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Photosystem II photoinhibition-repair cycle protects Photosystem I from irreversible damage

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ABSTRACT

Photodamage of Photosystem II (PSII) has been considered as an unavoidable and harmful reaction that decreases plant productivity. PSII, however, has an efficient and dynamically regulated repair machinery, and the PSII activity becomes inhibited only when the rate of damage exceeds the rate of repair. The speed of repair is strictly regulated according to the energetic state in the chloroplast. In contrast to PSII, Photosystem I (PSI) is very rarely damaged, but when occurring, the damage is practically irreversible. While PSII damage is linearly dependent on light intensity, PSI gets damaged only when electron flow from PSII exceeds the capacity of PSI electron acceptors to cope with the electrons. When electron flow to PSI is limited, for example in the presence of DCMU, PSI is extremely tolerant against light stress. Proton gradient (ΔpH)-dependent slow-down of electron transfer from PSII to PSI, involving the PGR5 protein and the Cyt b6f complex, protects PSI from excess electrons upon sudden increase in light intensity. Here we provide evidence that in addition to the ΔpH-dependent control of electron transfer, the controlled photoinhibition of PSII is also able to protect PSI from permanent photodamage. We propose that regulation of PSII photoinhibition is the ultimate regulator of the photosynthetic electron transfer chain and provides a photoprotection mechanism against formation of reactive oxygen species and photodamage in PSI.

1. Introduction

Energy capture and electron transfer reactions are the basis of photosynthesis. These reactions perform high-potential redox chemistry and have side effects leading to photo-oxidative damage of the photosynthetic machinery. In order to minimize these harmful side reactions, the energy transfer reactions are dynamically regulated (see for reviews [1–3]). Such control is not, however, complete and chloroplasts also need an extensive antioxidant system to take care of produced reactive molecules [4,5]. It has been considered that the photo-oxidative damage and consequential photoinhibition of PSII as such are unavoidable and harmful reactions that plants must combat against [6–16]. However, controlled oxidative damage in one compartment of the photosynthetic machinery can protect the rest of the machinery against damage. A good example is the D1 protein of PSII, which has been considered as a "suicide protein" sacrificed to protect the rest of PSII against oxidative damage under excess light [17]. This concept can be expanded from PSII to comprise the entire machinery of photosynthetic light reactions [2,18]. Indeed, PSII feeds electrons to PSI, which becomes irreversibly photodamaged if the capacity of PSI electron acceptors becomes exceeded [19–22].

PSII and PSI have evolved opposing strategies to cope with photo-oxidative stress. PSII is very susceptible to light, the rate of damage being linearly dependent on the photon fluence rate [23]. The recovery mechanisms, however, function efficiently and the damage gets rapidly repaired [24–28]. Indeed, the PSII activity becomes inhibited only when the rate of damage exceeds the rate of repair. The speed of repair is dynamically controlled by environmental cues and the energetic state of the photosynthetic machinery, and slows down under excess light when the capacity of plant metabolism becomes limited to accept the energy from the light reactions [28]. In sharp contrast to PSII, the PSI centers are very efficiently protected against photodamage, and in a rare case of damage, the subsequent recovery of PSI is extremely slow [20,29]. Nevertheless, high light illumination is, in principle, very dangerous to PSI if the amount of electrons fed to the electron transfer chain (ETC) by PSII exceeds the capacity of electron acceptors on the reducing side of PSI [22]. When electron transfer to PSI from PSII is strictly controlled for example in the presence of DCMU, PSI is not only protected against photo-oxidative stress, but can also function as an extremely efficient quencher of excitation energy [30] captured by the light harvesting machinery (LHCI + LHCII).

The past few years have revealed the complexity and interaction of a number of different regulatory mechanisms of light-harvesting and electron transfer reactions in the thylakoid membrane (see for reviews [2,31–34]). In higher plant chloroplasts, PSI is protected from excess electrons via the control of LHCII-mediated excitation of PSII and PSI through NPQ and LHCII phosphorylation, and via regulation of the speed of electron transfer through the Cyt b6f complex [19,21,22,35]. LHCII phosphorylation prevents the accumulation of excess electrons

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in ETC under low light [35], which would be dangerous to PSI upon the first moments of sudden increase in the light intensity. ΔpH-dependent regulation of the Cyt b6f complex slows down the electron transfer from plastoquinone (PQ) pool to plastocyanin (PC) allowing rapid oxidation of PSI electron donors upon increase in light intensity. Concomitant induction of NPQ (PsbS-dependent qE) decreases the excitation energy transfer to PSII reaction centers thus allowing oxidation of the PQ-pool [2,22,35]. In the absence of the proton gradient-dependent control of Cyt b6f and induction of NPQ (the *pgr5* mutant), PSI centers are extremely sensitive to photoinhibition [22]. Likewise, an accumulation of excess electrons in ETC of the *stn7* mutant under low light, due to an excitation imbalance of the two photosystems, is very harmful to PSI upon subsequent exposure of leaves to high light [35].

Here we provide evidence that despite all the regulatory mechanisms discussed above, the control of the amount of active PSII centers functions as an ultimate regulator of the photosynthetic electron transfer chain. Strict control of active PSII centers via photoinhibition-repair cycle is demonstrated to rescue PSI from permanent damage and likely also allows the oxidized PSI centers to function as quenchers of excess excitation energy.

2. Material and methods

2.1. Plant material

Arabidopsis thaliana ecotype Colombia wild type *gl1* and the *pgr5* [36] were used for the experiments. The experiments were performed with mature leaves detached from 4- to 5-week-old rosettes. All plant materials were grown under the photoperiod of 8 h light/16 h dark. SRAM PowerStar HQIT 400/D metal halide lamps were used as a light source during the growth and high light treatments. The plants were grown under 120 μmol photons m^{-2} s^{-1} (GL) and 800 μmol photons m^{-2} s^{-1} was used in high light (HL) treatments.

2.2. PSII and PSI measurements

Dual-PAM-100 (Heinz Walz GmbH, Effeltrich, Germany) was used for the simultaneous measurement of PSII and PSI as described in Suorsa et al. and Grieco et al. [22,35]. Pm was recorded to estimate the amount of photo-oxidable PSI centers and Fm was measured to estimate the amount of active PSII centers. Pm and Fm were measured after at least 20 min of dark incubation. Fm was used to monitor PSII activity instead of Fv/Fm due to the fact that the damage of PSI increases F0 and therefore Fv/Fm is affected by photodamage of both PSII and PSI.

Measurements on the redox state of PSII and PSI were performed by using red actinic light (625 nm) of 58 (LA) and 533 (HA) μmol photons m^{-2} s^{-1}. In order to estimate the redox state of PSI under low and high actinic light, the P700 reaction center oxidation level (ND) was measured as (P − P0) / (Pm − P0) and the relative QA redox state was measured as F′ / Fm, where F′ is the fluorescence yield under actinic light as described in Gieco et al. [35].

2.3. Isolation, separation and detection of thylakoid proteins

Thylakoid membranes were isolated as described by Suorsa et al. [37]. The chlorophyll content was measured according to Porra et al. [38]. SDS-PAGE (15% polyacrylamide, 6 M urea) was used to separate proteins. After electrophoresis, the proteins were electroblotted to a polyvinylidene difluoride membrane (Millipore) and blocked with 5% milk (nonfat dry milk; BioRad). Immunoblotting using enhanced chemiluminescence detection was performed according to Kangasjarvi et al. [39]. PsaB antibody was purchased from Agrisera (Vännar, Sweden) and the D1 antibody was described previously [37,40]. Even loading of samples was confirmed by Coomassie Brilliant Blue G-250 (BioRad) staining of gels.

3. Results

3.1. Photoinhibition of PSII and PSI

In order to understand how the amount of active PSII centers affects the redox state of PSI, in the presence and absence of the PGR5-mediated regulation of linear electron transfer, the following experiments were performed. WT and *pgr5* plants were exposed to different light pre-treatments in the presence and absence of lincomycin, an inhibitor of chloroplast translation machinery preventing the de novo synthesis of D1 after photodamage and thus stopping the turnover of PSII. This made it possible to tune the amount of active PSII centers (Fm) and investigate the consequent effects on the function of PSI (Pm).

Detached WT and *pgr5* leaves were incubated overnight with or without lincomycin (1 mM) and then transferred to growth light (GL) for 2 h. In the absence of lincomycin, neither the Fm nor Pm were significantly affected either in WT or *pgr5* plants (Fig. 1A and B). In the presence of lincomycin, Fm dropped during 2 h of GL illumination to about 80% of the original value in WT as well as in the *pgr5* plants, whereas Pm remained unchanged both in WT and *pgr5* (Fig. 1C and D).

Transfer of the non-lincomycin treated leaves to HL led to gradual decrease in Fm, to about 75% of the original Fm value during the 2 h HL treatment in both WT and *pgr5* (Fig. 1A and B). In WT, Pm was not affected in the course of HL illumination (Fig. 1A). In sharp contrast to WT, Pm in the *pgr5* mutant decreased to 50% of the original value in the course of 2 h of HL illumination in the absence of lincomycin (Fig. 1B).

Next, the consequences of PSII photodamage on the performance of PSI, recorded as Pm, were investigated (Fig. 1). In WT, Pm remained unaffected during both the growth light and HL illumination in the presence and absence of lincomycin (Fig. 1A and C). The *pgr5* mutant behaved differently and showed distinct decrease in Pm when pre-illumination treatments were performed in the absence of lincomycin, the damage getting higher with increase in the HL pre-treatment. Presence of lincomycin and notable PSII photodamage during pre-illumination, however, changed the inhibition pattern of PSI completely and the decline in Pm was induced in *pgr5* only during the first 30 min of high light pre-treatment (Fig. 1D). Noteworthy, longer pre-treatments in HL did not enhance PSI photodamage, apparently because the electron flow from PSII was remarkably reduced.

3.2. Effect of PSII photoinhibition on the redox state of PSI

In order to elucidate further the relationships between photo-oxidation of P700, PGR5-dependent regulation of Cyt b6f and the functional ratio between PSII and PSI, the oxidation level of P700 was measured under low (LA) and high actinic light (HA) from leaves pretreated with light in the absence and presence of lincomycin as described in Fig. 1. At LA, the oxidation of P700 is possible only if the function of PSII is down-regulated while at HA also the photosynthetic control (PGR5-dependent) facilitates photo-oxidation. P700 in dark-acclimated plants shifted from darkness to LA remained reduced in both WT and *pgr5* plants (Fig. 2A and B). Sudden increase in the intensity of actinic light (shift from LA to HA) resulted in oxidation of P700 in WT (Fig. 2A). The *pgr5* mutant could not oxidize P700 under HA (Fig. 2B) as also reported earlier [21,22,36].

In lincomycin-treated leaves of both WT and *pgr5*, the oxidation level of P700, as measured under LA, gradually increased with the length of the preceding HL treatment (Fig. 2C and D). This occurred concomitantly with a decrease in the amount of photoactive PSII (Fm) (Fig. 1C and D) in WT and *pgr5*.

Upon shift to HA, the maximum amplitude of oxidized P700 was rapidly reached in WT and occurred independently of the pre-illumination treatment, both in the absence and presence of lincomycin (Fig. 2A and C). *pgr5* mutants behaved differently. Firstly, in lincomycin treated *pgr5* leaves (Fig. 2D) the amplitude of P700 oxidation was not

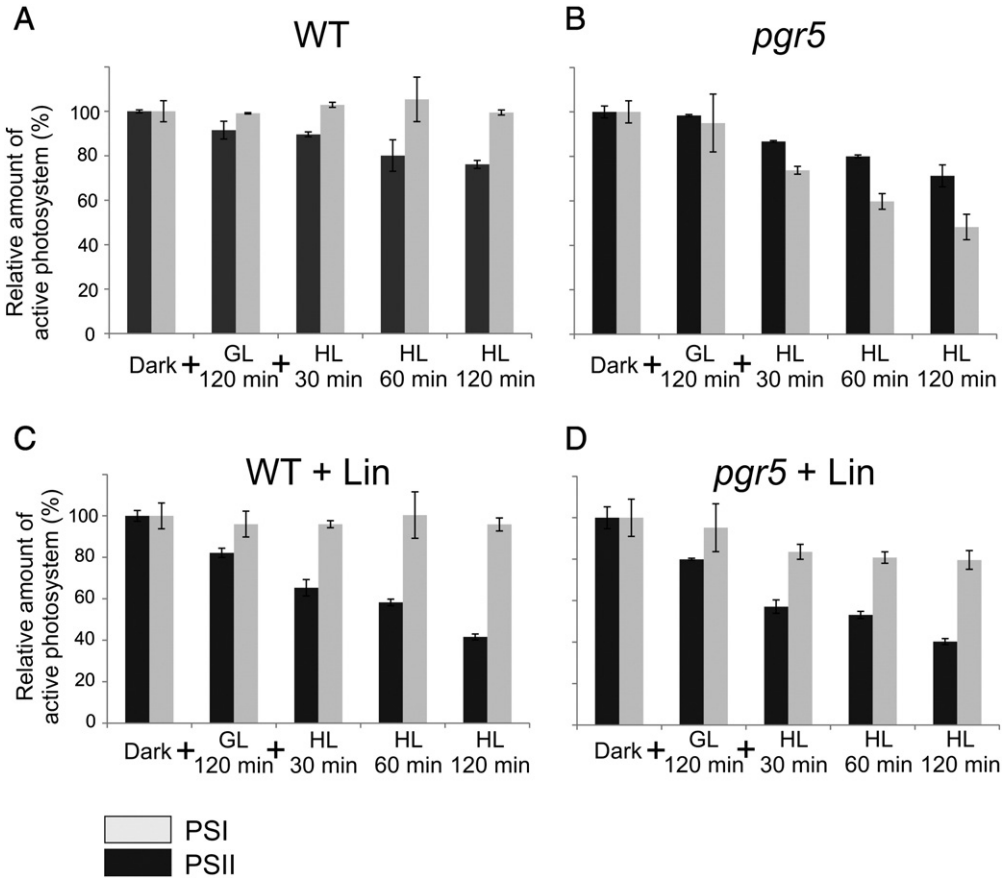


Fig. 1. Relationship between photoinhibition of PSII and PSI measured in the presence and absence of PGR5-dependent control of linear electron transfer. Leaves incubated in the presence or absence of lincomycin (1 mM) overnight in darkness were transferred to 120 μmol photons $m^{-2} s^{-1}$ (GL) for 2 h and subsequently to 800 μmol photons $m^{-2} s^{-1}$ for 30, 60 and 120 min. Fm was measured to estimate the amount of active PSII centers in dark incubated leaves, representing 100% of active PSII. Fm normalized by the leaf area was used instead of Fv/Fm because PSI photodamage has an effect on F0 (Fv = Fm − F0). Pm was measured to estimate the amount of photo-oxidable PSI, the dark incubated sample representing 100% of photo-oxidable P700. Averages and standard errors of five independent measurements are shown.

much affected by the intensity of actinic light (no big difference in shift from LA to HL). Secondly, the length of the pre-illumination treatment greatly modified the amplitude of P700 oxidation, which clearly increased when the preceding HL treatment was prolonged (Fig. 2D).

The data above provide strong support to the fact that the photodamage of PSI can be alleviated by down-regulation of PSII, which here was induced by mild lincomycin treatment.

Changes in photo-oxidation of PSI, as indicated in Fig. 2, are relative to the amount of photo-oxidable PSI (Fig. 1). As demonstrated in Fig. 1, the pretreatments did not decrease the amount of photo-oxidable PSI in WT and thus Fig. 2 represents the oxidation of the entire native PSI pool, as determined by the amount of photoactive PSII when measured in LA and in HL additionally by the photosynthetic control. In lincomycin treated *pgr5* the amount of photo-oxidable PSI was not affected under growth light but decreased to about 80% of the original value when leaves were exposed to HL (Fig. 1D). Due to this decrease in the relative amount of functional PSI, the real protective effect of PSII photoinhibition on the oxidation capacity of P700 is supposed to be even slightly higher than that indicated in Fig. 2D.

The degradation of the PSII reaction center protein D1 during different pretreatments (see Fig. 1) in the presence of lincomycin (no protein degradations were observed in the absence of lincomycin, data not shown) was monitored by immunoblotting (Fig. 3). 2-h treatment under growth light did not affect the amount of D1 protein in WT and *pgr5*. Upon 30 min, 60 min and 120 min of HL exposures D1 became gradually degraded (Fig. 3), which is in line with Fm measurements from the same time points (Fig. 1C and D). Interestingly, the D1 degradation seemed to be enhanced in *pgr5* as compared to WT. Only the proteins of the PSII core complex were affected by the treatment as shown by PsaB immunoblotting and Coomassie staining of the gel (Fig. 3).

4. Discussion

PSII photoinhibition has often been considered as an unavoidable and harmful reaction, decreasing plant productivity [6–16]. It is, however, conceivable that PSII photoinhibition-repair cycle is an active regulatory component of the photosynthetic electron transfer reactions. Here we provide evidence that the light sensitivity of PSII and dynamic regulation of D1 protein turnover, in concert with ΔpH- and PGR5-dependent control of Cyt b6f, enable the ultimate control of photosynthetic electron flow and thereby prevent irreversible damage of PSI.

Our experimental design was to decrease the amount of active PSII centers in a controlled manner and concomitantly follow the consequences of decreasing PSII activity to the activity of PSI, both in WT and in the *pgr5* mutant deficient in the ΔpH- and PGR5-dependent control of Cyt b6f (Fig. 1A and B vs. C and D). Decreasing the amount of photoactive PSII centers by HL pre-illumination in the presence of lincomycin (Fig. 1D) rescued the PGR5-independent photo-oxidation of P700 in PSI (measurement at LA in WT and LA and HA in *pgr5*) (Fig. 2D) and is thus capable of protecting PSI from permanent photodamage (Fig. 1D). The loss of PSII function down to 40% of the control value (Fig. 1) occurred only in lincomycin treated leaves together with degradation of the D1 protein (Fig. 3). The PSII photoinhibition occurred slightly faster than the degradation of D1 protein in both WT and *pgr5* leaves. Even though the photoinhibition of PSII was not faster

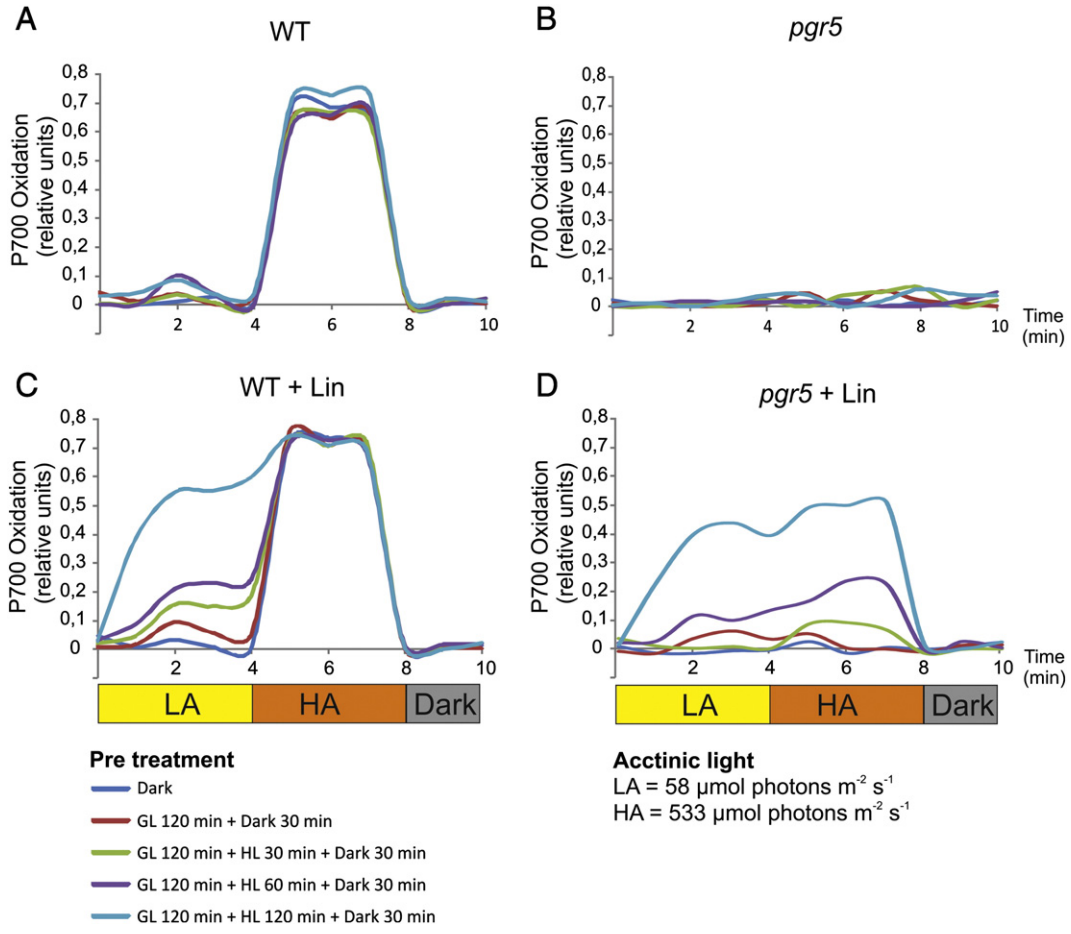
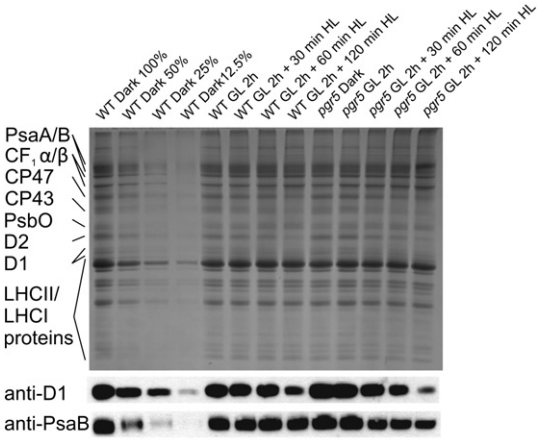


Fig. 2. Relationship between the amount of active PSII centers and the redox state of P700 electron acceptors. Leaves were treated as in Fig. 1. P700 oxidation (ND) was recorded as P700 reaction centers oxidation level (P — P0) / (Pm — P0). Curves represent averages of five independent measurements.

in *pgr5* as compared to WT (Fig. 2C and D), the D1 degradation was slightly accelerated in *pgr5* as compared to WT (Fig. 3). Degradation of the D1 protein after photodamage is dependent on fluent unpacking of damaged PSII–LHCII complexes and lateral migration of damaged PSII from grana to stroma membranes. This process is dependent on the STN8-dependent phosphorylation of PSII core proteins [41,42], yet the STN7-dependent phosphorylation of CP29 may also play a minor role [43]. Interestingly, under high light both the PSII–LHCII proteins are hyper phosphorylated in the *pgr5* mutant (Rao Mekala et al., manuscript under preparation) due to redox imbalance (Fig. 2B) disturbing the regulation of thylakoid protein kinases and phosphatases. It is possible that this hyperphosphorylation of PSII–LHCII phosphoproteins in *pgr5* allows more fluent unpacking of damaged PSII centers and accelerated degradation of the damaged D1 as compared to WT. Whatever the reasons are for slightly different rates of D1 protein degradation, the results collectively provide strong evidence that PSII photoinhibition protects PSI against photodamage.

The next question is why two distinct mechanisms, PSII photoinhibition-repair cycle as described above and the PGR5-mediated mechanism [22], are needed to protect PSI against photodamage? It seems likely that the ΔpH-dependent and PGR5-mediated control of Cyt b6f is operational upon short time scales, upon sudden shift to HL, in order to rapidly limit electron transfer to PSI (Fig. 2A vs. B). The generation of strong ΔpH resulting in photosynthetic control and NPQ has been considered as a transient reaction upon increase in light intensity [44]. Nevertheless, for proper protection of PSI against photodamage, there should be a possibility to always limit electron transfer upon a risk of too strong supply of electrons from PSII to PSI, which otherwise would exceed the capacity of PSI electron acceptors. In fact, this risk is the highest under prolonged high light stress when the transient protonation of the thylakoid lumen should already be relaxed. It is highly conceivable that the ΔpH-dependent and PGR5-mediated control of Cyt b6f (Fig. 2A vs. B) is a transient mechanism to limit electron transfer to PSI and in a longer time course the down-regulation of PSII activity (Fig. 2C and D) takes the main responsibility of preventing excess electron flow to PSI. Indeed, PSII turnover is dynamically regulated according to the energetic state of the thylakoid membrane, allowing the balancing of the amount of active PSII with the capacity of PSI electron acceptors [28].

Down-regulation of the repair of PSII from photoinhibition likely represents an efficient mechanism to protect PSI against photodamage upon prolonged high light stress (Fig. 1D). This mechanism can function above the co-operation of more dynamic, yet limited regulatory mechanisms: non-photochemical dissipation of excitation energy (NPQ), regulation of excitation energy distribution between PSII and PSI (LHCII phosphorylation) and the regulation of electron transfer by the Cyt b6f complex (see for review [2]). As described above, the dynamic regulation of PSII turnover after photodamage operates under strict control of the chloroplast redox network and is likely to function as the ultimate control of photosynthetic electron transfer allowing the maintenance of P700 optimally oxidized under excess light. Such sensing of chloroplast redox environment and capability for limitation of electron flow from PSII efficiently tune the electron flow and prevent exceeding the capacity of PSI electron acceptors shown to be extremely dangerous to PSI. Upon sudden and transient increase in the light intensity, the ΔpH-dependent and PGR5-mediated control of Cyt b6f leads to drastic oxidation of P700 [21,22,36]. Indeed, even more than 50% photoinhibition of PSII did not increase P700 oxidation upon shift from LA to HA (Figs. 1C and 2C) in



Sample	Relative amount of D1
WT Dark 100%	**100 %**
WT Dark 50%	65 %
WT Dark 25%	50 %
WT dark 12,5%	11 %
WT GL 2 h	87 %
WT GL 2 h + HL 30 min	87 %
WT 2h + HL 60 min	84 %
WT 2h + HL 120 min	55 %
pgr5 dark	**100 %**
pgr5 GL 2 h	93 %
pgr5 GL 2 h + HL 30 min	75 %
pgr5 2h + HL 60 min	62 %
pgr5 2h + HL 120 min	27 %

Fig. 3. Amount of the D1 protein in WT and *pgr5* thylakoids. Leaves were light-treated in the presence of lincomycin as described in the legend of Fig. 1. Amounts of D1 and PsaB were determined by immunoblotting with specific antibodies. All thylakoid proteins were visualized by Coomassie staining of the gel. Representative data from three independent experiments are shown. Relative densitometric values for D1 are presented below the figure. Dark adapted samples represent 100%.

WT indicating that the limitation of the electron transfer by the ΔpH-dependent mechanism is very strong. Such a drastic limitation of electron transfer is obviously needed to preserve PSI activity upon transient increase in the light intensity, but would likely start limiting the growth during longer time scales.

The mechanism of the PGR5-dependent protonation of the thylakoid lumen is not clear. Traditionally it has been explained by the PGR5 dependent enhancement of cyclic electron transfer around PSI [36], but this theory has some ambiguities leaving space for alternative explanations [2,45]. It is possible that a shift to HL enhances the q-cycle, leading to strong protonation of the lumen. This would, however, need q-cycle-independent electron transfer under low light, which is unlikely [46] yet possible [47]. The most probable explanation for the mechanism of PGR5 is that it generates strong ΔpH just by blocking the proton transport from lumen back to stroma via the ATP synthase upon increase in the light intensity. There is, however, no clear experimental evidence supporting any of these mechanisms. Whatever the mechanism to induce the PGR5-dependent ΔpH upon increase in the light intensity is, it would disturb the balance between NADPH and ATP production. Indeed, it seems obvious that plants need a mechanism to slow down the entire electron transfer chain, yet keep electron transfer and the ΔpH at optimal level for the balanced NADPH and ATP production. Basically, regulation of only the primary provider of electrons and protons, i.e., the PSII center would allow sustainable setting of the function of the photosynthetic light reactions to meet the capacity of PSI electron acceptors.

It is noteworthy that the ostensibly wasteful regulatory mechanisms of the photosynthetic machinery are prerequisites for survival under fluctuating light environment in nature [22,48]. This is especially important when designing better photosynthetic organisms for the future needs of mankind. Minimizing NPQ leads to high excitation pressure towards PSII and strong reduction of the PQ-pool upon shift to HL, yet the electron donors to PSI (PC) remain oxidized because of the photosynthetic control by Cyt b6f [35]. This leads to photoinhibition of PSII [49], which however does not seem to be very dangerous for the plant [35,50]. If additionally the control by Cyt b6f is removed (*pgr5*), an uncontrolled excitation energy transfer towards reaction centers and uncontrolled electron transfer from PSII to PSI lead first to an irreversible photodamage of PSI, subsequently to photoinhibition of PSII [22,36] and finally to the death of the plant [50]. Minimizing PSII photoinhibition would likely lead to a similar situation as the removal of the photosynthetic control and NPQ, with the exception that the consequences would be visible only after the saturation of the ΔpH-dependent mechanisms. Indeed, in light of our results the strategy to make plants grow faster by reducing the regulatory flexibility of the photosynthetic light reaction seems to not be functional, at least in natural environments. Instead, it would be crucial to understand how to redesign the plant metabolism and morphology towards more efficient use of the high capacity of the primary photosynthetic reactions.

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References

[1] K.K. Niyogi, Photoprotection revisited: genetic and molecular approaches, Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 50 (1999) 333–359.
[2] M. Tikkanen, M. Grieco, M. Nurmi, M. Rantala, M. Suorsa, E.M. Aro, Regulation of the photosynthetic apparatus under fluctuating growth light, Philos. Trans. R. Soc. Lond. B Biol. Sci. 367 (2012) 3486–3493.
[3] K.K. Niyogi, T.B. Truong, Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis, Curr. Opin. Plant Biol. 16 (2013) 307–314.
[4] J. Dat, S. Vandenabeele, E. Vranova, M. Van Montagu, D. Inze, F. Van Breusegem, Dual action of the active oxygen species during plant stress responses, Cell. Mol. Life Sci. 57 (2000) 779–795.
[5] K. Apel, H. Hirt, Reactive oxygen species: metabolism, oxidative stress, and signal transduction, Annu. Rev. Plant Biol. 55 (2004) 373–399.
[6] E. Ögren, M. Sjöström, Estimation of the effect of photoinhibition on the carbon gain in leaves of a willow canopy, Planta 181 (1990) 560–567.
[7] P.K. Farage, S.P. Long, The occurrence of photoinhibition in an over-wintering crop of oil-seed rape (*Brassica napus* L.) and its correlation with changes in crop growth, Planta 185 (1991) 279–286.
[8] N.R. Baker, P.K. Farage, C.M. Stirling, S.P. Long, Photoinhibition of crop photosynthesis in the field at low temperatures, in: N.R. Baker, J.R. Bowyer (Eds.), Photoinhibition of Photosynthesis From Molecular Mechanisms to the Field, Bios Scientific Publishers, Oxford, 1994, pp. 349–363.
[9] S.P. Long, S. Humphries, P.G. Falkowski, Photoinhibition of photosynthesis in nature, Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 45 (1994) 633–662.
[10] E. Ögren, The significance of photoinhibition for photosynthetic productivity, in: N.R. Baker, J.R. Bowyer (Eds.), Photoinhibition of Photosynthesis From Molecular Mechanisms to the Field, Bios Scientific Publishers, Oxford, 1994, pp. 433–447.
[11] C. Werner, R.J. Ryel, O. Correia, W. Beyschlag, Effects of photoinhibition on whole-plant carbon gain assessed with a photosynthesis model, Plant Cell Environ. 24 (2001) 27–40.
[12] P. Losciale, W.S. Chow, L. Corelli Grappadelli, Modulating the light environment with the peach 'asymmetric orchard': effects on gas exchange performances, photo-protection, and photoinhibition, J. Exp. Bot. 61 (2010) 1177–1192.
[13] J.A. Raven, The cost of photoinhibition, Physiol. Plant. 142 (2011) 87–104.
[14] S. Takahashi, M.R. Badger, Photoprotection in plants: a new light on Photosystem II damage, Trends Plant Sci. 16 (2011) 53–60.
[15] C. Goh, S. Ko, S. Koh, Y. Kim, H. Bae, Photosynthesis and environments: photoinhibition and repair mechanisms in plants, J. Plant Biol. (2011) 93–101.
[16] M. Reynolds, J. Foulkes, R. Furbank, S. Griffiths, J. King, E. Murchie, M. Parry, G. Slafer, Achieving yield gains in wheat, Plant Cell Environ. 35 (2012) 1799–1823.
[17] E.M. Aro, I. Virgin, B. Andersson, Photoinhibition of Photosystem II. Inactivation, protein damage and turnover, Biochim. Biophys. Acta 1143 (1993) 113–134.
[18] W.W. Adams III, O. Muller, C.M. Cohu, B. Demmig-Adams, May photoinhibition be a consequence, rather than a cause, of limited plant productivity? Photosynth. Res. (2013), http://dx.doi.org/10.1007/s11120-013-9849-7.
[19] K. Sonoike, I. Terashima, M. Iwaki, S. Itoh, Destruction of Photosystem I iron–sulfur centers in leaves of *Cucumis sativus* L. by weak illumination at chilling temperatures, FEBS Lett. 362 (1995) 235–238.
[20] K. Sonoike, Photoinhibition of Photosystem I, Physiol. Plant. 142 (2011) 56–64.
[21] P. Joliot, G.N. Johnson, Regulation of cyclic and linear electron flow in higher plants, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 13317–13322.

[22] M. Suorsa, S. Jarvi, M. Grieco, M. Nurmi, M. Pietrzykowska, M. Rantala, S. Kangasjarvi, V. Paakkarinen, M. Tikkanen, S. Jansson, E.M. Aro, PROTON GRADIENT REGULATION5 is essential for proper acclimation of *Arabidopsis* Photosystem I to naturally and artificially fluctuating light conditions, Plant Cell 24 (2012) 2934–2948.
[23] E. Tyystjarvi, E.M. Aro, The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 2213–2218.
[24] A.W. Russell, C. Critchley, S.A. Robinson, L.A. Franklin, G. Seaton, W.S. Chow, J.M. Anderson, C.B. Osmond, Photosystem II regulation and dynamics of the chloroplast D1 protein in *Arabidopsis* leaves during photosynthesis and photoinhibition, Plant Physiol. 107 (1995) 943–952.
[25] E.M. Aro, S. McCaffery, J.M. Anderson, Recovery from photoinhibition in peas (*Pisum sativum* L.) acclimated to varying growth irradiances (role of D1 protein turnover), Plant Physiol. 104 (1994) 1033–1041.
[26] S.I. Allakhverdiev, Y. Nishiyama, S. Takahashi, S. Miyairi, I. Suzuki, N. Murata, Systematic analysis of the relation of electron transport and ATP synthesis to the photodamage and repair of Photosystem II in *Synechocystis*, Plant Physiol. 137 (2005) 263–273.
[27] S.I. Allakhverdiev, N. Murata, Environmental stress inhibits the synthesis de novo of proteins involved in the photodamage-repair cycle of Photosystem II in *Synechocystis* sp. PCC 6803, Biochim. Biophys. Acta 1657 (2004) 23–32.
[28] N. Murata, S.I. Allakhverdiev, Y. Nishiyama, The mechanism of photoinhibition in vivo: re-evaluation of the roles of catalase, alpha-tocopherol, non-photochemical quenching, and electron transport, Biochim. Biophys. Acta 1817 (2012) 1127–1133.
[29] H.V. Scheller, A. Haldrup, Photoinhibition of Photosystem I, Planta 221 (2005) 5–8.
[30] V.V. Shubin, I.N. Terekhova, B.A. Kirillov, N.V. Karapetyan, Quantum yield of P700+ photodestruction in isolated Photosystem I complexes of the cyanobacterium *Arthrospira platensis*, Photochem. Photobiol. Sci. 7 (2008) 956–962.
[31] M. Tikkanen, M. Grieco, E.M. Aro, Novel insights into plant light-harvesting complex II phosphorylation and 'state transitions', Trends Plant Sci. 16 (2011) 126–131.
[32] C.H. Foyer, J. Neukermans, G. Queval, G. Noctor, J. Harbinson, Photosynthetic control of electron transport and the regulation of gene expression, J. Exp. Bot. 63 (2012) 1637–1661.
[33] P. Jahns, A.R. Holzwarth, The role of the xanthophyll cycle and of lutein in photoprotection of Photosystem II, Biochim. Biophys. Acta 1817 (2012) 182–193.
[34] H. van Amerongen, R. Croce, Light harvesting in Photosystem II, Photosynth. Res. 116 (2013) 251–263.
[35] M. Grieco, M. Tikkanen, V. Paakkarinen, S. Kangasjarvi, E.M. Aro, Steady-state phosphorylation of light-harvesting complex II proteins preserves Photosystem I under fluctuating white light, Plant Physiol. 160 (2012) 1896–1910.
[36] Y. Munekage, M. Hojo, J. Meurer, T. Endo, M. Tasaka, T. Shikanai, PGR5 is involved in cyclic electron flow around Photosystem I and is essential for photoprotection in *Arabidopsis*, Cell 110 (2002) 361–371.
[37] M. Suorsa, R.E. Regel, V. Paakkarinen, N. Battchikova, R.G. Herrmann, E.M. Aro, Protein assembly of Photosystem II and accumulation of subcomplexes in the absence of low molecular mass subunits PsbL and PsbJ, Eur. J. Biochem. 271 (2004) 96–107.
[38] R.J. Porra, W.A. Thompson, P.E. Kriedemann, Determination of accurate extinction coefficients and simultaneous-equations for assaying chlorophyll-a and chlorophyll-b extracted with 4 different solvents — verification of the concentration of chlorophyll standards by atomic-absorption spectroscopy, Biochim. Biophys. Acta 975 (1989) 384–394.
[39] S. Kangasjarvi, A. Lepisto, K. Hannikainen, M. Piippo, E.M. Luomala, E.M. Aro, E. Rintamaki, Diverse roles for chloroplast stromal and thylakoid-bound ascorbate peroxidases in plant stress responses, Biochem. J. 412 (2008) 275–285.
[40] Y. Allahverdiyeva, F. Mamedov, P. Maenpaa, I. Vass, E.M. Aro, Modulation of photosynthetic electron transport in the absence of terminal electron acceptors: characterization of the rbcL deletion mutant of tobacco, Biochim. Biophys. Acta 1709 (2005) 69–83.
[41] M. Tikkanen, M. Nurmi, S. Kangasjarvi, E.M. Aro, Core protein phosphorylation facilitates the repair of photodamaged Photosystem II at high light, Biochim. Biophys. Acta 1777 (2008) 1432–1437.
[42] R. Fristedt, A. Willig, P. Granath, M. Crevecoeur, J.D. Rochaix, A.V. Vener, Phosphorylation of Photosystem II controls functional macroscopic folding of photosynthetic membranes in *Arabidopsis*, Plant Cell 21 (2009) 3950–3964.
[43] R. Fristedt, A.V. Vener, High light induced disassembly of Photosystem II supercomplexes in *Arabidopsis* requires STN7-dependent phosphorylation of CP29, PLoS One 6 (2011) e24565.
[44] P.A. Joliot, G. Finazzi, Proton equilibration in the chloroplast modulates multiphasic kinetics of nonphotochemical quenching of fluorescence in plants, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 12728–12733.
[45] A.K. Livingston, J.A. Cruz, K. Kohzuma, A. Dhingra, D.M. Kramer, An *Arabidopsis* mutant with high cyclic electron flow around Photosystem I (hcef) involving the NADPH dehydrogenase complex, Plant Cell 22 (2010) 221–233.
[46] D.M. Kramer, A.G. Roberts, F. Muller, J. Cape, M.K. Bowman, Q-cycle bypass reactions at the Qo site of the cytochrome bc1 (and related) complexes, Methods Enzymol. 382 (2004) 21–45.
[47] A. Malnoe, F.A. Wollman, C. de Vitry, F. Rappaport, Photosynthetic growth despite a broken Q-cycle, Nat. Commun. 2 (2011) 301.
[48] C. Kulheim, J. Agren, S. Jansson, Rapid regulation of light harvesting and plant fitness in the field, Science 297 (2002) 91–93.
[49] X.P. Li, P. Muller-Moule, A.M. Gilmore, K.K. Niyogi, PsbS-dependent enhancement of feedback de-excitation protects Photosystem II from photoinhibition, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 15222–15227.
[50] M. Tikkanen, M. Grieco, S. Kangasjarvi, E.M. Aro, Thylakoid protein phosphorylation in higher plant chloroplasts optimizes electron transfer under fluctuating light, Plant Physiol. 152 (2010) 723–735.