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Review Article

Molecular mechanisms involved in plant photoprotection

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Photosynthesis uses sunlight to convert water and carbon dioxide into biomass and oxygen. When in excess, light can be dangerous for the photosynthetic apparatus because it can cause photo-oxidative damage and decreases the efficiency of photosynthesis because of photoinhibition. Plants have evolved many photoprotective mechanisms in order to face reactive oxygen species production and thus avoid photoinhibition. These mechanisms include quenching of singlet and triplet excited states of chlorophyll, synthesis of antioxidant molecules and enzymes and repair processes for damaged photosystem II and photosystem I reaction centers. This review focuses on the mechanisms involved in photoprotection of chloroplasts through dissipation of energy absorbed in excess.

Introduction

Light is essential for photosynthesis, which supports most life on earth. However, when in excess, light can damage the photosynthetic organisms. Photosynthesis is a complex mechanism including steps catalyzed in lifetimes spanning picosecond (10^{-12} s) to second time ranges: in photosystem II (PSII), charge separation occurs in picoseconds, while the slowest reaction is catalyzed by Rubisco in 10^{-1} s. In between, the time constant of plastoquinol (PQH_2) oxidation by cytochrome b_6f (Cytb_6f) is in the order of milliseconds (10^{-3} s). The time constants of these reactions are so different that a flux balance could only be reached by assembling excitation energy and electron transport chains with different stoichiometry of components in steady-state light conditions. Other environmental conditions affecting the rate of individual reactions should also be maintained constant. However, such conditions can only be found in growth cabinets: outdoor, light intensity and wavelength distribution rapidly change according to time of day (e.g. exposure to full sunlight at midday or sudden sunflecks under canopies), season, geography, climate and the position of the leaf within a canopy and of the cell within a leaf. In addition, growth is affected by temperature, nutrient, and water availability. This results in the light energy being often absorbed in excess with respect to the capacity for its utilization for photochemistry and electron transport. General mechanisms of photodamage are mainly two: (1) unquenched singlet chlorophyll excited states (^{1}Chl*), especially in PSII, undergo intersystem crossing to triplet chlorophyll excited states (^{3}Chl*). Chl triplets react with molecular oxygen (O_2), a triplet in its ground state, producing singlet oxygen ($^1O_2$*) and other reactive oxygen species (ROS), which damage thylakoid components [1–3]. Photo-oxidative damage, called 'photoinhibition,' decreases the efficiency of photosynthesis as well as undermines productivity [4–8]; (2) univalent electron transport reduces O_2 to superoxide ($O_2^{\cdot-}$) whenever final electron acceptors are limiting. Superoxide dismutase (SOD) plus peroxidase or flavodiiron proteins catalyze reductive detoxification to water (H_2O) [9,10]; yet scavenging fails in conditions such as low temperature and/or strong light excess causing photodamage [11,12].

Three sites in the photosynthetic apparatus are the major sources for generation of oxidizing, dangerous molecules: the PSII reaction center, the photosystem I (PSI) and the light-harvesting complex (LHC) of PSII.

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Solar energy is captured by Chls bound mainly to the LHC, which undergo transition to ^{1}Chls*. The energy of this state is excitonically transferred to the reaction center (RC) where it promotes photochemical reactions.

In PSII RC, *charge separation* equilibrates with the exciton density in the antenna. The 'special' Chl pair, P680 [13], operates with electrons being transferred to Pheophytin (Pheo) and, furthermore, to Q_A and Q_B sites. Double reduction at the Q_B site reduces plastoquinone (PQ) to PQH_2, while P680 transiently assumes a positive charge ($P680^+$), neutralized by electrons from splitting water, within 200 ns. While electron flow to PQ allows for efficient photochemical quenching of ^{1}Chls* in the antenna, accumulation of PQH_2 reduces quenching efficiency and promotes charge recombination from Q_{A-}/Q_{B-} to $P680^+$, restoring ^{1}P680*. Because of both recombination and equilibration with antenna pigments, the lifetime of ^{1}P680* increases allowing for intersystem crossing to triplet P680 (^{3}P680*) [14], which readily reacts with O_2 generating $^1O_2$*, causing photodamage in particular on the D1 subunit of PSII [4,15,16] and photoinhibition of photosynthetic rate [5]. Specific vulnerability of P680 to oxidation is caused by its proximity to the manganese (Mn) cluster of the oxygen evolving complex (OEC). The strong oxidants produced (+1 volt) could destroy carotenoids (Cars) [17] which, indeed, are located in every Chl-binding protein but specifically depleted around PSII RC [18]. Oxygenic photosynthesis is billion years old and yet high O_2 accumulated in the last 500 million years only [19] during which autotrophs evolved mechanisms for protection from oxidation.

Besides $^1O_2$*, PSII can generate both the superoxide anion ($O_2^{\cdot-}$) and the hydroxyl radical ($OH^{\cdot}$) [20]. Under high light, ROS production occurs by acceptor- and donor-side mechanisms in PSII: on the PSII electron-acceptor side, one-electron reduction of O_2 forms $O_2^{\cdot-}$, which dismutes to H_2O_2; the latter is reduced by the non-heme iron to $OH^{\cdot}$; on the PSII electron donor side, two-electron oxidation of H_2O results in the formation of H_2O_2 catalyzed by the Mn_4O_5Ca cluster in the OEC. When H_2O_2 is not properly scavenged by catalase (CAT), $HO^{\cdot}$ is formed by Fenton reactions [21] (Figure 1). Recently, specific oxidation of amino acid residues of the D1 and D2 proteins has been shown to be associated with the site-specific formation of $OH^{\cdot}$ and $O_2^{\cdot-}$ [22].

PSI can also experience photoinhibition, again caused by ROS [23–28] produced when PSI reducing activity exceeds the capacity of using reducing equivalents for CO_2 (carbon dioxide) fixation and other downstream

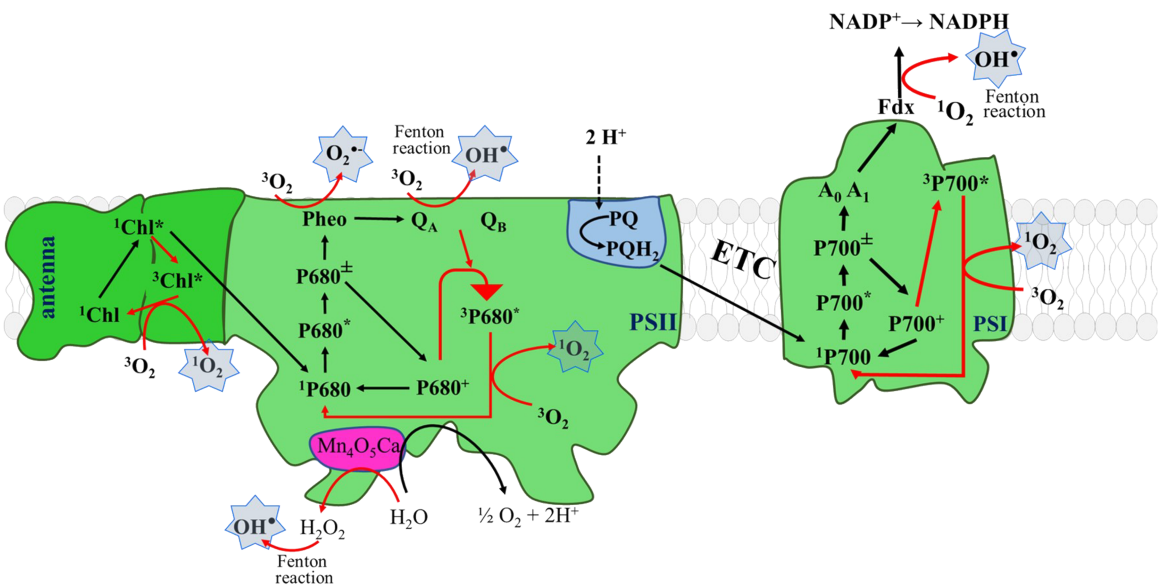

Figure 1. Scheme of ROS production and photoinhibition in PSII and PSI.

Black arrows represent the photoexcitation of RC and the ETC in steady-state conditions with all electrons generated being utilized for the CO_2 assimilation. Charge recombination occurs within PSII with increased frequency whenever the PQ acceptor is reduced to PQH_2. Red arrows represent pathways activated when the photon flux exceeds the capacity for electron transport and CO_2 assimilation. PSII and PSI: photosystems II and I; Chl, chlorophyll, ETC, electron transport chain; PQ/PQH_2, plastoquinone/plastoquinol; Pheo, pheophytin; Fdx, ferredoxin; $O_2^{\cdot-}$, superoxide anion; $OH^{\cdot}$, hydroxyl radical; $^1O_2$, singlet oxygen; Mn_4O_5Ca, water-splitting Mn complex.

reactions. Thus, production of $O_2^{\cdot-}$ occurs within PSI, probably at site A_1 [29]. $O_2^{\cdot-}$ can directly damage the peripheral component of PSI, or is converted into $OH^{\cdot}$ via the Fenton reaction [30,31], limiting electron transfer from P700 to Chl A_0 [32] inducing triplet excited P700 (^{3}P700*) [32,33] (Figure 1). Alike in PSII, ^{3}P700* reacts with O_2 to produce $^1O_2$, causing PSI photoinhibition [34], depletion in P700 Chls without protein degradation. While PSI recovers slowly, photoinhibited PSII recovers rapidly ($t_{1/2}$ is ∼60 min) [5,35].

Photo-oxidative stress (e.g. $^1O_2$* formation) also occurs from Chls bound to LHC proteins in excess light (EL) because closure of RCs decreases photochemical quenching of ^{1}Chl*, thus enhancing the probability of ^{3}Chl* [36] either in loosely coupled LHC subunits or in unbound Chls [37,38] during biogenesis of pigment-binding proteins [39] (Figure 1).

Constitutive photoprotection mechanisms

Photoprotection by carotenoids

Cars provide a crucial contribution to chloroplast photoprotection. Cars are present in thylakoids in two distinct forms: (i) free fraction of Cars (up to 15% of the total Car pool) performing their antioxidant function by scavenging ROS released from LHCs and RC complexes [40,41] and (ii) Cars bound to photosynthetic machinery where they are in close contact with Chl molecules. Despite the great diversity generated by evolution, the xanthophyll content of land plants is extremely well conserved with respect to both the overall composition and localization in chloroplast structures. The large majority of Cars is bound to the photosynthetic complexes and shows a constant distribution among their different components: β-carotene is bound to the RCs, while LHCs bind xanthophylls: Lutein (Lut), Violaxanthin (Viola), Neoxanthin (Neo) and, upon its accumulation under high light, Zeaxanthin (Zea). The conservation of Car composition and site of binding across a wide range of plant taxa suggest a unique role for each molecular species.

Three photoprotection mechanisms involve Cars.

Modulation of ^{3}Chl* yield

The strong coupling between Chls and Cars within Chl–protein complexes results in excitation energy transfer from the ^{3}Chl* to Cars, yielding the Car triplet excited states (^{3}Car*). Indeed, ^{3}Car* population increases with light intensity in leaves [42], thylakoids [43], isolated photosystems [44] and LHCs [45,46]. Quenching of ^{3}Chl* prevents $^1O_2$ formation. **Lut** is the most abundant xanthophyll species in the photosynthetic apparatus of plants and green algae. Its specific role is quenching of ^{3}Chl*. It also acts in scavenging of ROS produced by the reaction of ^{3}Chl* with O_2 [47].

$$^3\mathrm{Chl}^* + \mathrm{Car} \rightarrow {}^3\mathrm{Car}^* + {}^1\mathrm{Ch1}$$
$$^3\mathrm{Car}^* \rightarrow \mathrm{Car} + \mathrm{heat}$$

Quenching of ^{3}Chl* by Cars occurs in PSII antenna complexes predominantly by Lut [48], whereas in the PSII core complex is performed by β-carotene. Zea synthesis in EL enhances triplet quenching in LHC monomers [45]. The triplet–triplet energy transfer from the Chl to Car occurs efficiently because the triplet energy level of Car is below the triplet energy level of Chl [49,50]. Reaction is fast when the Chl to Car distance is less than 4 Å and takes place via the Dexter mechanism [20].

Scavenging of ROS

The excitation energy transfer from ROS to Car results in the formation of the ground triplet state of molecular oxygen ($^3O_2$) and ^{3}Car*. The Car triplet decays radiationless into the ground state, while the triplet excitation energy is converted effectively into heat [49].

$$\mathrm{ROS}^* + \mathrm{Car} \rightarrow \mathrm{Car}^* + O_2$$
$$^3\mathrm{Car}^* \rightarrow \mathrm{Car} + \mathrm{heat}$$

Neo, accounting for ∼15% of total Cars, has a specific function as a quencher of $^1O_2$ [51]; the specific role of **Viola** in photoprotection is $^1O_2$ scavenging. In EL, Viola is de-epoxidized to Zea, whose scavenging activity is enhanced [41,52,53], and also bears a ^{3}Chl* quenching activity [54]. In addition, binding of Zea decreases

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^{1}Chl* in pigment-binding complexes [55], and up-regulates non-photochemical quenching (NPQ) [56], thus undermining light-harvesting efficiency.

β-Car is a component of both PSI and PSII RC core complexes, thus suggesting a role in mitigating oxidative damage under EL conditions, especially in PSII [57,58]. Recently, $^{14}CO_2$ labeling studies showed that carbon flux is many times higher in β-Car with respect to downstream xanthophylls, implying that most of β-Car undergoes oxidative degradation within PSII core complexes and is replaced at high rates at binding sites of RC core complexes [59].

Modulation of ^{1}Chl* yield

$$^1\mathrm{Chl}^* + \mathrm{Car} \rightarrow {}^1\mathrm{Car}^* + {}^1\mathrm{Ch1}$$
$$^1\mathrm{Car}^* \rightarrow \mathrm{Car} + \mathrm{heat}$$

Xanthophyll composition, namely the binding of Zea *vs* Viola, modulates the ^{1}Chl* population in both isolated pigment-binding proteins and *in vivo* as shown by fluorescence analysis. This effect is stronger in monomeric LHCs *vs* trimeric LHCIIs, suggesting that this effect is related to occupancy of binding site L2, where Viola can be exchanged to Zea [60] rather than V1. Indeed, site V1 was found in LHCII and LHCSR only. The quenching effect of replacing Viola to Zea has been recently studied in the LHCSR1 protein in which Zea binding reduced fluorescence yield by 50% [61–63]. The underlying mechanism was energy transfer from the ^{1}Chl* to the Zea S1 state followed by rapid decay to the Zea ground state [62].

Other constitutive photoprotective components and agents

ROS production is unavoidable in plants. In addition to the excellent protective role fulfilled by the above mechanisms, other antioxidant species are present in the chloroplast in order to deactivate ROS and minimize photodamage.

The ROS detoxification systems include enzymatic and non-enzymatic antioxidant components [64].

Non-enzymatic antioxidant components

Non-enzymatic antioxidant components include:

a) **Prenylquinols** act as scavengers of $^1O_2$. Whereas Cars mediate physical scavenging by excitation energy transfer (quenching), prenylquinols, such as tocopherol [65–68] and plastoquinols mediate chemical scavenging by electron transport [20,69–71]. Cars are mainly bound by the pigment–protein complexes, while tocopherols are free in the thylakoid lipid matrix.

Tocopherols have two principal oxidation mechanisms: they can be oxidized to a tocopheryl radical in a one electron-transfer reaction or can react with $^1O_2$ to form a hydroperoxide, equivalent to a two electron-transfer reaction [72].

In plants, tocopherol co-operates with ascorbate: mutants with decreased ascorbate content show a compensatory increase in tocopherol [73,74]. Tocopherol and Cars have overlapping protection functions *in vivo*: the *Arabidopsis thaliana npq1* mutant, which lacks Zea, accumulates more α-tocopherol in young leaves exposed to EL, suggesting that high tocopherol levels can compensate for decreased scavenging of $^1O_2$ by Zea [75]. On the contrary, the *A. thaliana vte1* mutant, which is tocopherol-deficient, accumulates more Zea in EL respect to WT [68]. In *Chlamydomonas reinhardtii*, the *npq1 lor1* double mutant, lacking Lut and Zea, accumulates α-tocopherol [52]. Overproduction of tocopherol in *npq1 lor1* mutant by expression of homogentisate phytyltransferase vitamin E2 (*vte2*) from *Synechocystis* sp. PCC6803 made *C. reinhardtii* more resistant to other oxidative stresses [76]. Loss of α-tocopherol has been correlated with the loss of photosynthesis and of the D1 protein in EL [66]. Both Cars and prenylquinols are involved in scavenging lipid radicals [77].

b) **Ascorbate** (vitamin C) is the most abundant soluble antioxidant in chloroplasts where it can reach very high concentrations (20–300 mM) during acclimation to EL. Ascorbate acts (i) in preventing oxidative damage through direct quenching of $^1O_2$, $O_2^{\cdot-}$, and $OH^{\cdot}$, (ii) in regenerating α-tocopherol from α-tocopheryl radicals, (iii) as a cofactor of violaxanthin de-epoxidase (VDE), (iv) as electron donor to PSII, and (v) as $OH^{\cdot}$ scavenger though ascorbate peroxidase (APX) [78]. *In vivo* supporting data include the phenotype of *A. thaliana* ascorbate-deficient mutants (*vtc*), which are hypersensitive to many oxidative stresses such as ozone, ultraviolet

B radiation and high light and salt treatments [78], possibly because of a reduced de-epoxidation rate [79]. In contrast, ascorbate-overproducing mutants (*miox4*) prevent PSII damage in heat-stressed leaves [80].

c) **Glutathione** has a key role in detoxifying $^1O_2$ and $OH^{\cdot}$, and is involved in regeneration of both α-tocopherol and ascorbate, by the glutathione–ascorbate cycle [81].

Enzymatic antioxidant components

Enzymatic antioxidant components include SOD, APX, CAT, glutathione peroxidase, and peroxiredoxin. These enzymes are present in all subcellular compartments. Usually, an organelle has more than one enzyme acting in scavenging individual ROS [64,82,83]. The main oxidant produced by PSI is $O_2^{\cdot-}$, which is rapidly turned to H_2O_2 by SOD. The hydrogen peroxide-detoxification system in chloroplasts is operated by the ascorbate–glutathione cycle, in which APX is a key enzyme [84]. APX utilizes ascorbate as a specific electron donor to reduce H_2O_2 to H_2O. In this context, the water–water cycle is essential to avoid the photodamage in PSI. In EL, photoreduction of O_2 in PSI can occur, thus generating $O_2^{\cdot-}$ as the primary product [28], which can be enzymatically converted into H_2O_2 by SOD. Then, H_2O_2 is converted into H_2O by APX [85]. These reactions consume excess electrons, reducing the excitonic pressure on PSI, but contributes to generation of a ΔpH without concomitant utilization of ATP. In algae and mosses, flavodiiron proteins catalyze $O_2^{\cdot-}$ to H_2O_2 reaction in a single step [9,86], suggesting that their expression in crops is a possible strategy for improving resistance to abiotic stresses.

Cyclic electron flow

Cyclic electron flow (CEF) around PSI prevents photoinhibition of PSII. CEF increases the electron transfer from PSI back to PQ without production of O_2 or accumulation of NADPH: this results in the generation of a ΔpH across the thylakoid membrane which in turn drives the synthesis of ATP and the induction of thermal dissipation [87].

CEF appears to be important in cyanobacteria, in unicellular algae, in C4 plants [88–90] and, at least under certain stressful conditions (drought, high light, or low CO_2), also in C3 plants [91–93].

CEF occurs via two redundant pathways: the major one requires a complex involving at least two proteins, PGR5 (proton gradient regulation 5) and PGRL1 (PGR5-like photosynthetic phenotype 1) [94–96], while the minor pathway requires an NAD(P)H dehydrogenase-like or NDH complex [97–99]. Both of these pathways receive electrons from ferredoxin (Fdx) [96,100].

The NDH pathway requires the presence of a large, multi-subunit complex and was identified as a homolog of the mitochondrial complex I (NADH dehydrogenase) [101]. NDH complex mediates the electron transport from stromal reductants to PQ based on tobacco *ndh* mutants [102,103]; *ndh* mutants are sensitive to abiotic stresses, suggesting that this pathway is essential for photoprotection [104,105]. NDH complex most probably accepts electrons from Fdx rather than NAD(P)H [100]. Similar phenotypes are exhibited by *pgr5*, *pgrl1 and crr* (*chlororespiratory reduction*) mutants, implying that PGR5 and PGRL1 are necessary for NPQ induction and protection of PSI from photoinhibition [94,95], with PGRL1 representing the docking site for PGR5 [95]. The *pgr5* mutant is sensitive to fluctuating light levels [106]. The double-mutant *crr pgr5* (deficient of both pathways) impairs plant growth and performance even in low light (LL), suggesting that a complete disruption of CEF has strong photoinhibitory activity [107].

PGR5 is present in all photosynthetic organisms, whereas PGRL1 was acquired by green algae and plants [95]. In *C. reinhardtii*, PGRL1, but not PGR5, has been shown to become associated with PSI, LHCI, LHCII, Cytb_6f, and FNR (ferredoxin:$NADP^+$-oxidoreductase) in a complex not including PGR5 [108]. However, the recently characterized *pgr5* and *pgrl1* mutants in *C. reinhardtii* [109–112] showed similar characteristics of *A. thaliana* mutants, suggesting that PGR5 could have a role in CEF in *C. reinhardtii*.

Chlororespiration

The respiratory electron transport pathway within the chloroplast is defined as chlororespiration, which transfers electrons from NAD(P)H to O_2 via the plastoquinone pool [113]. This is a mechanism to prevent the complete oxidation of the PQ pool in the dark as well as to prevent its complete reduction in excess light. The components involved in this process are a chloroplast NAD(P)H dehydrogenase [102,103] and a chloroplast-targeted plastoquinol terminal oxidase (PTOX) [114–116]. PTOX shares sequence similarity with the

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mitochondrial alternative oxidase and was suggested to act in diverting the electron flow from PQH_2 to O_2, producing H_2O [117]. PTOX plays an essential role also in Car biosynthesis and plastid development [118].

Under control condition, the PTOX level is low but under stressing conditions its level increases [119–121].

In chlororespiration, both the NDH complex and PTOX work together providing and removing electrons, respectively, thus balancing the redox state of electron transporters [122,123]; in fact, it was proposed that chlororespiration tightly controls the rate of PSI-CEF *in vivo* by changing the redox state of intersystem electron carriers [92]; in particular, CEF around PSI and chlororespiration are co-ordinated to alleviate photoinhibition during heat stress [124].

Interestingly, diatoms are able to trigger qE (energy quenching) in the dark: weak ΔpH produced by chlororespiration through the plastoquinol pool is sufficient to induce diadinoxanthin de-epoxidation, thus converting diadinoxanthin (DD) in diatoxanthin (DT) [125,126]. Diadinoxanthin de-epoxidase is active at a neutral pH and although weak, acidification of the lumen NPQ occurs [126,127].

Long-term photoprotective mechanisms

When plants are exposed for a long time to stress conditions, long-term photoprotective mechanisms are activated, leading to acclimation to stress consisting in tuning of the composition of the photosynthetic apparatus, through the expression or repression of specific genes, the accumulation of antioxidant metabolites, and changes in plant, leaf and chloroplast architecture. A detailed description of acclimation is beyond the scope of this text and has been reviewed recently [128].

Acclimation to excess light includes the decrease in the light-harvesting antenna size through changes in LHC gene expression and/or LHC protein degradation [129,130], the increase in the capacity for photosynthetic electron transport and CO_2 fixation. These mechanisms involve the regulation of nuclear and chloroplast gene expression synergically by redox potentials and/or ROS levels [131].

Excess light-inducible photoprotective mechanisms

Non-photochemical quenching

Since $^3Chl^*$ production, derived from excess $^1Chl^*$, is an intrinsic property of Chls, the capacity to control its formation is essential for plant survival. A set of inducible mechanisms exists for quenching excess $^1Chl^*$ and dissipating the energy harmlessly as heat. These mechanisms are referred to as 'non-photochemical quenching' and are measured from the decrease in Chl fluorescence excited by a saturating light pulse (F_{max}). NPQ is triggered by EL and requires ΔpH across the thylakoid membrane. The proton concentration into the *lumen* is determined by balance between the rate of photosynthetic electron transport and the dissipation of the pH gradient by the activity of the ATP synthase complex. In EL conditions, the Calvin–Basham–Benson cycle (CBB cycle) is saturated and the ATPase activity is progressively decreased by lack of substrates, P_i and ADP leading to lumen acidification which triggers NPQ in the antenna system [132]. Within NPQ, three major components have been distinguished based on the timescales of their induction and relaxation upon exposure of dark adapted leaves to excess light: (i) fast, reversible quenching caused by build-up of the *trans*-thylakoid ΔpH gradient on a tenth of seconds timescale (qE) and (ii) a slower component, activated within a few minutes relying on the synthesis of Zea, which increases qE quenching. Upon return to the dark, this quenching (qZ, zeaxanthin-dependent) relaxes slowly (within minutes to hours). Slower components (iii) can be either photoprotective or a consequence of photoinhibitory damage. The former (qH, sustained quenching) consists of a sustained decrease in fluorescence yield of the major LHCII antenna complexes catalyzed by a plastid lipocalin (LCNP) [133]. qI is the photoinhibitory quenching caused by the photodamage of RC complexes and relaxes within several hours, relying on repair of damaged D1 subunits of PSII [134,135]. In addition to these components common to most plants and algae, fluorescence emission of plants is also down-regulated by the chloroplast light avoidance response (qM, chloroplast movement) [136] and, particularly in unicellular algae, by the displacement of the LHCII antenna from PSII to PSI (State 1–State 2 transition, qT) [137]. Although all these mechanisms affect fluorescence yield of leaves/cells, only qE, qZ, qH, and qI can be correctly defined as NPQ, while qM derives from a decreased absorption and qT is obtained through photochemical quenching by PSI (Figure 2).

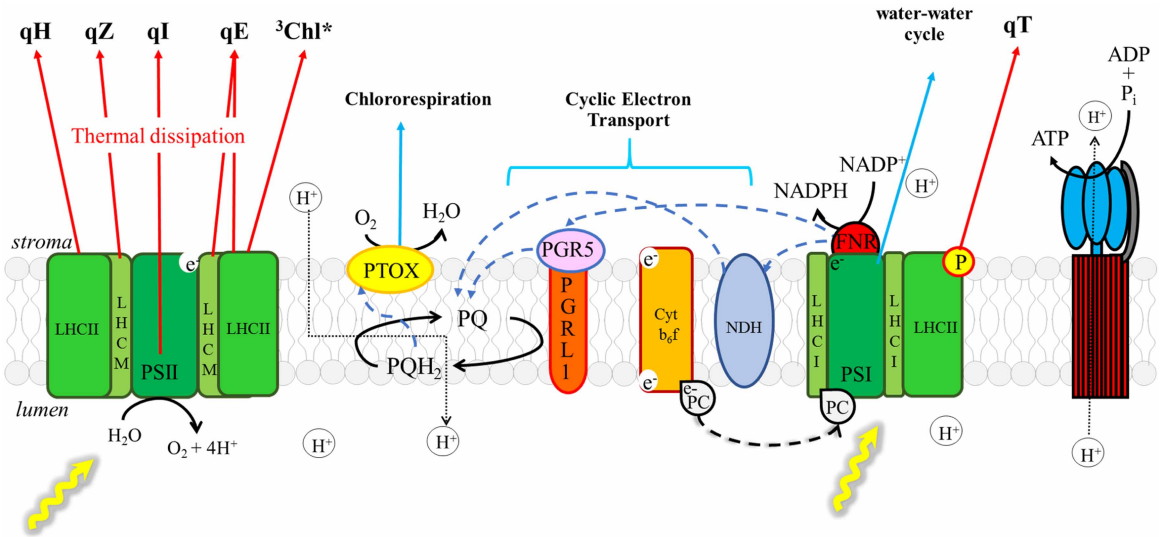

Figure 2. Localization of photoprotection mechanisms acting in conditions of excess excitons (red arrows) or excess electrons (blue arrows).

NPQ components (qE, qZ, qI, qH, and qT) and ^{3}Chls* catalyze thermal dissipation. Chlororespiration, cyclic electron transport and water–water cycle dissipate reducing power in excess. PSI/PSII, photosystems I and II; LHCM, light harvest complex monomers; LHCII, light-harvesting complex trimers; FNR, ferredoxin:NADP$^+$-oxidoreductase; PQ/PQH$_2$, plastoquinone/plastoquinol; PC, plastocyanin; NDH, NADH dehydrogenase-like complex; PTOX, plastoquinol terminal oxidase; PGR5, proton gradient regulation 5; PGRL1, PGR5-like photosynthetic phenotype 1.

The fastest component of NPQ:qE

Energy quenching, qE, develops within a tenth of a second upon an increase in light intensity [122] and relaxes within 1–2 min upon return to darkness or to a sub-saturating light intensity [138,139]. The signal for qE triggering is thylakoid lumen acidification caused by accumulation of ATP/consequent to saturation of the CBB cycle. Depletion of ADP + P$_i$ limits ATPase activity, thus the return of H$^+$ to the stromal membrane side and hence lumen acidification. qE triggering occurs by protonation of lumen-exposed acidic residues carried by specific gene products, namely LHCSR in algae and PSBS in plants, with mosses using both [140–142], converting the antenna from a light-harvesting mode into a light energy dissipative mode [143–146]. The mode by which this conversion occurs is slightly different depending on whether LHCSR or PSBS is involved: LHCSR undergoes conformational change from a long lifetime from 3.7 ns to 80 ps, which efficiently quenches the PSII antenna. PSI as well is quenched, although to a lesser extent [147]. Thus, LHCSR is both a pH detector and hosts quenching reactions catalyzed by its Chls and xanthophyll pigments [61,148]. PSBS, on the contrary, does not bind pigments [149,150], implying that quenching occurs in interacting LHC proteins, mainly CP29 [151], catalyzing a prompt component (1–2 min), and LHCII, catalyzing a slower quenching within several minutes [152]. The quenching site in monomeric LHCs relies on formation of Zea radical cations [153,154], while the LHCII site acts through excitation energy transfer from Chl *a* to Cars followed by rapid decay to the ground state [155]. Both these mechanisms have been found active in LHCSR and, yet, the radical cation activity was due to Lut rather than Zea [62,148]. Reorganization of thylakoid membrane domains is involved in the plant qE with PSII–LHCII supercomplexes dissociating in two distinct domains, one including the PSII core complex containing CP29, CP26, and the LHCII-S trimer, and the other made of the most peripheral antenna subunits (CP24, LHCII-M, and LHCII-L) [156,157]. Membrane reorganization in algae is less studied; nevertheless, LHCSR quenching activity requires, as interaction partners, specific LHCB antenna proteins [158–161]. In diatoms, NPQ mainly relies on qE, which is controlled by the build-up of a Δp across thylakoid membranes, the presence of the LHC antenna named Lhcx and the xanthophyll cycle including one-step de-epoxidation of DD in DT [162–166]. In diatoms, qE can be four to five times higher than in plants, making it the most important rapid photoprotective process [167,168]. In addition, diatoms are characterized by the absence of PSBS as well as CP29 and CP26, which are involved in NPQ in plants [169].

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Xanthophylls and modulation of qE

Plant and algae Car composition undergoes changes depending on environmental conditions. The fastest response involves three xanthophylls: Viola, Antheraxanthin (Anthera), and Zea whose interconversion forms the xanthophyll cycle. Viola is the only species found in LL conditions, while its mono and bis de-epoxidated forms, Anthera and Zea, accumulate in EL by the activity of the VDE enzyme [170] using ascorbate as an electron donor [171]. Monomeric, inactive VDE is located in the *lumen* and, like qE, is activated by acidification, upon which it dimerizes and attaches to the thylakoid membranes where it acts on its lipid-soluble substrate [172,173]. In LL, Zea is converted back into Viola by a stromal enzyme: zeaxanthin-epoxidase [174,175]. The Xanthophyll cycle has a central role in energy dissipation activity over the entire spectrum of the possible light environment including sunflecks: Zea is rapidly synthesized for photoprotection during the sunflecks and rapidly reconverted into Viola upon return to LL in order to permit high levels of carbon fixation [176].

The effect of the xanthophyll cycle on NPQ is species-dependent: NPQ of *C. reinhardtii* is Zea-independent, while LHCSR-dependent NPQ of mosses is strongly up-regulated by Zea [61] through binding to both V1- and L2-binding sites [63] whose occupancy is synergic with pH in switching between energy-conservative and -dissipative conformations [63,177].

In plants, NPQ activity is modulated by Zea, and constitutive Zea accumulation, as in the *npq2* mutant, makes the onset of qE faster, implying that Zea is required for the full activation of qE [56,178]. Lut is also a player in NPQ: Lut-deficient (*lut2*) mutants have reduced and slower NPQ, while Lut over-accumulation in part compensates for the lack of Zea as in the *szl1xnpq1* double mutant [179]. Consistently, lack of both Lut and Zea, as in the *npq1xlut2* KO, yields a null NPQ phenotype mimicking the *PSBS*-less (*npq4*) phenotype.

Zeaxanthin-dependent NPQ (qZ)

Zea accumulation is also responsible for a slower component of NPQ occurring in parallel with reconversion of Zea into Viola, which takes up to 1 h in laboratory experiments [180–182]. This Zea-dependent mechanism is present in some higher plants as *A. thaliana* and is independent from PSBS and ΔpH. qZ is probably due to Zea binding to monomeric LHC antenna complexes, with CP26 being essential for establishing this slow component [180].

Sustained quenching (qH)

A sustained decrease in fluorescence yield of the major LHCII antenna complex was detected by suppressor analysis identifying a suppressor of quenching called SOQ1. qH itself is catalyzed by a plastidial lipocain (LCNP) whose still unknown activity is controlled by SOQ1. It is likely that additional gene products will be identified in the future with particular reference to sustained quenching occurring during winter in evergreens [183] or upon desiccation [184].

Photoinhibitory quenching (qI)

The slowest quenching component, qI, is attributed to processes involving a decrease in active RC of PSII upon photodamage [185]. D1 protein of the RC is more susceptible to photodamage [4,186]. However, photodamaged D1 is degraded and repaired by an efficient and dynamically regulated repair machinery of PSII [4]; photoinhibition of PSII only occurs if the rate of damage overtakes the rate of repair [187]. Recently, highly quenched pigment–protein complexes have been involved in protecting PSII during assembly/repair which may maintain damaged PSII in its quenched state [188].

State transitions (qT)

PSII and PSI have different performance in light capture, depending on both light quality and quantity. Both PSI and PSII have an absorption peak in the blue and the red region of the spectrum, but PSII does not absorb in the far-red region. Under canopy or through shading the photon absorption rate of PSII vs. PSI is affected and, in these conditions, state transitions (qT) redistribute excitation energy between PSII and PSI.

State 1 refers to the antenna arrangement favoring PSII excitation, whereas in State 2 PSI is preferentially excited. This process is reversible: upon preferential excitation of PSII, the PQ pool is reduced and PQH_2 docks to Cytb_6f. This leads to the activation of a protein kinase — Stt7 in algae [189–191] and STN7 in plants [192,193] which phosphorylates LHCII. The latter dissociates from PSII and migrates to PSI [194–196]; upon preferential excitation of PSI, the PQ pool is oxidized, the kinase is inactivated and a phosphatase (TAP38/

PPH1) dephosphorylates the mobile LHCII, which moves back to PSII [197,198]. As a result of this rearrangement, a fraction of the LHCII antenna is transferred from PSII, a shallow trap, to PSI, which is a stronger quencher, yielding into an overall fluorescence quenching of the chloroplasts.

Chloroplast movement, qM

The other component is qM, which decreases the fluorescence yield of leaves upon exposure to EL. Rather than a genuine quenching, this component depends on the lower photon absorption caused by the chloroplast movement away from excess light and toward the cell walls aligned parallel to the incident light direction, thus increasing leaf transmission. This effect contributes to photoprotection [136,199,200].

Molecular mechanisms for quenching

As for the physical mechanism of quenching in LHC proteins, several mechanisms have been proposed:

1. *Aggregation-dependent LHCII quenching:* In this model, qE occurs upon aggregation of LHCII, which causes a conformational change within the protein and promotes energy transfer from Chl *a* to a low-lying carotenoid excited state of Lut bound to site L1 of LHCII [155,201]. Recently, the same quenching channel was proposed for LHCSR1 of mosses and it is achieved by fast energy transfer from excited Chl *a* to the S1 state of Zea [62], while in LHCSR of algae this involved Lut [148].
2. *CT (Charge-Transfer) quenching mechanism:* According to this model, qE activation involves a charge separation between a Chl–Zea heterodimer producing a transient Zea radical cation (Zea+) [154,202]. The process is located in monomeric LHC proteins of plants, does not occur in LHCII, and involves Chl pair (Chl A5 and Chl B5) located in close proximity to the carotenoid-binding site L2. Lutein can also be active in this process [179]. This quenching mechanism was reported in LHCSR1 of mosses where it involves Lut [62].
3. *Chl–Chl interaction quenching*: This mechanism suggests that Cars are not a major player in quenching, which is rather produced by the establishment of Chl–Chl excitonic coupling which becomes visible as a red-shifted emission at 700 nm [203].
4. *Chl–Car interaction quenching*: This is similar to [3] but hypothesizes that excitonic interactions are rather established between Chl and Cars [204].

It should be underlined that quenching mechanisms occurring *in vivo* do not need to be mutually exclusive. Rather more than one mechanism might well contribute to establish the overall quenching state. In at least one case, energy transfer from Chl *a* to S1 Car with thermal dissipation (mechanism 1) and formation of the Lut radical cation (mechanism 2) have been observed to occur within the same pigment–protein complex [62].

Abbreviations

Anthera, antheraxanthin; Car(s), carotenoid(s); CEF, cyclic electron flow; Chl(s), chlorophyll(s); ^{1}Chl*/^{3}Chl*, singlet/triplet chlorophyll excited state; CO_2, carbon dioxide; Cytb_6f, cytochrome b_6f; EL, excess light; LHC, light-harvesting complex; LHCII, trimeric LHC; LL, low light; Lut, lutein; Neo, neoxanthin; NPQ, non-photochemical quenching; O_2, oxygen; $^1O_2$, singlet oxygen; $O_2^{\cdot-}$, superoxide anion; $OH^{\cdot}$, hydroxyl radical; P680/P700, 'special' Chl pair in the PSII/PSI reaction center; PQ/PQH_2, plastoquinone/plastoquinol; PSI and PSI, photosystems I and II; qE, energy quenching; qH, sustained quenching; qI, photoinhibitory quenching; qM, chloroplast movement; qT, state transition; qZ, zeaxanthin-dependent; RC, reaction center; ROS, reactive oxygen species; VDE, Viola de-epoxidase; Viola, violaxanthin; Zea, zeaxanthin.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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References

1 Prasil, O., Adir, N. and Ohad, I. (1992) Dynamics of photosystem II: mechanism of photoinhibition and recovery processes. In *The photosystems: structure, function and molecular biology* (Barber, J., ed.), pp. 295–348, Elsevier

2 Tjus, S.E., Scheller, H.V., Andersson, B. and Møller, B.L. (2001) Active oxygen produced during selective excitation of photosystem I is damaging not only to photosystem I, but also to photosystem II. *Plant Physiol.* **125**, 2007–2015 https://doi.org/10.1104/pp.125.4.2007

3 Tjus, S.E., Møller, B.L. and Scheller, H.V. (1998) Photosystem I is an early target of photoinhibition in barley illuminated at chilling temperatures. *Plant Physiol.* **116**, 755–764 https://doi.org/10.1104/pp.116.2.755

4 Aro, E.M., Virgin, I. and Andersson, B. (1993) Photoinhibition of photosystem II. inactivation, protein damage and turnover. *Biochim. Biophys. Acta* **1143**, 113–134 https://doi.org/10.1016/0005-2728(93)90134-2

5 Melis, A. (1999) Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage? *Trends Plant Sci.* **4**, 130–135 https://doi.org/10.1016/S1360-1385(99)01387-4

6 Hideg, E., Kálai, T., Hideg, K. and Vass, I. (1998) Photoinhibition of photosynthesis in vivo results in singlet oxygen production detection via nitroxide-induced fluorescence quenching in broad bean leaves. *Biochemistry* **37**, 11405–11411 https://doi.org/10.1021/bi972890+

7 Powles, S.B. and Björkman, O. (1982) Photoinhibition of photosynthesis: effect on chlorophyll fluorescence at 77 K in intact leaves and in chloroplast membranes of *Nerium oleander*. *Planta* **156**, 97–107 https://doi.org/10.1007/BF00395424

8 Kok, B. (1956) On the inhibition of photosynthesis by intense light. *Biochim. Biophys. Acta* **21**, 234–244 https://doi.org/10.1016/0006-3002(56)90003-8

9 Gerotto, C., Alboresi, A., Meneghesso, A., Jokel, M., Suorsa, M., Aro, E.-M. et al. (2016) Flavodiiron proteins act as safety valve for electrons in *Physcomitrella patens*. *Proc. Natl Acad. Sci. U.S.A.* **113**, 12322–12327 https://doi.org/10.1073/pnas.1606685113

10 Murata, N., Takahashi, S., Nishiyama, Y. and Allakhverdiev, S.I. (2007) Photoinhibition of photosystem II under environmental stress. *Biochim. Biophys. Acta, Bioenerg.* **1767**, 414–421 https://doi.org/10.1016/j.bbabio.2006.11.019

11 Öquist, G. and Huner, N.P.A. (2003) Photosynthesis of overwintering evergreen plants. *Annu. Rev. Plant Biol.* **54**, 329–355 https://doi.org/10.1146/annurev.arplant.54.072402.115741

12 Li, Z., Wakao, S., Fischer, B.B. and Niyogi, K.K. (2009) Sensing and responding to excess light. *Annu. Rev. Plant Biol.* **60**, 239–260 https://doi.org/10.1146/annurev.arplant.58.032806.103844

13 Nelson, N. and Ben-Shem, A. (2004) The complex architecture of oxygenic photosynthesis. *Nat. Rev. Mol. Cell Biol.* **5**, 971–982 https://doi.org/10.1038/nrm1525

14 Rutherford, A. and Thurnauer, M. (1982) Radical pair state in photosystem II. *Proc. Natl Acad. Sci. U.S.A* **79**, 7283–7287 https://doi.org/10.1073/pnas.79.23.7283

15 Asada, K. and Takahashi, M. (1987) Production and scavenging of active oxygen in chloroplasts. In *Photoinhibition* (Kyle, D.J., Osmond, C.B. and Arntzen, C.J., eds.), pp. 227–228, Elsevier, Amsterdam

16 Vass, I. (2011) Role of charge recombination processes in photodamage and photoprotection of the photosystem II complex. *Physiol. Plant* **142**, 6–16 https://doi.org/10.1111/j.1399-3054.2011.01454.x

17 Telfer, A., De Las Rivas, J. and Barber, J. (1991) β-Carotene within the isolated photosystem II reaction centre: photooxidation and irreversible bleaching of this chromophore by oxidised P680. *Biochim. Biophys. Acta, Bioenerg.* **1060**, 106–114 https://doi.org/10.1016/S0005-2728(05)80125-2

18 Umena, Y., Kawakami, K., Shen, J.-R. and Kamiya, N. (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* **473**, 55–60 https://doi.org/10.1038/nature09913

19 Crowe, S.A., Døssing, L.N., Beukes, N.J., Bau, M., Kruger, S.J., Frei, R. et al. (2013) Atmospheric oxygenation three billion years ago. *Nature* **501**, 535–538 https://doi.org/10.1038/nature12426

20 Pospíšil, P. and Prasad, A. (2014) Formation of singlet oxygen and protection against its oxidative damage in photosystem II under abiotic stress. *J. Photochem. Photobiol. B* **137**, 39–48 https://doi.org/10.1016/j.jphotobiol.2014.04.025

21 Pospíšil, P. (2016) Production of reactive oxygen species by photosystem II as a response to light and temperature stress. *Front. Plant Sci.* **7**, 1950 https://doi.org/10.3389/fpls.2016.01950

22 Kale, R., Hebert, A.E., Frankel, L.K., Sallans, L., Bricker, T.M. and Pospíšil, P. (2017) Amino acid oxidation of the D1 and D2 proteins by oxygen radicals during photoinhibition of Photosystem II. *Proc. Natl Acad. Sci. U.S.A.* **114**, 2988–2993 https://doi.org/10.1073/pnas.1618922114

23 Takagi, D., Takumi, S., Hashiguchi, M., Sejima, T. and Miyake, C. (2016) Superoxide and singlet oxygen produced within the thylakoid membranes both cause photosystem I photoinhibition. *Plant Physiol.* **171**, 1626–1634 https://doi.org/10.1104/pp.16.00246

24 Sonoike, K. and Terashima, I. (1994) Mechanism of photosystem-I photoinhibition in leaves of *Cucumis sativus* L. *Planta* **194**, 287–293 https://doi.org/10.1007/BF01101690

25 Terashima, I., Funayama, S. and Sonoike, K. (1994) The site of photoinhibition in leaves of *Cucumis sativus* L. at low temperatures is photosystem I, not photosystem II. *Planta* **193**, 300–306 https://doi.org/10.1007/BF00192544

26 Sejima, T., Takagi, D., Fukayama, H., Makino, A. and Miyake, C. (2014) Repetitive short-pulse light mainly inactivates photosystem I in sunflower leaves. *Plant Cell Physiol.* **55**, 1184–1193 https://doi.org/10.1093/pcp/pcu061

27 Asada, K., Kiso, K. and Yoshikawa, K. (1974) Univalent reduction of molecular oxygen by spinach chloroplasts on illumination. *J. Biol. Chem.* **249**, 2175–2181 PMID:4362064

28 Mehler, A. (1951) Studies on reactivities of illuminated chloroplasts. I. Mechanism of the reduction of oxygen and other Hill reagents. *Arch. Biochem. Biophys.* **33**, 65–77 https://doi.org/10.1016/0003-9861(51)90082-3

29 Kozuleva, M.A., Petrova, A.A., Mamedov, M.D., Semenov, A.Y. and Ivanov, B.N. (2014) O_2 reduction by photosystem I involves phylloquinone under steady-state illumination. *FEBS Lett.* **588**, 4364–4368 https://doi.org/10.1016/j.febslet.2014.10.003

30 Takahashi, M. and Asada, K. (1988) Superoxide production in aprotic interior of chloroplast thylakoids. *Arch. Biochem. Biophys.* **267**, 714–722 https://doi.org/10.1016/0003-9861(88)90080-X

31 Sonoike, K., Terashima, I., Iwaki, M. and Itoh, S. (1995) Destruction of photosystem I iron-sulfur centers in leaves of *Cucumis sativus* L. by weak illumination at chilling temperatures. *FEBS Lett.* **362**, 235–238 https://doi.org/10.1016/0014-5793(95)00254-7

32 Shuvalov, V., Nuijs, A., van Gorkom, H., Smit, H. and Duysens, L. (1986) Picosecond absorbance changes upon selective excitation of the primary electron donor P-700 in photosystem I. *Biochim. Biophys. Acta, Bioenerg.* **850**, 319–323 https://doi.org/10.1016/0005-2728(86)90187-8
33 Rutherford, A.W., Osyczka, A. and Rappaport, F. (2012) Back-reactions, short-circuits, leaks and other energy wasteful reactions in biological electron transfer: redox tuning to survive life in O_2. *FEBS Lett.* **586**, 603–616 https://doi.org/10.1016/j.febslet.2011.12.039
34 Cazzaniga, S., Li, Z., Niyogi, K.K., Bassi, R. and Dall'Osto, L. (2012) The *Arabidopsis* szl1 mutant reveals a critical role of β-carotene in photosystem I photoprotection. *Plant Physiol.* **159**, 1745–1758 https://doi.org/10.1104/pp.112.201137
35 Sonoike, K. (2011) Photoinhibition of photosystem I. *Physiol. Plant* **142**, 56–64 https://doi.org/10.1111/j.1399-3054.2010.01437.x
36 Triantaphylidès, C. and Havaux, M. (2009) Singlet oxygen in plants: production, detoxification and signaling. *Trends Plant Sci.* **14**, 219–228 https://doi.org/10.1016/j.tplants.2009.01.008
37 Santabarbara, S. and Jennings, R.C. (2005) The size of the population of weakly coupled chlorophyll pigments involved in thylakoid photoinhibition determined by steady-state fluorescence spectroscopy. *Biochim. Biophys. Acta, Bioenerg.* **1709**, 138–149 https://doi.org/10.1016/j.bbabio.2005.06.001
38 Pospíšil, P. (2012) Molecular mechanisms of production and scavenging of reactive oxygen species by photosystem II. *Biochim. Biophys. Acta* **1817**, 218–231 https://doi.org/10.1016/j.bbabio.2011.05.017
39 Keren, N., Liberton, M. and Pakrasi, H.B. (2005) Photochemical competence of assembled photosystem II core complex in cyanobacterial plasma membrane. *J. Biol. Chem.* **280**, 6548–6553 https://doi.org/10.1074/jbc.M410218200
40 Havaux, M., Dall'Osto, L., Cuiné, S., Giuliano, G. and Bassi, R. (2004) The effect of zeaxanthin as the only xanthophyll on the structure and function of the photosynthetic apparatus in *Arabidopsis thaliana*. *J. Biol. Chem.* **279**, 13878–13888 https://doi.org/10.1074/jbc.M311154200
41 Dall'Osto, L., Cazzaniga, S., Havaux, M. and Bassi, R. (2010) Enhanced photoprotection by protein-bound vs free xanthophyll pools: a comparative analysis of chlorophyll b and xanthophyll biosynthesis mutants. *Mol. Plant* **3**, 576–593 https://doi.org/10.1093/mp/ssp117
42 Witt, H.T. (1971) Coupling of quanta, electrons, fields, ions and phosphorylation in the functional membrane of photosynthesis. Results by pulse spectroscopic methods. *Q. Rev. Biophys.* **4**, 365 https://doi.org/10.1017/S0033583500000834
43 Jávorfi, T., Garab, G. and Naqvi, K.R. (2000) Reinvestigation of the triplet-minus-singlet spectrum of chloroplasts. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **56A**, 211–214 https://doi.org/10.1016/S1386-1425(99)00226-7
44 Mathis, P., Butler, W.L. and Satoh, K. (1979) Carotenoid triplet state and chlorophyll fluorescence quenching in chloroplasts and subchloroplast particles. *Photochem. Photobiol.* **30**, 603–614 https://doi.org/10.1111/j.1751-1097.1979.tb07187.x
45 Mozzo, M., Dall'Osto, L., Hienerwadel, R., Bassi, R. and Croce, R. (2008) Photoprotection in the antenna complexes of photosystem II: role of individual xanthophylls in chlorophyll triplet quenching. *J. Biol. Chem.* **283**, 6184–6192 https://doi.org/10.1074/jbc.M708961200
46 Peterman, E.J., Dukker, F.M., van Grondelle, R. and van Amerongen, H. (1995) Chlorophyll a and carotenoid triplet states in light-harvesting complex II of higher plants. *Biophys. J.* **69**, 2670–2678 https://doi.org/10.1016/S0006-3495(95)80138-4
47 Formaggio, E., Cinque, G. and Bassi, R. (2001) Functional architecture of the major light-harvesting complex from higher plants. *J. Mol. Biol.* **314**, 1157–1166 https://doi.org/10.1006/jmbi.2000.5179
48 Dall'Osto, L., Lico, C., Alric, J., Giuliano, G., Havaux, M. and Bassi, R. (2006) Lutein is needed for efficient chlorophyll triplet quenching in the major LHCII antenna complex of higher plants and effective photoprotection in vivo under strong light. *BMC Plant Biol.* **6**, 32 https://doi.org/10.1186/1471-2229-6-32
49 Edge, R., McGarvey, D.J. and Truscott, T.G. (1997) The carotenoids as anti-oxidants — a review. *J. Photochem. Photobiol. B* **41**, 189–200 https://doi.org/10.1016/S1011-1344(97)00092-4
50 Polívka, T. and Sundström, V. (2004) Ultrafast dynamics of carotenoid excited states — from solution to natural and artificial systems. *Chem. Rev.* **104**, 2021–2072 https://doi.org/10.1021/cr020674n
51 Dall'Osto, L., Fiore, A., Cazzaniga, S., Giuliano, G. and Bassi, R. (2007) Different roles of alpha- and beta-branch xanthophylls in photosystem assembly and photoprotection. *J. Biol. Chem.* **282**, 35056–35068 https://doi.org/10.1074/jbc.M704729200
52 Baroli, I., Do, A.D., Yamane, T. and Niyogi, K.K. (2003) Zeaxanthin accumulation in the absence of a functional xanthophyll cycle protects *Chlamydomonas reinhardtii* from photooxidative stress. *Plant Cell* **15**, 992–1008 https://doi.org/10.1105/tpc.010405
53 Havaux, M., Dall'Osto, L. and Bassi, R. (2007) Zeaxanthin has enhanced antioxidant capacity with respect to all other xanthophylls in *Arabidopsis* leaves and functions independent of binding to PSII antennae. *Plant Physiol.* **145**, 1506–1520 https://doi.org/10.1104/pp.107.108480
54 Dall'Osto, L., Holt, N.E., Kaligotla, S., Fuciman, M., Cazzaniga, S., Carbonera, D. et al. (2012) Zeaxanthin protects plant photosynthesis by modulating chlorophyll triplet yield in specific light-harvesting antenna subunits. *J. Biol. Chem.* **287**, 41820–41834 https://doi.org/10.1074/jbc.M112.405498
55 Dall'Osto, L., Bressan, M. and Bassi, R. (2015) Biogenesis of light harvesting proteins. *Biochim. Biophys. Acta* **1847**, 861–871 https://doi.org/10.1016/j.bbabio.2015.02.009
56 Niyogi, K.K., Grossman, A.R. and Björkman, O. (1998) *Arabidopsis* mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. *Plant Cell* **10**, 1121–1134 https://doi.org/10.1105/tpc.10.7.1121
57 Telfer, A., Bishop, S., Phillips, D. and Barber, J. (1994) Isolated photosynthetic reaction center of photosystem II as a sensitizer for the formation of singlet oxygen. Detection and quantum yield determination using a chemical trapping technique. *J. Biol. Chem.* **269**, 13244–13253 PMID:8175754
58 Telfer, A. (2005) Too much light? How beta-carotene protects the photosystem II reaction centre. *Photochem. Photobiol. Sci.* **4**, 950–956 https://doi.org/10.1039/b507888c
59 Beisel, K.G., Schurr, U. and Matsubara, S. (2011) Altered turnover of -carotene and Chl a in *Arabidopsis* leaves treated with lincomycin or norflurazon. *Plant Cell Physiol.* **52**, 1193–1203 https://doi.org/10.1093/pcp/pcr069
60 Morosinotto, T., Baronio, R. and Bassi, R. (2002) Dynamics of chromophore binding to Lhc proteins in vivo and in vitro during operation of the xanthophyll cycle. *J. Biol. Chem.* **277**, 36913–36920 https://doi.org/10.1074/jbc.M205339200
61 Pinnola, A., Dall'Osto, L., Gerotto, C., Morosinotto, T., Bassi, R. and Alboresi, A. (2013) Zeaxanthin binds to light-harvesting complex stress-related protein to enhance nonphotochemical quenching in *Physcomitrella patens*. *Plant Cell* **25**, 3519–3534 https://doi.org/10.1105/tpc.113.114538
62 Pinnola, A., Staleva-Musto, H., Capaldi, S., Ballottari, M., Bassi, R. and Polívka, T. (2016) Electron transfer between carotenoid and chlorophyll contributes to quenching in the LHCSR1 protein from *Physcomitrella patens*. *Biochim. Biophys. Acta* **1857**, 1870–1878 https://doi.org/10.1016/j.bbabio.2016.09.001

63 Pinnola, A., Ballottari, M., Bargigia, I., Alcocer, M., D'Andrea, C., Cerullo, G. et al. (2017) Functional modulation of LHCSR1 protein from *Physcomitrella patens* by zeaxanthin binding and low pH. *Sci. Rep.* **7**, 11158 https://doi.org/10.1038/s41598-017-11101-7
64 Scandalios, J.G. (2005) Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz. J. Med. Biol. Res.* **38**, 995–1014 https://doi.org/10.1590/S0100-879X2005000700003
65 Krieger-Liszkay, A. and Trebst, A. (2006) Tocopherol is the scavenger of singlet oxygen produced by the triplet states of chlorophyll in the PSII reaction centre. *J. Exp. Bot.* **57**, 1677–1684 https://doi.org/10.1093/jxb/erl002
66 Trebst, A., Depka, B. and Holländer-Czytko, H. (2002) A specific role for tocopherol and of chemical singlet oxygen quenchers in the maintenance of photosystem II structure and function in *Chlamydomonas reinhardtii*. *FEBS Lett.* **516**, 156–160 https://doi.org/10.1016/S0014-5793(02)02526-7
67 Trebst, A. (2003) Function of beta-carotene and tocopherol in photosystem II. *Z. Naturforsch. C* **58**, 609–620 https://doi.org/10.1515/znc-2003-9-1001
68 Havaux, M., Eymery, F., Porfirova, S., Rey, P. and Dörmann, P. (2005) Vitamin E protects against photoinhibition and photooxidative stress in *Arabidopsis thaliana*. *Plant Cell* **17**, 3451–3469 https://doi.org/10.1105/tpc.105.037036
69 Kruk, J. and Trebst, A. (2008) Plastoquinol as a singlet oxygen scavenger in photosystem II. *Biochim. Biophys. Acta* **1777**, 154–162 https://doi.org/10.1016/j.bbabio.2007.10.008
70 Nowicka, B. and Kruk, J. (2010) Occurrence, biosynthesis and function of isoprenoid quinones. *Biochim. Biophys. Acta* **1797**, 1587–1605 https://doi.org/10.1016/j.bbabio.2010.06.007
71 Yadav, D.K., Kruk, J., Sinha, R.K. and Pospíšil, P. (2010) Singlet oxygen scavenging activity of plastoquinol in photosystem II of higher plants: electron paramagnetic resonance spin-trapping study. *Biochim. Biophys. Acta* **1797**, 1807–1811 https://doi.org/10.1016/j.bbabio.2010.07.003
72 Neely, W.C., Martin, J.M. and Barker, S.A. (1988) Products and relative reaction rates of the oxidation of tocopherols with singlet molecular oxygen. *Photochem. Photobiol.* **48**, 423–428 https://doi.org/10.1111/j.1751-1097.1988.tb02840.x
73 Müller-Moulé, P., Havaux, M. and Niyogi, K.K. (2003) Zeaxanthin deficiency enhances the high light sensitivity of an ascorbate-deficient mutant of *Arabidopsis*. *Plant Physiol.* **133**, 748–760 https://doi.org/10.1104/pp.103.026252
74 Müller-Moulé, P., Golan, T. and Niyogi, K.K. (2004) Ascorbate-deficient mutants of *Arabidopsis* grow in high light despite chronic photooxidative stress. *Plant Physiol.* **134**, 1163–1172 https://doi.org/10.1104/pp.103.032375
75 Havaux, M., Bonfils, J.P., Lütz, C. and Niyogi, K.K. (2000) Photodamage of the photosynthetic apparatus and its dependence on the leaf developmental stage in the npq1 *Arabidopsis* mutant deficient in the xanthophyll cycle enzyme violaxanthin de-epoxidase. *Plant Physiol.* **124**, 273–284 https://doi.org/10.1104/pp.124.1.273
76 Li, Z., Keasling, J.D. and Niyogi, K.K. (2012) Overlapping photoprotective function of vitamin E and carotenoids in *Chlamydomonas*. *Plant Physiol.* **158**, 313–323 https://doi.org/10.1104/pp.111.181230
77 Munné-Bosch, S. and Alegre, L. (2002) The function of tocopherols and tocotrienols in plants. *Crit. Rev. Plant Sci.* **21**, 31–57 https://doi.org/10.1080/0735-260291044179
78 Smirnoff, N. (2000) Ascorbate biosynthesis and function in photoprotection. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **355**, 1455–1464 https://doi.org/10.1098/rstb.2000.0706
79 Azzabi, G., Pinnola, A., Betterle, N., Bassi, R. and Alboresi, A. (2012) Enhancement of non photochemical quenching in the bryophyte *Physcomitrella patens* during acclimation to salt and osmotic stress. *Plant Cell Physiol.* **53**, 1815–1825 https://doi.org/10.1093/pcp/pcs124
80 Tóth, S.Z., Nagy, V., Puthur, J.T., Kovács, L. and Garab, G. (2011) The physiological role of ascorbate as photosystem II electron donor: protection against photoinactivation in heat-stressed leaves. *Plant Physiol.* **156**, 382–392 https://doi.org/10.1104/pp.110.171918
81 Foyer, C.H., Descourvieres, P. and Kunert, K.J. (1994) Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. *Plant Cell Environ.* **17**, 507–523 https://doi.org/10.1111/j.1365-3040.1994.tb00146.x
82 Mittler, R. (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* **7**, 405–410 https://doi.org/10.1016/S1360-1385(02)02312-9
83 Mittler, R., Vanderauwera, S., Gollery, M. and Van Breusegem, F. (2004) Reactive oxygen gene network of plants. *Trends Plant Sci.* **9**, 490–498 https://doi.org/10.1016/j.tplants.2004.08.009
84 Asada, K. (1992) Ascorbate peroxidase — a hydrogen peroxide-scavenging enzyme in plants. *Physiol. Plant* **85**, 235–241 https://doi.org/10.1111/j.1399-3054.1992.tb04728.x
85 Asada, K. (1999) The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 601–639 https://doi.org/10.1146/annurev.arplant.50.1.601
86 Jokel, M., Kosourov, S., Battchikova, N., Tsygankov, A.A., Aro, E.M. and Allahverdiyeva, Y. (2015) *Chlamydomonas flavodiiron* proteins facilitate acclimation to anoxia during sulfur deprivation. *Plant Cell Physiol.* **56**, 1598–1607 https://doi.org/10.1093/pcp/pcv085
87 Heber, U. and Walker, D. (1992) Concerning a dual function of coupled cyclic electron transport in leaves. *Plant Physiol.* **100**, 1621–1626 https://doi.org/10.1104/pp.100.4.1621
88 Herbert, S.K., Fork, D.C. and Malkin, S. (1990) Photoacoustic measurements in vivo of energy storage by cyclic electron flow in algae and higher plants. *Plant Physiol.* **94**, 926–934 https://doi.org/10.1104/pp.94.3.926
89 Finazzi, G., Furia, A., Barbagallo, R.P. and Forti, G. (1999) State transitions, cyclic and linear electron transport and photophosphorylation in *Chlamydomonas reinhardtii*. *Biochim. Biophys. Acta* **1413**, 117–129 https://doi.org/10.1016/S0005-2728(99)00089-4
90 Rumeau, D., Peltier, G. and Cournac, L. (2007) Chlororespiration and cyclic electron flow around PSI during photosynthesis and plant stress response. *Plant Cell Environ.* **30**, 1041–1051 https://doi.org/10.1111/j.1365-3040.2007.01675.x
91 Johnson, G.N. (2005) Cyclic electron transport in C3 plants: fact or artefact? *J. Exp. Bot.* **56**, 407–416 https://doi.org/10.1093/jxb/eri106
92 Joët, T., Cournac, L., Peltier, G. and Havaux, M. (2002) Cyclic electron flow around photosystem I in C(3) plants. In vivo control by the redox state of chloroplasts and involvement of the NADH-dehydrogenase complex. *Plant Physiol.* **128**, 760–769 https://doi.org/10.1104/pp.010775
93 Joliot, P. and Joliot, A. (2002) Cyclic electron transfer in plant leaf. *Proc. Natl Acad. Sci. U.S.A.* **99**, 10209–10214 https://doi.org/10.1073/pnas.102306999
94 Munekage, Y., Hojo, M., Meurer, J., Endo, T., Tasaka, M. and Shikanai, T. (2002) PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*. *Cell* **110**, 361–371 https://doi.org/10.1016/S0092-8674(02)00867-X

95 DalCorso, G., Pesaresi, P., Masiero, S., Aseeva, E., Schünemann, D., Finazzi, G. et al. (2008) A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in *Arabidopsis*. *Cell* **132**, 273–285 https://doi.org/10.1016/j.cell.2007.12.028
96 Hertle, A.P., Blunder, T., Wunder, T., Pesaresi, P., Pribil, M., Armbruster, U. et al. (2013) PGRL1 is the elusive ferredoxin-plastoquinone reductase in photosynthetic cyclic electron flow. *Mol. Cell* **49**, 511–523 https://doi.org/10.1016/j.molcel.2012.11.030
97 Ifuku, K., Endo, T., Shikanai, T. and Aro, E.-M. (2011) Structure of the chloroplast NADH dehydrogenase-like complex: nomenclature for nuclear-encoded subunits. *Plant Cell Physiol.* **52**, 1560–1568 https://doi.org/10.1093/pcp/pcr098
98 Peng, L., Yamamoto, H. and Shikanai, T. (2011) Structure and biogenesis of the chloroplast NAD(P)H dehydrogenase complex. *Biochim. Biophys. Acta, Bioenerg.* **1807**, 945–953 https://doi.org/10.1016/j.bbabio.2010.10.015
99 Endo, T., Mi, H., Shikanai, T. and Asada, K. (1997) Donation of electrons to plastoquinone by NAD(P)H dehydrogenase and by ferredoxin-quinone reductase in spinach chloroplasts. *Plant Cell Physiol.* **38**, 1272–1277 https://doi.org/10.1093/oxfordjournals.pcp.a029115
100 Yamamoto, H., Peng, L., Fukao, Y. and Shikanai, T. (2011) An Src homology 3 domain-like fold protein forms a ferredoxin binding site for the chloroplast NADH dehydrogenase-like complex in *Arabidopsis*. *Plant Cell* **23**, 1480–1493 https://doi.org/10.1105/tpc.110.080291
101 Matsubayashi, T., Wakasugi, T., Shinozaki, K., Yamaguchi-Shinozaki, K., Zaita, N., Hidaka, T. et al. (1987) Six chloroplast genes (ndhA-F) homologous to human mitochondrial genes encoding components of the respiratory chain NADH dehydrogenase are actively expressed: determination of the splice sites in ndhA and ndhB pre-mRNAs. *Mol. Gen. Genet.* **210**, 385–393 https://doi.org/10.1007/BF00327187
102 Burrows, P.A., Sazanov, L.A., Svab, Z., Maliga, P. and Nixon, P.J. (1998) Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid ndh genes. *EMBO J.* **17**, 868–876 https://doi.org/10.1093/emboj/17.4.868
103 Shikanai, T., Endo, T., Hashimoto, T., Yamada, Y., Asada, K. and Yokota, A. (1998) Directed disruption of the tobacco ndhB gene impairs cyclic electron flow around photosystem I. *Proc. Natl Acad. Sci. U.S.A.* **95**, 9705–9709 https://doi.org/10.1073/pnas.95.16.9705
104 Endo, T., Shikanai, T., Takabayashi, A., Asada, K. and Sato, F. (1999) The role of chloroplastic NAD(P)H dehydrogenase in photoprotection. *FEBS Lett.* **457**, 5–8 https://doi.org/10.1016/S0014-5793(99)00989-8
105 Horváth, E.M., Peter, S.O., Joët, T., Rumeau, D., Cournac, L., Horváth, G.V. et al. (2000) Targeted inactivation of the plastid ndhB gene in tobacco results in an enhanced sensitivity of photosynthesis to moderate stomatal closure. *Plant Physiol.* **123**, 1337–1350 https://doi.org/10.1104/pp.123.4.1337
106 Suorsa, M., Jarvi, S., Grieco, M., Nurmi, M., Pietrzykowska, M., Rantala, M. et al. (2012) PROTON GRADIENT REGULATION5 is essential for proper acclimation of *Arabidopsis* photosystem I to naturally and artificially fluctuating light conditions. *Plant Cell* **24**, 2934–2948 https://doi.org/10.1105/tpc.112.097162
107 Munekage, Y., Hashimoto, M., Miyake, C., Tomizawa, K., Endo, T., Tasaka, M. et al. (2004) Cyclic electron flow around photosystem I is essential for photosynthesis. *Nature* **429**, 579–582 https://doi.org/10.1038/nature02598
108 Iwai, M., Takizawa, K., Tokutsu, R., Okamuro, A., Takahashi, Y. and Minagawa, J. (2010) Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis. *Nature* **464**, 1210–1213 https://doi.org/10.1038/nature08885
109 Petroutsos, D., Terauchi, A.M., Busch, A., Hirschmann, I., Merchant, S.S., Finazzi, G. et al. (2009) PGRL1 participates in iron-induced remodeling of the photosynthetic apparatus and in energy metabolism in *Chlamydomonas reinhardtii*. *J. Biol. Chem.* **284**, 32770–32781 https://doi.org/10.1074/jbc.M109.050468
110 Tolleter, D., Ghysels, B., Alric, J., Petroutsos, D., Tolstygina, I., Krawietz, D. et al. (2011) Control of hydrogen photoproduction by the proton gradient generated by cyclic electron flow in *Chlamydomonas reinhardtii*. *Plant Cell* **23**, 2619–2630 https://doi.org/10.1105/tpc.111.086876
111 Dang, K.-V., Plet, J., Tolleter, D., Jokel, M., Cuiné, S., Carrier, P. et al. (2014) Combined increases in mitochondrial cooperation and oxygen photoreduction compensate for deficiency in cyclic electron flow in *Chlamydomonas reinhardtii*. *Plant Cell* **26**, 3036–3050 https://doi.org/10.1105/tpc.114.126375
112 Johnson, X., Steinbeck, J., Dent, R.M., Takahashi, H., Richaud, P., Ozawa, S.-I. et al. (2014) Proton gradient regulation 5-mediated cyclic electron flow under ATP- or redox-limited conditions: a study of ΔATpase pgr5 and ΔrbcL pgr5 mutants in the green alga *Chlamydomonas reinhardtii*. *Plant Physiol.* **165**, 438–452 https://doi.org/10.1104/pp.113.233593
113 Bennoun, P. (1982) Evidence for a respiratory chain in the chloroplast. *Proc. Natl Acad. Sci. U.S.A.* **79**, 4352–4356 PMID:16593210
114 Wu, M., Nie, Z.Q. and Yang, J. (1989) The 18-kD protein that binds to the chloroplast DNA replicative origin is an iron-sulfur protein related to a subunit of NADH dehydrogenase. *Plant Cell* **1**, 551–557 https://doi.org/10.1105/tpc.1.5.551
115 Carol, P., Stevenson, D., Bisanz, C., Breitenbach, J., Sandmann, G., Mache, R. et al. (1999) Mutations in the *Arabidopsis* gene IMMUTANS cause a variegated phenotype by inactivating a chloroplast terminal oxidase associated with phytoene desaturation. *Plant Cell* **11**, 57–68 https://doi.org/10.1105/tpc.11.1.57
116 Cournac, L., Josse, E.-M., Joet, T., Rumeau, D., Redding, K., Kuntz, M. et al. (2000) Flexibility in photosynthetic electron transport: a newly identified chloroplast oxidase involved in chlororespiration. *Philos. Trans. R Soc. B Biol. Sci.* **355**, 1447–1454 https://doi.org/10.1098/rstb.2000.0705
117 Josse, E.-M., Alcaraz, J.-P., Labouré, A.-M. and Kuntz, M. (2003) In vitro characterization of a plastid terminal oxidase (PTOX). *Eur. J. Biochem.* **270**, 3787–3794 https://doi.org/10.1046/j.1432-1033.2003.03766.x
118 Wu, D., Wright, D.A., Wetzel, C., Voytas, D.F. and Rodermel, S. (1999) The IMMUTANS variegation locus of *Arabidopsis* defines a mitochondrial alternative oxidase homolog that functions during early chloroplast biogenesis. *Plant Cell* **11**, 43–55 https://doi.org/10.1105/tpc.11.1.43
119 Quiles, M. (2006) Stimulation of chlororespiration by heat and high light intensity in oat plants. *Plant Cell Environ.* **29**, 1463–1470 https://doi.org/10.1111/j.1365-3040.2006.01510.x
120 Stepien, P. and Johnson, G.N. (2009) Contrasting responses of photosynthesis to salt stress in the glycophyte *Arabidopsis* and the halophyte thellungiella: role of the plastid terminal oxidase as an alternative electron sink. *Plant Physiol.* **149**, 1154–1165 https://doi.org/10.1104/pp.108.132407
121 Ivanov, A.G., Rosso, D., Savitch, L.V., Stachula, P., Rosembert, M., Oquist, G. et al. (2012) Implications of alternative electron sinks in increased resistance of PSII and PSI photochemistry to high light stress in cold-acclimated *Arabidopsis thaliana*. *Photosynth. Res.* **113**, 191–206 https://doi.org/10.1007/s11120-012-9769-y
122 Niyogi, K.K. (2000) Safety valves for photosynthesis. *Curr. Opin. Plant Biol.* **3**, 455–460 https://doi.org/10.1016/S1369-5266(00)00113-8

123 Streb, P., Josse, E., Gallouet, E., Baptist, F., Kuntz, M. and Cornic, G. (2005) Evidence for alternative electron sinks to photosynthetic carbon assimilation in the high mountain plant species *Ranunculus glacialis*. *Plant Cell Environ.* **28**, 1123–1135 https://doi.org/10.1111/j.1365-3040.2005.01350.x

124 Li, Q., Yao, Z.-J. and Mi, H. (2016) Alleviation of photoinhibition by co-ordination of chlororespiration and cyclic electron flow mediated by NDH under heat stressed condition in tobacco. *Front. Plant Sci.* **7**, 285 https://doi.org/10.3389/fpls.2016.00285

125 Caron, L., Berkaloff, C., Duval, J.C. and Jupin, H. (1987) Chlorophyll fluorescence transients from the diatom *Phaeodactylum tricornutum*: relative rates of cyclic phosphorylation and chlororespiration. *Photosynth. Res.* **11**, 131–139 https://doi.org/10.1007/BF00018271

126 Grouneva, I., Jakob, T., Wilhelm, C. and Goss, R. (2009) The regulation of xanthophyll cycle activity and of non-photochemical fluorescence quenching by two alternative electron flows in the diatoms *Phaeodactylum tricornutum* and *Cyclotella meneghiniana*. *Biochim. Biophys. Acta,Bioenerg.* **1787**, 929–938 https://doi.org/10.1016/j.bbabio.2009.02.004

127 Derks, A., Schaven, K. and Bruce, D. (2015) Diverse mechanisms for photoprotection in photosynthesis. Dynamic regulation of photosystem II excitation in response to rapid environmental change. *Biochim. Biophys. Acta, Bioenerg.* **1847**, 468–485 https://doi.org/10.1016/j.bbabio.2015.02.008

128 Moejes, F.W., Matuszyńska, A., Adhikari, K., Bassi, R., Cariti, F., Cogne, G. et al. (2017) A systems-wide understanding of photosynthetic acclimation in algae and higher plants. *J. Exp. Bot.* **68**, 2667–2681 https://doi.org/10.1093/jxb/erx137

129 Escoubas, J.M., Lomas, M., LaRoche, J. and Falkowski, P.G. (1995) Light intensity regulation of cab gene transcription is signaled by the redox state of the plastoquinone pool. *Proc. Natl Acad. Sci. U.S.A.* **92**, 10237–10241 https://doi.org/10.1073/pnas.92.22.10237

130 Ballottari, M., Dall'Osto, L., Morosinotto, T. and Bassi, R. (2007) Contrasting behavior of higher plant photosystem I and II antenna systems during acclimation. *J. Biol. Chem.* **282**, 8947–8958 https://doi.org/10.1074/jbc.M606417200

131 Pfannschmidt, T., Nilsson, A., Tullberg, A., Link, G. and Allen, J.F. (1999) Direct transcriptional control of the chloroplast genes psbA and psaAB adjusts photosynthesis to light energy distribution in plants. *IUBMB Life* **48**, 271–276 https://doi.org/10.1080/713803507

132 Joliot, P.A. and Finazzi, G. (2010) Proton equilibration in the chloroplast modulates multiphasic kinetics of nonphotochemical quenching of fluorescence in plants. *Proc. Natl Acad. Sci. U.S.A.* **107**, 12728–12733 https://doi.org/10.1073/pnas.1006399107

133 Malnoë, A., Schultink, A., Shahrasbi, S., Rumeau, D., Havaux, M. and Niyogi, K.K. (2018) The plastid lipocalin LCNP is required for sustained photoprotective energy dissipation in *Arabidopsis*. *Plant Cell* **30**, 196–208 https://doi.org/10.1105/tpc.17.00536

134 Murchie, E.H. and Lawson, T. (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *J. Exp. Bot.* **64**, 3983–3998 https://doi.org/10.1093/jxb/ert208

135 Rochaix, J.-D. (2014) Regulation and dynamics of the light-harvesting system. *Annu. Rev. Plant Biol.* **65**, 287–309 https://doi.org/10.1146/annurev-arplant-050213-040226

136 Cazzaniga, S., Dall' Osto, L., Kong, S.-G., Wada, M. and Bassi, R. (2013) Interaction between avoidance of photon absorption, excess energy dissipation and zeaxanthin synthesis against photooxidative stress in *Arabidopsis*. *Plant J.* **76**, 568–579 https://doi.org/10.1111/tpj.12314

137 Allorent, G., Tokutsu, R., Roach, T., Peers, G., Cardol, P., Girard-Bascou, J. et al. (2013) A dual strategy to cope with high light in *Chlamydomonas reinhardtii*. *Plant Cell* **25**, 545–557 https://doi.org/10.1105/tpc.112.108274

138 Horton, P. (1996) Nonphotochemical quenching of chlorophyll fluorescence. In *Light as an Energy Source Inf Carr Plant Physiol NATO ASI Ser.* (Jennings, R.C., Zucchelli, G., Ghetti, F. and Colombetti, G., eds.), vol. 287, pp. 99–111, Springer, Boston, MA

139 Demmig-Adams, B., Gilmore, A.M. and Adams, W.W. (1996) Carotenoids 3: in vivo function of carotenoids in higher plants. *FASEB J.* **10**, 403–412 https://doi.org/10.1096/fasebj.10.4.8647339

140 Li, X.P., Björkman, O., Shih, C., Grossman, A.R., Rosenquist, M., Jansson, S. et al. (2000) A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature* **403**, 391–395 https://doi.org/10.1038/35000131

141 Peers, G., Truong, T.B., Ostendorf, E., Busch, A., Elrad, D., Grossman, A.R. et al. (2009) An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. *Nature* **462**, 518–521 https://doi.org/10.1038/nature08587

142 Alboresi, A., Gerotto, C., Giacometti, G.M., Bassi, R. and Morosinotto, T. (2010) *Physcomitrella patens* mutants affected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization. *Proc. Natl Acad. Sci. U.S.A.* **107**, 11128–11133 https://doi.org/10.1073/pnas.1002873107

143 Goss, R. and Lepetit, B. (2015) Biodiversity of NPQ. *J. Plant Physiol.* **172**, 13–32 https://doi.org/10.1016/j.jplph.2014.03.004

144 Zaks, J., Amarnath, K., Sylak-Glassman, E.J. and Fleming, G.R. (2013) Models and measurements of energy-dependent quenching. *Photosynth. Res.* **116**, 389–409 https://doi.org/10.1007/s11120-013-9857-7

145 Ruban A, V., Johnson, M.P. and Duffy, C.D.P. (2012) The photoprotective molecular switch in the photosystem II antenna. *Biochim. Biophys. Acta* **1817**, 167–181 https://doi.org/10.1016/j.bbabio.2011.04.007

146 de Bianchi, S., Ballottari, M., Dall'Osto, L. and Bassi, R. (2010) Regulation of plant light harvesting by thermal dissipation of excess energy. *Biochem. Soc. Trans.* **38**, 651–660 https://doi.org/10.1042/BST0380651

147 Pinnola, A., Cazzaniga, S., Alboresi, A., Nevo, R., Levin-Zaidman, S., Reich, Z. et al. (2015) Light-harvesting complex stress-related proteins catalyze excess energy dissipation in both photosystems of *Physcomitrella patens*. *Plant Cell* **27**, 3213–3227 https://doi.org/10.1105/tpc.15.00443

148 Bonente, G., Ballottari, M., Truong, T.B., Morosinotto, T., Ahn, T.K., Fleming, G.R. et al. (2011) Analysis of LhcSR3, a protein essential for feedback de-excitation in the green alga *Chlamydomonas reinhardtii*. *PLoS Biol.* **9**, e1000577 https://doi.org/10.1371/journal.pbio.1000577

149 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 https://doi.org/10.1074/jbc.M200604200

150 Fan, M., Li, M., Liu, Z., Cao, P., Pan, X., Zhang, H. et al. (2015) Crystal structures of the PsbS protein essential for photoprotection in plants. *Nat. Struct. Mol. Biol.* **22**, 729–735 https://doi.org/10.1038/nsmb.3068

151 de Bianchi, S., Dall'Osto, L., Tognon, G., Morosinotto, T. and Bassi, R. (2008) Minor antenna proteins CP24 and CP26 affect the interactions between photosystem II subunits and the electron transport rate in grana membranes of *Arabidopsis*. *Plant Cell* **20**, 1012–1028 https://doi.org/10.1105/tpc.107.055749

152 Dall'Osto, L., Cazzaniga, S., Bressan, M., Paleček, D., Žídek, K., Niyogi, K.K. et al. (2017) Two mechanisms for dissipation of excess light in monomeric and trimeric light-harvesting complexes. *Nat. Plants* **3**, 17033 https://doi.org/10.1038/nplants.2017.33

153 Holt, N.E., Zigmantas, D., Valkunas, L., Li, X.-P., Niyogi, K.K. and Fleming, G.R. (2005) Carotenoid cation formation and the regulation of photosynthetic light harvesting. *Science* **307**, 433–436 https://doi.org/10.1126/science.1105833
154 Ahn, T.K., Avenson, T.J., Ballottari, M., Cheng, Y.-C., Niyogi, K.K., Bassi, R. et al. (2008) Architecture of a charge-transfer state regulating light harvesting in a plant antenna protein. *Science* **320**, 794–797 https://doi.org/10.1126/science.1154800
155 Ruban, A.V., Berera, R., Ilioaia, C., van Stokkum, I.H.M., Kennis, J.T.M., Pascal, A.A. et al. (2007) Identification of a mechanism of photoprotective energy dissipation in higher plants. *Nature* **450**, 575–578 https://doi.org/10.1038/nature06262
156 Betterle, N., Ballottari, M., Zorzan, S., de Bianchi, S., Cazzaniga, S., Dall'Osto, L. et al. (2009) Light-induced dissociation of an antenna hetero-oligomer is needed for non-photochemical quenching induction. *J. Biol. Chem.* **284**, 15255–15266 https://doi.org/10.1074/jbc.M808625200
157 Johnson, M.P., Goral, T.K., Duffy, C.D.P., Brain, A.P.R., Mullineaux, C.W. and Ruban, A.V. (2011) Photoprotective energy dissipation involves the reorganization of photosystem II light-harvesting complexes in the grana membranes of spinach chloroplasts. *Plant Cell* **23**, 1468–1479 https://doi.org/10.1105/tpc.110.081646
158 Elrad, D., Niyogi, K.K. and Grossman, A.R. (2002) A major light-harvesting polypeptide of photosystem II functions in thermal dissipation. *Plant Cell* **14**, 1801–1816 https://doi.org/10.1105/tpc.002154
159 Tokutsu, R. and Minagawa, J. (2013) Energy-dissipative supercomplex of photosystem II associated with LHCSR3 in *Chlamydomonas reinhardtii*. *Proc. Natl Acad. Sci. U.S.A.* **110**, 10016–10021 https://doi.org/10.1073/pnas.1222606110
160 Ferrante, P., Ballottari, M., Bonente, G., Giuliano, G. and Bassi, R. (2012) LHCBM1 and LHCBM2/7 polypeptides, components of major LHCII complex, have distinct functional roles in photosynthetic antenna system of *Chlamydomonas reinhardtii*. *J. Biol. Chem.* **287**, 16276–16288 https://doi.org/10.1074/jbc.M111.316729
161 Girolomoni, L., Ferrante, P., Berteotti, S., Giuliano, G., Bassi, R. and Ballottari, M. (2017) The function of LHCBM4/6/8 antenna proteins in *Chlamydomonas reinhardtii*. *J. Exp. Bot.* **68**, 627–641 https://doi.org/10.1093/jxb/erw462
162 Depauw, F.A., Rogato, A., Ribera d'Alcala, M. and Falciatore, A. (2012) Exploring the molecular basis of responses to light in marine diatoms. *J. Exp. Bot.* **63**, 1575–1591 https://doi.org/10.1093/jxb/ers005
163 Zhu, S.-H. and Green, B.R. (2010) Photoprotection in the diatom *Thalassiosira pseudonana*: role of LI818-like proteins in response to high light stress. *Biochim. Biophys. Acta, Bioenerg.* **1797**, 1449–1457 https://doi.org/10.1016/j.bbabio.2010.04.003
164 Bailleul, B., Rogato, A., de Martino, A., Coesel, S., Cardol, P., Bowler, C. et al. (2010) An atypical member of the light-harvesting complex stress-related protein family modulates diatom responses to light. *Proc. Natl Acad. Sci. U.S.A.* **107**, 18214–9 https://doi.org/10.1073/pnas.1007703107
165 Lepetit, B., Goss, R., Jakob, T. and Wilhelm, C. (2012) Molecular dynamics of the diatom thylakoid membrane under different light conditions. *Photosynth. Res.* **111**, 245–257 https://doi.org/10.1007/s11120-011-9633-5
166 Lavaud, J. (2007) Fast regulation of photosynthesis in diatoms: mechanisms, evolution and ecophysiology. In *Functional Plant Science and Biotechnology* vol. 1, Global Science Books Ltd., UK, Ikenobe, Japan
167 Ruban, A., Lavaud, J., Rousseau, B., Guglielmi, G., Horton, P. and Etienne, A.-L. (2004) The super-excess energy dissipation in diatom algae: comparative analysis with higher plants. *Photosynth. Res.* **82**, 165–175 https://doi.org/10.1007/s11120-004-1456-1
168 Lavaud, J., Rousseau, B., van Gorkom, H.J. and Etienne, A.-L. (2002) Influence of the diadinoxanthin pool size on photoprotection in the marine planktonic diatom *Phaeodactylum tricornutum*. *Plant Physiol.* **129**, 1398–1406 https://doi.org/10.1104/pp.002014
169 Armbrust, E.V., Berges, J.A., Bowler, C., Green, B.R., Martinez, D., Putnam, N.H. et al. (2004) The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. *Science* **306**, 79–86 https://doi.org/10.1126/science.1101156
170 Yamamoto, H.Y. and Kamite, L. (1972) The effects of dithiothreitol on violaxanthin de-epoxidation and absorbance changes in the 500-nm region. *Biochim. Biophys. Acta* **267**, 538–543 https://doi.org/10.1016/0005-2728(72)90182-X
171 Yamamoto, H.Y., Wang, Y. and Kamite, L. (1971) A chloroplast absorbance change from violaxanthin de-epoxidation. A possible component of 515 nm changes. *Biochem. Biophys. Res. Commun.* **42**, 37–42 https://doi.org/10.1016/0006-291X(71)90358-5
172 Hager, A. and Holocher, K. (1994) Localization of the xanthophyll-cycle enzyme violaxanthin de-epoxidase within the thylakoid lumen and abolition of its mobility by a (light-dependant) pH decrease. *Planta* **192**, 581–589 https://doi.org/10.1007/BF00203597
173 Arnoux, P., Morosinotto, T., Saga, G., Bassi, R. and Pignol, D. (2009) A structural basis for the pH-dependent xanthophyll cycle in *Arabidopsis thaliana*. *Plant Cell* **21**, 2036–2044 https://doi.org/10.1105/tpc.109.068007
174 Bugos, R.C., Hieber, A.D. and Yamamoto, H.Y. (1998) Xanthophyll cycle enzymes are members of the lipocalin family, the first identified from plants. *J. Biol. Chem.* **273**, 15321–15324 https://doi.org/10.1074/jbc.273.25.15321
175 Jahns, P., Latowski, D. and Strzalka, K. (2009) Mechanism and regulation of the violaxanthin cycle: the role of antenna proteins and membrane lipids. *Biochim. Biophys. Acta, Bioenerg.* **1787**, 3–14 https://doi.org/10.1016/j.bbabio.2008.09.013
176 Adams, III, W.W., Demmig-Adams Logan, B.A., Barker, D.H. and Osmond, C.B. (1999) Rapid changes in xanthophyll cycle-dependent energy dissipation and photosystem II efficiency in two vines, *Stephania japonica* and *Smilax australis*, growing in the understory of an open eucalyptus forest. *Plant Cell Environ.* **22**, 125–136 https://doi.org/10.1046/j.1365-3040.1999.00369.x
177 Kondo, T., Pinnola, A., Chen, W.J., Dall'Osto, L., Bassi, R. and Schlau-Cohen, G.S. (2017) Single-molecule spectroscopy of LHCSR1 protein dynamics identifies two distinct states responsible for multi-timescale photosynthetic photoprotection. *Nat. Chem.* **9**, 772–778 https://doi.org/10.1038/nchem.2818
178 Li, X.-P., Gilmore, A.M. and Niyogi, K.K. (2002) Molecular and global time-resolved analysis of a psbS gene dosage effect on pH- and xanthophyll cycle-dependent nonphotochemical quenching in photosystem II. *J. Biol. Chem.* **277**, 33590–33597 https://doi.org/10.1074/jbc.M204797200
179 Li, Z., Ahn, T.K., Avenson, T.J., Ballottari, M., Cruz, J.A., Kramer, D.M. et al. (2009) Lutein accumulation in the absence of zeaxanthin restores nonphotochemical quenching in the *Arabidopsis thaliana* npq1 mutant. *Plant Cell* **21**, 1798–1812 https://doi.org/10.1105/tpc.109.066571
180 Dall'Osto, L., Caffarri, S. and Bassi, R. (2005) A mechanism of nonphotochemical energy dissipation, independent from PsbS, revealed by a conformational change in the antenna protein CP26. *Plant Cell* **17**, 1217–1232 https://doi.org/10.1105/tpc.104.030601
181 Reinhold, C., Niczyporuk, S., Beran, K.C. and Jahns, P. (2008) Short-term down-regulation of zeaxanthin epoxidation in *Arabidopsis thaliana* in response to photo-oxidative stress conditions. *Biochim. Biophys. Acta* **1777**, 462–469 https://doi.org/10.1016/j.bbabio.2008.03.002

Biochemical Society Transactions (2018) **46** 467–482
https://doi.org/10.1042/BST20170307

182 Nilkens, M., Kress, E., Lambrev, P., Miloslavina, Y., Müller, M., Holzwarth, A.R. et al. (2010) Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated under steady-state conditions in *Arabidopsis*. *Biochim Biophys Acta* **1797**, 466–475 https://doi.org/10.1016/j.bbabio.2010.01.001
183 Verhoeven, A. (2014) Sustained energy dissipation in winter evergreens. *New Phytol.* **201**, 57–65 https://doi.org/10.1111/nph.12466
184 Bilger, W. (2014) *Desiccation-induced quenching of chlorophyll fluorescence in cryptogams*, Springer, Dordrecht, pp. 409–420
185 Krause, G., Somersalo, S., Zumbusch, E., Weyers, B. and Laasch, H. (1990) On the mechanism of photoinhibition in chloroplasts. relationship between changes in fluorescence and activity of photosystem II. *J. Plant Physiol.* **136**, 472–479 https://doi.org/10.1016/S0176-1617(11)80038-6
186 Ohad, I., Kyle, D.J. and Arntzen, C.J. (1984) Membrane protein damage and repair: removal and replacement of inactivated 32-kilodalton polypeptides in chloroplast membranes. *J. Cell Biol.* **99**, 481–485 https://doi.org/10.1083/jcb.99.2.481
187 Takahashi, S. and Murata, N. (2005) Interruption of the Calvin cycle inhibits the repair of photosystem II from photodamage. *Biochim. Biophys. Acta, Bioenerg.* **1708**, 352–361 https://doi.org/10.1016/j.bbabio.2005.04.003
188 Staleva, H., Komenda, J., Shukla, M.K., Šlouf, V., Kaňa, R., Polívka, T. et al. (2015) Mechanism of photoprotection in the cyanobacterial ancestor of plant antenna proteins. *Nat. Chem. Biol.* **11**, 287–291 https://doi.org/10.1038/nchembio.1755
189 Rochaix, J.-D. (2007) Role of thylakoid protein kinases in photosynthetic acclimation. *FEBS Lett.* **58**, 2768–2775 https://doi.org/10.1016/j.febslet.2007.04.038
190 Wollman, F.A. (2001) State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. *EMBO J.* **20**, 3623–3630 https://doi.org/10.1093/emboj/20.14.3623
191 Rochaix, J.-D., Lemeille, S., Shapiguzov, A., Samol, I., Fucile, G., Willig, A. et al. (2012) Protein kinases and phosphatases involved in the acclimation of the photosynthetic apparatus to a changing light environment. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **367**, 3466–3474 https://doi.org/10.1098/rstb.2012.0064
192 Bellafiore, S., Barneche, F., Peltier, G. and Rochaix, J.-D. (2005) State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. *Nature* **433**, 892–895 https://doi.org/10.1038/nature03286
193 Bonardi, V., Pesaresi, P., Becker, T., Schleiff, E., Wagner, R., Pfannschmidt, T. et al. (2005) Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. *Nature* **437**, 1179–1182 https://doi.org/10.1038/nature04016
194 Allen, J.F. (1992) Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta* **1098**, 275–335 https://doi.org/10.1016/S0005-2728(09)91014-3
195 Drop, B., Yadav, K.N.S., Boekema, E. and Croce, R. (2014) Consequences of state transitions on the structural and functional organization of photosystem I in the green alga *Chlamydomonas reinhardtii*. *Plant J.* **78**, 181–191 https://doi.org/10.1111/tpj.12459
196 Kouril, R., Zygadlo, A., Arteni, A.A., de Wit, C.D., Dekker, J.P., Jensen, P.E. et al. (2005) Structural characterization of a complex of photosystem I and light-harvesting complex II of *Arabidopsis thaliana*. *Biochemistry* **44**, 10935–10940 https://doi.org/10.1021/bi051097a
197 Pribil, M., Pesaresi, P., Hertle, A., Barbato, R. and Leister, D. (2010) Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow. *PLoS Biol.* **8**, e1000288 https://doi.org/10.1371/journal.pbio.1000288
198 Shapiguzov, A., Ingelsson, B., Samol, I., Andres, C., Kessler, F., Rochaix, J.-D. et al. (2010) The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in *Arabidopsis*. *Proc. Natl Acad. Sci. U.S.A.* **107**, 4782–4787 https://doi.org/10.1073/pnas.0913810107
199 Kasahara, M., Kagawa, T., Oikawa, K., Suetsugu, N., Miyao, M. and Wada, M. (2002) Chloroplast avoidance movement reduces photodamage in plants. *Nature* **420**, 829–832 https://doi.org/10.1038/nature01213
200 Dall'Osto, L., Cazzaniga, S., Wada, M. and Bassi, R. (2014) On the origin of a slowly reversible fluorescence decay component in the *Arabidopsis* npq4 mutant. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **369**, 20130221 https://doi.org/10.1098/rstb.2013.0221
201 Horton, P., Wentworth, M. and Ruban, A. (2005) Control of the light harvesting function of chloroplast membranes: the LHCII-aggregation model for non-photochemical quenching. *FEBS Lett.* **579**, 4201–4206 https://doi.org/10.1016/j.febslet.2005.07.003
202 Avenson, T.J., Ahn, T.K., Zigmantas, D., Niyogi, K.K., Li, Z., Ballottari, M. et al. (2008) Zeaxanthin radical cation formation in minor light-harvesting complexes of higher plant antenna. *J. Biol. Chem.* **283**, 3550–3558 https://doi.org/10.1074/jbc.M705645200
203 Miloslavina, Y., Wehner, A., Lambrev, P.H., Wientjes, E., Reus, M., Garab, G. et al. (2008) Far-red fluorescence: a direct spectroscopic marker for LHCII oligomer formation in non-photochemical quenching. *FEBS Lett.* **582**, 3625–3631 https://doi.org/10.1016/j.febslet.2008.09.044
204 Bode, S., Quentmeier, C.C., Liao, P.-N., Hafi, N., Barros, T., Wilk, L. et al. (2009) On the regulation of photosynthesis by excitonic interactions between carotenoids and chlorophylls. *Proc. Natl Acad. Sci. U.S.A.* **106**, 12311–12316 https://doi.org/10.1073/pnas.0903536106