ANNUAL REVIEWS

Annual Review of Plant Biology

Dissipation of Light Energy Absorbed in Excess: The Molecular Mechanisms

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Annu. Rev. Plant Biol. 2021. 72:47–76

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-071720-015522

Keywords

photosynthesis, photoinhibition, oxidative stress, antioxidant, thermal energy dissipation

Abstract

Light is essential for photosynthesis. Nevertheless, its intensity widely changes depending on time of day, weather, season, and localization of individual leaves within canopies. This variability means that light collected by the light-harvesting system is often in excess with respect to photon fluence or spectral quality in the context of the capacity of photosynthetic metabolism to use ATP and reductants produced from the light reactions. Absorption of excess light can lead to increased production of excited, highly reactive intermediates, which expose photosynthetic organisms to serious risks of oxidative damage. Prevention and management of such stress are performed by photoprotective mechanisms, which operate by cutting down light absorption, limiting the generation of redox-active molecules, or scavenging reactive oxygen species that are released despite the operation of preventive mechanisms. Here, we describe the major physiological and molecular mechanisms of photoprotection involved in the harmless removal of the excess light energy absorbed by green algae and land plants. In vivo analyses of mutants targeting photosynthetic components and the enhanced resolution of spectroscopic techniques have highlighted specific mechanisms protecting the photosynthetic apparatus from overexcitation. Recent findings unveil a network of multiple interacting elements, the reaction times of which vary from a millisecond to weeks, that continuously maintain photosynthetic organisms within the narrow safety range between efficient light harvesting and photoprotection.

Contents

INTRODUCTION

Photoprotection: set of mechanisms that limit generation of oxidizing molecules, detoxify reactive oxygen species, and repair oxidative damage of photosynthetic structures

Excess light: light energy absorbed in excess with respect to the capacity of downstream metabolism to use it

Why do plants and algae depend on light for their autotrophic metabolism and yet need photoprotection against excess light? There are at least three answers. First, the dynamic range of light supply is very high. The available light on the earth's surface changes every day from low intensity at dawn to very high at midday. Although photosynthetic organisms can adapt their functional architecture to level out the light absorption by modulating the size of their photosynthetic antenna systems through regulatory tuning (referred to as acclimation), this requires several days (41), which makes it impossible to cope with the diurnal change in photon fluxes. Too fast an increase in irradiance leads to the absorption of too much light, exceeding the capacity of plants and algae to use the photons in reaction centers (RCs) for photochemistry (specifically, charge separation) and, later, for CO_2 reduction, which is orders of magnitude slower than charge separation (**Figure 1**). When the capacity for charge separation is exceeded (referred to as overexcitation), the excited pigments return to the ground state by other mechanisms, possibly resulting in the generation of reactive compounds, which cause photooxidative damage to the components of the photosystems, and, ultimately, inhibiting photosynthesis (or photoinhibition). The second answer is the high pigment content of leaves. Plants accumulate a large surplus of chlorophylls (Chls) in their leaf mesophyll, relative to the optimal amount needed for light harvesting, in order to compete with shading competitors (29). This creates steep light gradients in canopies, further exacerbating the impact of sudden changes in light intensity and quality (spectrum). Third, plants grow continuously, requiring continuous synthesis of the photosynthetic apparatus, which involves the

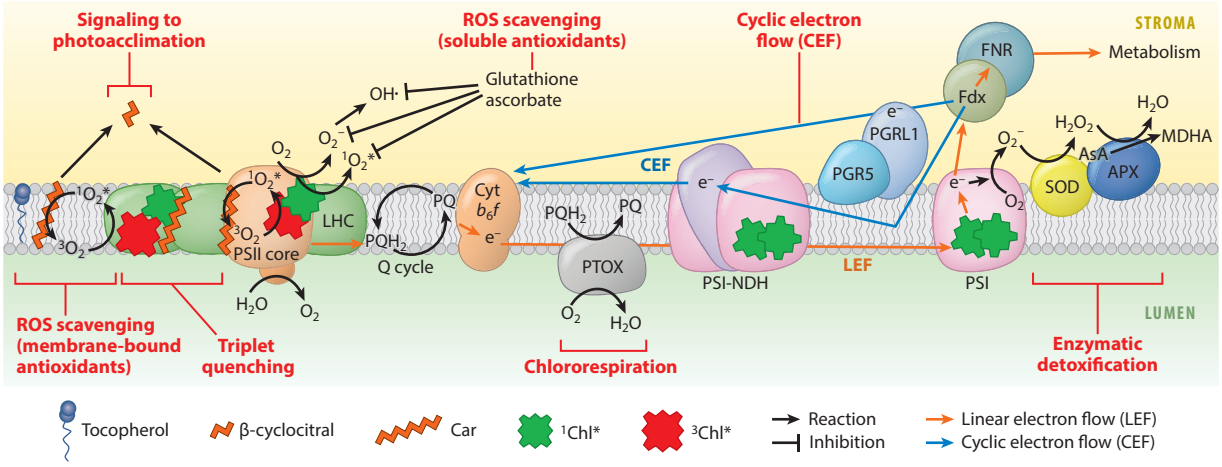

Figure 1

Photoprotective mechanisms in thylakoid membranes. The main protein complexes involved in the pathways of electron transport in the thylakoid membranes are depicted schematically, showing the sites of ROS production and the mechanisms that prevent light energy from inducing damage. In pigment-binding complexes, intersystem crossing $^1Chl^* \rightarrow {}^3Chl^*$ occurs. Excitation energy transfer from $^3Chl^*$ to O_2 in the triplet ground state generates excited singlet O_2 ($^1O_2^*$). Electron transfer to O_2 (univalent reduction of O_2) produces O_2^- (superoxide anion), which in turn can generate hydroxyl radical (OH·). CEF pathways (PGR5/PGRL1, NDH) and PTOX balance the redox state of electron carriers. Cars scavenge ROS, modulate $^3Chl^*$ yield, and regulate $^1Chl^*$ lifetime; moreover, Cars generate stress-signaling compounds that feed the pathways by activating acclimation to excess light. Other antioxidant systems include the compounds tocopherols, Asa, glutathione, and the enzymatic components SOD, APX, and glutathione peroxidase. Abbreviations: AsA, ascorbate; Car, carotenoid; CEF, cyclic electron flow; Chl, chlorophyll; Cyt, cytochrome; Fdx, ferredoxin; FNR, Fdx-$NADP^+$ oxidoreductase; LHC, light-harvesting complex; MDHA, monodehydroascorbate; PQ/PQH_2, plastoquinone/plastoquinol; PSII, photosystem II; PTOX, plastid terminal oxidase; ROS, reactive oxygen species.

coordinated assembly of proteins, lipids, and pigments. Less than perfect coordination between biosynthetic pathways and the assembly steps can lead to an imbalance between pigment and protein accumulation. Among the pigments are Chls, which absorb photons to generate the above-mentioned reactive excited states. Pigment-protein breakdown also occurs continuously during the life span of plants, and this also poses risks with respect to the exposure of assembly intermediates to light, and hence the prospect for photodamage. Therefore, most photosynthetic organisms must deal with excess light daily or seasonally. The amount of light that is defined as excess is modified by environmental conditions (116). If the capacity of the system to use light energy or the products of the light reactions are reduced because of environmental stress, such as cold, drought, salinity, heat, or nutrient deprivation, the utilization of the incident light is limited, and stress is worsened (182).

The importance of photoprotection is highlighted by the large number of mechanisms by which chloroplasts sense excess light and modify the molecular architecture of their photosynthetic apparatus in order to maintain photosynthetic efficiency. Many excellent reviews have been published on general aspects (91, 113) or that focus on the more specific aspects of acclimation (180), biogenesis (133), redox signaling (52) and the role of photoreceptors in activating photoprotection mechanisms (4). Here, we review the diverse photoprotective mechanisms that are activated to limit overreduction of photosynthetic electron chains and to sense and dissipate excitation pressure. The molecular physiology of photoprotective processes is discussed in light of recent insights provided by molecular biological, genetic, and spectroscopic approaches.

Antenna system: an array of membrane-embedded, light-harvesting complexes (LHCs) binding chlorophylls and carotenoids that form a modular system providing excitation energy to photosystem core complexes

PHOTOINHIBITION AND ITS CAUSES

Reaction center (RC): a transmembrane complex, containing polypeptides, electron donors, and acceptors, catalyzing the charge separation reaction, which fuels photosynthetic electron transport, using excitation energy transferred from antenna systems

Excitation pressure: the concentration of excited states in the antenna system of a photosystem; high excitation pressure increases the probability of PSII RC shifting to closed state

Reactive oxygen species (ROS): oxygen redox states intermediate between H_2O and O_2, including superoxide, hydrogen peroxide, and hydroxyl radical

P680: a pair of chlorophylls, present in the core complexes of PSII, which undergoes oxidation upon photon excitation ($P680^* \rightarrow P680^+$)

Photochemical quenching: a process that brings back excited P680* to the ground state P680, through a photochemical reaction, thus quenching ^{1}Chls* in the antenna

Photosynthesis is a complex mechanism that includes steps catalyzed in lifetimes over a wide time range. In both photosystem (PS) I and PSII, charge separation occurs in picoseconds, while the slowest reaction in photosynthetic metabolism 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 10^{-3} s. The time constants of these reactions are so different that a flux balance could only be reached in steady-state light conditions by assembling electron transport chains with components in vastly different stoichiometries. Under natural conditions, the dynamic range and spectral distribution of irradiance, however, exceed the capacity of both the assimilatory reactions and regulation mechanisms, thus potentially giving way to overexcitation and photodamage. The major mechanisms of photodamage are two: (*a*) reduction of O_2 to superoxide anion (O_2^-), hydroxyl radical (OH·) (99), and reactive oxygen species (ROS) and (*b*) the release of singlet oxygen ($^1O_2^*$) (164, 165). Although not technically a ROS, singlet oxygen is often included in this group, because of its similar oxidative properties. Both species lead to photoinhibition and undermine productivity (9).

The major sources for the generation of ROS are the PSII RC, PSI, and the light-harvesting complex (LHC) of PSII (110), LHCII. Solar energy is captured mainly by Chls bound to the LHCs, which undergo transition to ^{1}Chls*. Energy is then excitonically transferred to the RC, where it promotes photochemical reactions. In PSII RC, the special Chl pair P680 is excited to P680* and transfers electrons to pheophytin, which, in turn, reduces quinone molecules, Q_A and then Q_B. Reduced Q_B transfers electrons to plastoquinone (PQ). P680 transiently assumes a positive charge ($P680^+$), neutralized, within 30 μs, by electrons from a specific tyrosine, named Z, in the D1 polypeptide of PSII, linked to a water-splitting reaction (143). While electron flow from P680* to PQ enables efficient photochemical quenching of ^{1}Chls* in the antenna, the accumulation of large amounts of reduced plastoquinol (PQH_2) promotes the reverse reaction, which is charge recombination from Q_A^-/Q_B^- to $P680^+$, restoring P680*. Charge recombination and also the rapid equilibration of P680 with antenna pigments can generate P680* beyond the capacity of the electron transfer chain. Therefore, the lifetime of ^{1}P680* increases, enhancing the probability of intersystem crossing to ^{3}P680* (139). ^{3}P680* readily reacts with O_2 in the triplet ground state ($^3O_2$), generating $^1O_2^*$, which will damage nearby protein and lipid molecules, in particular the D1 subunit of PSII (174). In addition to $^1O_2^*$, PSII can generate both O_2^- and OH· under excess light (127).

PSI can also experience photoinhibition by ROS (159) produced when PSI-reducing activity exceeds the capacity for the use of reducing equivalents for downstream reactions. Thus, production of O_2^- occurs within PSI (80); O_2^- can directly damage the peripheral subunits of PSI, limiting electron transfer from the excited state of the special Chl pair P700 (^{1}P700*) to acceptor Chl and enhancing triplet excited P700 (^{3}P700*) formation (138), $^1O_2^*$ release, and PSI photoinhibition (25).

Photooxidative stress also occurs due to Chls bound to LHC proteins since RC closure in excess light decreases photochemical quenching of ^{1}Chl* and enhances ^{3}Chl* yield (169), either in loosely coupled LHC subunits or in unbound Chls (126), during biogenesis of pigment-binding proteins (76). While oxygenic photosynthesis has been functioning for a billion years, high O_2 levels only accumulated in the last 500 million years (30). Release of ROS is an unavoidable consequence of Chl excited states and their reaction with O_2. The protection mechanisms were developed by photoautotrophs during a long evolutionary process in order to react to enhanced photooxidation.

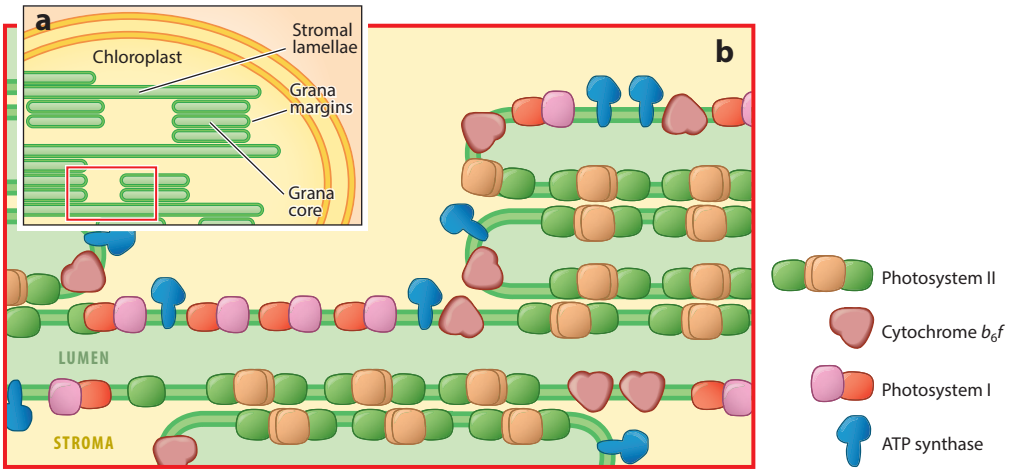

Figure 2

Chloroplasts (*a*) are organelles found in the cells of plants and algae, in which photosynthesis takes place. They are enclosed by a double membrane, the so-called envelope, while a third membrane system, the thylakoids, hosts all of the pigment–protein complexes and electron carriers that drive light reactions. The thylakoids consist of stacks of vesicles, which form the grana regions. Grana include two distinct functional domains, core and margins. Grana are connected by unstacked domains called stromal lamellae. The volume delimited by the thylakoids is the lumenal space (*b*, *red border detail*). The photosynthetic complexes are unevenly distributed in the membrane system: Photosystem II localizes in the grana core and is segregated from Photosystem I, which is exclusively found in the stromal lamellae and grana margins. Cytochrome b_6f and ATP synthase populate both the grana margins and the stromal lamellae (78).

REGULATION OF PHOTOSYNTHETIC ELECTRON TRANSPORT AND PHOTOPROTECTION

Under light-limited conditions, most of the energy absorbed by the antenna pigments is used in photochemistry, which drives linear electron flow (LEF) from H_2O to NADPH, resulting in O_2 evolution, assimilatory $CO_2/NO_3^{1-}/SO_4^{2-}$ reduction and support to cell functions. The complexes PSII, PSI, and Cytb_6f cooperate to drive LEF and to generate the *trans*-thylakoid ΔpH. The thylakoid membranes fold to form distinct compartments: the grana partition, forming paired vesicles further stacked into piles, and the stromal lamellae, unstacked thylakoids connecting the granas. Grana formation establishes two functionally distinct domains, namely the grana core surrounded by the grana margins. The distribution of photosynthetic complexes within the thylakoid membranes is heterogeneous: Functional PSII resides mainly in the grana core, PSI in the stromal domains, and ATPase and Cytb_6f are present in both grana margins and stromal lamellae (**Figure 2**).

In order to maintain photosynthetic electron transport in an environment of contrasting light conditions, the tuning of excitation pressure versus partitioning of reducing power is a crucial factor (132). The assimilatory rate of photosynthesis is optimized to the prevailing light conditions by long-term acclimation responses, which involve changes in gene expression, metabolic fluxes, and enzyme activities (167). Regulatory mechanisms, in addition, affect a number of alternative electron transport pathways, which were shown to be relevant for preventing photoinhibition (**Figure 1**).

Cyclic electron flow (CEF) around PSI prevents the photoinhibition of PSII: CEF increases electron transfer from PSI back to PQ without the 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

Intersystem crossing: photophysical event of electron spin inversion from the low-lying singlet ^{1}Chl* to the triplet excited state (^{3}Chl*), which is favored by long lifetimes of ^{1}Chl* states

P700: a pair of chlorophylls, present in the core complexes of PSI, which undergoes oxidation upon photon excitation ($P700^* \rightarrow P700^+$)

synthesis of ATP and the induction of energy dissipation (61) (see the section titled Nonphotochemical Quenching).

CEF occurs via two redundant pathways: The major one requires a complex involving proton gradient regulation 5 (PGR5) and PGR5-like photosynthetic phenotype 1 (PGRL1) (63), while the minor pathway requires an NAD(P)H dehydrogenase-like complex (NDH) (120). Tobacco *ndh* mutants (145), which are sensitive to heat and drought stresses, suggest that this pathway is essential for photoprotection (69, 177). Similar phenotypes are exhibited by *pgr5*, *pgrl1*, and *crr* (chlororespiratory reduction) mutants (31, 108). In addition to the linear photosynthetic electron flow, a chloroplast respiratory electron transport pathway, defined as chlororespiration, transfers electrons from NAD(P)H to O_2 via the PQ pool, thus preventing the complete oxidation of the PQ pool in the absence of light as well as its full reduction in excess light. The chloroplastic components involved in this process are an NAD(P)H dehydrogenase and a plastoquinol terminal oxidase (PTOX), the latter deployed to divert electrons from PQH_2 to O_2, producing H_2O (171). Together, these provide and remove electrons, respectively, thus balancing the redox state of electron carriers (83).

RC closure: condition of an RC holding a positive charge; it cannot undergo further charge separation (until it is reduced) and is thus defined as closed

^{1}Chl*: chlorophyll singlet excited state, arising upon absorption of a light quantum

Xanthophylls: oxygen-containing carotenoids

Excitation energy transfer: the mechanism by which excitation energy is rapidly transferred between antenna pigments on its way to the RC

THE CHLOROPLAST ANTIOXIDANT NETWORK

A complex antioxidant network in the chloroplast deactivates ROS and minimizes photodamage. The detoxification systems include ROS scavengers and enzymatic antioxidant components (**Figure 1**).

Carotenoids (Cars), including oxygenated derivatives called xanthophylls, make a crucial contribution to chloroplast photoprotection. Cars are present in thylakoid membranes in two distinct pools: (*a*) a fraction free in lipid phase, which scavenges ROS released from LHCs and RC complexes, and (*b*) Cars bound to photosynthetic machinery, where they are in close contact with Chls (36). Despite the great diversity generated by evolution, the carotenoid content of land plants is well conserved in both the overall composition and the localization in the photosynthetic complexes: β-Carotene is bound to the RC, while LHCs bind xanthophylls lutein (Lut), violaxanthin (Vio), neoxanthin (Neo), and, once accumulated under excess light, zeaxanthin (Zea). While free Cars undergo fast photooxidation (181) due to the effect of ROS produced by the reaction of ^{3}Chl* with O_2, pigment-protein complexes are far more resistant due to synergic effects, including ROS scavenging, modulation of ^{3}Chl* yield, and regulation of ^{1}Chl* lifetime (53).

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*. ^{3}Car* decays into the ground state, while the excitation energy is safely converted into heat (48).

Neo has a specific function as a quencher of O_2^- (37), while Vio's photoprotection role is $^1O_2$* scavenging (39). In excess light, Vio is de-epoxidized to Zea, with enhanced antioxidant and ^{3}Chl*-quenching activity. In addition, the binding of Zea to LHC proteins upregulates thermal energy dissipation, thereby undermining light-harvesting efficiency (38). β-Carotene is a component of both PSI and PSII core complexes, suggesting it has a role in mitigating oxidative damage under excess light conditions (161). Recently, $^{14}CO_2$-labeling studies showed that carbon flux is far greater in β-carotene compared to downstream xanthophylls, implying that most of the β-carotene undergoes oxidative degradation within complexes and is turned over at high rates at its binding sites (15).

The degradation of β-carotene has been shown to produce β-cyclocitral, both through direct oxidation by $^1O_2$* or, enzymatically, by carotenoid cleavage dioxygenases (CCDs). The observation that knocking out CCDs in *Arabidopsis* did not significantly affect the β-cyclocitral level (128)

suggests that β-cyclocitral is mostly produced by direct oxidation from $^1O_2^*$, particularly under excess light stress (32). Exposure of *Arabidopsis* leaves to β-cyclocitral changed transcription in a similar way to that observed during $^1O_2^*$ stress and increased stress resistance to excess light and drought, implying that β-cyclocitral is a stress-signaling compound downstream of the primary events of oxidative stress. Sensitivity to β-cyclocitral and the enhancement of stress resistance require the MBS1 protein (146), without which ROS response is deregulated. Other genes induced by β-cyclocitral encode xenobiotic detoxifying enzymes, which act by modifying these signal molecules and compartmentalizing them in the vacuole (130). Loss of SCL14 (SCARECROW-like 14) regulative factor, which is required for the transcriptional activation of genes involved in the detoxification of xenobiotics, results in plants that do not respond to β-cyclocitral. The plants remain sensitive to excess light stress, suggesting that detoxification mechanisms, activated by endogenous harmful metabolites that are products of the decomposition of lipid peroxides (98), are key components of the excess light stress resistance.

In this way, Cars, while participating in chemical and photophysical reactions related to photoprotection, also feed the signaling pathways and activate the defense mechanisms involved in the acclimation to excess light. Nevertheless, the full landscape of apocarotenoid signaling in unicellular algae still needs to be defined.

The strong coupling between Chl and Car within pigment-protein complexes results in excitation energy transfer from ^{3}Chl* to Car, yielding the Car triplet excited states (^{3}Car*). Indeed, the ^{3}Car* population increases with light intensity in leaves, thylakoid membranes, and isolated complexes (38). Quenching of ^{3}Chl* prevents $^1O_2^*$ formation. Lut, the most abundant xanthophyll species in the photosynthetic apparatus of plants and green algae, promotes ^{3}Chl* quenching and also has a role in scavenging of ROS (39).

Moreover, Cars modulate the ^{1}Chl* population in photosynthetic complexes. Under excess excitation energy, excited Chls cannot be quenched by P680 because it is in the closed state. Hence ^{1}Chl* builds up, with the potential to generate reactive species causing photodamage. Alternatively, ^{1}Chl* can relax to the ground state by thermal degradation thereby decreasing the probability of intersystem crossing to generate ^{3}Chl*. This process can be observed through an acceleration of Chl fluorescence decay. Cars are involved in this process as quenchers of the ^{1}Chl* population in photosynthetic complexes.

In organic solvent, the fluorescence lifetime of ^{1}Chl* is 6 ns but decreases in detergent-solubilized LHC antenna proteins to 3.7–4 ns (105) and drops further to 2.8 ns in a more physiological environment, such as in thylakoid membranes or lipid nanodiscs, thereby decreasing the probability of ^{3}Chl* formation (149). The Chl fluorescence lifetime of the LHCII antenna, binding most of the Chl in leaves, depends on the properties of the Chl-xanthophyll interactions.

In addition to Car, other nonenzymatic antioxidant components include the following:

1. Prenylquinols, such as tocopherol and plastoquinols, mediate chemical scavenging of $^1O_2^*$ (60, 82). There are free tocopherols in the thylakoid lipid matrix, where they can be oxidized as a tocopheryl radical in a one-electron-transfer reaction or can react with $^1O_2^*$ to form a hydroperoxide, which is the equivalent to a two-electron-transfer reaction (109) (**Figure 3**). In plants, tocopherol, ascorbate, and Cars have overlapping functions of protection. Mutants with decreased ascorbate or low Cars content display a compensatory increase in tocopherol (106, 107).
2. Ascorbate is the most abundant soluble antioxidant in chloroplasts. Ascorbate acts (*a*) through the direct quenching of $^1O_2^*$, O_2^-, and OH·, (*b*) in regenerating α-tocopherol from α-tocopheryl radicals, (*c*) as a cofactor for Vio-Zea conversion, (*d*) as an electron donor

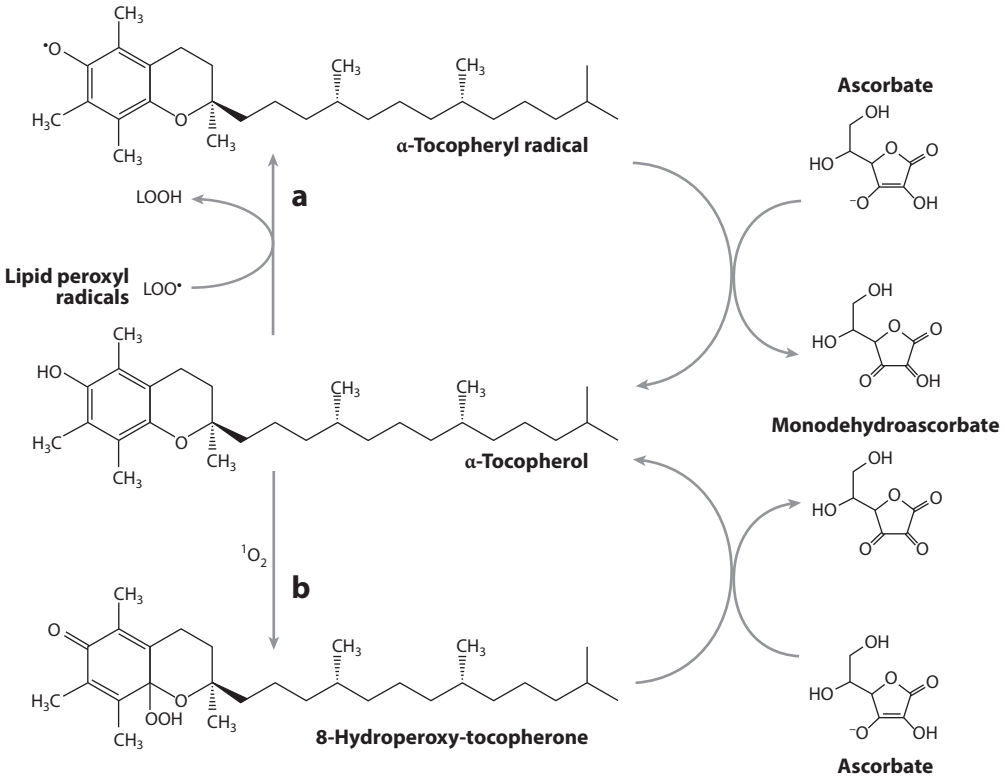

Figure 3

Oxidation mechanisms of tocopherol in the thylakoid membranes. (*a*) Tocopherol can be oxidized to a tocopheryl radical in a one-electron-transfer redox reaction; tocopherol acts as electron donor in reactions with ROS-generated lipid peroxyl radicals. (*b*) Tocopherol is converted to hydroperoxides upon reaction with $^1O_2$ in a two-electron-transfer mechanism. Both of these products can be rereduced to tocopherol by ascorbate. Abbreviation: ROS, reactive oxygen species.

to PSII, and (*e*) as an OH· scavenger though ascorbate peroxidase (APX) (147). *Arabidopsis* ascorbate-deficient mutants *vtc* are hypersensitive to many oxidative stresses (147), while the ascorbate-overproducing mutant *miox4* prevents PSII damage in heat-stressed leaves (168).

3. Glutathione plays a key role in detoxifying $^1O_2$* and OH· and is involved in the regeneration of both α-tocopherol and ascorbate by the glutathione–ascorbate cycle (44).

Enzymatic antioxidant components include superoxide dismutase (SOD), APX, catalase, glutathione reductase, and peroxiredoxins (101). In this context, the water–water cycle is essential to avoid photodamage in PSI: In excess light, photoreduction of O_2 in PSI can occur, thereby generating O_2^-, which can be enzymatically converted into H_2O_2 by SOD. The hydrogen peroxide detoxification system in chloroplasts is operated by the ascorbate–glutathione cycle, in which APX utilizes ascorbate to reduce H_2O_2 to H_2O. These reactions consume excess electrons, reducing the excitation pressure on PSI, but contribute to the generation of a ΔpH without concomitant utilization of ATP. In algae and mosses, flavodiiron proteins catalyze O_2 to H_2O_2 reactions in a single step (3), suggesting that their expression in crops is a possible strategy for improving resistance to abiotic stresses.

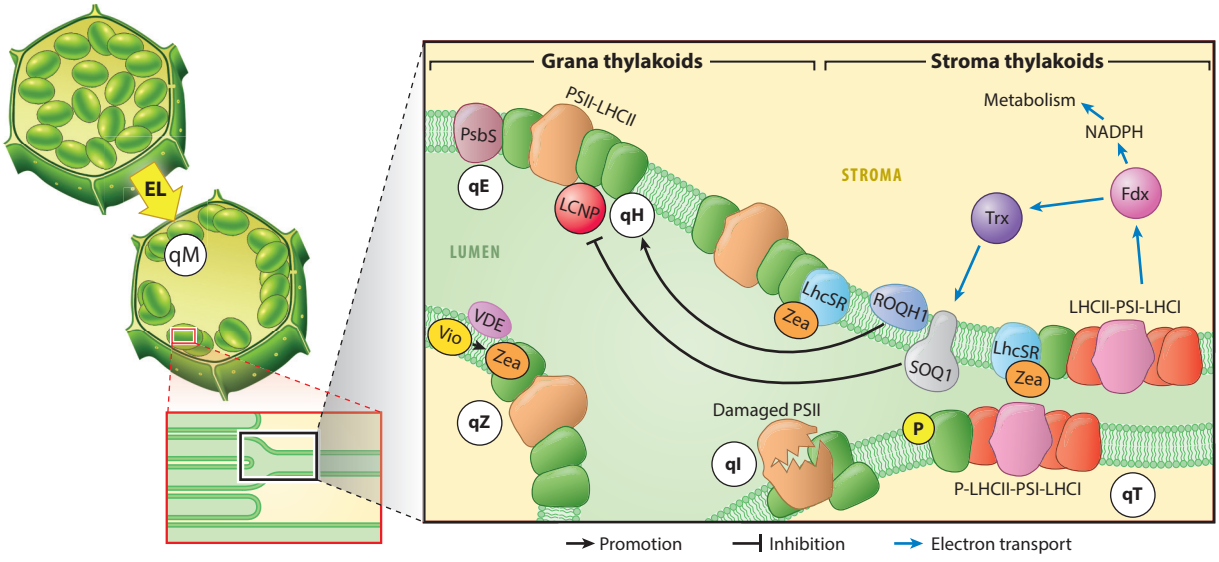

Figure 4

Model for the localization of different EL-induced photoprotective processes at the cellular and molecular levels. In a plant cell, light induces chloroplast movements: Chloroplasts align to the upper and lower surfaces of cells in low light, while they align along the sides of cells under EL (light avoidance response). In the thylakoid membranes, rapidly induced energy quenching (qE) is triggered in the PSII outer antenna system by protonation of lumen-exposed acidic residues in LhcSR (algae), PsbS (plants), or both (mosses). LhcSR is localized in the stroma membranes. It quenches both PSI and PSII antenna systems. Its interaction with PSII supercomplexes occurs at the grana margins. These responses require the xanthophyll Zea, produced from preexisting Vio under EL. Zea accumulation is also responsible for qZ, a slower component of NPQ independent from both PsbS and lumen acidification. LCNP is a lipocalin localized in the thylakoid lumen where it induces sustained quenching (qH) in the peripheral antenna of PSII. Its activity is regulated depending on both ROQH1 and the redox poise of stromal electron donors: Excess reducing power under stress reduces SOQ1 inhibition of LCNP activity. The slowest quenching component, qI, is attributed to photodamaged PSII RC, possibly induced by the binding of one-helix LHC-like proteins with quenching properties. Fluorescence emission is also decreased by the displacement of the LHCII antenna from PSII to PSI upon state transitions (qT) and by the chloroplast light avoidance response (qM). Notice that only qE, qZ, qH, and qI are genuine NPQ processes. Abbreviations: EL, excess light; Fdx, ferredoxin; LHC, light-harvesting complex; NPQ, nonphotochemical quenching; P-LHCII, phosphorylated LHCII; PS, photosystem; RC, reaction center; Trx, thioredoxin; Vio, violaxanthin; Zea, zeaxanthin.

EXCESS LIGHT–INDUCIBLE PHOTOPROTECTIVE MECHANISMS

Nonphotochemical Quenching

Nonphotochemical quenching (NPQ): quenching of chlorophyll fluorescence caused by processes alternative to charge separation; under excess light, NPQ is photoprotective by safely dissipating excitation energy in excess as heat

Since ^{3}Chl* production is an intrinsic property of Chls, the capacity to control its formation is essential for plant survival and is operated by several inducible mechanisms that are effective in quenching excess ^{1}Chl* (that cannot be used for photochemistry) and safely dissipating excited states as heat. Collectively, these mechanisms are referred to as nonphotochemical quenching (NPQ) in contrast to photochemical quenching, which refers to quenching of the excited ^{1}Chl* in the RC by charge separation. NPQ was originally measured as a decrease in the Chl fluorescence of leaves when illuminated with enough actinic light to saturate photosynthesis: Since photochemistry is saturated, the quenching of the excited Chl can only be nonphotochemical. Within NPQ, a number of components (depicted in **Figure 4**) have been identified, based on the timescales of their rise in excess light and relaxation on return to a dark state. These are triggered by acidification of the thylakoid lumen, which is a consequence of the saturation of photosynthesis. The proton concentration in the lumen results from the balance between the rate of photosynthetic

Actinic light: a light source able to excite Chl for photochemical reactions, such as photosynthetic electron transport, or nonphotochemical reactions, such as NPQ

electron transport and the dissipation of the pH gradient by the activity of the ATPase: In excess light, the saturation of the Calvin-Benson-Bassham cycle limits the regeneration of Pi and ADP, the substrates of the ATP synthase. Lumen acidification occurs both directly and through the activation of CEF, which enhances H^+ transport to the lumen and triggers NPQ (75). In plants and algae, other mechanisms modulate the fluorescence yield. The antenna systems of PSII and PSI differ in their light absorption properties, while changes in the quality of incident light can lead to unequal excitation of the two PSs, thus impairing photosynthetic yield. Photoautotrophs can balance the light energy absorbed by the PSs in a process known as state transition, which redistributes antenna proteins between PSs to optimize photosynthetic electron flow during short-term light quality acclimation (5). As a result of this rearrangement, the displacement of the LHCII antenna from PSII to PSI results in fluorescence quenching called qT (see the section titled State Transition-Induced Fluorescence Quenching (qT) and Spillover). In addition to these components, fluorescence emission is also decreased by the chloroplast light avoidance response (23).

Although the dissection of different fluorescence decline processes is not a trivial matter, it can be performed empirically. The use of pulse amplitude modulation (PAM) fluorometry is widespread in plant physiology laboratories. In this procedure, a supersaturating light pulse is directed at dark-adapted leaves or cells in order to determine the maximal level of fluorescence (F_m). When actinic light is switched on to build up quenching(s), additional supersaturating flashes are delivered to determine F_m', at different times, and the NPQ is then calculated as $(F_m - F_m')/F_m'$ (12). However, this simple and effective procedure cannot, for example, disentangle quenching from decreased fluorescence due to the bleaching of the sample or other processes. Alternatively, direct determination of fluorescence lifetime, irrespective of the amplitude, can be carried out by applying a PAM-like protocol but using fast pulsed light rather than continuous sources, coupled with time-gated detection within the short dark windows of actinic light (157). This measurement through fluorescence lifetime snapshots produces lifetime values that are reliable indicators of quenchers and are insensitive (within limits) to changes in fluorescence amplitude. For example, PSII fluorescence kinetics have a quenching component dependent on lumen acidification, which develops within 1–2 min, superimposed on a slower component due to chloroplast light avoidance, which develops within tens of minutes (23). Fluorescence lifetime selectively detects the first component while remaining insensitive to the latter, since it does not involve a genuine quenching reaction (157) (**Table 1**).

The Relation Between Energy Quenching, Reactive Oxygen Species Release, and Photodamage

When studying quenching processes and their photoprotective effects, the researcher is faced with the problem of separating quenching from loss of PSII maximal quantum yield. Increase in minimal Chl fluorescence F_0 can be due to an inefficient connection of the antenna to the PSII core as in the *no minor* (*NoM*) mutant (35), sustained quenching in LHCII as in the *suppressor of quenching 1* (*soq1*) mutant (97), or PSII damage (18), all resulting in a decrease of the photochemical yield of PSII, measured by the Chl fluorescence parameter F_v/F_m, calculated as $(F_m - F_0)/F_m$ (where F_0 is the very low fluorescence emitted from dark-adapted leaves that have all of their PSII RCs in the open state and therefore are excellent quenchers). The kinetic analysis of fluorescence parameters during dark relaxation is therefore a better indicator of photochemical and quenching processes than the F_v/F_m ratio (96).

Fluorescence is the technique of choice when a reliable protocol is available. Measuring the photochemical quenching parameter in the dark (qPd) following a series of lighting setups with increasing levels of light was proposed to identify the photon flux at which qPd is less than 1 due to a higher measured dark fluorescence level (F_0') with respect to the true F_0' value [calculated as

Table 1 Mechanisms that contribute to NPQ

Energy dissipation mechanism	Description	Mutant/gene affected (organism)	Reference(s)
qE	It is the strongest NPQ component, rapidly forming in high light (rise time ~0.5–2 min) and rapidly reversible in low light. It has been localized in the PSII antenna and assigned in plants to a synergistic action of the lumen acidification, the activation of the pH sensor PsbS, and the conversion of the xanthophyll violaxanthin to zeaxanthin. In green algae, qE is triggered by protonation of lumen-exposed acidic residues in the LhcSR proteins. Because of its dependence on *trans*-thylakoid pH gradient, qE is also defined as energy quenching.	*npq1*/VDE (*Arabidopsis thaliana*)	115
		npq1/CVDE (*Chlamydomonas reinhardtii*)	90
		npq2/ZE (*A. thaliana*, *C. reinhardtii*)	114, 115
		npq4/PSBS (*A. thaliana*)	86
		npq4/LHCSR (*C. reinhardtii*)	118
qZ	Its formation (rise time ~8–10 min) correlates with the synthesis and accumulation of zeaxanthin. Comparative analysis of NPQ mutant *npq1*, unable to form zeaxanthin, versus WT showed that qZ is a zeaxanthin-dependent NPQ component.	*npq1*/VDE (*A. thaliana*)	112, 115
qT	This quenching component is due to the movement of phosphorylated LHC proteins away from PSII and their transfer to PSI. Since PSI is a stronger quencher than PSII, this results in a decreased fluorescence yield. The fluorescence decline time of this component is ~10–12 min.	*stn7*/STN7 (*A. thaliana*)	5, 16
		stt7/STT7 (*C. reinhardtii*)	84
qH	qH is a sustained Chl fluorescence quenching component; i.e., it relaxes very slowly (decay time > 30 min). This ΔpH-independent form of NPQ has been reported to be independent from PSII RC damage.	*soq1*/SOQ1 (*A. thaliana*)	21
		lcnp/LCNP (*A. thaliana*)	97
		roqh1/ROQH1 (*A. thaliana*)	7
qI	Photoinhibitory quenching qI comprises all processes that relax extremely slowly (hours or longer). These Chl fluorescence quenching components originate from photoinhibition, namely the EL-induced decrease of the quantum yield of photosynthesis due to inactivation and/or degradation of the PSII RC.	*lqy1*/LQY1 (*A. thaliana*)	95
		hhl1/HHL1 (*A. thaliana*)	73
		mph2/MPH2 (*A. thaliana*)	93
qM	It is a decline in Chl fluorescence yield (decay time ~20–30 min), which arises from the light-induced chloroplasts photorelocation within the cell: The chloroplasts align parallel to the anticlinal cell wall and shade each other with respect to the incident light, thus decreasing the fluorescence yield from the underlying chloroplast layers. The fluorescence decrease is thus due to decreased light absorption, i.e., a light-avoidance response, rather than to a genuine quenching reaction.	*phot2*/PHOT2 (*A. thaliana*)	23, 176

[3]Chl* formations by intersystem crossing and release of $^1O_2$* are harmful events, which are counteracted by the photoprotective, multicomponent mechanism NPQ, which prevents production of harmful excited states through de-excitation of excess ^{1}Chl* and thermal energy dissipation. All of these processes have been classified according to the kinetics of chlorophyll fluorescence quenching under EL. The different processes that contribute to NPQ, and the mutants affecting the quenching response, are listed above.

Abbreviations: Chl, chlorophyll; EL, excess light; LHC, light-harvesting complex; NPQ, nonphotochemical quenching; PS, photosystem; RC, reaction center; WT, wild type.

State transitions: migration of antenna proteins between PSII and PSI, induced by unequal excitation of the two PSs and aimed to optimize photosynthetic electron flow to the light environment

$1/(1/F_0 - 1/F_m - 1/F_m')$] (137). Experience with this method is still limited, and results are not always consistent with independent methods of analysis, as in the case of the *phototropin 2* (*phot2*) mutant, which appears to be insensitive to photoinhibition, as assessed by this method, (179), and yet undergoes photobleaching upon excess light exposure (23). Although more challenging experimentally, the direct measurement of ROS release by fluorescent probes (66) or electron paramagnetic resonance spectroscopy (54) was efficient in revealing the performance of wild type (WT) versus the *npq4* mutant, devoid of qE (131). ROS react with proteins and lipids, producing covalent modifications that can be detected either biochemically (10), by quantifying products from lipid peroxidation such as malonyldialdehyde (170), or by the autoluminescence from the decomposition of lipid peroxides (47). Finally, the best assessment of photodamage/photoprotection can be obtained from a physiological characterization of electron flow, either on the whole chain or through individual thylakoid complexes by probing electrochromic shift at different wavelengths (11).

The Landscape of Photoprotection Mechanisms: The Light-Harvesting Systems

The core complexes of the two PSs are similar in different organisms while the antenna complexes are far more diverse. In green algae and plants, LHC proteins are encoded by the multigenic *Lhc* family of three membrane-spanning α-helices (33, 94), which evolved from the high-light-inducible proteins (HLIPs) of cyanobacteria (45), likely protecting intermediates during the biosynthesis of Chl-binding proteins (26). Within the dimeric PSII core, each monomer binds one copy of the monomeric antenna proteins Lhcb4 (CP29) and Lhcb5 (CP26). An additional interactor of Lhcb4, in plants but not in algae, is Lhcb6 (CP24), which, in turn, coordinates M-LHCII (moderately bound) trimers. Additional L-LHCII (loosely bound) trimers accumulate in low light conditions (144, 154). Finally, Lhcb4 is an interacting partner of PSBS, the pH sensor protein essential for triggering NPQ (55). Lhcb5 (CP26) is bound to CP43, together with the S-LHCII (strongly bound) trimer (154) (**Figure 5**). LHCII forms trimers in a homo- or heterotrimeric composition. Each LHCII monomer binds eight Chl *a*, six Chl *b*, and four xanthophylls—two Lut, one Neo, and one Vio/Zea (see 94), bound to sites named L1, L2, N1, and V1, respectively. While L1, L2, and N1 are active in excitation energy transfer to Chl *a*, V1 is not, and its Vio ligand is exchanged for Zea in excess light (22). In *Arabidopsis thaliana*, LHCII is encoded by nine genes belonging to three groups, *Lhcb1–Lhcb3* (72). In the green alga *Chlamydomonas reinhardtii*, LHCII is also encoded by nine genes. Notice that there is no correspondence with the *Lhcb* genes in *A. thaliana*, indicating that these evolved after the separation of green algae and land plants. In the case of green algae and land plants, the LHC-type antenna system of PSI is named light-harvesting complex I (LHCI). LHCI consists of two heterodimers, Lhca1/Lhca4 and Lhca2/Lhca3 (**Figure 5**) (117). Two additional *Lhca* genes are expressed in *A. thaliana*: The corresponding Lhca5 and Lhca6 proteins are essential for the assembly of large and rare PSI-NDH complexes catalyzing CEF (119). The 10 LHCI subunits are distributed between two different locations of the supercomplex (155). Eight of them form a double moon–shaped arc with four Lhca proteins each, while two additional Lhca subunits are bound to an opposite site with respect to the PSI core complex. The PSI-LHCI supercomplex in *C. reinhardtii* is depleted of red-shifted absorption forms ($\lambda > 700$ nm) compared to plants. Far-red light is absorbed by water and is therefore a poor energy source in aquatic environments. LHCII is also known to bind to PSI, either upon state transition or in steady-state light conditions, and increases the total amount of Chl by approximately 20% with a minimal quantum efficiency loss (178). In green algae, besides LHCII, Lhcb4 and Lhcb5 also migrate between PSs during state transitions, forming an even larger complex than the one found in plants (166).

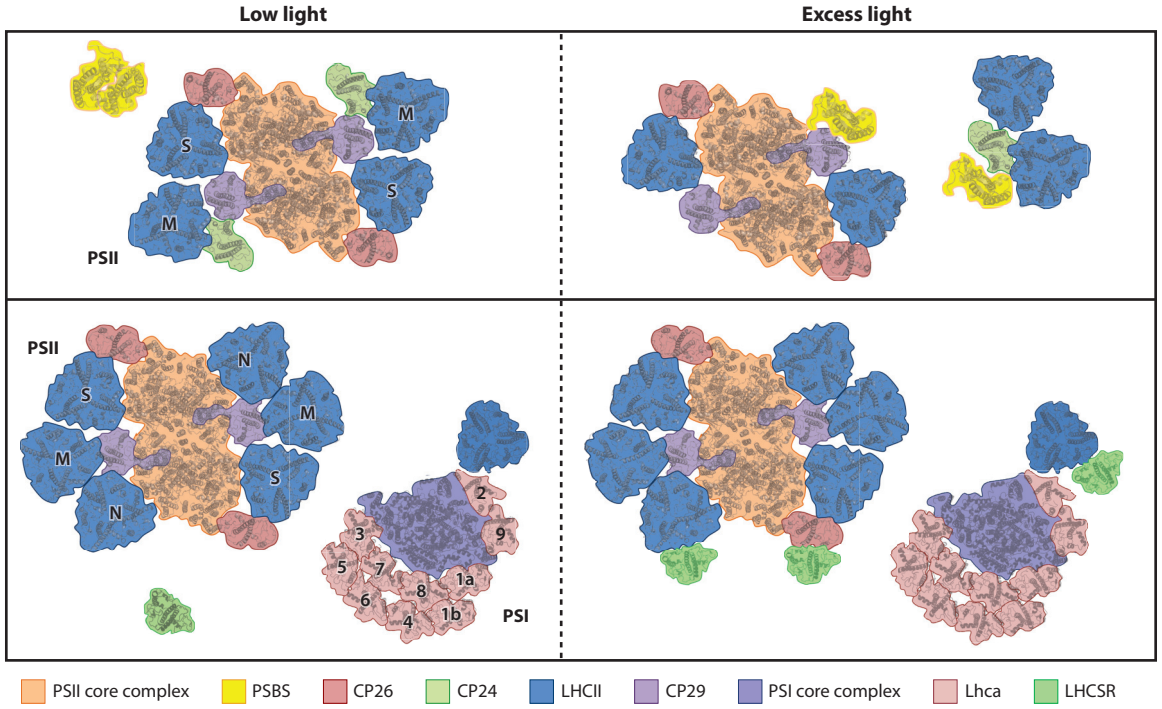

Figure 5

Models of the supramolecular reorganization of PSs in land plants and green algae before and upon exposure to EL. (*Upper panel*) In land plants, monomerization of PSBS under EL controls the association/dissociation of a membrane complex composed of CP29, CP24, and the trimeric M-LHCII. Dissociation exposes the PsbS binding sites on CP29. (*Lower panel*) In green algae, LHCSR-dependent quenching requires both CP26 and LHCII. LHCSR is located in stroma membranes and quenches the PSI antenna system, in addition to PSII. PSII structural models have been assembled based on the 3D structures of *Pisum sativum* $C_2S_2M_2$ (PDB ID 5XNM) (154) and *Chlamydomonas reinhardtii* $C_2S_2M_2N_2$ (PDB ID 6KAF) (144). The *C. reinhardtii* model of the PSI supercomplex has been assembled using the 3D structure of PSI-LHCI (PDB ID 6JO5) (155) and the projection map of PSI upon state transitions (46); for the Lhc subunits, the structures of CP29 and LHCII (PDB ID 6KAF) (144) and PSBS (PDB ID 4RI2) (49) have been used. M, N, and S refer to subunits of LHCII, while numbers refer to Lhca subunits. Abbreviations: 3D, three-dimensional; EL, excess light; LHC, light-harvesting complex; PDB ID, Protein Data Bank identification code; PS, photosystem.

Energy Quenching, the Fast Component of Nonphotochemical Quenching

Energy quenching (qE) develops within a tenth of a second after a sudden increase in light intensity and relaxes within 1–2 min at subsaturating light (68). qE is triggered by protonation of lumen-exposed acidic residues carried by specific gene products—either LHCSR in algae, PSBS in plants, or both, as in mosses (2, 86, 118)—switching the antenna system from light-harvesting mode to dissipative mode (136). The occurrence of both LHCSR and PSBS in mosses has been of major importance in comparing the properties of distinct qE mechanisms in the same physiologic and structural background (2).

Light-harvesting mode and dissipative mode: the biophysical states of the antenna system in which the functions of efficiently collecting light and fuel photochemistry or dissipating excitation energy are tuned by NPQ

Quenching Reactions by LHCSR Proteins

In the green alga *C. reinhardtii*, LHCSR is encoded by three genes, two of which are deleted in the *npq4* mutant, which displays a qE-deficient phenotype. Mutation of the last *LHCSR1* gene results in a complete lack of qE activity (172). Like PSBS, LHCSR belongs to the LHC superfamily,

and its responsiveness to low pH depends on lumen-exposed protonatable residues (20, 92). Two LHCSR isoforms, LHCSR1 and LHCSR3, are active in qE. The former is constitutively weakly expressed at low light while the latter strongly accumulates in excess light (104).

Critically, LHCSR differs from PSBS for binding pigments, namely Chl *a*, Lut, and Vio (20). Consequently, LHCSR is, potentially, the site of both the pH-sensing and the quenching reactions.

Functional analysis of LHCSR proteins has mainly been performed on the LHCSR1 protein from *Physcomitrella patens* (123). Fluorescence lifetime analysis of recombinant ppLHCSR1 in detergent solution showed that the protein responds to acidification by undergoing conformational transition from a 3.7-ns excited state to a short-lived 80-ps state (122), thus quenching the excited states (77). These results were observed with the protein binding Zea, consistent with the extreme dependence of quenching on Zea observed in vivo (124). It has been reported that the quenching reactions in land plant antennae rely on the formation of Zea radical cations in monomeric LHCs (35) or on excitation energy transfer from Chl *a* to Cars in trimeric LHCII (135). Surprisingly, both these mechanisms were found to be active in LHCSR1 (125). Interestingly, the radical cation activity was dependent on Lut rather than Zea (125), suggesting xanthophyll site occupancy might be different in LHCSR compared to plant LHCs.

While LHCSR proteins undergo quenching during acidification in vitro, the drag of the excitation energy requires interaction(s) with the PSII core and/or antennae. LHCSR proteins have been localized in stroma membranes and grana margins but depleted in grana partitions (123). Nevertheless, PSII fluorescence is efficiently quenched, implying that interaction between LHCSR and PSII supercomplexes at the grana margins and cooperativity between PSII units in delocalizing excitation energy (158) are effective in photoprotecting PSII. In addition to PSII, the PSI antenna system is quenched by LHCSR (123). There are at least two answers to the question of whether LHCSR-photosystems interaction occurs through a specific pigment-protein complex. First, single-particle electron microscopy (EM) analysis revealed that a dimeric LHCSR was located in PSII supercomplexes near the CP26 position (141), consistent with the observation that LHCSR-dependent quenching in transgenic *Arabidopsis* requires monomeric LHCs (43); second, in CP26-less *Chlamydomonas* NPQ was strongly decreased (24). Since the CP26-less mutant still retains substantial NPQ activity, at least one additional interaction subunit can be identified as LHCII, it being the only antenna accumulated in stroma membranes (57) (**Figure 5**).

PSBS-Dependent Quenching

The PSBS protein is missing in the *Arabidopsis* qE-less *npq4* mutant (86). Mutation analysis showed that pH-dependent PSBS activation requires two lumen-exposed glutamate residues (87). Despite this, PSBS is not a pigment-binding protein (49), implying that whatever the effect of pH on its conformation, it must be transduced to a Chl-xanthophyll-binding protein in order for quenching to be catalyzed. The identity of PSBS interactors has been studied extensively. Reverse genetics has shown that knocking out CP29 does affect qE amplitude (59), that the deletion of CP26 has no consequence, and that the direct involvement of CP24 is difficult to assess due to pleiotropic effects on PQH_2 diffusion (40). The strongest contribution to qE is provided by trimeric LHCII (35), mainly through its Lhcb1 components (121), while Lhcb2 and Lhcb3 reportedly play a minor role, possibly due to their lower abundance (111).

While LHCII is readily accessible from membrane-embedded subunits due to its high abundance and peripheral location in PSII, CP29 is buried deep within supercomplexes, implying its interaction with PSBS might be hindered. However, triggering qE involves PSBS-dependent reorganization of thylakoid membrane domains (17), which might well expose CP29 to interaction

with PSBS (**Figure 5**). Cross-linking PSBS within *Arabidopsis* thylakoid membranes upon induction of quenching revealed that the most represented covalent adducts were PSII core and Lhcb1 (28). Consistent results were obtained through affinity chromatography of histidine-tagged PSBS in moss (55).

Models other than specific protein-protein interactions were also proposed for triggering quenching reactions: It has been suggested that quenching is generated on the formation of aggregated LHCII-only domains, undergoing quenching due to cooperative interactions and the *trans*-thylakoid pH gradient (136). In this scenario, however, any mutation that overaccumulates LHCII or disrupts PSII supercomplexes (35) is expected to enhance NPQ activity, rather than enhancing fluorescence yield, as observed.

Chl Q_y: one of the four bands that compose the absorption spectra of chlorophylls, corresponding to the low-energy electronic transition from ground state to first excited singlet state

Modulation of Energy Quenching by Xanthophylls

Plant and algae Car composition undergoes changes depending on environmental conditions. The fastest response involves Vio to Zea interconversion, which, in excess light, forms the xanthophyll cycle through the activity of the violaxanthin de-epoxidase (VDE) enzyme; in low light, Zea is converted back into Vio by a stromal zeaxanthin-epoxidase (71). The effect of the xanthophyll cycle on NPQ is species-dependent: NPQ of *C. reinhardtii* is Zea-independent, while LHCSR-dependent NPQ of *P. patens* is strongly upregulated by Zea (124) through binding to both V1 and L2 binding sites in synergy with low pH (122). In plants, quenching activity is modulated by Zea, and its constitutive accumulation, as in the *npq2* mutant, makes the onset of qE faster (115). Lut also plays a role in NPQ: Lut-deficient mutants have reduced and slower NPQ, while Lut overaccumulation in part compensates for the lack of Zea (89). Consistently, the lack of both Lut and Zea results in a null NPQ phenotype mimicking the PSBS-less mutation. Owing to the involvement of multiple LHCs, which have different xanthophyll compositions, the amplitude and kinetics of quenching in vivo are the result of different contributions. The NPQ catalyzed in LHCII was only dependent on Zea since it was unaffected by *lut2* mutation. Since a lack of Lut severely affects NPQ in WT backgrounds (39), it is suggested that NPQ reactions in monomeric LHCs are Lut-dependent (35).

Zea accumulation is also responsible for a slower component of NPQ developing in parallel with the reconversion of Zea into Vio and is therefore called qZ (112). This Zea-dependent mechanism is independent of PSBS and lumenal pH and is associated with Zea binding to monomeric LHCs (34), while its binding to the trimeric LHCII does not affect Chl fluorescence lifetime, implying that qZ is specific to the inner antenna closely connected to PSII core (150).

Molecular Mechanisms for Quenching

Several physical mechanisms underlying the quenching processes have been proposed, localized in either LHCs or RCs of PSII (**Figure 6**):

1. Chl Q_y to Car S1 energy transfer: According to this model, qE occurs upon aggregation of LHCII, which causes a conformational change that promotes energy transfer from Chl *a* to a low-lying excited state of Lut bound to site L1 of LHCII (135). Recently, the same quenching channel was observed in moss LHCSR1, where it is activated through fast energy transfer from excited Chl *a* to the S1 state of Zea (125), while a similar process in algae LHCSR involves Lut (20).
2. CT (charge-transfer) quenching: According to this model, qE activation involves a charge separation within a Chl-Zea heterodimer, producing a transient Zea radical cation (Zea^+)

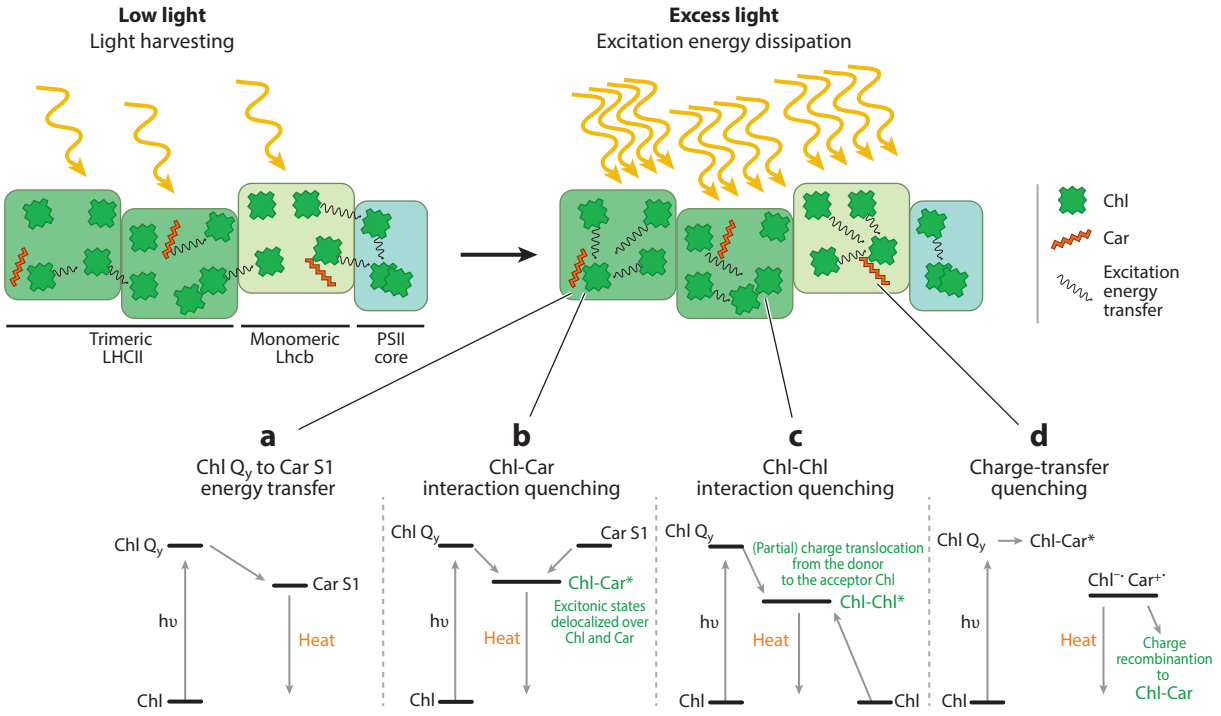

Figure 6

Molecular mechanisms for Chl fluorescence quenching in the PSII. In low-light conditions, PSII efficiently harvests sunlight and transfers excitation energy to the RC. Excess light conditions promote the closure of all RCs and the activation of quenching center(s), which act as local energy traps that consume excitation energy. (*a*) In the Chl Q_y to Car S1 energy transfer, aggregation of LHCII causes a conformational change promoting energy transfer from Chl *a* to Lut bound to site L1. (*b*) In Chl-Car interaction quenching, short-living, low excitonic Car S1-Chl interactions serve as dissipation valves for excess excitation energy. (*c*) In Chl-Chl interaction quenching, Chl-Chl excitonic coupling would originate low-level dissipative electronic states. (*d*) In charge-transfer quenching, a charge separation event within a Chl-Zea heterodimer produces a transient Zea^+ whose fast relaxation time would ensure efficient quenching. Abbreviations: Car, carotenoid; Chl, chlorophyll; hν, light energy; LHC, light-harvesting complex; Lut, lutein; PS, photosystem; RC, reaction center; Zea, zeaxanthin.

(67). The process has been located in monomeric LHC proteins of plants, although it does not occur in LHCII, and involves the Chl pair (Chl A5 and Chl B5) located in close proximity to the Car-binding site L2 (59) (**Figure 7**).

3. Chl-Chl interaction quenching: This mechanism was proposed in the belief that Cars might not play a major role in photoprotective quenching processes. Rather, it was suggested that low-level dissipative electronic states were produced by Chl–Chl excitonic coupling, which becomes visible as a red-shifted emission at 700 nm (100).
4. Chl-Car interaction quenching: This is similar to mechanism 3 but instead, excitonic interactions are established between Chl and Car (19).

It should be emphasized that quenching mechanisms occurring in vivo are not necessarily mutually exclusive, and more than one process might well contribute to establishing the overall quenching state. In at least one case, the occurrence of energy transfer from Chl *a* to Car S1 with thermal dissipation (mechanism 1) and formation of the Lut radical cation (mechanism 2) have been observed within the same pigment-protein complex (125).

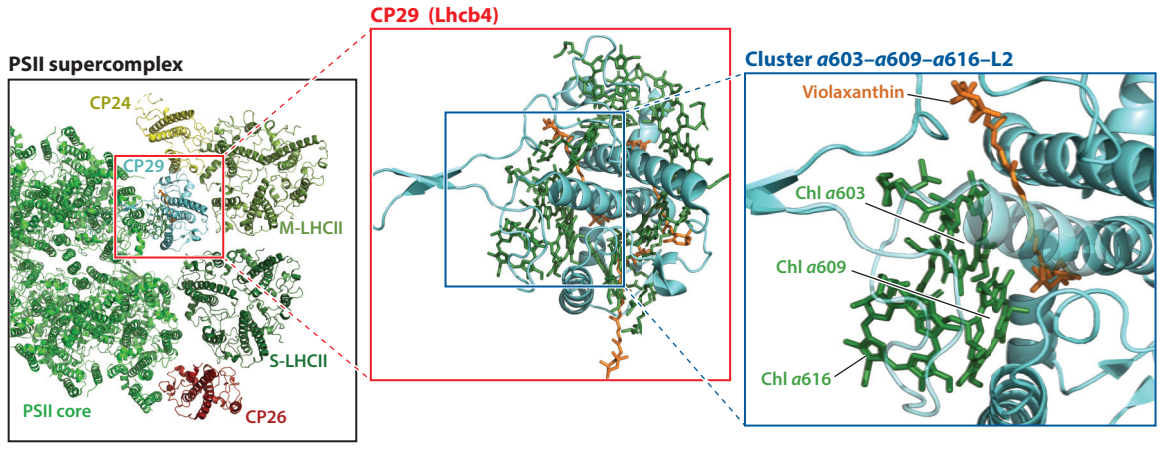

Figure 7

Stereo view of the *a*603–*a*609–*a*616–L2 Chl-xanthophyll cluster of CP29 illustrating the structure of the PSII supercomplex $C_2S_2M_2$ (PDB ID 5XNM) (154), showing the position of CP29 (*left panel*), a view of the overall structure of CP29 from spinach showing Chl and xanthophyll ligands (*central panel*), and a detailed view of the pigment cluster involved in the quenching reaction by the radical pair (*right panel*). For the sake of clarity, the Chl phytyl chains are not shown. Abbreviations: Chl, chlorophyll; LHC, light-harvesting complex; PDB ID, Protein Data Bank identification code; PS, photosystem.

Transition from LHCSR- to PSBS-Dependent Energy-Quenching Modes

PsbS catalyzes qE in mosses and land plants. Genes that encode PSBS-like proteins are found in the genome of unicellular algae, including the model species *C. reinhardtii*. Transcription analysis of synchronized cells displayed strong induction upon dark to light transition (153), and the PSBS protein was transiently detected, although it was unable to induce NPQ in the absence of LHCSR (162). Nevertheless, PSBS overexpressors in a LHCSR3-less background exhibited enhanced photoprotection towards excess light, possibly by regulating LHCSR1 activity. In mosses, the ancillary PSBS quenching activity evolved in a self-sufficient NPQ activity (2) and became the only form of quenching in land plants, since LHCSR-encoding genes were lost (58). The question of why LHCSR was replaced by PSBS is interesting and potentially useful for translational research: If the two proteins are endowed with nonredundant photoprotective functions, crops expressing LHCSR might exhibit enhanced stress resistance. The major difference between PSBS and LHCSR lies in the latter's ability to bind two Zea ligands upon excess light exposure and possibly enhance its ROS scavenging activity. A second difference is the localization of PSBS in grana partitions (123), while LHCSR resides in stroma membrane domains where most biogenetic machineries are located, including the PSII repair cycle. Grana width and stacking level increased during evolution from unicellular algae to land plants (8). In land plants, a typical grana partition disc contains ~600 PSII supercomplexes (79), while quenching can be propagated over ~8–10 PSII RCs (6). Therefore, activity from the periphery of grana discs provided by LHCSR can only quench ~30% of PSII, leaving those at the center unprotected. According to this theory, segregation of PSII in large grana partitions has favored quenching mechanisms catalyzed by PSBS, which is located in this membrane domain. In the presence of PSBS, LHCSR residual function (e.g., in mosses) consisted of quenching LHCII, a component of the large PSI antenna, until the evolution of red-shifted chlorophyll absorption forms in LHCI (102) made both LHCII and LHCSR complexes redundant in the process of providing sufficient photons to PSI and regulating this activity.

State Transition-Induced Fluorescence Quenching (qT) and Spillover

Spillover: the drag of the excitation energy from the slower trap, PSII, by the fast energy trap, PSI

PSII and PSI perform differently in terms of light capture, depending on both light spectrum and fluence. PSII cannot absorb far-red radiation ($\lambda > 685$ nm); therefore, the photon absorption rate of PSII is limited under canopy compared to PSI because of the enrichment in far-red radiation of these environmental niches (13). Under these conditions, the process of state transition redistributes part of the LHCII antenna, i.e., the excitation energy supply between PSII and PSI. State 1 refers to the antenna arrangement that favors PSII excitation, whereas, in state 2, PSI is endowed with additional LHCII. This reversible process is controlled by reducing the PQ pool and activating a protein kinase, STT7 in algae and STN7 in plants (134), which phosphorylates specific gene products of the LHCII antenna (85, 166) and promotes its migration to PSI. On preferential excitation of PSI, the PQ pool becomes oxidized, the kinase is inactivated, and a phosphatase (TAP38/PPH1) dephosphorylates the mobile LHCII, which moves back to PSII (142). As a result of this rearrangement, a fraction of the LHCII antenna is transferred from PSII, a shallow trap, to PSI, which is a more effective quencher, resulting in a fluorescence quenching called qT. The photoprotective relevance of this process is unclear since the STT7/STN7 kinases are inhibited in excess light (84).

Since PSII is a shallow trap for excitons while PSI has a far shorter fluorescence lifetime, a LHCII complex connected to both PSI and PSII RC will be quenched photochemically by the former. PSI is very sensitive to highly variable irradiance, owing to the photoinhibitory effect of excess electrons from PSII on Fe-S centers (156). Under these conditions, concomitant overreduction of PQ activates state transitions while damaged PSI remains a trap for excitation energy, thereby leading to thermal dissipation with the outcome of both reducing PSII antenna size and quenching LHCII in stroma domains (163). While the occurrence of spillover between PSII and PSI has been reported in diatoms (51), its activity in plants is controversial, owing to the extreme lateral heterogeneity of thylakoid membranes and segregation of PSI versus PSII.

Sustained Quenching

Sustained quenching has been identified in evergreens (56), either deployed for the maintenance of low lumenal pH (175) or independent of lumen acidification (42). A sustained decrease in fluorescence yield was linked to a suppressor of quenching called SOQ1 (21). qH itself is catalyzed by a plastidial lipocalin (LCNP), the as-yet-unknown action of which on the major LHCII antenna complex is inhibited by SOQ1 (97). SOQ1 spans the thylakoid membrane, exposing a thioredoxin (TRX) domain to the lumen where it may act to regulate LCNP. CCDA, a TRX-like subunit of thylakoid membrane, is also involved in the transfer of reducing equivalents from the stroma to the lumenal compartment (103). Further genetic analysis identified ROQH1, a reductase that catalyzes the relaxation of quenching induced in LHCII by LCNP (7). qH appears to be independent of lumenal pH and is instead controlled by the redox state of the stroma compartment, which becomes reducing upon the saturation of CO_2 fixation. Consistent with this hypothesis, the *Arabidopsis* mutant devoid of NADPH thioredoxin reductase C enhances NPQ, boosting its qH component. Both LCNP and VDE are lumen-localized lipocalins that have multiple thiol residues, potential targets for TRX.

Photoinhibitory Quenching

The slowest quenching component, qI, is attributed to processes involving a decrease in active RCs of PSII upon photodamage (148). Quenching in photoinactivated RCs occurs due to charge recombination between Q_A^- and $P680^+$, but other quenching reactions of an unknown nature are

probably involved (27, 50). D1 protein of the RC is more susceptible to photodamage, and photodamaged D1 can accumulate in high levels under conditions such as cold and excess light before being degraded and replaced (9); photoinhibition of PSII only occurs if the rate of damage overtakes the rate of repair (160). Recently, highly quenched pigment-protein complexes have been found to be involved in protecting PSII during assembly/repair, which may maintain damaged PSII in its quenched state (151).

Photoprotection During Biogenesis and Repair

While most three-helix LHC proteins are abundant, some LHC-like proteins only accumulate in small amounts or are undetectable under normal conditions but can be strongly and transiently induced under specific stress conditions. This is particularly true for early light-induced proteins (ELIPs) (62). These proteins accumulate transiently in thylakoid membranes, mostly under conditions of light stress. ELIPs have three transmembrane domains, the first and third helices of which display high sequence identity with the corresponding helices of LHC proteins. In comparison with other LHC proteins, ELIPs have an unusual pigment composition that consists mostly of Chl *a* and large amounts of Lut and have a low excitonic coupling between Chls (1). ELIPs have been localized in the stroma-exposed thylakoid membranes where PSII repair takes place. Also, constitutive expression of ELIPs was shown to restore the photosensitivity of *chaos*, a mutant deficient in the signal recognition particle (SRP) pathway and unable to rapidly accumulate ELIPs under excess light and chilling conditions and therefore highly photosensitive. Together, these findings strongly suggest that ELIPs have a photoprotective role (70).

Constitutive expression of ELIP2 in *Arabidopsis* led to a 50% decrease in leaf Chl content and photosystems (173), which suggests that ELIPs may act as sensors that modulate Chl synthesis in order to prevent the accumulation of free pigments and, thereby, photooxidative stress. Among ELIP-like proteins, the MSF1 protein of *Chlamydomonas* (183) is structurally related to ELIPs and accumulates only under stress conditions. MSF1 is required for the stability and maintenance of PSI since the loss of MSF1 leads to a fourfold decrease in PSI abundance. MSF1 appears to be linked to the Chl biosynthetic pathway, based on the observation that it interacts with CTH1, which catalyzes the synthesis of protochlorophyllide. This suggests that MSF1 might link Chl biosynthesis to the maintenance of photosynthetic Chl-protein complexes under specific stress conditions. In the absence of both LIL3 (a protein with two transmembrane domains) isoforms of *Arabidopsis*, the supply of the two metabolites chlorophyllide and phytyl pyrophosphate, which are required for the final steps of Chl synthesis, is compromised (65). In barley seedlings, LIL3 specifically accumulates during the de-etiolation stage and assembles as a Chl-binding protein complex (129). LIL3 could thus play an important role in efficiently channeling tetrapyrroles to their final destination within the photosynthetic complexes, thereby preventing photooxidative damage.

Originally identified in cyanobacteria, the small cab-like proteins (SCPs), also called one-helix proteins (OHPs) or HLIPs, are small Chl binding-like proteins containing one transmembrane domain that binds Chl in vitro (152). In land plants, OHP1 appears to deliver Chl to the PSII core complex, and loss of either OHP1 or OHP2 leads to a pale green phenotype and reduction of the accumulation of the photosystems (14). Thylakoid membrane organization is altered in mutants lacking OHP1, with an almost complete absence of stromal lamellae and swelling of the marginal thylakoid membranes (88). Moreover, OHP2, the stromal part of which interacts with HCF244, is required for the stability of OHP1 and HCF244 and is specifically involved in the assembly of the PSII complex (64). Formation of the PSII RC complex is specifically inhibited in the absence of OHP1 and OHP2 in *Arabidopsis*, and it was shown they form a PSII-RC-like complex at an

early stage of PSII assembly as well as during PSII repair (88). Ultrafast absorption spectroscopy revealed that this process occurs through a direct energy transfer from the Chl *a* Q_y state to the β-carotene S1 state (151). Taken together, these studies indicate that the mechanisms governing PSII RC assembly are highly conserved in both prokaryotic and eukaryotic photosynthetic organisms and there is a close coordination between Chl synthesis and the assembly of the photosystems. Despite this control, low levels of unbound Chl are probably present in the membranes, which have been reported to cause photoinhibition (140). This suggests that the excess Chl content of leaves, functional to competition by shading competitors, might have negative effects on photoprotection and plant productivity.

OUTLOOK

The photoprotective processes described above clearly have beneficial effects for the survival of photosynthetic eukaryotes in the natural environment. However, most of these mechanisms have not been optimized for productivity. Rather, evolution has selected and honed them for survival and reproductive success. In particular, processes that prevent photooxidation have a cost in terms of energy and are not optimized for carbon gain. Most of them are potential targets for manipulation aimed at enhancing productivity. Photosynthesis in crops has not been improved during the Green Revolution, and it is currently far below its theoretical limit. Recent advances in genome engineering provide direct proof that photosynthetic performance can be significantly improved by tuning phototolerance. Such exciting evidence makes clear that understanding the photoprotective responses is critical in order to identify molecular targets for enhanced light-use efficiency. To bring such ambitious goals within reach, the following will be crucial: (*a*) theoretical modelling for predicting targets in photosynthetic machinery, the redesign of which could enhance yield in either suboptimal or controlled conditions, and (*b*) monitoring the effects of chloroplast manipulation in real canopy conditions, since the complex genotype-environment interactions are what determine biomass yield.

SUMMARY POINTS

1. In plants and algae, the dynamic of irradiance under natural conditions easily exceeds the capacity of the assimilatory reactions, which results in overexcitation of the photosynthetic machinery, reactive oxygen species (ROS) release and photodamage.
2. In the chloroplast, an antioxidant network that includes ROS scavengers and enzymatic antioxidant components aims to minimize photodamage.
3. Nonphotochemical quenching (NPQ) involves a number of inducible mechanisms, which are effective in safely dissipating excess excitation energy into heat.
4. The major, rapidly induced energy-quenching component qE is triggered in the photosystem II (PSII) outer antenna system by protonation of lumen-exposed acidic residues in LhcSR (algae), PsbS (plants), or both (mosses).
5. Beside qE, other excess light–inducible photoprotective mechanisms include state transitions (qT), sustained quenching (qH), chloroplast light avoidance response (qM), and Zea binding to light-harvesting complex (LHC) proteins (qZ).
6. Understanding the molecular targets that mediate photoprotection is critical in order to plan engineering strategies for improving plant and alga light-use efficiency.

FUTURE ISSUES

Despite the long time since its discovery, photoprotection by energy dissipation is far from elucidated in terms of both the underlying molecular and biophysical mechanisms as well as its consequences for plant productivity.

1. Among the major open questions is the role of thylakoid membrane reorganization in quenching reactions. While PSBS-dependent domain reorganization during development of NPQ has been assessed (17, 74) it is unclear whether the quenching reactions are triggered upon the establishment of specific interactions between PSBS and individual LHC subunits or whether PSBS merely acts as a promoter of membrane reorganization. While the authors of this text favor the first hypothesis, the possibility that some level of quenching can be obtained through the clustering of antenna proteins without the direct involvement of PSBS cannot be excluded. Dissection of the complex supramolecular thylakoid machinery into smaller domains with defined and simple composition is needed in order to verify this hypothesis. To this end, liposomes and lipid nanodiscs should be employed to host LHC proteins and PSBS and/or other components such as xanthophylls, as recently pioneered (149).
2. A second open problem is the relation between fluorescence quenching and photoprotection. Given that more than a single LHC complex is involved in qE (35), it is still not clear whether individual quenching events have the same photoprotective effect. The use of genome editing technology will be instrumental in obtaining genotypes suitable to answer these questions despite the high complexity of the LHC multigenic family that hosts quenching reactions.
3. Last but not least is the question of whether mechanisms underlying the photoprotective roles of LHCSR and PSBS are the same or whether they are only partially redundant, thus opening the possibility of enhancing protection against environmental stress by expressing LHCSR in crops.

Regulation of photosynthesis by energy dissipation is one of the most complex chapters of plant physiology, and only because of that, it is worth studying and devising new instrumentations and methods of analysis. Nevertheless, the translation of knowledge into engineering crops for enhanced resistance and productivity is a target of high importance for humankind. The seminal work by Kromdijk and coworkers (81) has shown that NPQ reactions are a target for improving productivity. It is likely that a more detailed knowledge of the mechanisms involved will allow for even more precise and effective engineering work.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors acknowledge the support of MIUR (grant 201795SBA3-PRIN2017 to L.D.) and EEC (grant 675006-SE2B to R.B.). Dr. Roberto Caferri of the University of Verona is thanked for help in the preparation of **Figures 5** and **7**.

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