Safety valves for photosynthesis

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Recent studies have provided new insights into the ways that plants may dissipate excess photons and electrons, thereby protecting the photosynthetic apparatus against light-induced damage. These 'safety valves' include nonphotochemical mechanisms for quenching excited chlorophylls, as well as alternative electron acceptors such as oxygen.

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Abbreviations

Chl	chlorophyll
im	*immutans*
LHC	light-harvesting complex
NPQ	nonphotochemical quenching
qE	ΔpH-dependent quenching
qI	photoinhibitory quenching
PS II	photosystem II
PsbS	PS II subunit S
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase

Introduction

The photosynthetic apparatus in plants performs the essential function of converting light energy into chemical energy that is used for CO_2 fixation and other assimilatory reactions, but this complex machinery is susceptible to light-induced damage caused by the inevitable generation of reactive intermediates and by-products. Diurnal and seasonal fluctuations in light intensity, as well as environmental stresses such as cold, drought, salinity, and nutrient deficiency, that limit CO_2 fixation can result in the absorption of more light energy than can be utilized productively by photosynthesis. Under these conditions, safe dissipation of excess photons and electrons is necessary to protect the photosynthetic apparatus from light-induced damage.

Maintenance of photosynthesis is critical for plant fitness and survival, as evidenced by the light sensitivity of many photosynthetic mutants and antisense plants, and therefore plants have evolved numerous photoprotective mechanisms [1•]. These include several antioxidant molecules and enzymes [1•], repair processes for lipid peroxidation [2] and damaged photosystem II (PS II) reaction centers [3], and even systemic signaling and acclimation processes [4]. This short review will focus on the various 'safety valves' that may be involved in photoprotection through dissipation of excess photons and electrons (Figure 1).

The constitutive triplet chlorophyll valve

Light is absorbed by chlorophyll (Chl) and carotenoid molecules that are bound to light-harvesting complex (LHC)-proteins in the thylakoid membranes of chloroplasts [5], resulting in singlet state excitation of the pigment molecules. Excitation energy is then transferred to reaction centers to drive electron transport, which oxidizes H_2O, reduces $NADP^+$, and generates a ΔpH that is harnessed for ATP synthesis. In some plants, leaf movements allow the plant to adjust the angle of a leaf relative to the sun in order to optimize (or minimize) light absorption. However, during the course of a typical day most plants are simply unable to regulate light absorption on a timescale that is fast enough to cope with the diurnal and other rapid fluctuations in light intensity that occur in natural environments. Instead of changing the capacity for light absorption, plants rely on mechanisms for dissipating photons that have already been absorbed.
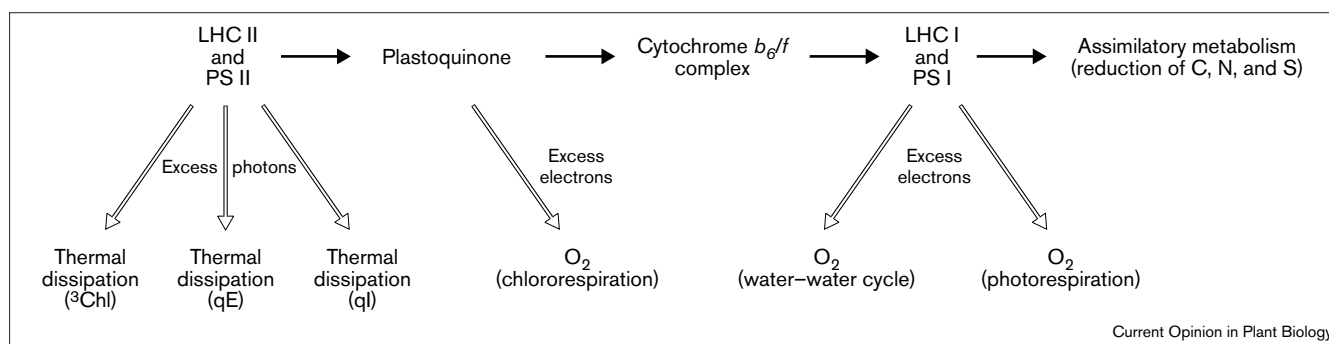
Absorption of excess photons can potentially cause accumulation of excitation energy in the LHCs and thereby increase the lifetime of singlet Chl (^{1}Chl). Through a physical process known as intersystem crossing, ^{1}Chl can be converted into triplet Chl (^{3}Chl), a long-lived state that is incapable of initiating photosynthetic electron transfer. Generation of ^{3}Chl results in the dissipation of absorbed photons because ^{3}Chl is efficiently quenched by energy transfer to carotenoids, which are closely associated with Chls in the photosynthetic apparatus. It has been estimated that between 4% and 25% of the photons absorbed by PS II can be dissipated via this triplet valve. The proportion of photons dissipated in this way is correlated to the average lifetime of ^{1}Chl [6]. However, because the lifetime of ^{1}Chl is kept relatively low and constant by regulated de-excitation (see below), dissipation via the triplet valve is usually limited to the lower end of the estimated 4–25% range.

Although ^{3}Chl itself is not harmful, it can interact with ground state molecular O_2, which normally exists as a triplet, converting it into singlet oxygen ($^1O_2$), a highly reactive oxygen species. Production of $^1O_2$ has been detected in leaves illuminated with intense light [7], and it can directly damage the proteins, pigments, and lipids that comprise the photosynthetic apparatus. Quenching of $^1O_2$ is also accomplished by carotenoids, which explains why these pigments are essential for photosynthesis in the presence of oxygen.

Rapidly inducible dissipation of excess photons

Whereas the de-excitation of ^{1}Chl via the triplet valve is basically an unregulated, constitutive process, plants and most eukaryotic algae also possess a rapidly inducible nonphotochemical quenching (NPQ) mechanism, termed ΔpH-dependent quenching (qE), for the harmless thermal dissipation of excess absorbed photons in PS II [8] (Figure 1). The qE mechanism can be considered as a feedback-regulated safety valve for photosynthesis because it is controlled primarily by the magnitude of the thylakoid ΔpH that is generated by photosynthetic electron transport.

Figure 1

Schematic depiction of safety valves for excess photons and electrons that are discussed in the text. Thermal dissipation of excess photons is accomplished by the triplet chlorophyll valve ([3]Chl), inducible qE, and sustained qI. Oxygen-dependent alternative electron sinks include photorespiratory metabolism, photoreduction of O_2 in the water–water cycle, and a chlororespiratory alternative oxidase.

Absorption of excess photons causes the buildup of a high ΔpH, and the resulting decrease in lumen pH is essential for qE [8]. The lowering of the lumen pH activates violaxanthin de-epoxidase, a lumenal enzyme that converts violaxanthin to zeaxanthin as part of the xanthophyll cycle [9]. A strong correlation between zeaxanthin accumulation and qE has been demonstrated for a wide variety of plants under both natural and laboratory conditions [10]. Low lumen pH may also result in the protonation of one or more protein(s) in the LHC that is associated with PS II [8]. Together, binding of protons and zeaxanthin is postulated to cause a conformational change, which can be assayed as an absorbance change at 535 nm in leaves or isolated thylakoids, that effectively switches the LHC into a state in which efficient nonphotochemical de-excitation of [1]Chl and concomitant thermal dissipation of the excitation energy occur. The switch to thermal dissipation is accompanied by a discrete change in Chl-fluorescence-lifetime distributions [11]. The conformational change and the conversion of the LHC into a quenched state are rapidly reversible when light is no longer excessive and the magnitude of the ΔpH decreases.

The recent isolation and characterization of mutants are providing new insights into qE [12–15,16[••]]. For example, the role of xanthophylls in qE has been addressed using mutations that affect xanthophyll synthesis. The *npq1* mutant of the common laboratory weed, *Arabidopsis thaliana*, is defective in the gene encoding violaxanthin de-epoxidase, so it is unable to convert violaxanthin to zeaxanthin in the xanthophyll cycle [13]. Measurements of qE in this mutant showed that de-epoxidation of violaxanthin to zeaxanthin is necessary for most of the qE *in vivo* in leaves of *Arabidopsis*. Similar results were reported recently with violaxanthin de-epoxidase antisense tobacco plants [17]. *Arabidopsis lutein deficient 2* (*lut2*) mutants that lack lutein exhibit a partial defect in qE [18] and *npq1* ; *lut2* double mutants completely lack qE [19], suggesting a possible role for lutein in qE. Transgenic *Arabidopsis* plants with elevated lutein have been generated by overexpression of lycopene ε-cyclase, resulting in an increase in qE [20]. It is possible that lutein has a direct role in the qE mechanism or that the absence of lutein could affect qE indirectly, perhaps through a perturbation of LHC assembly and structure [21].

To identify factors besides lumen pH and xanthophylls that are necessary for qE, mutants have been isolated that lack qE but have normal pigment composition [15,16[••]]. In particular, the *Arabidopsis npq4* mutant is completely defective in qE and also lacks the conformational change that is monitored by measuring the absorbance change at 535 nm in leaves [16[••]]. Map-based cloning revealed that the *NPQ4* gene encodes photosystem II subunit S (PsbS), an integral-membrane-protein component of PS II that is a member of the LHC-protein superfamily. Like other LHC proteins, PsbS (also known as CP22 [for *22*-kiloDalton chlorophyll-protein complex]) is thought to bind Chls and xanthophylls, although the stoichiometry and affinity of this binding have not been firmly established [22]. Despite the absence of PsbS protein in the *npq4* mutant, the light harvesting and photosynthetic oxygen evolution of this mutant are not impaired, suggesting that PsbS functions specifically in qE [16[••]]. It has been proposed that proton binding to PsbS triggers a conformational change and [1]Chl de-excitation either in the PsbS protein itself or in neighboring LHC proteins [16[••]].

Despite these advances in understanding the genetics of qE, the actual biophysical mechanism of [1]Chl de-excitation in qE is still unknown. An attractive proposal has been that zeaxanthin is a quencher of excess photons through a reverse transfer of excitation energy from [1]Chl to the singlet state of zeaxanthin [23], and that the xanthophyll cycle converts a light-harvesting pigment (violaxanthin) into a quencher (zeaxanthin) [24]. Until recently, the feasibility of this proposal has been unclear, because the energy levels of the lowest singlet state of the carotenoids had not been measured directly. Using the techniques of transient absorption spectroscopy and fluorescence spectroscopy, both zeaxanthin and violaxanthin have recently been found

to have low-energy singlet states that could potentially accept energy from ^{1}Chl [25••,26••]. Although it is possible that zeaxanthin could be acting as a quencher, the difference in energy between zeaxanthin and violaxanthin is not sufficient to explain why zeaxanthin but not violaxanthin is involved in qE [26••]. As suggested by experiments with isolated LHC proteins, it is likely that the distinct structural features of zeaxanthin and violaxanthin are important in explaining their different roles [27,28].

Long-term strategies for excess photons

In addition to coping with excess photons during short-term fluctuations in incident sunlight, many plants are confronted with environmental stresses, often on a seasonal basis, that limit their productive utilization of light energy and necessitate long-term mechanisms to deal with excess photons. Common acclimation responses to excess light include increasing the capacity for photosynthetic electron transport and CO_2 fixation, as well as decreasing the size of the light-harvesting antennae that are associated with the photosystems. This acclimation may involve the regulation of nuclear and chloroplast gene expression by redox potential [29–31], and/or the proteolytic degradation of existing LHCs [32]. In terms of safety valves, there are also mechanisms for the sustained (rather than rapidly reversible) dissipation of excess photons. Because sustained thermal dissipation results in a decrease in the quantum efficiency of photosynthesis, these mechanisms have been collectively termed photoinhibitory quenching (qI) (Figure 1). It is possible that at least part of qI may be mechanistically similar to qE [33,34], but some types of qI do not appear to depend on lumen pH [35,36].

qI has been studied extensively in overwintering evergreen plants that retain Chl despite prolonged cold-stress conditions that limit photosynthesis. These plants exhibit persistent thermal dissipation of excess photons that is reversed only slowly, even upon warming of their leaves [37]. Interestingly, zeaxanthin accumulation is associated with qI, as with qE, and recovery from qI has been correlated with the conversion of zeaxanthin back to violaxanthin in the xanthophyll cycle [38]. A possible role for protein phosphorylation in zeaxanthin epoxidation and recovery from qI has been indicated by the effects of phosphatase inhibitors on these processes [39,40].

New insights into qI have come from spectroscopic studies of overwintering evergreen (snow gum) leaves [41••]. In their winter-acclimated state, these leaves were characterized by the presence of a novel band at 710-715 nm in their Chl fluorescence emission spectra and a short-lifetime Chl-fluorescence component, indicating that they had increased thermal dissipation. Slow recovery from qI was correlated with the loss of these spectral and lifetime components and with the epoxidation of zeaxanthin to violaxanthin. These results are consistent with the idea that winter acclimation in evergreens involves significant changes in the organization and/or composition of Chl-containing complexes. It is interesting to note that increased levels of several proteins, including PsbS, are associated with the winter acclimation of pine trees [42].

Alternative sinks for excess electrons

In linear photosynthetic electron transport, the reducing side of PS I is a key branchpoint from which electrons can be transferred for use in the major chloroplast assimilatory reactions, namely the reduction of C, N, and S. Limited availability of acceptors, especially of CO_2, can result in an excess of electrons in the photosynthetic electron-transport chain, despite the efficient dissipation of excess photons in PS II. Overreduction of electron-transport carriers may sensitize PS II to photoinhibition [3] and increase the production of other reactive oxygen species such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) [1•].

Alternative electron acceptors may serve as sinks for excess electrons, thereby helping to prevent overreduction of electron carriers. Although the contribution of alternative sinks appears to be limited under steady-state conditions, they may be especially important in fluctuating light, for example during the induction phase of photosynthesis following a dark to light transfer [43]. NADPH-dependent reduction of oxaloacetate to malate has been suggested to provide such a safety valve for excess electrons, although experiments with antisense malate dehydrogenase plants have not supported a major role for this potential electron sink under steady-state conditions [44]. Thioredoxin is an electron acceptor that has important regulatory functions in chloroplasts [45], but its capacity as a sink for excess electrons is limited.

The best characterized alternative sink for electrons is photorespiratory metabolism (Figure 1). Oxygenation of ribulose-1,5-bisphosphate by Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) results in the formation of one molecule of 3-phosphoglycerate and one molecule of a toxic byproduct, 2-phosphoglycolate. Photorespiratory metabolism utilizes both NADPH and ATP in reactions occurring in chloroplasts, mitochondria, and peroxisomes to recover fixed C that would otherwise be lost. Thus, although it may initially appear to be a wasteful process that is a relic of Rubisco's evolutionary history, photorespiration can help to prevent light-induced damage to the photosynthetic apparatus [46].

Photoreduction of oxygen by Photosystem I: the water–water cycle

Besides the oxygenase activity of Rubisco, O_2 can be directly reduced by PS I (Figure 1). Univalent electron transfer to O_2 results in the generation of O_2^-, which can subsequently be converted to H_2O_2 by superoxide dismutase. Ascorbate peroxidase activity in the chloroplast is responsible for the reduction of H_2O_2 to H_2O; the resulting monodehydroascorbate radical can be re-reduced directly by PS I. Together, these reactions are termed the water–water cycle, because electrons generated from the oxidation of water at PS II are used to reduce O_2 to water at PS I [47•]. The water–water

cycle consumes excess electrons but contributes to generation of a ΔpH without concomitant utilization of ATP.

The electron transport capacity of the water–water cycle is unclear and the extent to which it operates *in vivo* is controversial [46,48•]. Much of the uncertainty is due to difficulty in measuring flux through the water–water cycle, because there is no net O_2 exchange. Mass spectrometric measurements of gross $^{16}O_2$ evolution and $^{18}O_2$ uptake have been used to study the contribution of the water–water cycle as a safety valve in Rubisco antisense plants [49••]. A decrease in Rubisco levels would be expected to affect both the CO_2 fixation and photorespiration sinks for excess electrons, resulting in enhanced electron flow via proposed safety valves such as the water–water cycle. However, measurements of gross $^{18}O_2$ uptake by Rubisco antisense plants under steady-state non-photorespiratory conditions and at the CO_2 compensation point did not provide any indication of increased water–water cycle activity [49••]. Whether the water–water cycle plays a more significant role under other conditions, such as during photosynthetic induction, requires further investigation.

A chlororespiratory pathway involved in photoprotection

Chlororespiration is yet another type of O_2-dependent electron transport that appears to be possible in chloroplasts (Figure 1). Nearly 20 years ago, Bennoun [50] proposed that chloroplasts contain a respiratory chain that transfers electrons from NAD(P)H to O_2 via the plastoquinone pool. Several genes encoding subunits of an NADH-plastoquinone oxidoreductase (NDH)-complex are located in the chloroplast genomes of some plants, and the corresponding enzyme activity has been found in chloroplasts [51]. Tobacco mutants lacking the NDH complex appear normal under favorable growth conditions, but they have a subtle cyclic-electron-transport defect [52,53] and increased sensitivity to photoinhibition induced by extremely strong light [54].

Until recently, there was little evidence for the existence of a chlororespiratory oxidase. However, two groups studying the *immutans* (*im*) variegation mutant of *Arabidopsis* have recently found that the *IM* gene encodes a chloroplast protein that is similar to the alternative oxidase of mitochondria [55••,56••]. Mutations in the *IM* gene act 'plastid autonomously' to produce white leaf sectors in which the chloroplasts accumulate phytoene [57], a colorless carotenoid precursor that cannot protect against photooxidation. Phytoene desaturation is thought to require oxidized plastoquinone as an electron acceptor [58], suggesting that the oxidation of reduced plastoquinol by the IM protein is linked to phytoene desaturation [55••,56••].

The existence of a chloroplast alternative oxidase has also been demonstrated in studies of mutants of the unicellular green alga *Chlamydomonas reinhardtii* that lack PS I. The PS-I-less mutants are unable to perform photosynthesis, but they are viable because of the ability of *Chlamydomonas* to grow heterotrophically using acetate as a source of fixed C.

Despite the lack of PS I, mass spectrometric measurements revealed that these algal mutant cells exhibit a limited but significant PS-II-dependent O_2 evolution that is matched by equivalent O_2 uptake [59,60•]. The activity persists in double mutants lacking both PS I and the cytochrome b_6/f complex, and in isolated chloroplasts, suggesting the involvement of a thylakoid plastoquinone oxidase that is homologous to the *Arabidopsis* IM protein [61••]. Consistent with this hypothesis, the O_2 exchange activity of *Chlamydomonas* thylakoids was inhibited by propyl gallate, which also inhibits oxidase activity in *Escherichia coli* cells expressing the *Arabidopsis* IM protein, and a *Chlamydomonas* thylakoid protein of the expected size was detected using an antibody raised against IM [61••].

Thus, chloroplasts appear to have an alternative oxidase activity that can remove electrons from the plastoquinone pool. The oxidase function may be especially critical for efficient carotenoid biosynthesis during the early stages of chloroplast development when PS-I-dependent oxidation of plastoquinol is less efficient [55••,56••]. Carotenoid biosynthesis is also necessary in fully greened chloroplasts for the repair of photodamaged PS II [62], raising the possibility that the chlororespiratory oxidase may have a significant role in providing a safety valve for excess electrons in intense light, thereby allowing carotenoid biosynthesis to proceed when the photosynthetic electron-transport chain becomes overreduced.

Conclusions

Constitutive (i.e. the triplet valve), inducible (i.e. qE), and sustained (i.e. qI) mechanisms are involved in eliminating excess photons in the Chl-containing LHCs, whereas several oxygen-dependent electron-transport processes are potential safety valves for excess electrons. Analysis of an *Arabidopsis* mutant has uncovered a role for the PsbS protein in qE [16••], raising new questions while some old questions remain. For example, future studies will address the possible involvement of proton- and pigment-binding by PsbS, and the location of PsbS relative to other PS II subunits, but the ultimate goal is still to elucidate the structural and biophysical basis of qE. At the same time, progress can be expected in understanding the mechanism of sustained qI, which is associated with a reorganization of Chl that results in fluorescence spectral and lifetime changes [41••]. It will be interesting to compare and contrast findings for qE and qI, especially because of the changes in PsbS levels that have been observed in overwintering pine needles [42]. Although the water–water cycle does not appear to be a major contributor to dissipation of excess electrons during steady-state photosynthesis [49••], its significance under rapidly fluctuating conditions warrants further investigation. A newly discovered chloroplast alternative oxidase is important for photoprotection during chloroplast development [55••,56••] and perhaps also in mature chloroplasts. Undoubtedly, the isolation of new mutants and antisense plants will allow further assessment of the physiological significance of various safety valves for photosynthesis. However, because several processes contribute to

the dissipation of excess photons and electrons from the photosynthetic apparatus, one must consider that the phenotypes of mutants may be affected by the compensatory engagement of alternative protective mechanisms.

Acknowledgements

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