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REVIEW PAPER

Photoprotection of photosystems in fluctuating light intensities

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Abstract

Oxygenic photosynthetic organisms experience strong fluctuations in light intensity in their natural terrestrial and aquatic growth environments. Recent studies with both plants and cyanobacteria have revealed that Photosystem (PS) I is the potential target of damage upon abrupt changes in light intensity. Photosynthetic organisms have, however, developed powerful mechanisms in order to protect their photosynthetic apparatus against such potentially hazardous light conditions. Although the electron transfer chain has remained relatively unchanged in both plant chloroplasts and their cyanobacterial ancestors, the photoprotective and regulatory mechanisms of photosynthetic light reactions have experienced conspicuous evolutionary changes. In cyanobacteria, the specific flavodiiron proteins (Flv1 and Flv3) are responsible for safeguarding PSI under rapidly fluctuating light intensities, whilst the thylakoid located terminal oxidases are involved in the protection of PSII during 12 h diurnal cycles involving abrupt, square-wave, changes from dark to high light. Higher plants such as *Arabidopsis thaliana* have evolved different protective mechanisms. In particular, the PGR5 protein controls electron flow during sudden changes in light intensity by allowing the regulation mostly via the Cytochrome *b6f* complex. Besides the function of PGR5, plants have also acquired other dynamic regulatory mechanisms, among them the STN7-related LHCII protein phosphorylation that is similarly responsible for protection against rapid changes in the light environment. The green alga *Chlamydomonas reinhardtii*, as an evolutionary intermediate between cyanobacteria and higher plants, probably possesses both protective mechanisms. In this review, evolutionarily different photoprotective mechanisms under fluctuating light conditions are described and their contributions to cyanobacterial and plant photosynthesis are discussed.

Key words: Cyanobacteria, flavodiiron proteins, green algae, PGR5, PGRL1, photodamage, photosynthesis, Photosystem I (PSI), plant, terminal oxidases.

Introduction

In natural growth environments, in addition to diurnal light-dark cycles, plants and phytoplankton are often exposed to rapid fluctuations in light intensity. The position of the sun and clouds, and the wind-induced movement of leaves, lead to conditions where the photon flux reaching the vegetation can increase and decrease with high frequency and several orders of magnitude (e.g. Ganeteg *et al.*, 2004; Hirth *et al.*, 2013). In many forests with closed canopies the situation is even more complicated and only a small fraction of light (0.5–5%) can penetrate the canopy and reach the understory vegetation. The dim and diffuse light environment of forest understory vegetation may, however, be frequently interrupted by short and strong sunflecks.

Light conditions of aquatic organisms are also highly heterogeneous and dynamic. In aquatic environments, strong fluctuations of light are mainly caused by movements of waves, clouds, and the vertical migration of phytoplankton (for review, see Iluz *et al.*, 2012). A lens effect of waves, by

simultaneously focusing and diffusing light in the few upper metres, creates a high frequency of light fluctuations for phytoplankton. Due to the lens effect, the light intensity in aquatic environments could be increased by up to five times, reaching 9000 µmol photons m^{-2} s^{-1} with frequency higher than 1 Hz (Schubert *et al.*, 2001).

Nevertheless, in laboratory conditions, experimental plants and microalgae are generally cultivated under constant illumination or dark/light regimes that do not represent a natural light environment. Therefore, until very recently, more thorough information about physiological effects of sudden changes in light intensity on the photosynthetic apparatus of terrestrial plants and phytoplankton has been largely missing.

Photoprotection mechanisms of cyanobacteria under fluctuating light intensity

Cyanobacteria, comprising a large proportion of marine and freshwater phytoplankton, have been subjected to thorough investigation of mechanisms responsible for successfully coping with abrupt fluctuations in light intensity (Ibelings *et al.*, 1994; Nicklisch, 1998; Fietz and Nicklisch, 2002; MacKenzie and Campbell, 2005; Allahverdiyeva *et al.*, 2013; Lea-Smith *et al.*, 2013). Cyanobacteria represent the progenitors of plant chloroplasts and acquired in their early evolution specific mechanisms to cope with abrupt fluctuations in light intensity. The key components of cyanobacterial photosynthetic apparatus responsible for survival under rapidly fluctuating light have so far been demonstrated to include specific members of the flavodiiron protein (FDP) family and specific respiratory terminal oxidases. As to the FDPs, their absence has been shown to induce lethality under fluctuating light intensity conditions in the unicellular, non-N_2-fixing model cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter referred as to *Synechocystis*) as well as in the filamentous, N_2-fixing *Anabaena* sp. PCC 7120 (also named *Nostoc* sp. PCC7120, and hereafter referred as to *Anabaena*) (Allahverdiyeva *et al.*, 2013). On the other hand, the respiratory terminal oxidases, cytochrome *bd*-type quinol oxidase (Cyd) and *aa3*-type cytochrome *c* oxidase (Cox), were shown to be crucial for *Synechocystis* during diurnal dark/high-light square-wave cycles (Lea-Smith *et al.*, 2013). The FDP and Cyd/Cox mechanisms essential for cyanobacteria under rapidly changing light intensities are briefly discussed below.

FDP proteins in cyanobacteria

FDPs, previously also called A-type flavoproteins (Flvs), were originally found in anaerobic Archaea, bacteria, and some eukaryotes. FDPs in these organisms protect the cells from NO and O_2 toxicity (Wasserfallen *et al.*, 1998; Vicente *et al.*, 2008). Bioinformatics analysis of the cyanobacterial genomes so far sequenced revealed genes with high homology to genes encoding FDPs in anaerobic bacteria (Zhang *et al.*, 2009, Peltier *et al.*, 2010). *Synechocystis* possesses four genes encoding FDPs, Flv1–Flv4. BLAST and complete genome sequence analyses demonstrated that filamentous N_2-fixing cyanobacteria contain two extra copies of the hypothetical genes encoding the Flv1 and Flv3 proteins (Zhang *et al.*, 2009). Fluorescence protein tagging of the four *Anabaena* FDPs revealed that the duplicates of the Flv1 and Flv3 proteins are spatially segregated in the filaments, so that Flv1A and Flv3A are strictly present only in vegetative cells, while Flv1B and Flv3B are expressed only after combined nitrogen step-down and exclusively in heterocysts, where they are involved in protection of the N_2-fixation apparatus (Ermakova *et al.*, 2013, 2014).

The Flv1 and Flv3 proteins function during illumination and dissipate excess electrons by reducing O_2 directly to water on the reducing side of Photosystem (PS) I. As a consequence, mutants lacking the Flv1 and/or Flv3 protein do not show light-induced O_2 uptake under standard growth conditions (Helman *et al.*, 2003, 2005; Allahverdiyeva *et al.*, 2011). It is well known that during different stress conditions, the photoreduction of O_2 on the reducing side of PSI also takes place in the chloroplasts of higher plants and green algae, in a process called the Mehler reaction (Mehler, 1957). Importantly, the Mehler reaction involves a production of reactive oxygen species (ROS). In contrast to the Mehler reaction, FDP-mediated O_2 photoreduction in cyanobacteria does not generate ROS (Vicente *et al.*, 2002). For this reason it is called a Mehler-like reaction or a cyanobacterial-type Mehler reaction (Allahverdiyeva *et al.*, 2013). Recent studies demonstrated that the Flv1(A) and Flv3(A) proteins are crucial for survival of cyanobacteria under fluctuating light, i.e. when growth light is repeatedly interrupted with strong high-light phases (Allahverdiyeva *et al.*, 2013). The Flv2 and Flv4 proteins are not involved in O_2 photoreduction (Helman *et al.*, 2003) and their absence does not show any phenotype different from the wild type under fluctuating light (Allahverdiyeva *et al.*, 2013). Instead, they function as a heterodimer in photoprotection of PSII under ambient levels of CO_2 by passing excess electrons from the acceptor side of PSII to an as yet unknown electron acceptor (Zhang *et al.*, 2009, 2012; Bersanini *et al.*, 2014). Since the heterocyst-specific Flv1B and Flv3B proteins (Ermakova *et al.*, 2013) are not involved in photoprotection of cyanobacteria under fluctuating light (Allahverdiyeva *et al.*, 2013; Ermakova *et al.*, 2014), they are not discussed here.

Mechanisms leading to damage of photosynthetic apparatus under fluctuating light in cyanobacteria

Abruptly fluctuating light conditions, e.g. an FL20/500 regime when 20 µmol photons m^{-2} s^{-1} background light is punctured for 30 s with 500 µmol photons m^{-2} s^{-1} every 5 min, completely inhibit the growth of the Δ*flv1*, Δ*flv3*, and Δ*flv1*/Δ*flv3* mutants of *Synechocystis* and promote cell death. Exposure of cells to milder fluctuating light, e.g. an FL50/500 regime when 50 µmol photons m^{-2} s^{-1} background light is punctured for 30 s with 500 µmol photons m^{-2} s^{-1} every 5 min, results in an arrest of growth (Allahverdiyeva *et al.*, 2013). Thus, the severity of consequences upon exposure of *Synechocystis* cells to fluctuating light is determined, at least in these particular

cases, by the intensity difference between the background light and high light phase. Similar behaviour was observed with the Δ*flv1A* and Δ*flv3A* mutants of *Anabaena*, lacking the functional Flv1A and Flv3A proteins (Allahverdiyeva *et al.*, 2013) localized in the vegetative cells (Ermakova *et al.*, 2013). The Δ*flv1*/Δ*flv3* mutant of *Synechocystis* treated under an FL20/500 regime for 2–3 days exhibited low net photosynthesis, impaired CO_2 concentration/fixation capacity, and diminished PSI level, evidenced by a decrease in the PsaA protein content as well as in the maximum oxidizable P700, P_M (Allahverdiyeva *et al.*, 2013).

Monitoring the performance of PSII and PSI in the Δ*flv1*/Δ*flv3* mutants grown under constant light, using DUAL-PAM and mimicking of the fluctuating growth light-regime by actinic light, led to the identification of primary targets behind malfunction of the photosynthetic apparatus in these conditions. It was clear that upon a shift from constant to fluctuating light, always at the onset of high light illumination, the Δ*flv1*/Δ*flv3* mutant cells experienced a significant delay in oxidation of P700, due to a strong acceptor-side limitation of PSI (Helman *et al.*, 2003; Allahverdiyeva *et al.*, 2013). It was hypothesized that blockage of electron transport on the reducing-side of PSI possibly generates ROS production, damages PSI, and/or initiates a signalling cascade resulting in downregulation of PSI proteins. After 2–3 days of incubation under fluctuating light, a preferential loss of the PSI complex led to an increase in the ratio of PSII to PSI. Thus, the over-reduction of the electron-transport chain during the high-light phase kept P700 completely reduced and limited net photosynthesis in the Δ*flv1*/Δ*flv3* mutant. Malfunction of the PSI complex and strongly induced ROS production, apparently via the true Mehler reaction in the absence of Flv1 and Flv3, led to irreversible cell damage and death upon prolonged incubation of the Δ*flv1*/Δ*flv3* cells under severe fluctuating light conditions (Allahverdiyeva *et al.*, 2013). In line with this, it has been previously documented that superoxide can induce growth arrest in *Synechocystis* (Kim *et al.*, 2009).

Respiratory terminal oxidases as important components involved in safeguarding the photosynthetic apparatus under fluctuating light

In cyanobacteria, respiratory electron transport components reside in both the cytoplasmic and thylakoid membranes. Therefore, in the thylakoid membrane, the two main bioenergetic pathways, oxygenic photosynthesis and respiration, share some redox components in a close functional relationship. *Synechocystis* possesses three respiratory terminal oxidases: Cyd, Cox, and alternative respiratory terminal oxidase (ARTO). Although there are a vast number of studies regarding the terminal oxidases in cyanoabacteria, a solid consensus about localization, function, and regulation is still missing (Howitt and Vermaas, 1998; Pils *et al.*, 1997; Pils and Schmetterer, 2001; Berry *et al.*, 2002; Hart *et al.*, 2005). BLAST analysis of sixty sequenced cyanobacterial genomes demonstrated that all cyanobacterial strains contain at least one set of the genes encoding Cox (Lea-Smith *et al.*, 2013). Cox is regarded as a major terminal oxidase and was suggested to function in the thylakoid membrane downstream of the Cytochrome *b6f* (Cyt*b6f*) complex (Howitt and Vermaas, 1998). Another terminal oxidase, Cyd has been localized to the cytoplasmic membrane (Howitt and Vermaas, 1998) and to the thylakoid membrane and has been suggested to compete with the Cyt*b6f* complex to oxidase the plastoquinone (PQ) pool (Berry *et al.*, 2002). ARTO, in turn, has a main location in the cytoplasmic membrane and seems not to play a significant role in cell metabolism (Howitt and Vermaas, 1998).

Interestingly, *Synechocystis* Δ*flv1*/Δ*flv3* cells incubated under fluctuating light demonstrated significant light-induced O_2 uptake, which was sensitive to KCN, an inhibitor of the respiratory chain (Allahverdiyeva *et al.*, 2013). It is highly conceivable that the malfunction of PSI under fluctuating light triggers the activity of terminal oxidases in the Δ*flv1*/Δ*flv3* mutant. This was, to our knowledge, the first direct evidence on the existence of light-induced O_2 uptake by terminal oxidases in cyanobacteria, being based on use of $^{18}O_2$ isotope in membrane-inlet mass spectrometry (MIMS) analysis. However, from this experiment it is not possible to evaluate which one of the terminal oxidases is contributing to photoreduction of O_2 in the Δ*flv1*/Δ*flv3* mutant under fluctuating light. Thus, the exact origin of light-induced O_2 uptake in Δ*flv1*/Δ*flv3* requires further investigation.

In line with the findings described above, Lea-Smith *et al.* (2013) demonstrated the significance of Cyd and Cox terminal oxidases during diurnal dark/high light square-wave cycles, where the ΔCox/ΔCyd double mutant demonstrated a lethal phenotype. Importantly, the presence of ARTO could not rescue the double mutant from the harmful effects of these abrupt changes in light intensity. Thus, it seems that the existence of at least one thylakoid membrane-localized terminal oxidase (Cox or Cyd) is an absolute prerequisite for the survival of *Synechocystis* during abrupt shifts from dark to high light. The severity of the phenotype was tightly dependent on the promptness and intensity of applied light: at 12 h dark/high light, sinusoidal-wave conditions, mimicking diurnal light changes, the mutant demonstrated a less severe phenotype, and at constant growth or high light the cell growth was similar to that of the wild-type cells. The lack of both thylakoid-localized terminal oxidases, Cox and Cyd, was demonstrated to result in: (i) over-reduction of the photosynthetic electron transfer chain, which in turn induced photoinhibition; (ii) over-reduction of the respiratory chain and production of ROS in the dark; (iii) and insufficient dark ATP production essential for repair of impaired components of the photosynthetic apparatus, particularly PSII damaged during the high-light phase (Lea-Smith *et al.*, 2013). More precise evidence about the function of the terminal oxidases as a safety valve in photosynthetic electron transport during abrupt and periodic changes of light intensity remains to be elucidated. Nevertheless, based on the current literature on cyanobacterial mechanisms of survival under fluctuating light, it can be concluded that protection of PSII is based on activity of the terminal oxidases (Lea-Smith *et al.*, 2013), whereas photoprotection of PSI relies on the function of the Flv1 and Flv3 proteins on the acceptor side of PSI safely

directing electrons to molecular oxygen (Allahverdiyeva *et al.*, 2013).

Green algae and lower land plants also have the Flv1 and Flv3 proteins

Phylogenetic analysis has demonstrated that green algae and lower land plants also possess genes showing a homology to *flv* genes (Zhang *et al.*, 2009; Peltier *et al.*, 2010). The genome of the model green alga *Chlamydomonas reinhardtii* (hereafter *Chlamydomonas*) holds two genes, *flvA* (Cre12.g531900) and *flvB* (Cre16.g691800), which are homologues of cyanobacterial *flv1* and *flv3*. Enhanced accumulation of the FLVA and FLVB proteins, together with increased O_2 photoreduction activity in the *pgrl1* mutant, has suggested a possible function of these proteins in a Mehler-like reaction in *Chlamydomonas* (Dang *et al.*, 2014). It is conceivable that the FLVA and FLVB proteins also play a safeguarding role under fluctuating light in *Chlamydomonas,* similarly to their homologues, the Flv1 and Flv3 proteins, in cyanobacteria. Nevertheless, more detailed studies are required, including the generation and characterization of *flvA* and/or *flvB* mutants of *Chlamydomonas* and in-depth analysis of their behaviour under fluctuating light.

Photoprotection mechanisms of higher plant photosystems under light intensity fluctuations

Contrary to cyanobacteria, microalgae, and lower terrestrial land plants, the seed plants do not have FDPs to protect their photosystems. Consequently, they have developed different mechanisms to cope with abruptly changing light intensities in the prevailing oxygenic environment.

PGR5 as a major component of acclimation to fluctuating light in plant chloroplasts

The *pgr5* mutant of *Arabidopsis thaliana* (hereafter, *Arabidopsis*), previously characterized as a high-light sensitive and proton gradient-deficient mutant (Munekage *et al.*, 2002, 2004; Okegawa *et al.*, 2007), turned out in subsequent experiments to be lethal under strongly fluctuating light conditions (Tikkanen *et al.*, 2010; Suorsa *et al.*, 2012).

Originally, the *pgr5* mutant was identified in a mutant screen based on its inability to perform non-photochemical quenching (NPQ) upon exposure to high light (Munekage *et al.*, 2002). Furthermore, and in contrast to the wild type, *pgr5* was unable to oxidize P700 under high intensities of actinic light. This was reported to be mostly due to limited electron acceptance from PSI rather than to a nonfunctional PSI. Importantly, PSI in *pgr5* was shown to be prone to photodamage upon exposure to high light. The corresponding mutation was subsequently identified as an amino acid substitution in a previously unknown chloroplast protein of low molecular weight, named PROTON GRADIENT REGULATION5, which was suggested to have a role as a mediator of antimycin A-sensitive cyclic electron flow (CET) around PSI (Munekage *et al.*, 2002; Sugimoto *et al.* 2013). Although the *pgr5* mutant shows a wild-type-like phenotype under constant light conditions, the *pgr5 crr2* double mutant, which also lacks another CET route mediated by the NAD(P)H dehydrogenase [NDH]-like complex, revealed a stunted phenotype and impaired photosynthetic parameters. Thus, the PSI CET is important not only for C4 plants but also for C3 plants (Munekage *et al.*, 2004). Yet another protein, PGR5-LIKE PHOTOSYNTHETIC PHENOTYPE1 (PGRL1), has been characterized and shown to interact with PGR5 and with PSI (Dal Corso *et al.*, 2008). PGRL1 has demonstrated a capacity to accept electrons from ferredoxin and reducing quinones, thus strongly suggesting that PGRL1 is actually the long-sought ferredoxin-plastoquinone reductase, FQR (Hertle *et al.*, 2013). On the other hand, the roles of the PGR5 and PGRL1 proteins in *Arabidopsis* PSI CET have also been heavily debated. Some reports provide evidence that, to some extent, the *pgr5* and *pgrl1* mutants are also capable of performing CET and only under specific conditions; for example, under high light or CO_2 limitation, the CET was found to be impaired in these mutants (Nandha *et al.*, 2007; Dal Corso *et al.*, 2008; Joliot and Johnson, 2011; Kono *et al.*, 2014). Thus, it is likely that rather than being absolute requirements, the PGR5 and PGRL1 proteins are needed to regulate and facilitate CET.

Although growth of the *pgr5* mutant was shown to be hampered under high light (Munekage *et al.*, 2008), the mutant plants still demonstrate viability under constant high-light growth conditions (Grieco *et al.*, 2012; Suorsa *et al.*, 2012). The fact that the lethal phenotype of the *pgr5* single mutant appears only under fluctuating light (Tikkanen *et al.*, 2010; Suorsa *et al.*, 2012) points to a unique function of the PGR5 protein that enables plants to survive under fluctuating light conditions. To address the function of the photosynthetic electron transfer reactions under fluctuating light in more detail, the wild-type and *pgr5* plants were grown under mildly fluctuating light (FL50/350, i.e. 50 µmol photons m^{-2} s^{-1} background light interrupted every 5 min by a 1 min high-light pulse of 350 µmol photons m^{-2} s^{-1}) that partially rescued the growth of the *pgr5* mutant. Plants were then subjected to measurements of PSII and PSI functionality by DUAL-PAM, applying the actinic light that mimics the strongly fluctuating light conditions that kill the *pgr5* mutant in the longer term (FL50/500, 50 µmol photons m^{-2} s^{-1} background light interrupted every 5 min by 1 min high light pulse of 500 µmol photons m^{-2} s^{-1}) (Suorsa *et al.*, 2012). In wild-type plants, application of the 1 min high-light phases in the fluctuating light induced efficient NPQ, oxidized P700, and generated high proton motive force, whereas the *pgr5* plants were not able to induce NPQ, and proton motive force and P700 remained reduced (Suorsa *et al.*, 2012), as was the case with the Δ*flv1*/Δ*flv3* mutant of cyanobacteria under similar light intensity fluctuations (Allahverdiyeva *et al.*, 2013).

Acceptor-side limitation of PSI (Nandha *et al.*, 2007; Kono *et al.*, 2014) and uncontrolled electron flow from PSII during high-light phases causes damage to PSI in *pgr5*, demonstrated by a decline in P_M, the maximum oxidizable amount of P700, and by a strong depletion of the PSI reaction centre protein PsaB, particularly under fluctuating light conditions (Joliot and Johnson, 2011; Suorsa *et al.*, 2012). When

electron transfer from PSII was blocked with DCMU, the high-light-induced damage to PSI was also largely abolished in *pgr5*. Indeed, it has been shown that 30–40% reduction in the amount of active PSII is enough to protect PSI against photodamage upon exposure of the *pgr5* mutant to high light (Tikkanen *et al.*, 2014). It is conceivable that a high amount of PSII is needed in plants for sufficient electron flow under low light conditions, whereas under high light conditions this potentially leads to damage of the PSI complex.

The safeguarding of PSI against photodamage is particularly important under fluctuating light. Such protection involves redox poising of stromal components downstream of PSI via PGR5-dependent control of linear electron flow through the Cyt *b6f* complex (Suorsa *et al.*, 2012). The exact mechanisms of this 'photosynthetic control', i.e. regulation of linear electron flow, are not known. Changes in lumenal pH that lead to transthylakoid ΔpH have been shown to regulate linear electron flow (Joliot and Johnson, 2011; Rott *et al.*, 2011; Suorsa *et al.*, 2012). It has also previously been shown that the redox poise of stroma, particularly the NADPH pool, is responsible for regulation of Cyt*b6f* control (Johnson, 2005; Hald *et al.*, 2008).

In the wild type, rapid induction of NPQ upon increase in light intensity avoids over-reduction of the PQ-pool. However, the absence of NPQ seems only to play an indirect role in acclimation to fluctuating light. Although the *pgr5* mutant is incapable of triggering proper NPQ (Munekage *et al.*, 2002; Suorsa *et al.*, 2012; Sato *et al.*, 2014), the NPQ mutants *npq4* and *npq1* do not show the *pgr5*-phenotype under artificially (Tikkanen *et al.*, 2010) or naturally fluctuating light conditions in the field (Kühlheim *et al.*, 2002; Frenkel *et al.*, 2007).

Interestingly, the deleterious effect of fluctuating light on PSI depends on plant developmental stage, as young leaves were found to be more susceptible to damage than mature ones (Suorsa *et al.*, 2012). The *pgr5* seeds germinating and developing under strongly fluctuating light were able to develop only cotyledons and small first true leaves before withering, presumably after using up the seed endosperm energy. In contrast, when wild-type and *pgr5* plants were first grown under constant light until development of the mature rosettes and only thereafter transferred to fluctuating light, *pgr5* survived for weeks under fluctuating light, even though both the functionality and the amount of PSI were compromised (Suorsa *et al.*, 2012). It is thus conceivable that the young, fast-growing tissues are particularly vulnerable to alterations in the level of PSI. Indeed, it is known that the level of PSI in mature leaves remains rather stable, but varies more in young leaves (Schöttler *et al.*, 2011); moreover, the levels of PSI assembly factors Ycf4 and PPD1 have been shown to be higher in young leaves than in mature leaves (Krech *et al.*, 2012; Liu *et al.*, 2012).

Recently, the effect of fluctuating light on the *pgr5* mutant plants was further addressed by exposing the wild-type and *pgr5*, grown under constant light, to fluctuating light-intensity regimes of low light (30 µmol photons m^{-2} s^{-1}) and high light (240 µmol photons m^{-2} s^{-1}) every 2 min (Kono *et al.*, 2014). Drastic photoinhibition of PSI was evident in *pgr5* as demonstrated earlier (Suorsa *et al.*, 2012), but the wild-type plants also exhibited some PSI acceptor-side limitation (Kono *et al.*, 2014). It has been shown that the wild-type plants grown under fluctuating light accumulate foliar H_2O_2 as well as ROS-scavenging superoxide dismutase (Alter *et al.*, 2012; Suorsa *et al.*, 2012) and show a pronounced increase in the amount of the PsaB protein as compared to wild-type plants grown under constant light (Suorsa *et al.*, 2012). It seems likely that the acclimation strategy of wild-type plants to fluctuating light involves not only the controlled regulation of electron flow by the PGR5 protein, but also triggers an enhanced capacity to cope with ROS and to adjust the photosystem stoichiometry. This strategy is also evident upon variations in the duration and intensity of sunflecks, e.g. brief and strong sunflecks generally enhance photoprotection and energy dissipation, whereas longer periods of high light allow upregulation of electron-transport rate in *Arabidopsis* (Alter *et al.*, 2012).

Other components of the chloroplast thylakoid membrane involved in coping with fluctuating light

Several thylakoid regulatory proteins, besides PGR5, have been tested for a fluctuating light phenotype (Tikkanen *et al.*, 2010; Grieco *et al.*, 2012). The most striking phenotypes, beyond *pgr5*, have been identified for the *stn7* and *tlp18.3* mutants.

The STN7 kinase (Bellafiore *et al.*, 2005; Bonardi *et al.*, 2005) is responsible for phosphorylation of light-harvesting complex II (LHCII) proteins Lhcb1 and Lhcb2. LHCII becomes phosphorylated upon a shift of plants from darkness to light or from high light to low light (Rintamäki *et al.*, 1997, 2000). The *stn7* mutant plants were shown to have a growth retardation phenotype when the low and high light intensities were fluctuating in 1-hour intervals (Bellafiore *et al.*, 2005) but the underlying molecular mechanism was not investigated further. As the reversible LHCII phosphorylation changes are rather slow, occurring in several-minute and tens-of-minute time scales, it is conceivable that malfunction of such reversible changes resulted in the stunted phenotype of *stn7*. The frequency of light intensity changes in natural environments caused, for example, by movement of clouds or by sunflecks, is often extremely high. Under such conditions, the phosphorylation level of the LHCII proteins remains fairly constant (Grieco *et al.*, 2012). Consequently, the protective role of steady state LHCII protein phosphorylation was addressed by exposing plants to abrupt short-term fluctuations in light intensity (Tikkanen *et al.*, 2010, 2011). When the *stn7* plants were exposed to a fluctuating light regime (50 µmol photons m^{-2} s^{-1} background light interrupted every 5 min by a 1 min high-light pulse of 500 µmol photons m^{-2} s^{-1}), a strong growth phenotype appeared (Tikkanen *et al.*, 2010, 2011). These experiments with the *stn7* mutant demonstrated a redox imbalance of the PQ-pool, especially upon rapid relaxation of NPQ during a shift of leaves from high to low light, suggesting the importance of the STN7 kinase and steady-state LHCII phosphorylation during relaxation of NPQ (Tikkanen *et al.*, 2010, 2011). Subsequent in-depth analyses on the functionality of PSII and PSI upon fluctuations in light intensity revealed the molecular mechanisms behind the protective role

of steady-state LHCII protein phosphorylation (Grieco *et al.*, 2012). It was revealed that when the STN7 kinase is missing (*stn7* and *npq4 stn7*), the shift from low to high light always causes a short acceptor-side limitation of PSI (Grieco *et al.*, 2012). It was further shown that in the absence of steady-state LHCII phosphorylation, there is a functional imbalance between PSII and PSI, always in the low-light phase (PSII excitation is higher than that of PSI), leading to accumulation of electrons in the electron-transport chain. Particularly, the electrons accumulating between the Cyt*b6f* and PSI appeared dangerous to PSI upon a subsequent high-light pulse. It is highly conceivable that a burst of electrons from plastocyanin, located beyond the control of the Cyt*b6f* complex, was detrimental to PSI, inducing photodamage similar to that observed in the *pgr5* mutant (Grieco *et al.*, 2012).

It is important to note that the *stn7* mutant grows like the wild type in constant light, due to the fact that it compensates for the lack of steady-state LHCII phosphorylation in energy transfer to PSI by increasing the number of PSI complexes in relation to the PSII complexes (Grieco *et al.*, 2012). Based on this compensation mechanism, the *stn7* plants, first grown under constant light and thereafter transferred to fluctuating light, do not suffer from PSI damage. Recently, a model was presented to explain how the two delta-pH regulated mechanisms, the thermal energy dissipation and the control of electron flow via the Cyt*b6f* complex, co-operate with redox-controlled thylakoid protein phosphorylation and collectively enable maintenance of the functional balance of photosynthetic light reactions during fluctuations in light intensity (Tikkanen and Aro, 2014).

Depletion of TLP18.3 protein induces a fluctuating light phenotype

The acidic phosphatase protein TLP18.3 is involved in regulation of the PSII repair cycle via its effect on degradation of the photodamaged D1 protein (Sirpiö *et al.*, 2007; Wu *et al.*, 2011). Interestingly, the *tlp18.3* mutant was also shown to suffer from reduced growth as compared to the wild type under fluctuating light (Sirpiö *et al.*, 2007). Thus, it seems likely that besides the PGR5 and STN7 proteins, the proper regulation of PSII function via a controlled PSII turnover or photoinhibition (Tikkanen *et al.*, 2014) also represents one of the important factors ensuring proper growth under fluctuating light. Currently, the variety of acclimation mechanism(s) that the wild-type plants use in coping with fluctuating light is being investigated at transcriptional, proteomic, and functional levels (Aro *et al.*, in preparation).

Different strategies are applied by photosynthetic organisms to cope with fluctuating light intensities

During the past few years it has become apparent that the PSI complex requires specific safeguarding in all oxygenic photosynthetic organisms under abrupt changes in light intensity. Today, the molecular mechanisms of such protective strategies are under extensive investigation and it has become evident that they have undergone conspicuous changes during evolution.

In plants, the PGR5 protein plays an essential role during the high-light phases of fluctuating light, while the STN7 kinase, by maintaining steady-state LHCII phosphorylation, is important during low-illumination phases to keep the electron-transport chain oxidized (Tikkanen *et al.*, 2010; Suorsa *et al.*, 2012; Grieco *et al.*, 2012; Tikkanen and Aro, 2014). Genome analyses of 71 sequenced cyanobacteria showed no sequences similar to the *Arabidopsis* gene encoding STN7, whereas 13 cyanobacteria contain a gene encoding a PGR5-like protein with sequence similarity from 50.77 to 66.15% when compared to *Arabidopsis* PGR5 (Table 1). In particular, *Synechocystis* possesses an open reading frame, *ssr2016*, encoding a protein with low sequence similarity to

Table 1. *BLAST analysis of the* pgr5 *gene in sequenced cyanobacteria genomes*

Gene ID	Locus tag	Product name	Scaffold ID	Genome
641252325	AM1_3236	PGR5 involved in CET	NC_009925	*Acaryochloris marina* MBIC11017
641611487	SYNPCC7002_A1477	Hypothetical protein	NC_010475	*Synechococcus* sp. PCC 7002
641676146	cce_1663	Hypothetical protein	NC_010546	*Cyanothece* sp. BH68, ATCC 51142
643580580	Cyan7425_5437	PGR5 involved in CET	NC_011880	*Cyanothece* sp. PCC 7425
651078550	SYNGTS_0815	Hypothetical protein	AP012205	*Synechocystis* sp. GT-S, PCC 6803
2022829473	CYJSC1_DRAFT_08460	Hypothetical protein	CYJSC1_DRAF_scaffold00069	*Leptolyngbya* sp. JSC-1
2503797998	Sta7437_4735	Hypothetical protein	Scya7437_Contig545	*Stanieria cyanosphaera* PCC 7437
2503887471	Lepto7376_1857	Hypothetical protein	Lsp7376_Contig1125	*Leptolyngbya* sp. PCC 7376
2509429018	Syn6312_0977	Hypothetical protein	Syn6312_Contig100.1	*Synechococcus* sp. PCC 6312
2509573793	Ple7327_1910	Hypothetical protein	Ple7327_Contig355.1	*Pleurocapsa* sp. PCC 7327
2509811040	Nos7524_3471	hypothetical protein	Nos7524_Contig213.1	*Nostoc* sp. PCC 7524
2558009256	Osccy1DRAFT_02323	Hypothetical protein	Osccy1DRAFT_CYJSC1_DRAF_scaffold00069.1	*Leptolyngbya* sp. JSC-1
2562386077	MYO_18200	Hypothetical protein	CP003265	*Synechocystis* sp. PCC 6803

PGR5 in *Arabidopsis* and which putatively participates in FQR-mediated CET around PSI (Yeremenko *et al.*, 2005). In contrast to the PGR5 mutant of higher plants, the *ssr2016* deletion mutant of *Synechosystis* did not demonstrate a fluctuating-light phenotype, implying that the PGR5-like protein in cyanobacteria is not important for survival under fluctuating light (Allahverdiyeva *et al.*, 2013). Moreover, cyanobacterial genomes do not contain a *pgrl1* gene, which has been shown to be important for FQR-mediated CET in chloroplasts of plants (DalCorso et. al., 2008, Hertle *et al.*, 2013) and green algae (Petroutsos *et al.*, 2009; Tolleter *et al.*, 2011).

Interestingly, several microalgae genomes possess both the *flv* and *pgr5* genes, showing high similarity with cyanobacterial and *Arabidopsis* genes, respectively (Zhang *et al.*, 2009; Peltier *et al.*, 2010). The possible involvement of these proteins in safeguarding the photosynthetic apparatus of green algae under fluctuating light intensities has not yet been studied. However, it is highly possible that in green algae, particularly in *Chlamydomonas*, both pathways are functional.

A *Chlamydomonas pgrl1* knock-out mutant, deficient in PGRL1-mediated CET, under an FL50/800 regime (50 µmol photons·m^{-2}·s^{-1} background light punctured for 1 min with 800 µmol photons·m^{-2}·s^{-1} every 5 min) and ambient CO_2 conditions, demonstrated reduced growth compared to the wild type. Such a decrease in growth rate is explained by the incapability of *pgrl1* mutant cells to take advantage of the applied high-light pulses, without the possible damage of the PSI complex (Dang *et al.*, 2014). The PGRL1-related CET seems to be essential in *Chlamydomonas* during low-to-high-light transition, not during steady-state growth under different light intensities. Thus, at least in this respect, the function of PGRL1 *Chlamydomonas* is analogous to that of the Flv1 and Flv3 proteins in cyanobacteria and the PGR5 protein in *Arabidopsis*.

From the experiments performed so far, it is too early to evaluate whether the FLV- or PGRL1-mediated route is more important for *Chlamydomonas* under fluctuating light, and how these two routes are regulated. Compared to the *pgr5* mutant of *Arabidopsis* (Tikkanen *et al.* 2010, Suorsa *et al.* 2012), it is obvious that the *pgrl1* mutant of *Chlamydomonas* demonstrates only a mild growth phenotype under fluctuating light. This might be related to the fact that *Chlamydomonas* FLVA and FLVB proteins also possibly function in the dissipation of excess electrons and, in the absence of one mechanism, the other one is upregulated as a compensating mechanism (Dang *et al.*, 2014). Further research by comparative analyses of the *pgr5* and *pgrl1* mutants of *Chlamydomonas* and *Arabidopsis* under fluctuating light will shed light on many open questions.

Concluding remarks

When considering abrupt and rapid fluctuations in light intensity, it is clear that the PSI complex is the most vulnerable component of the photosynthetic electron transfer chain (Suorsa *et al.*, 2012; Grieco *et al.*, 2012; Allahverdiyeva *et al.*, 2013; Kono *et al.*, 2014, Sejima *et al.*, 2014). Nevertheless, cyanobacteria, algae, and plants have evolved sophisticated mechanisms, with intriguing evolutionary differences, to cope with rapid changes in light intensity (Fig. 1).

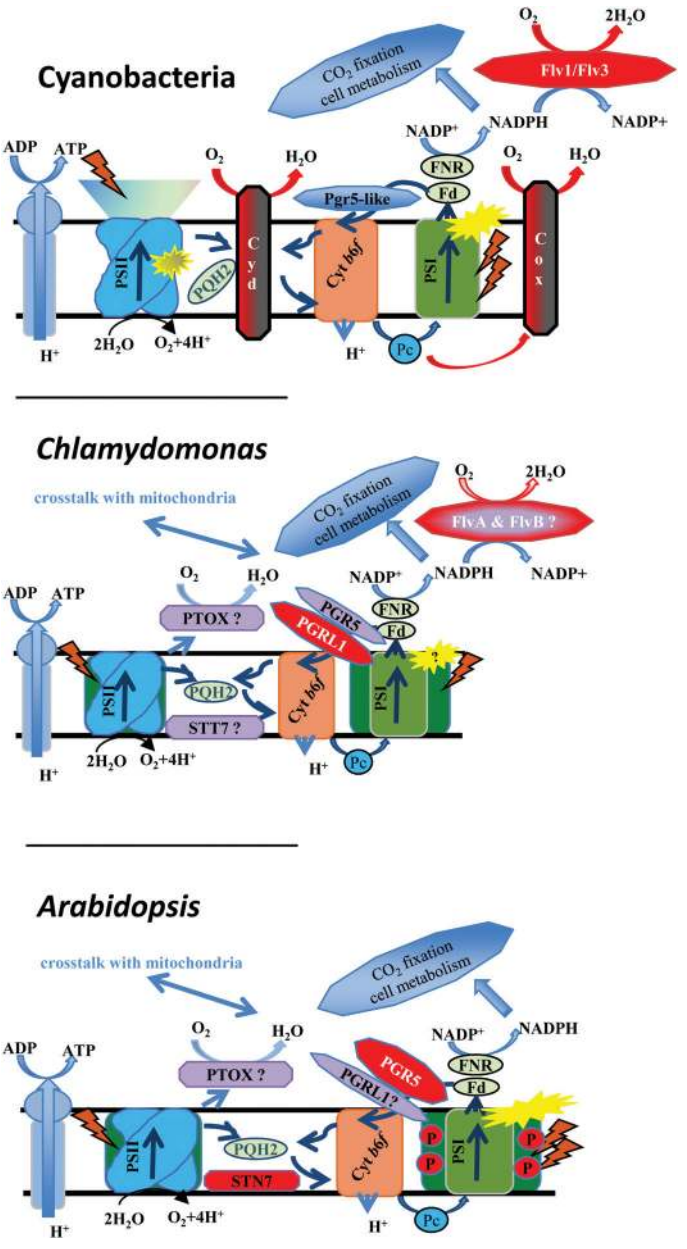

Fig 1. A schematic view of electron-transfer components operating in different photosynthetic organisms under fluctuating light conditions. The main components of the photosynthetic electron transport chain participating in safeguarding the photosynthetic apparatus upon rapidly changing light conditions are marked with red. The target of the damage caused by fluctuating light is shown as a yellow explosion. Other components also possibly involved in photoprotection are marked in violet. The FDP proteins Flv1 and Flv3 in cyanobacteria and the PGR5 and STN7 proteins in *Arabidopsis* play an essential role in protection of the photosynthetic machinery, particularly PSI, under fluctuating light conditions. Terminal oxidases (Cyd and Cox, marked as red/black) are involved in protection of cyanobacterial cells during dark-to-high light cycles, but their role upon frequent interruption of background low light (e.g. sunflecks) has not been studied so far. In *Chlamydomonas* the PGRL1 protein is essential under fluctuating light. *Chlamydomonas* also possesses the PGR5, STT7, FLVA, and FLVB proteins. It is conceivable that these proteins play a safeguarding role under fluctuating light conditions similarly to their homologues in cyanobacteria and plants. Nevertheless, the involvement of these proteins in acclimation of *Chlamydomonas* to fluctuating light remains to be further investigated. PTOX might also have a protective function under fluctuating light in *Arabidopsis* and *Chlamydomonas*. However, any direct evidence for the role of this protein in safeguarding either PSII or PSI under fluctuating light is completely missing.

Cyanobacteria possess several regulatory elements facilitating acclimation of the cells to abrupt fluctuations in the light environment. These include: (i) a high PSI/PSII ratio that is characteristic of cyanobacteria, enabling a rapid oxidation of the PQ-pool during illumination and creating favourable conditions under high-light phases; (ii) high traffic in cyanobacterial thylakoid membranes, due to the significant input and output of electrons via coexisting respiratory chains and the presence of terminal oxidases which provide additional, flexible mechanisms for the regulation of photosynthetic electron transport; (iii) the Flv1 and Flv3 proteins as a powerful safety valve, redirecting from 20–25% (Helman *et al.*, 2005) up to 60% of electrons to O_2 under specific conditions, thus playing a crucial role in controlling linear electron flow under fluctuating light conditions (Allahverdiyeva *et al.*, 2011).

In contrast to cyanobacteria, the protection of *Arabidopsis* photosynthetic apparatus under fluctuating light mainly occurs via the PGR5-related mechanism when the intersystem electron flow through the Cyt *b6f* complex is strictly controlled and thus the PSI complex is protected from excess electron flow upon abrupt increase in the light intensity. This is of particular importance for protection of PSI in plant chloroplasts when the electron acceptors in the stroma are reduced. Plants have also acquired additional dynamic mechanisms that respond to fast changes in the light environment. Specifically, the PsbS-mediated NPQ mechanism and the STN7-related steady-state phosphorylation of the LHCII proteins are important in providing additional flexibility for tuning the ATP/NADPH ratio. It is highly possible that in green algae, particularly in *Chlamydomonas*, both FLV and PGRL1 pathways are functional. Moreover, it is important to note that lower land plants like mosses and lycophytes also have genes related to *flv1* and *flv3*.

The reason behind the gradual disappearance of FDPs during higher plant evolution and replacement with different mechanisms is not clear. Several hypothetical reasons, however, can be suggested. (i) It is possible that during the evolution of oxygenic photosynthetic organisms, the 'NADPH-wasting' FDPs have been replaced with more energy-efficient mechanisms, such as the PGR5- and STN7-related regulation of linear electron transport. (ii) It is also conceivable that the movement of life from oceans to land, accompanied by modified light fluctuation patterns and gradients of O_2 exposure, as well as a lack of carbon-concentrating mechanisms in C3 plants, pushed evolution against FDPs in land plants. (iii) Taking into account that the diffusion coefficient of O_2 is about 10 000-fold greater in air than in water, it is possible that FDPs act efficiently on O_2 concentration gradients in aquatic environments, but less efficiently in the air. Thus, the replacement of FDPs with other photoprotection mechanisms in higher plants might have provided more efficiently functioning photosynthetic machinery under an oxygenic atmosphere.

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