +30°C and between +35° to +40°C respectively. In the presence of DCMU, the B-bands are significantly reduced and replaced with a new emission band at between 0° and +10°C called the Q-band which has been shown to be associated with $S_2Q_A^-$ recombinations (Inoue, 1996; Ke, 2001). The C-band assigned to $Y_D^+Q_A^-$recombinations is detected at temperatures of about +50°C.

An upshift in the peak temperature for a particular recombination event indicates an increase in the activation energy required for that recombination event. This implies a change in the PS II reaction center that has increased the depth of the trap between the charged pairs making it energetically less favourable for charge recombination. Alternatively, a downshift in the peak temperature for a particular recombination event indicates a decrease in the activation energy required for that recombination event. This implies a shallower trap between the charged pairs of the PS II reaction center making it energetically more favourable for the recombination event.

Since the free energy of activation for recombination is related to the redox midpoint potential difference between the recombining species (Devault and Govindjee, 1990), an upward shift in the temperature for a TL peak emission maximum has been interpreted to indicate an increase in the redox potential difference between the recombining species. Conversely, a downward shift in the temperature for a TL emission maximum has been interpreted to reflect a decrease in the redox potential difference between recombining species (Devault and Govindjee, 1990). Thus, changes in the temperature maxima for TL emission have usually been discussed in terms of changes in redox potentials of recombining species within PS II reaction centers (Mayes et al., 1993; Nixon et al., 1995; Minagawa et al., 1999; Sane et al., 2002, 2003). However, through crystallographic analyses of reaction centers of *Rhodobacter sphaeroides*, Stowell et al. (1997) showed light-induced protein conformational changes within these reaction centers that altered the rate of electron transfer between Q_A and Q_B with no changes in redox potential. Thus, an alternative explanation for the shifts in the TL temperature maxima may be that they reflect alterations in the activation energy required to alter PS II reaction center protein conformation rather than changes in redox potential per se. Although changes in T_M have been primarily interpreted to indicate changes in the redox potential of PS II reaction center components, this alternative explanation should not be ignored.

V. Photoprotection through Reaction Center Quenching

The over-reduction of Q_A has been suggested as a major prerequisite for efficient dissipation of the excess light within the reaction center of PS II (Krause, 1988; Walters and Horton, 1993; Bukhov et al., 2001; Öquist and Huner, 2003). Non-radiative charge recombination between Q_A^- and the donor side of PS II has been suggested as a mechanism for the dissipation of excitation energy by PS II reaction center quenching (Briantais et al., 1979; Weis and Berry, 1987; Vavilin and Vermaas, 2000). This is consistent with the recent reports that the overwintering evergreens, snow gum and mistletoe, exhibit a distinctive 'cold-hard-band' (CHB)

in their 77K fluorescence emission spectrum that is associated with Chl aggregation and dissipates excess energy as heat from PS II while simultaneously decreasing the quantum yield of PS II (Gilmore and Ball, 2000; Gilmore et al., 2003). Below, we summarize recent data for the direct estimation of the redox properties of the acceptor side of PS II (Q_A and Q_B) using thermoluminescence and its implications for reaction center quenching as an alternative mechanism for the non-radiative dissipation of excess light energy during cold stress, cold acclimation, and high light stress of the cyanobacterium, *Synechococcus* sp. PCC 7942, the conifer, *Pinus sylvestris*, the model plant species, *Arabidopsis thaliana*, and the model green alga, *Chlamydomonas reinhardtii*. Since the effects of low temperature on the TL peak temperatures can be mimicked by high light, we suggest that the alterations in the activation energies for PS II charge recombination pairs reflect a response to excitation pressure, the relative reduction state of Q_A measured either as $1 - q_P$ (Huner et al., 1998; Bukhov et al., 2001) or $1 - qL$ (Kramer et al., 2004). This is consistent with earlier theoretical considerations of reaction center quenching in barley (Walters and Horton, 1993).

A. Synechococcus *sp. PCC 7942*

Unlike eukaryotic photosynthetic organisms that contain a single chloroplastic *psbA* gene encoding the PS II reaction center polypeptide, D1, the cyanobacterium *Synechococcus* sp. PCC 7942 possesses three genes that are differentially regulated by light (Golden et al., 1986; Schaefer and Golden, 1989). Under normal growth light conditions, *Synechococcus* sp. PCC 7942 exhibits the presence of form one of the D1 reaction center polypeptide (D1:1). However, upon exposure to high light, D1:1 is exchanged for form two of the PS II reaction center polypeptide (D1:2). Cells expressing D1:2 exhibit decreased susceptibility to photoinhibition compared to those expressing D1:1 (Krupa et al., 1990, 1991). However, Campbell et al. (1995) reported that low temperature stress mimicked the effects of high light in inducing the exchange of D1:1 for D1:2 in *Synechococcus* sp. PCC 7942. Furthermore, they showed that this PS II reaction center polypeptide exchange was a transient phenomenon.

The effect of D1 replacement on the charge recombination events between the acceptor and donor sides of PS II were examined recently in *Synechococcus* sp. PCC 7942 cells exposed to short term low temperature stress (Sane et al., 2002). The TL data demonstrate that exposing *Synechococcus* cells grown at 36°C to 25°C

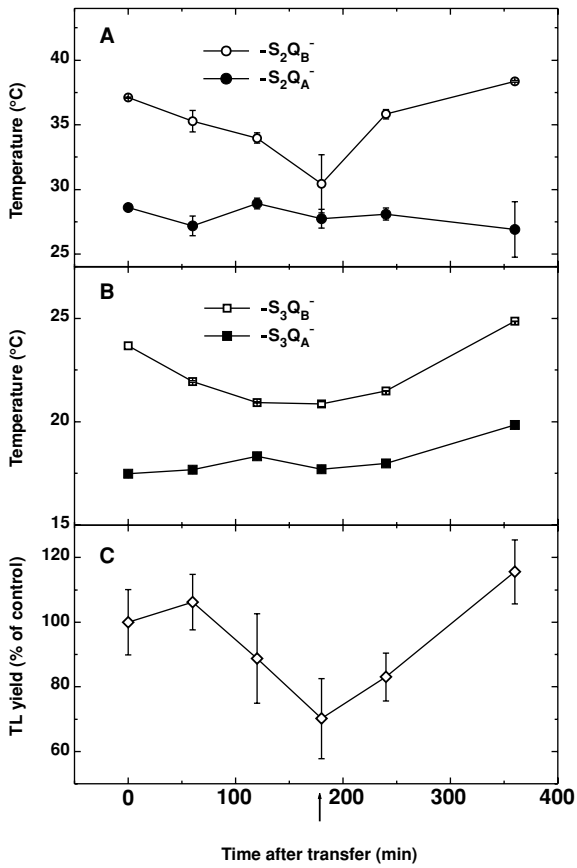

Fig. 2. Time course of $S_2Q_B^-$ and $S_2Q_A^-$ (A) and $S_3Q_B^-$ and $S_3Q_A^-$ (B) characteristic peaks in wild type *Synechococcus* sp. PCC 7942 cells during the temperature shift from the growth temperature of 36°C to 25°C for the first 180 min and back to 36°C for the second part of the curve. C – Relative TL yield measured as the total area under the experimental glow curves. The peak positions were estimated by decomposition analysis of the experimental TL curves after illumination with continuous white light. The presented mean values ± SE are calculated from 6–8 measurements in 3–5 independent experiments. ↑ – shift from 25°C back to 36°C. (From Sane et al., 2002)

shifted the recombination temperatures of $S_2Q_B^-$ and $S_3Q_B^-$ pairs closer to those of $S_2Q_A^-$ and $S_3Q_A^-$ pairs (Fig. 2A). The characteristic T_M of $S_2Q_B^-$ decreased gradually from about 40°C to 30°C after 180 min of low temperature stress. Transferring the cells from 25°C back to the normal growth temperature of 36°C caused a shift of the $S_2Q_B^-$ peak back to about 40°C, indicating that the shifts in T_M are completely reversible. A similar trend was observed for $S_3Q_B^-$ recombinations. In contrast, the T_M for $S_2Q_A^-$ and $S_3Q_A^-$ recombinations remained fairly constant during the temperature shifts indicating the changes in T_M are specific for Q_B^- recombinations (Fig. 2B). Furthermore, the overall TL yield

also decreased by 30% after the 180 min exposure of the cells to low temperature, and this effect was also fully reversible after shifting the cultures back to 36°C (Fig. 2C; Sane et al., 2002). The reversible exchange of D1:1 for D1:2 followed the kinetics observed for the reversible changes in T_M (Fig. 3). These data indicate that in cold-stressed cells exhibiting D1:2, the redox potential of Q_B becomes lower approaching that of Q_A. A similar shift in the redox potential of Q_B was confirmed independently of growth temperature by examining the *Synechococcus* sp. PCC 7942 inactivation mutants R2S2C3 and R2K1 which possess either D1:1 or D1:2 respectively (Sane et al., 2002).

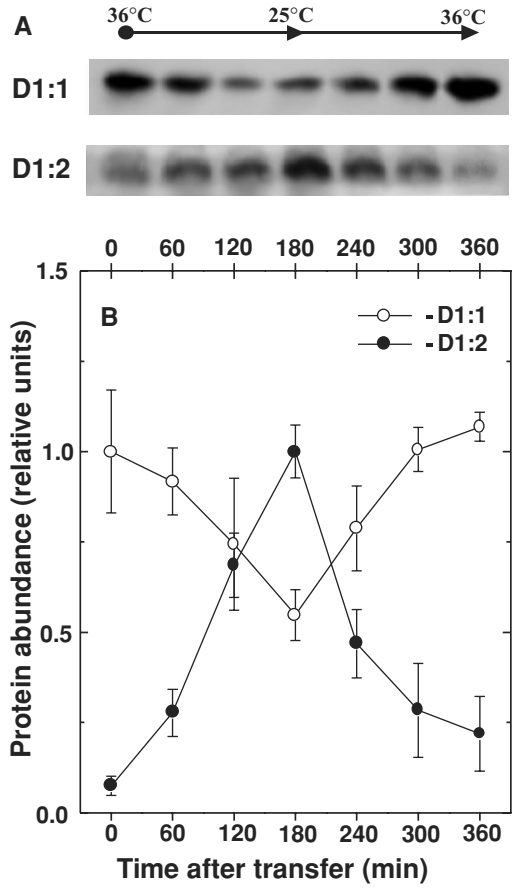

Fig. 3. Representative immunoblots (A) and densitometric analysis (B) of D1:1 and D1:2 polypeptides of PS II during the temperature shift of *Synechococcus* sp. PCC 7942 cells from 36°C to 25°C for 180 min and back to 36°C. Polypeptide abundance was detected by immunoblotting after SDS-PAGE with D1:1 and D1:2 specific antibodies. Mean values ± SE were calculated from 5–7 independent experiments. The data for relative abundance of D1:1 was normalized to its maximal values in control non-treated cells and for D1:2 to its maximal values after 180 min at 25°C. (From Sane et al., 2002)

We suggest that PS II reaction center protein exchange of D1:1 for D1:2 changes the redox properties of Q_B creating an altered charge equilibrium in favour of Q_A (Sane et al., 2002). This would increase the accumulation of Q_A^- and enhance the probability of non-radiative PS II reaction center quenching. Cyanobacteria synthesize zeaxanthin de novo from β-carotene in response to excess light (Adams et al., 1993; Ibelings et al., 1994; Masamoto and Furukawa, 1997), and although these prokaryotes lack xanthophyll cycle-dependent antenna quenching, they do possess the capability of utilizing zeaxanthin-dependent antenna quenching (Demmig-Adams et al., 1990). PS II reaction center quenching associated with the D1:1 / D1:2 exchange may, in part, contribute to the enhanced resistance to photoinhibition induced either by high light or low temperature in cyanobacteria (Krupa et al., 1990, 1991; Campbell et al., 1995).

B. Pinus sylvestris

The acceptor side of PS II has generally been considered to be a primary target for photoinhibition of photosynthesis (Powles, 1984; Krause, 1988; Öquist et al., 1992; Aro et al. 1993; Long et al., 1994; Keren and Ohad, 1998; Melis, 1999). The winter-induced inhibition of PS II photochemistry in Scots pine in vivo has been ascribed also to low temperature-induced photoinhibition of PS II (M. Strand and Öquist, 1985). Thus, the effects of photoinhibition on the charge recombination events between the acceptor and donor sides of PS II in Scots pine needles under both controlled environment as well as natural field conditions were assessed by TL (Ivanov et al., 2001, 2002).

As expected, TL glow emission curves of control, non-hardened pine needles pre-illuminated with continuous light were resolved into six distinct peaks with characteristic T_M corresponding to Z_v ($P680^+Q_A^-$ recombination), and A ($S_3Q_B^-$) bands below 0°C and to Q ($S_2Q_A^-$), B_1 ($S_2Q_B^-$), B_2 ($S_3Q_B^-$), and C ($TyrD^+Q_A^-$) bands above 0°C (Fig. 1A). In contrast, the cold hardened pine TL glow curves were best fitted with only three emission bands with T_M corresponding to the Z_V, Q, and B peaks with a concomitant decrease in the total TL emission (Fig. 1B; Ivanov et al., 2001). These effects on TL emission were reversible upon recovery of the pine needles from low temperature photoinhibition under laboratory conditions. In contrast to non-hardened needles, the treatment of cold hardened needles with DCMU to block the electron transfer from Q_A to Q_B, did not cause any significant changes in either the TL yield, the $T_{M,}$ or the relative contribution

of each peak to the overall glow curve. Furthermore, needles from cold hardened pine exhibited a significant inhibition of electron transfer from Q_A to Q_B relative to summer pine needles (Ivanov et al., 2001). This is consistent with the thesis that Q_A^- accumulates in PS II reaction centers of cold hardened pine needles to a greater extent than that in non-hardened pine needles due to an over-reduced PQ pool. These results are in agreement with earlier reports indicating that DCMU mimics the effects of low temperature photoinhibition of PS II in pine (Öquist and Martin, 1980), *Pisum sativum* (Farineau, 1993), and *Chlamydomonas reinhardtii* (Ohad et al., 1988). The lower total TL emission from winter pine needles and the relatively strong Z_v band accounting for almost 60% of the total luminescence is indicative of a preferred back reaction of Q_A with primary donors and a low probability of transfer of electrons from Q_A to Q_B (Ivanov et al., 2001). In addition, the $S_2Q_B^-$ charge recombinations were shifted to lower temperatures in cold hardened pine needles than non-hardened pine with little change in the T_M for $S_2/S_3Q_A^-$ recombinations (Ivanov et al., 2001; 2002). Thus, cold hardening conditions appear to cause major changes in the redox properties on the acceptor-side of PS II in *Pinus sylvestris*.

Seasonal dynamics of TL in Scots pine needles under natural field conditions showed that between November and April the contribution of the Q- and B-bands to the overall TL emission was less than 5%. During spring, the relative contribution of the Q- and B-bands, corresponding to charge recombination events between the acceptor and donor sides of PS II, rapidly increased, reaching maximal values in late July. Clearly, the reversible changes in the TL emission bands are observed both under controlled laboratory conditions as well as on a seasonal basis in *Pinus sylvestris* under natural conditions (Ivanov et al., 2002). Thus, the winter inhibition of photosynthesis in Scots pine is associated with major changes on the acceptor-side of PS II. We suggest that exposure to winter conditions narrows the redox potential gap between Q_B and Q_A causing the accumulation of Q_A^- and enhancing the probability for charge recombination within PS II reaction centers through a non-radiative pathway (Vavilin and Vermaas, 2000). This reaction center quenching may enhance the protection of PS II reaction centers through the dissipation of excess absorbed energy and complement the capacity for antenna quenching under conditions where the enzyme-dependent xanthophyll cycle is thermodynamically restricted resulting in a sustained non-photochemical quenching (Öquist and Huner, 2003).

C. Arabidopsis thaliana

Relative to winter wheat and winter rye, cold acclimation of *Arabidopsis thaliana* results in an incomplete recovery of photosynthetic capacity (Savitch et al., 2001). Thus, the possibility of cold induced alterations of PS II was also addressed by TL measurements for direct estimation and comparison of the redox properties of PSII in control, non-hardened, cold-stressed, and cold-acclimated *Arabidopsis* plants (Sane et al., 2003). As observed in *Synechococcus* and *Pinus sylvestris*, cold stress and cold acclimation of *Arabidopsis thaliana* resulted in significant shifts in the T_M for the flash induced TL-bands. However, in contrast to results for pine and *Synechococcus*, cold acclimated *Arabidopsis* exhibited a characteristic upshift of the T_M associated with $S_2Q_A^-$ recombination with a concomitant downshift in the T_M associated with $S_2Q_B^-$ recombinations relative to control plants (Fig. 4) (Sane et al., 2003). These data were confirmed by assessing the time course for the shift in the $S_2Q_A^-$ and $S_2Q_B^-$ peak temperatures when non-hardened control plants were transferred from 23°C to 5°C. The characteristic T_M of $S_2Q_B^-$ peak exhibited a gradual downshift while the T_M of $S_2Q_A^-$ exhibited a gradual upshift such that the initial gap of 55°C between $S_2Q_A^-$ and $S_2Q_B^-$ in non-hardened *Arabidopsis* was narrowed to about 36°C over a period of 4 weeks of cold acclimation (Sane et al., 2003). In addition, the relative TL yield measured as the integrated area under the glow curves also decreased in non-hardened plants transferred to 5°C such that after 24 days at low temperature, the non-hardened plants exhibited overall TL luminescence close to that in fully cold acclimated *Arabidopsis* (Sane et al., 2003). This has been interpreted to indicate major alterations in the redox properties of the acceptor-side of PS II during cold stress and cold acclimation in *Arabidopsis*, increasing the probability of non-radiative dissipation through PS II reaction center quenching and complementing the capacity for antenna quenching to protect PS II from over excitation.

To assess the relative contributions of antenna quenching to total non-photochemical quenching in non-acclimated and cold acclimated *Arabidopsis*, q_O, a measure of antenna quenching (Bukhov et al., 2001; Krause and Jahns, 2003), was plotted as a function of q_N (Fig. 5). If all of the non-photochemical quenching was due to antenna quenching, one would expect a positive, straight line relationship between q_O and q_N (Fig. 5). However, there is a clear curvilinear relationship between q_O and q_N in non-hardened *Arabidopsis* (Fig. 5), indicating significant contributions to q_N that

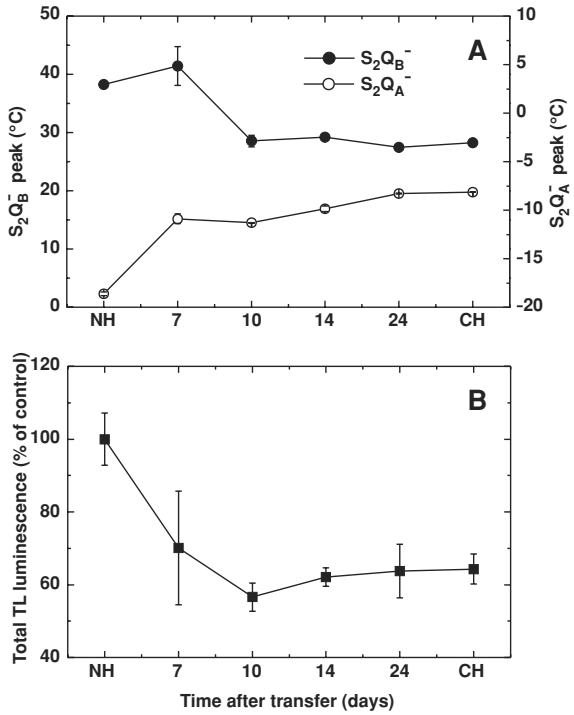

Fig. 4. A – Time course of the characteristic T_M of $S_2Q_B^-$ and $S_2Q_A^-$ peaks in *Arabidopsis* leaves during the temperature shift of control (NH) plants from the growth temperature of 23°C and 250 μmol photons m^{-2} s^{-1} to 5°C and the same irradiance. The peak positions were estimated by decomposition analysis of the experimental curves in control and in DCMU treated leaves. B – Relative TL yield measured as the total area under the experimental glow curves. The mean values ± SE were calculated from 6–8 measurements in 3 independent experiments. NH – control (non-hardened) plants grown at 23°C, 250 μmol photons m^{-2} s^{-1}, and a 16h photoperiod; CH – fully cold-acclimated plants grown at 5°C, 250 μmol photons m^{-2} s^{-1}, and a 16h photoperiod. (From Sane et al., 2003)

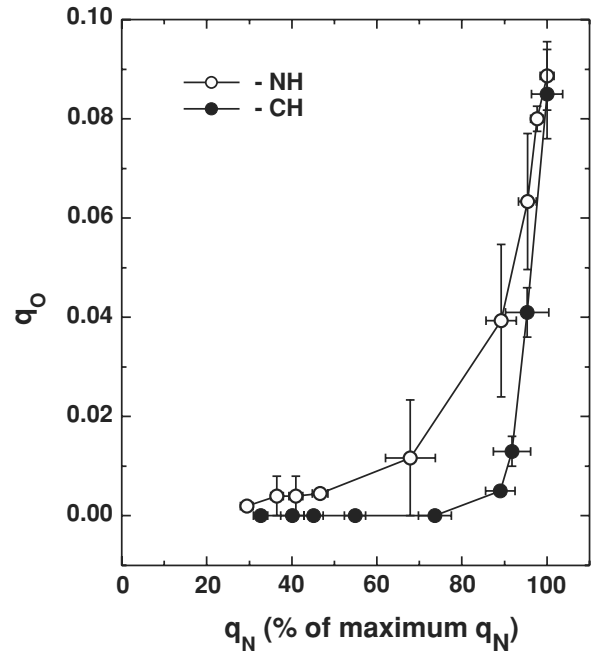

Fig. 5. Non-photochemical (q_N) versus q_O chlorophyll fluorescence quenching measured at different actinic light intensities in non-hardened (open symbols) and cold acclimated (closed symbols) *Arabidopsis* leaves. Mean values ± SE were calculated from 3–4 independent experiments. q_N values are presented as percentage of maximal q_N registered during illumination with 1600 μmol photons m^{-2} s^{-1} actinic white light. (From Sane et al., 2003)

do not originate from antenna quenching. This curvilinear relationship is accentuated in cold acclimated *Arabidopsis* where up to 75% of the non-photochemical quenching appears to be due to a quenching component(s) that is/are independent of q_O, that is, independent of the antenna. It has been suggested that PS II reaction center quenching represents the source of this additional quenching capacity (Buhkov et al., 2001; Sane et al., 2003).

D. Chlamydomonas reinhardtii

Photoinhibition and PS II photodamage has been studied extensively in green algae such as *Dunaliella salina* (Melis, 1999) and the model green alga, *Chlamydomonas reinhardtii* (Keren and Ohad, 1998). The consensus is that a continuous repair mechanism that requires the de novo synthesis of D1 is operative during photoinhibition and recovery in this green alga (Falk et al., 1990; Keren and Ohad, 1998; Melis, 1999). Thus, protection from photoinhibition is thought to reflect the relative rates of photodamage versus the rates of repair (Aro et al., 1993; Keren and Ohad, 1998; Melis, 1999). Similar to *Synechococcus* (Krupa et al., 1990, 1991), *Chlorella vulgaris* (Huner et al., 1998) and several plant species (Somersalo and Krause, 1990; Huner et al., 1993; Savitch et al., 2001), growth of *Chlamydomonas reinhardtii* at low temperature (12°C) increases the resistance of these cells to high light regardless of the temperature (Falk et al., 1990). It has been proposed that this increased resistance to photoinhibition in *Chlamydomonas reinhardtii* is, at least in part, due to an increased rate of repair of damaged D1 (Falk et al., 1990; Keren and Ohad, 1998).

Through the generation of NPQ mutants in *Chlamydomonas reinhardtii*, the important role of antenna quenching in photoprotection has been documented in this model green alga (Niyogi, 1999). Results on

photoprotection in the *npq5* mutant of *Chlamydomonas reinhardtii* indicate that non-photochemical dissipation through antenna quenching is mediated by trimeric LHCII (Elrad et al., 2002). Furthermore, suppressors of the *npq1 lor1* double mutant in this green alga indicate that zeaxanthin acts as an antioxidant in the quenching of $^1O_2$ and free radicals in addition to its role in antenna quenching through the xanthophyll cycle (Baroli et al., 2003).

What role, if any, does reaction center quenching play in photoprotection of PS II of *Chlamydomonas reinhardtii*? The data in Table 1 illustrate that at a constant growth temperature of 29°C, an increase in growth irradiance from 20 to 500 μmol photons m^{-2} s^{-1} resulted in a decrease in the temperature gap between $S_2Q_B^-$ and $S_2Q_A^-$ from 21.5 to 7.5°C. This 14°C downward shift in the T_M for the $S_2Q_B^-$ recombination occurred with minimal changes in the $S_2Q_A^-$ recombinations. This indicates that exposure of *Chlamydomonas reinhardtii* to high light narrows the redox potential gap between Q_A and Q_B causing the accumulation of Q_A^- that increases the probability for charge recombination and PS II reaction center quenching. This apparent light-dependent effect on TL emission was reversed when high light-grown cells were shifted back to low light.

However, the downward shift in the T_M for $S_2Q_B^-$ can not be a simple high light effect since growth of *Chlamydomonas reinhardtii* at low temperature and moderate irradiance (15°C and 150 μmol photons m^{-2} s^{-1}) also caused a 15°C downward shift in the T_M for $S_2Q_B^-$, the extent of which was comparable to that observed for cells grown at high light with minimal changes in the T_M for $S_2Q_A^-$ recombinations (Table 1). Furthermore, cells grown at low temperature and low irradiance (15°C and 20 μmol photons m^{-2} s^{-1}) exhibited a comparable downward shift to the cells grown at 29°C and moderate irradiance (150 μmol photons m^{-2} s^{-1}). These results are consistent with the fact that either increased irradiance or low temperature does indeed increase the accumulation of Q_A^- in *Chlamydomonas reinhardtii*. Since low temperature can mimic high light effects due to comparable modulation of the relative redox state of Q_A (Huner et al., 1998), we conclude that reaction center quenching is modulated by excitation pressure in *Chlamydomonas reinhardtii*.

Table 1. The effects of growth irradiance and growth temperature on thermoluminescence T_M in *Chlamydomonas reinhardtii.*

Growth Regime (°C / μmol m^{-2} s^{-1})	Temperature Gap (°C)[a]
29 / 20	21.5
29 / 150	10.3
29 / 500	7.5
15 / 150	6.2
15 / 20	10.3

[a] The temperature gap was calculated as $T_M(S_2Q_B^-)$–$T_M(S_2Q_A^-)$. The light- and temperature-dependent changes in the temperature gap were due to downshifts in the T_M for $S_2Q_B^-$ since the T_M for $S_2Q_A^-$ remained fairly constant under all conditions tested.

VI. Bioenergetics of Reaction Center Quenching

Summarizing the experimental data discussed above, it is evident that exposure to low temperatures causes major alterations in the redox properties of the acceptor side of PS II in various photosynthetic organisms. The shifts in the characteristic T_M of $S_2Q_A^-$ and $S_2Q_B^-$ recombinations in cold-acclimated *Arabidopsis thaliana* with the Q_A- and Q_B-associated peaks appearing at higher and lower temperatures, respectively, imply substantial changes in the activation energies associated with de-trapping of the electron from reduced Q_A and Q_B (Fig. 6A, B). Similar changes in the redox properties of PS II have been observed by Briantais et al. (1992), who reported a shift towards lower temperatures for the $S_2Q_B^-$ peak in cold acclimated spinach compared to non-hardened plants. More recently, a downshift in the T_M of the B-band ($S_2Q_B^-$) was observed in low temperature grown maize (Janda et al., 2000). Because the activation energies have been shown to be directly related to the redox potentials of the participating species (Devault and Govindjee, 1990), narrowing the temperature gap between the characteristic T_M for Q_A and Q_B may reflect a narrowing of the redox potential gap between Q_A and Q_B as a result of cold acclimation (Fig. 6).

The high temperature shift in the T_M of $S_2Q_A^-$ corresponding to increased activation energy of Q_A/Q_A^- in *Arabidopsis thaliana* would increase the free energy gap between Q_A^- and $P680^+$. This could cause stabilization of $S_2Q_A^-$ and decrease the probability for the back reaction through $P680^+Pheo^-$ (Minagawa et al., 1999; Vavilin and Vermaas, 2000). Moreover, the preferential localization of the electron on Q_A in cold acclimated *Arabidopsis* could also result from a change in the redox potential of Q_B. Lowering the redox potential of Q_B will narrow the gap between the redox potentials between Q_A and Q_B even further, and will decrease the probability for electron transfer between the two quinone acceptors by shifting the redox equilibrium

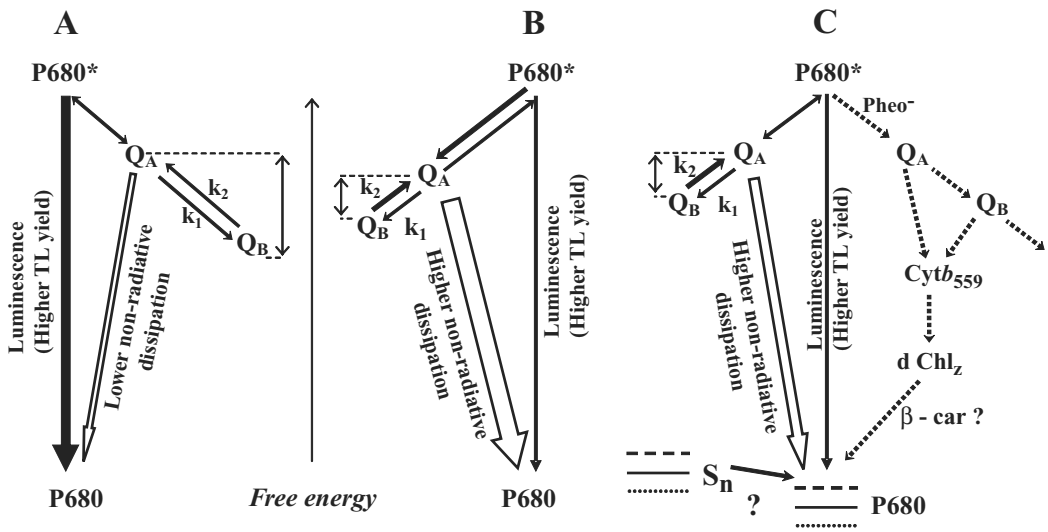

Fig. 6. Schematic diagram of the free energy levels explaining the differences in radiative vs non-radiative energy dissipation pathways in control (A) and cold acclimated (B), (C) plants. A - In control leaves, radiative energy dissipation pathway characterized by higher TL yield is predominant and probably involves the back-reaction *via* the $P680^+Pheo^-$ radical pair. B – In cold acclimated plants the increased free energy gap between $P680^+$ and Q_A^- would decrease the probability for a charge recombination pathway involving $P680^+Pheo^-$ and will cause stabilization of $S_2Q_A^-$ pair. In addition, shifting the redox potential of Q_B toward Q_A favors the k_2 rate constant and also results in an increased steady state proportion of reduced Q_A. It is proposed that this will increase the probability for direct recombination of Q_A^- with $P680^+$ *via* non-radiative interaction resulting in low TL yield without generating chlorophyll triplet. C – In some cases when there is no apparent shift of $S_2Q_A^-$ recombination to higher temperatures, but the shift of Q_B towards Q_A implies increased proportion of reduced Q_A and the low TL yield suggests increased non-radiative dissipation, the cyclic electron pathway: Cytb_{559} → dChl$_z$ → β-Car → P_{680}^+ might be involved. In this case, the redox properties of the donor side might also be modified during cold acclimation. In both types of plants the radiative charge recombination occurs, but is proportionally less in cold acclimated plants (Ivanov et al., 2003).

between $Q_A^-Q_B$ and $Q_AQ_B^-$ towards $Q_A^-Q_B$ (Minagawa et al., 1999). The retention of electrons preferentially on Q_A through a modification of the redox potentials of Q_A and Q_B in opposite directions would inhibit the reoxidation of Q_A^- (Mäenpää et al., 1995). The slower re-oxidation kinetics of Q_A^- in both overwintering Scots pine (Ivanov et al., 2001) and cold acclimated *Arabidopsis* leaves (Sane et al., 2003) are consistent with this interpretation. This would ensure that the Q_B site remains occupied by a quinone, which would protect PS II from photoinhibition and D1 degradation (Ohad and Hirschberg, 1992). Supporting evidence for this argument comes from experiments in which addition of DCMU had a protective effect on D1 turnover under photoinhibitory conditions (Komenda and Masojidek, 1998). When the Q_B site is occupied in the presence of DCMU and Q_A is in a reduced state, PS II shows increased resistance to photoinhibition.

A possible back reaction of the reduced Q_A with $P680^+$ has been suggested from earlier data (Prasil et al., 1996; Krieger-Liszkay and Rutherford, 1998), and this may be enhanced when Q_A remains reduced (Vavilin and Vermass, 2000). The accumulation of Q_A^- has been shown to inhibit the formation of the radical pair $P680^+Pheo^-$, thus preventing P680 triplet formation (Schatz et al., 1988; Vass et al., 1992). In addition, it has been suggested that there is a non-radiative pathway of charge recombination between Q_A^- and the donor side of PS II (Briantais et al., 1979; Weis and Berry, 1987; Vavilin and Vermaas, 2000). Such a pathway would increase the probability for non-radiative dissipation of excitation energy within the reaction center of PS II (Weis and Berry, 1987; Bukhov et al., 2001). The significantly lower total TL emission observed in winter pine (Ivanov et al., 2001, 2002), cold stressed *Synechococcus* (Sane et al., 2002), and in cold acclimated *Arabidopsis* (Sane et al., 2003) is consistent with such a non-radiative pathway within the PS II reaction center.

The reduction of Q_A has been suggested to be a major requirement for efficient reaction center quenching (Krause, 1988; Krause and Weis, 1991; Walters and Horton, 1993; Huner et al., 1993; Bukhov et al., 2001). In this regard, it is important to note that acclimation to low temperatures is strongly correlated with an increased proportion of reduced Q_A at the given growth temperature (Huner et al., 1993, 1998). Hence, it seems

very likely that the increased population of Q_A^- due to the altered redox potential of Q_A and Q_B during the shift and acclimation to low temperature in *Arabidopsis* may enhance the dissipation of excess light within the reaction center of PS II via non-radiative $P680^+Q_A^-$ recombination, protecting the Q_A site from excessive excitation pressure (Huner et al., 1998; Öquist and Huner, 2003). Similar trends were reported for both winter Scots pine (Ivanov et al., 2001) and low temperature stressed *Synechococcus* (Sane et al., 2002), which are consistent with the results of Bukhov et al. (2001) and Grasses et al. (2002). The importance of excitation pressure and the relative redox state of Q_A in regulating reaction center quenching is further supported by the data for *Chlamydomonas reinhardtii* (Table 1). The low temperature-induced downshift in the T_M for S_2QB^- recombination was mimicked by exposing cells to moderate temperatures but high light. Exposure to low temperature but moderate irradiance induces PS II closure because of a slower rate of Q_A^- oxidation relative to the rate of its reduction, whereas exposure to high light at moderate temperatures induces PS II closure due to a higher rate of Q_A reduction relative to the rate of its oxidation (Huner et al., 1998). This is consistent with the notion that the equilibrium Q_A^- : Q_A / Q_B^- : Q_B controls charge recombination within PS II (Keren and Ohad, 1998).

Cyclic electron transport around PS II (Fig. 6C) has been suggested as an alternative photoprotective mechanism operating within the PS II reaction centre (Telfer et al., 1991; Barber and De Las Rivas, 1993), and the role of the high potential form of Cyt b_{559} in this process has been discussed (Stewart and Brudwig, 1998). Allakhverdiev et al. (1997) provided direct evidence for the involvement of cyclic electron transport around PS II in protection against photoinhibitory damage. It was suggested that Cytb_{559} may protect PS II by acting as a secondary donor to $P680^+$ via the electron donation pathway: Cyt$b_{559} \rightarrow dChl_z \rightarrow \beta$-Car $\rightarrow P680^+$, where Chl_z is a chlorophyll molecule coordinated to His118 of the B trans-membrane helix of the D1 protein (Barber and De Las Rivas, 1993; Nield et al., 2000b). The accumulation of Chl_Z^+ as a result of over-oxidation of P680 has been suggested as a site for photoprotection (Stewart and Brudvig, 1998). A simplified model illustrating the cyclic electron transport around PS II is presented in Figure 6. It seems reasonable to suggest that such a mechanism for photoprotection is a possible alternative to non-radiative reaction center quenching. Such a mechanism may take place in cases when there is no apparent upward shift of $S_2Q_A^-$ recombination to higher temperatures.

VII. Molecular Mechanisms Regulating Reaction Center Quenching

As discussed above, the prokaryotic and eukaryotic species examined exhibit significant downshifts in the T_M for the $S_2Q_B^-$ and $S_3Q_B^-$ recombinations whereas, in addition, *Arabidopsis thaliana* exhibits an upshift in the T_M for the $S_2Q_A^-$ and the $S_3Q_A^-$ recombinations (Fig. 4). Since these shifts in T_M can occur in response to the reduction state of Q_A induced either by high light or low temperature, we suggest that excitation pressure regulates reaction center quenching by altering the redox potentials of Q_A and Q_B in PS II reaction centers. What is/are the molecular mechanism(s) underlying these alterations in redox potentials of Q_A and Q_B in response to excitation pressure?

A. D1 Exchange

In the case of *Synechococcus* PCC 7942, excitation pressure and reaction center quenching are associated with D1 protein exchange; the D1:1 is exchanged for D1:2. We propose that, in *Synechococcus* sp. PCC 7942, it is primarily reaction center polypeptide exchange that results in a change in the microenvironment of the Q_B-binding site inducing a change in its redox properties. In support of this proposal, it has been shown that a single change in the crucial amino acid residue of D1 (Ohad and Hirschberg, 1992; Minagawa et al., 1999) or a deletion of the PEST-like sequence of D1 (Nixon et al., 1995) results in a shift of the $S_2Q_B^-$ TL peak towards lower temperatures.

However, growth temperature and growth irradiance also have a significant impact on the lipid and fatty acid composition of thylakoid membranes of cyanobacteria (Nishida and Murata, 1996; Los and Murata, 2002). The increased fatty acid unsaturation observed in cyanobacteria at low temperature appears to be a prerequisite for efficient D1 repair upon exposure to low temperature photoinhibition (Nishida and Murata, 1996). Recently, Sakurai et al. (2003) used the *pgsA* mutant of the cyanobacterium *Synechocystis* sp. PCC 6803 to show that the absence of the phospholipid, phosphatidylglycerol (PG), increased the susceptibility of the mutant cells to photoinhibition. Although *pgsA* cells exhibited comparable rates of D1 synthesis and degradation to those observed in the wild type cells, the mutant cells were impaired with respect to the dimerization of PS II core monomers and the reactivation of photoinhibited PS II core complexes (Sakurai et al., 2003). Thus, it is conceivable that changes in the thylakoid lipid and fatty acid composition could also

alter the microenvironment of PS II reaction centers by altering lipid-protein interactions causing a shift in the T_M for the Q_B recombinations in *Synechococcus* sp. PCC 7942. However, since the R2S2C3 mutant of *Synechococcus* sp. PCC 7942 exhibiting D1:1 only and the R2K1 mutant exhibiting only D1:2 showed comparable changes in the T_M for $S_2Q_B^-$ and $S_3Q_B^-$ recombinations independent of any temperature change and presumably any changes in thylakoid lipid and fatty acid composition, we conclude that the observed changes in the redox properties of Q_B are most likely a consequence of D1 protein exchange rather than changes in lipid-protein interactions with PS II reaction centers of *Synechococcus* sp PCC 7942.

B. Posttranslational Modification of D1

In higher plants, the D1 polypeptide of PS II is subject to at least five post-translational modifications: C-terminal processing in the conversion of 34 kDa precursor polypeptide to the 32 kDa mature polypeptide; removal of the initiating methionine residue; N-acetylation of N-terminal threonine residue; covalent palmitoylation mapped to the N-terminal two thirds of the D1 polypeptide, and finally, reversible phosphorylation of the N-terminal threonine catalyzed by a light-dependent, redox-regulated kinase (Mattoo et al., 1993; Rintamäki and Aro, 2001). Although the functional role of D1 palmitoylation remains unknown, palmitoylation has been shown to regulate signal transduction through G-protein linked receptors by regulating protein-protein interactions (Milligan et al., 1995). There is no evidence for the role of D1 palmitoylation in altering the T_M for $S_2/S_3 - Q_A^-/Q_B^-$ recombinations. However, alterations in protein-protein interactions within PS II may be important since *Arabidopsis thaliana npq4-1* mutant lacking only the PsbS protein within PS II complexes exhibit significant downshifts in the T_M for $S_2/S_3 - Q_A^-/Q_B^-$ recombinations under normal growth conditions relative to wild-type. We hypothesize that this may be due to changes in protein-protein interactions within PS II reaction centers induced by the absence of PsbS. Further experimentation is ongoing to test this hypothesis.

In higher plants, both the D1 and D2 reaction center polypeptides undergo reversible phosphorylation during the PS II damage-repair cycle. The extent of D1 phosphorylation appears to be regulated by excitation pressure (Rintamäki and Aro, 2001) as well as by an endogenous circadian rhythm (Booij-James et al., 2002). Site-directed mutagenesis of *PsbA* in *Synechocystis* PCC 6803 indicates that alterations in a single amino acid can result in significant changes in the T_M for $S_2/S_3 - Q_A^-/Q_B^-$ recombinations (Minagawa et al., 1999; Vavilin and Vermaas, 2000). Thus, it is conceivable that post-translational modification of D1 and / or D2 PS II reaction center polypeptides by either palmitoylation or phosphorylation may alter the conformation of these polypeptides. This, in turn, may result in shifts in the T_M for $S_2/S_3 - Q_A^-/Q_B^-$ recombinations and hence the changes in the redox potentials of Q_A and Q_B. Consistent with the thesis that protein phosphorylation may convert PS II active centers into PS II quenching centers is the suggestion that the CHB observed in overwintering snow gum and mistletoe represents a Chl-protein complex containing PS II quenching centers formed as a result of PS II core protein phosphorylation (Gilmore et al. (2003).

Unlike in seed plants, no phosphorylation of the D1 polypeptide has been detected in *Chlamydomonas reinhardtii* (Keren and Ohad, 1998; Rintamäki and Aro, 2001). Clearly, this potential post-translational modification mechanism of the D1 polypeptide can not account for shifts in the T_M for $S_2/S_3 - Q_A^-/Q_B^-$ recombinations induced by excitation pressure in this green alga. However, D2 is phosphorylated in *Chlamydomonas reinhardtii* (Keren and Ohad, 1998) and may account for the shifts in the T_M for $S_2/S_3 - Q_A^-/Q_B^-$ recombinations and hence the changes in the redox protentials of Q_A and Q_B. In addition, the decrease in HCO_3^- concentrations in the chloroplast under saturating irradiance has also been shown to affect the redox potentials of Q_A and Q_B (Govindjee, 1993; Demeter et al., 1995). Thus, regulation of chloroplastic HCO_3^- concentrations may also contribute to modulating the redox potentials of Q_A and Q_B in this model green alga.

C. Thylakoid Lipids and Fatty Acids

Unlike *Synechococcus* sp PCC 7942, pine, *Arabidopsis thaliana*, and *Chlamydomonas reinhardtii* have only one *PsbA* gene coding for the D1 protein. Thus, a protein exchange mechanism similar to that observed for *Synechococcus* sp PCC 7942 cannot account for the modulation of the T_M for $S_2/S_3 - Q_A^-/Q_B^-$ recombinations and hence the changes in the redox potentials of Q_A and Q_B. However, the lipid and fatty acid compositions of thylakoid membranes of higher plants are sensitive to growth temperature and growth irradiance (Harwood, 1998; Vijayan et al., 1998; Selstam, 1998). PG and its fatty acid

composition are important in regulating the oligomerization of LHCII in many higher plants as well as *Chlamydomonas reinhardtii* (Trémolières and Siegenthaler, 1998). Furthermore, Dobrikova et al. (1997) showed that the asymmetric surface charge distribution and electric polarizability of thylakoid membranes are significantly altered in the *fad*B and the *fad*C *Arabidopsis* mutants deficient in lipid fatty acid desaturases compared to wild type. Thus, it is possible that light- and temperature-induced changes in the thylakoid lipid / fatty acid composition may result in alterations in lipid-protein interactions within PS II reaction centers in pine, *Arabidopsis thaliana*, and *Chlamydomonas reinhardtii*. This, in turn, may result in the observed shifts in the T_M for $S_2/S_3 - Q_A^-/Q_B^-$ recombinations and hence changes in the redox potentials of Q_A and Q_B.

Although excitation pressure may be critical in regulating the shifts in the T_M for $S_2/S_3 - Q_A^-/Q_B^-$ recombinations and hence the redox potentials of Q_A and Q_B, the molecular alterations required to induce these shifts in T_M may be species dependent and vary with the environmental changes to which an organism is exposed. Whereas D1:1/D1:2 polypeptide exchange appears to be the primary molecular mechanism regulating the shifts in T_M for $S_2/S_3 - Q_A^-/Q_B^-$ recombinations in *Synechococcus* PCC 7942, other species such as *Pinus sylvestris*, *Arabidopsis thaliana*, and *Chlamydomonas reinhardtii* may use any one or a combination of the molecular mechanisms outlined above. We conclude that, as originally suggested by Krause and Weis (1991), both reaction center and antenna quenching function in vivo to different extents to protect PS II from photodamage depending on the species as well as the environmental conditions. However, further research is required not only to assess the contribution of any one of these mechanisms to the shifts in the T_M for $S_2/S_3 - Q_A^-/Q_B^-$ recombinations and hence reaction center quenching, but also to assess the timing for the onset of reaction center quenching versus antenna quenching associated with NPQ during exposure to increased excitation pressure.

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References

Adams III WW, Demmig-Adams B and Lange OL (1993) Carotenoid composition and metabolism in green and blue algal lichens in the field. Oecologia 94: 576–584

Adams III WW, Demmig-Adams B, Rosenstiel TN and Ebbert V (2001) Dependence of photosynthesis and energy dissipation upon growth form and light environment during the winter. Photosynth Res 67: 51–62

Adams WW III, Demmig-Adams B, Rosenstiel TN, Brightwell AK and Ebbert V (2002) Photosynthesis and photoprotection in overwintering plants. Plant Biol 4: 545–557

Adams WW III, Zarter CR, Ebbert V and Demmig-Adams B (2004) Photoprotective strategies of overwintering evergreens. BioScience 54: 41–49

Allakhverdiev SI, Klimov VV and Carpentier R (1997) Evidence for the involvement of cyclic electron transport in the protection of photosystem II against photoinhibition: Influence of a new phenolic compound. Biochem 36: 4149–4154

Aspinall-O'Dea M, Wentworth M, Pascal A, Robert B, Ruban A and Horton P (2002) *In vitro* reconstitution of the activated zeaxanthin state associated with energy dissipation in plants. Proc Natl Acad Sci USA 99: 16331–16335

Aro E-M, Virgin I and Andersson B (1993) Photoinhibition of photosystem II: inactivation, protein damage and turnover. Biochim Biophys Acta 1143: 113–134

Barber J and De Las Rivas J (1993) A functional model for the role of cytochrome b_{559} in the protection against donor and acceptor side photoinhibition. Proc Natl Acad Sci USA 90:10942–10946

Baroli I, Do AD, Yamane T and Niyogi KK (2003) Zeaxanthin accumulation in the absence of a functional xanthophyll cycle protects *Chlamydomonas reinhardtii* from photooxidative stress. Plant Cell 15: 992–1008

Bassi R, Croce R, Cugini D and Sandona D (1999) Mutational analysis of a higher plant antenna protein provides identification of chromophores bound in multiple sites. Proc Natl Acad Sci USA 96: 10056–10061

Booij-James IS, Swegle WM, Edelman M and Mattoo AK (2002) Phosphorylation of the D1 Photosystem II reaction center protein is controlled by an endogenous circadian rhythm. Plant Physiol 130: 2069–2075

Briantais J-M, Vernotte C, Picaud M and Krause GH (1979) A quantitative study of the slow decline of chlorophyll *a* fluorescence in isolated chloroplasts. Biochim Biophys Acta 548: 128–138

Briantais J-M, Ducruet J-M, Hodges M and Krause GH (1992) The effects of low temperature acclimation and photoinhibitory treatments on photosystem 2 studied by thermoluminescence and fluorescence decay kinetics. Photosynth Res 31: 1–10

Bukhov NG, Heber U, Wiese C and Shuvalov VA (2001) Energy dissipation in photosynthesis: does the quenching of chlorophyll fluorescence originate from antenna complexes of photosystem II or from the reaction center? Planta 212: 749–758

Butler WG (1978) Energy distribution in the photochemical apparatus of photosynthesis. Ann Rev Plant Physiol 29: 345–378

Campbell D, Zhou G, Gustafsson P, Öquist G and Clarke AK (1995) Electron transport regulates exchange of two forms of photosystem II D1 protein in the cyanobacterium *Synechococcus*. EMBO J 14: 5457–5466

Delrieu MJ (1998) Regulation of thermal dissipation of absorbed excitation energy and violaxanthin deepoxidation in the thylakoids of *Lactuca sativa*. Photoprotective mechanism of a population of photosystem II centers. Biochim Biophys Acta 1363: 157–173

Demeter S, Janda T, Kovacs L, Mende D and Wiessner W (1995) Effects of in vivo CO_2-depletion on electron transport and photoinhibition in the green algae *Chlamydobotrys stellata* and *Chlamydomonas reinhardtii*. Biochim Biophys Acta 1229: 166–174

Demmig-Adams B and 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 and Adams WW III (2002) Antioxidants in photosynthesis and human nutrition. Science 298: 2149–2153

Demmig-Adams B, Adams WW III, Czygan F-C, Schreiber U and Lange OL (1990) Differences in the capacity for radiationless energy dissipation in the photochemical apparatus of green and blue-green algal lichens associated with differences in carotenoid composition. Planta 180: 582–589

Demmig-Adams B, Gilmore AM and Adams WW III (1996) In vivo functions of carotenoids in higher plants. FASEB J 10: 403–412

Demmig-Adams B, Adams WW, Ebbert V and Logan BA (1999) Ecophysiology of the xanthophyll cycle. In: Frank HA, Young AJ, Britton G and Cogdell RJ (eds) The Photochemistry of Carotenoids, Advances in Photosynthesis, Vol 8, pp 245–269. Kluwer Academic Publishers, Dordrecht

Devault D and Govindjee (1990) Photosynthetic glow peaks and their relationship with the free energy changes. Photosynth Res 24: 175–181

Dobrikova A, Taneva SG, Busheva M, Apostolova E and Petkanchin I (1997) Surface electric properties of thylakoid membranes from *Arabidopsis thaliana* mutants. Biophys Chem 67: 239–244

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

Dreuw A, Fleming GR and Head-Gordon M (2003a) Charge-transfer state as a possible signature of a zeaxanthin-chlorophyll dimmer in the non-photochemical quenching process in green plants. J Phys Chem B 107: 6500–6503

Dreuw A, Fleming GR and Head-Gordon M (2003b) Chlorophyll fluorescence quenching by xanthophylls. Phys Chem Physics 5: 3247–3256

Ducruet J-M (2003) Chlorophyll thermoluminescence of leaf discs: simple instruments and progress in signal interpretation open the way to new ecophysiological indicators. J Exp Bot 54: 2419–2430

Elrad D, Niyogi KK and Grossman AR (2002) A major light harvesting polypeptide of photosystem II functions in thermal dissipation. Plant Cell 14: 1801–1816

Falk S, Samuelsson G and Öquist G (1990) Temperature-dependent photoinhibition and recovery of photosynthesis in the green alga *Chlamydomonas reinhardtii* acclimated to 12 and 27C. Physiol Plant 78: 173–180

Farineau J (1993) Compared thermoluminescence characteristics of pea thylakoids studied in vitro and in situ (in leaves). The effect of photoinhibitory treatments. Photosynth Res 36: 25–34

Finazzi G, Johnson GN, Dall'Osto L, Joliot P, Wollman F-A and Bassi R (2004) A zeaxanthin-independent nonphotochemical quenching mechanism localized in the photosystem II core complex. Proc Natl Acad Sci USA 101: 12375–12380.

Frank HA, Cua A, Chynwat V, Young A, Gosztola D and Wasielewski MR (1994) Photophysics of the carotenoids associated with the xanthophyll cycle in photosynthesis. Photosyn Res 41: 389–395

Funk C (2001) The PsbS protein: a Cab-protein with a function of its own. In: Aro E-M and Andersson B (eds) Regulation of Photosynthesis, Advances in Photosynthesis and Respiration, Vol 11, pp 453–467. Kluwer Academic Publishers, Dordrecht

Gilmore AM (1997) Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. Physiol Plant 99: 197–209

Gilmore AM (2000) Mechanistic role of xanthophyll-dependent photoprotection in higher plant chloroplasts and leaves. Physiol Plant 99: 197–209

Gilmore AM and Ball MC (2000) Protection and storage of chlorophyll in overwintering evergreens. Proc Natl Acad Sci USA 97: 11098–11101

Gilmore AM, Matsubara S, Ball MC, Barker DH and Itoh S (2003) Excitation energy flow at 77K in the photosynthetic apparatus of overwintering evergreens. Plant Cell Environ 26: 1021–1034

Golden SS, Brussan J and Haselkorn R (1986) Expression of a family of *psbA* genes encoding a photosystem II polypeptide in the cyanobacterium *Anacystsis nidulans* R2. EMBO J 5: 2789–2798

Govindjee (1993) Bicarbonate reversible inhibition of plastoquinone reduction in photosystem II. Z Naturforsch 48c: 251–258

Grasses, T, Pesaresi P, Schiavon F, Varotto C, Salamini F, Jahns P and Leister D (2002) The role of ΔpH-dependent dissipation of excitation energy in protecting photosystem II against light-induced damage in *Arabidopsis thaliana*. Plant Physiol Biochem 40: 41–49

Greer DH, Berry J and Björkman O (1986) Photoinhibition of photosynthesis in intact bean leaves: role of light and temperature and requirement of chloroplast-protein synthesis during recovery. Planta 168: 253–260

Harwood JL (1998) Involvement of chloroplast lipids in the reaction of plants submitted to stress. In: Siegenthaler P-A (ed) Lipids in Photosynthesis: Structure, Function and Genetics, Advances in Photosynthesis and Respiration, Vol 6, pp 287–302. Kluwer Academic Publishers, Dordrecht

Havaux M and Kloppstech K (2001) The protective functions of carotenoids and flavonoid pigments against excess visible radiation at chilling temperature investigated in *Arabidopsis npq* and *tt* mutants. Planta 213: 95–966

Havaux M and Niyogi KK (1999) The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. Proc Natl Acad Sci USA 96: 8762–8767

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

Horton P, Ruban AV and Young AJ (1999) Regulation of the structure and function of the light harvesting complexes of photosystem II by the xanthophyll cycle. In: Frank HA, Young AJ, Britton G and Cogdell RJ (eds) The Photochemistry of

Carotenoids, Advances in Photosynthesis , Vol 8, pp 271–291. Kluwer Academic Publishers, Dordrecht

Huner NPA, Öquist G, Hurry VM, Król M, Falk S and Griffith M (1993) Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. Photosynth Res 37: 19–39

Huner NPA, Öquist G and Sarhan F (1998) Energy balance and acclimation to light and cold. Trends Plant Sci 3: 224–230

Huner NPA, Öquist G and Melis A (2003) Photostasis in plants, green algae and cyanobacteria: the role of light harvesting antenna complexes. In: Green BR and Parson WW (eds) Light Harvesting Antennas in Photosynthesis, Advances in Photosynthesis and Respiration, Vol 13, pp 401–421. Kluwer Academic Publishers, Dordrecht

Hurry VM, Strand Å, Tobiæson M, Gardeström P and Öquist G (1995) Cold hardening of spring and winter-wheat and rape results in differential-effects on growth, carbon metabolism, and carbohydrate content. Plant Physiol 109: 697–706

Hurry VM, Anderson JM, Chow WS and Osmond CB (1997) Accumulation of zeaxanthin in abscisic acid-deficient mutants of *Arabidopsis* does not affect chlorophyll fluorescence quenching or sensitivity to photoinhibition *in vivo*. Plant Physiol 113: 639–648

Ibelings BW, Kroon BMA and Mur LR (1994) Acclimation of photosystem II in a cyanobacterial photosynthetic apparatus and a eukaryotic green alga to high and fluctuating photosynthetic photon flux densities simulating light regimes induced by mixing in lakes. New Phytol 128: 407–424

Inoue Y (1996) Photosynthetic luminescence as a simple probe of photosystem II electron transport. In: Amesz J and Hoff AJ (eds) Biophysical Techniques in Photosynthesis, Advances in Photosynthesis, Vol 3, pp 93–107. Kluwer Academic Publishers, Dordrecht

Ivanov AG, Sane PV, Zeinalov Y, Malmberg G, Gardeström P, Huner NPA and Öquist G (2001) Photosynthetic electron transport adjustments in overwintering Scots pine (*Pinus sylvestris* L.). Planta 213: 575–585

Ivanov AG, Sane PV, Zeinalov Y, Simidjiev I, Huner NPA and Öquist G (2002) Seasonal responses of photosynthetic electron transport in Scots pine (*Pinus sylvestris* L.) studied by thermoluminescence. Planta 215: 457–465

Ivanov AG, Sane PV, Hurry V, Krol M, Sveshnikov D, Hunter NPA, Öquist G (2003) Low temperature modulation of the redok properties of the acceptor side of photosystem II: Photoprotection through reaction center quenching of excess energy. Physiol Plant 119: 376–383.

Janda T, Szalai G and Páldi E (2000) Thermoluminescence investigation of low temperature stress in maize. Photosynthetica 38: 635–639

Ke B (2001) Charge recombination in photosystem II and thermoluminescence. In: Ke B. Photobiochemistry and Photobiophysics, Advances in Photosynthesis, Vol 10, pp 407–418. Kluwer Academic Publishers, Dordrecht

Keren N and Ohad I (1998) State transitions and photoinhibition. In: Rochaix J-D, Goldschmidt-Clermont M and Merchant S (eds) Molecular Biology of Chloroplasts and Mitochondria in *Chlamydomonas*, Advances in Photosynthesis, Vol 7, pp 569–598. Kluwer Academic Publishers, Dordrecht

Komenda J and Masojidek J (1998) The effect of photosystem II inhibitors DCMU and BNT on the light-induced D1 turnover in two cyanobacterial strains *Synechocystis* PCC 6803 and *Synechococcus* PCC 7942. Photosynth Res 57: 193–202

Kramer DM, Johnson G, Kiirats O and Edwards GE (2004) New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. Photosyn Res 79: 209–218.

Krause GH (1988) Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiol Plant 74: 566–574

Krause GH and Jahns P (2003) Pulse amplitude modulated chlorophyll fluorometry and its application in plant science. In: Green BR and Parson WW (eds) Light Harvesting Antennas in Photosynthesis, Advances in Photosynthesis and Respiration, Vol 13, pp 373–399. Kluwer Academic Publishers, Dordrecht

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

Krieger-Liszkay A and Rutherford AW (1998) Influence of herbicide binding on the redox potential of the quinone acceptor in photosystem II: relevance to photodamage and phototoxicity. Biochemistry 37: 17339–17344

Król M, Ivanov AG, Jansson S, Kloppstech K and Huner NPA (1999) Greening under high light and cold temperature affects the level of xanthophyll-cycle pigments, early light-inducible proteins, and light-harvesting polypeptides in wild-type barley and the *Chlorina f2* mutant. Plant Physiol 120: 193–203

Krupa Z, Öquist G and Gustafsson P (1990) Photoinhibition and recovery of photosynthesis in *psbA* gene-inactivated strains of the cyanobacterium *Anacystsis nidulans*. Plant Physiol 93: 1–6

Krupa Z, Öquist G and Gustafsson P (1991) Photoinhibition of photosynthesis and growth responses at different light levels in *psbA* gene mutants of the cyanobacterium *Synechococcus*. Physiol Plant 82: 1–8

Kruse O, Zheleva D and Barber J (1997) Stabilization of photosystem two dimers by phosphorylation: Implication for the regulation of the turnover of D1 protein. FEBS Lett 408: 276–280

Kühlbrandt W, Wang DN and Fujiyoshi Y (1994) Atomic model of plant light-harvesting complex by electron crystallography. Nature 367: 614–621

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

Lee H-Y, Hong Y-N and Chow WS (2001) Photoinactivation of photosystem II complexes and photoprotection by nonfunctional neighbors in *Capsicum annuum* L. leaves. Planta 212: 332–342

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

Li X-P, Phippard A, Pasari J and Niyogi KK (2002) Structure-function analysis of photosystem II subunit S (PsbS) in vivo. Func Plant Biol 29: 1131–1139

Lindahl M, Funk C, Webster J, Bingsmark S, Adamska I and Andersson B (1997) Expression of Elips and PsbS protein in spinach during acclimative reduction of the photosystem II antenna in response to increased light intensities. Photosynth Res 54: 227–236

Long SP, Humphries S and Falkowski PG (1994) Photoinhibition of photosynthesis in nature. Ann Rev Plant Physiol Plant Mol Biol 45: 633–662

Los DA and Murata N (2002) Sensing and responses to low temperature in cyanobacteria. In: Storey KB and Storey JM

(eds) Sensing, Signalling and Cell Adaptation, pp 139–153. Elsevier Science BV, Amsterdam

Ma YZ, Holt NE, Li X-P, Niyogi KK and Fleming GR (2003) Evidence for direct carotenoid involvement in the regulation of photosynthetic light harvesting. Proc Natl Acad Sci USA 100: 4377–4382

Mäenpää P, Miranda T, Tyystjarvi E, Govindjee, Ducruet J-M and Kirilovsky D (1995) A mutation in the D-*de* loop of D1 modifies the stability of the $S_2Q_A^-$ and $S_2Q_B^-$ states in photosystem II. Plant Physiol 107: 187–197

Masamoto K and Furukawa K (1997) Accumulation of zeaxanthin in cells of the cyanobacterium, *Synechococcus* sp strain PCC 7942 under high irradiance. J Plant Physiol 151: 257–261

Matsubara S and Chow WS (2004) Populations of photoinactivated photosystem II reaction centers characterized by chlorophill a fluroscence life time in vivo. Proc Natl Acad Sci USA 101: 18234–18239.

Mattoo AK, Elich TD, Ghirardi ML, Callahan FE and Edelman M (1993) Post-translational modification of chloroplast proteins and the regulation of protein turnover. In: Battey NH, Dickinson HG and Hetherington AM (eds) Post-translational Modification in Plants, Society for Experimental Biology Seminar Series, Vol 53, pp 65–78. Cambridge University Press, Cambridge UK

Mayes SR, Dubbs JM, Vass I, Nagy L and Barber J (1993) Further characterization of the *psbH* locus of *Synechocystis* sp PCC 6803: inactivation of *psbH* impairs Q_A to Q_B electron transport in photosystem 2. Biochemistry 32: 1454–1465

Melis A (1999) Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage *in vivo*? Trends Plant Sci 4: 130–135

Melis A and Homann PH (1976) Heterogeneity of the photochemical centers in system II chloroplasts. Photochem Photobiol 23: 343–350

Meyer G and Kloppstech K (1984) A rapidly light-induced chloroplast protein with high turnover coded for by pea nuclear DNA. Eur J Biochem 138: 201–207

Milligan G, Parenti M and Magee AI (1995) The dynamic role of palmitoylation in signal transduction. Trends Biochem Sci 20: 181–185

Minagawa J, Narusaka Y, Inoue Y and Satoh K (1999) Electron transfer between Q_A and Q_B in photosystem II is thermodynamically perturbed in phototolerant mutants of *Synechocystis sp*. PCC 6803. Biochemistry 38: 770–775

Montane M-H and Kloppstech K (2000) The family of light-harvesting-related proteins (LHCs, ELIPs, HLIPs): was the harvesting of light their primary function? Gene 258: 1–8

Nield J, Orlova E, Morris E, Cowen B, Van Heel M and Barber J (2000a) 3D map of the plant photosystem two supercomplex obtained by cryoelectron microscopy and single particle analysis. Nature Struct Biol 7: 44–47

Nield J, Funk C and Barber J (2000b) Supramolecular structure of photosystem II and location of the PsbS protein. Phil Trans Royal Soc B 355: 1337–1343

Nishida I and Murata N (1996) Chilling sensitivity in plants and cyanobacteria: the crucial contribution of membrane lipids. Ann Rev Plant Physiol Plant Mol Biol 47: 541–568

Nixon PJ, Komenda J, Barber J, Deak ZS, Vass I and Diner BA (1995) Deletion of the PEST-like region of photosystem two modifies the QB–binding pocket but does not prevent rapid turnover of D1. J Biol Chem 270: 14919–14927

Niyogi KK (1999) Photoprotection revisited: genetic and molecular approach. Annu Rev Plant Physiol Plant Mol Biol 50: 333–359

Niyogi KK, Li X-P, Rosenberg V and Jung H-S (2005) Is PsbS the site of non-photochemical quenching in photosynthesis? J Exp Bot 56: 375–382.

Norén H, Svensson P, Stegmark R, Funk C, Adamska I and Andersson B (2003) Expression of the early light-induced protein but not the PsbS protein is influenced by low temperature and depends on the developmental stage of the plant in field-grown cultivars. Plant Cell Environ 26: 245–253

Ohad I and Hirschberg J (1992) Mutations in the D1 subunit of photosystem II distinguish between quinone and herbicide binding sites. Plant Cell 4: 273–282

Ohad I, Koike H, Shochat S and Inoue Y (1988) Changes in the properties of reaction center II during the initial stages of photoinhibition as revealed by thermoluminescence measurements. Biochim Biophys Acta 933: 288–298

Öquist G and Huner N PA (2003) Photosynthesis of overwintering evergreen plants. Annu Rev Plant Biol 54: 329–355

Öquist G and Martin B (1980) Inhibition of photosynthetic electron transport and formation of inactive chlorophyll in winter-stressed *Pinus sylvestris*. Physiol Plant 48: 33–38

Öquist G, Chow WS and Anderson JM (1992) Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosynthesis. Planta 186: 450–460

Ort DR (2001) When there is too much light. Plant Physiol 125: 29–32

Peterson RB and Havir EA (2001) Photosynthetic properties of an *Arabidopsis thaliana* mutant possessing a defective PsbS gene. Planta 214: 142–152

Peterson RB and Havir EA (2003) Contrasting modes of regulation of PSII light utilization with changing irradiance in normal and PsbS mutant leaves of *Arabidopsis thaliana*. Photosynth Res 75: 57–70

Polivka T, Herek JL, Zigmantis D and Åkerlund H-E (1999) Direct observation of the (forbidden) S1 state in carotenoids. Proc Natl Acad Sci USA 96: 4914–4917

Polivka Zigmantis D and Sundström V (2002) Carotenoid S1 state in a recombinant light-harvesting complex of photosystem II. Biochemistry 41: 439–450

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

Prasil O, Kolber Z, Berry JA and Falkowski PG (1996) Cyclic electron flow around photosystem II *in vivo*. Photosynth Res 48: 395–410

Rintamäki E and Aro E M (2001) Phosphorylation of photosystem II proteins. In: Aro E-M and Andersson B (eds), Regulation of Photosynthesis, Advances in Photosynthesis and Respiration, Vol 11, pp 395–418. Kluwer Academic Publishers, Dordrecht

Ruban AV, Pascal AA, Robert B and Horton P (2002) Activation of zeaxanthin is an obligatory event in the regulation of photosynthetic light harvesting. J Biol Chem 277: 7785–7789

Sakurai I, Hagio M, Gombos Z, Tyystjarvi T, Paakkarinen V, Aro E-M and Wada H (2003) Requirement of phosphatidylglycerol for maintenance of photosynthetic machinery. Plant Physiol 133: 1376–1384

Sane PV and Rutherford AW (1986) Thermoluminescence from Photosynthetic Membranes. In: Govindjee, Amesz J and Fork DC (eds) Light Emission by Plants and Bacteria, pp 329–360. Academic Press, Orlando

Sane PV, Ivanov AG, Sveshnikov D, Huner NPA and Öquist G (2002) A transient exchange of the photosystem II reaction center protein D1:1 with D1:2 during low temperature stress of *Synechococcus* sp. PCC 7942 in the light lowers the redox potential of Q_B. J Biol Chem 277: 32739–32745

Sane PV, Ivanov AG, Hurry VM, Huner NPA and Öquist G (2003) Changes in the redox potential of Q_B confer increased resistance against photoinhibition in low temperature acclimated *Arabidopsis thaliana*. Plant Physiol 132: 2144–2151

Savitch LV, Massacci A, Gray GR and Huner NPA (2000) Acclimation to low temperature or high light mitigates sensitivity to photoinhibition: roles of the Calvin cycle and the Mehler reaction. Aust J Plant Physiol 27: 253–264

Savitch LV, Barker-Aström J, Ivanov AG, Hurry VM, Öquist G and Gardeström P (2001) Cold acclimation of *Arabidopsis thaliana* results in complete recovery of photosynthetic capacity and is associated with reduction of the chloroplast stroma. Planta 214: 295–303

Schaefer MR and Golden S (1989) Differential expression of members of a cyanobacterial *psbA* gene family in response to light. J Bacteriol 17: 3973–3981

Schatz GH, Brock H and Holzwarth AR (1988) Kinetic and energetic model for the primary processes in photosystem II. Biophys J 54: 397–405

Selstam E (1998) Development of thylakoid membranes with respect to lipids. In: Siegenthaler P-A and Murata N (eds) Lipids in Photosynthesis: Structure, Function and Genetics, Advances in Photosynthesis and Respiration, Vol 6, pp 209–224. Kluwer Academic Publishers, Dordrecht

Somersalo S and Krause GH (1990) Reversible photoinhibition of unhardened and cold-acclimated spinach leaves at chilling temperatures. Planta 180: 181–187

Stewart DH and Brudvig GW (1998) Cytochrome b559 of photosystem II. Biochim Biophys Acta 1367: 63–87

Stitt M and Hurry VM (2002) A plant for all seasons: alterations in photosynthetic carbon metabolism during cold acclimation in *Arabidopsis*. Curr Opinion Plant Biol 5: 199–206

Stowell MHB, McPhillips TM, Rees DC, Soltis SM, Abresch E and Feher G (1997) Light-induced structural changes in photosynthetic reaction center: implications for mechanism of electron-proton transfer. Science 276: 812–816

Strand Å, Hurry VM, Gustafsson P and Gardeström P (1997) Development of *Arabidopsis thaliana* leaves at low temperature releases the suppression of photosynthesis and photosynthetic gene expression despite the accumulation of soluble carbohydrates. Plant J 12: 605–614

Strand Å, Foyer CH, Gustafsson P, Gardeström P and Hurry VM (2003) Altering flux through the sucrose biosynthesis pathway in transgenic *Arabidopsis thaliana* modifies photosynthetic acclimation at low temperatures and the development of freezing tolerance. Plant Cell Environ 26: 523–535

Strand M and Öquist G (1985) Inhibition of photosynthesis by freezing temperatures and high light levels in cold-acclimated seedlings of Scots pine (*Pinus silvestris*). I. Effects of the light-limited and light saturated rates of CO_2 assimilation. Physiol Plant 64: 425–430

Swiatek M, Kuras R, Sokolenko A, Higgs D, Olive J, Cinque G, Müller B, Eichacker LA, Stern DB, Bassi R, Herrmann RG and Wollman F-A (2001) The chloroplast gene *ycf9* encodes a photosystem II (PSII) core subunit, PsbZ, that participates in PSII supramolecular architecture. Plant Cell 13: 1347–1367

Telfer A, De Las Rivas J and Barber J (1991) Beta-carotene within the isolated photosystem-II reaction center – photooxidation and irreversible bleaching of this chromophore by oxidized P680. Biochim Biophys Acta 1060: 106–114

Trémolières A and Siegenthaler P-A (1998) Reconstitution of photosynthetic structures and activities with lipids. In: Siegenthaler P-A (ed) Lipids in Photosynthesis: Structure, Function and Genetics, Advances in Photosynthesis and Respiration, Vol 6, pp 175–189. Kluwer Academic Publishers, Dordrecht

van Wijk KJ and van Hasselt PR (1993) Kinetic resolution of different recovery phases of photoinhibited photosystem II in cold-acclimated and non-acclimated spinach leaves. Physiol Plant 87: 187–198

Vass I and Govindjee (1996) Thermoluminescence from the photosynthetic apparatus. Photosynth Res 48: 117–126

Vass I, Styring S, Hundal T, Koivuniemi A, Aro E-M and Andersson B (1992) Reversible and irreversible intermediates during photoinhibition of photosystem II: Stable reduced Q_A species promote chlorophyll triplet formation. Proc Natl Acad Sci USA 89: 1408–1412

Vavilin DV and Vermass WFJ (2000) Mutations in the CD-loop region of the D2 protein in *Synechocystis* sp. PCC 6803 modify charge recombination pathways in photosystem II *in vivo*. Biochemistry 39: 14831–14838

Vijayan P, Routaboul JM and Browse J (1998) A genetic approach to investigating membrane lipid structure and photosynthetic function. In: Siegenthaler P-A (ed) Lipids in Photosynthesis: Structure, Function and Genetics, Advances in Photosynthesis and Respiration, Vol 6, pp 263–285. Kluwer Academic Publishers, Dordrecht

Walters RG and Horton P (1993) Theoretical assessment of alternative mechanisms for non-photochemical quenching of PSII fluorescence in barley leaves. Photosynth Res 36: 119–139

Weis E and 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 and Horton P (2003) Thermodynamic investigation into the mechanism of the chlorophyll fluorescence quenching in isolated photosystem II light harvesting complexes. J Biol Chem 278: 21845–21850