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Interacting short-term regulatory mechanisms enable the conversion of light energy to chemical energy in photosynthesis

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Highlights

This review synthesizes the regulatory networks of photosynthesis that drive plant acclimation. Key gaps are identified and future research needed to enhance crop performance in naturally dynamic environments is outlined.

Abstract

Photosynthesis is a complex sequence of physical, electrochemical, biochemical and physiological processes that convert light energy and carbon dioxide into sugars. These sugars then provide the energy and carbon backbone for all metabolic pathways involved in plant growth and development. However, if light energy is not managed effectively within the thylakoid membrane, it can destroy the photosynthetic apparatus in an oxygenic environment, generated by photosynthesis itself. Effective photoprotection requires a variety of partially overlapping regulatory mechanisms that control energy, electron and proton transport, and induce changes in the molecular, structural and functional features of the photosynthetic apparatus and the thylakoid architecture. This review focuses on vital regulatory mechanisms and how they cooperate to maintain effective photosynthesis and to protect the thylakoid-embedded photosystems (PSII and PSI) against fatal light-induced damage under fluctuating light conditions. The current understanding of plant light regulation is primarily based on studies conducted under stable laboratory conditions, which limits the physiological relevance of the findings. The need for light regulation is further amplified by its complex interactions with other environmental variables. To bridge the gap between laboratory insights and real-world applicability, new technologies are needed for multi-environmental plant growth and experimentation that leverage artificial intelligence and machine learning.

Keywords

Photosynthesis, regulation on photosynthesis, light acclimation, photoprotection, photoinhibition

Introduction

Plant photosynthesis takes place in chloroplasts, which contain the thylakoid membrane network that houses the photosynthetic electron transfer (PET) machinery responsible of photochemical reactions converting light energy into reducing power (NADPH) and proton motive force (pmf) essential for ATP production (Figure 1). The rate of photochemistry depends on the amount of light relative to the plant's ability to make use of

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collected light energy. Instead of photochemistry, the collected light energy may also be lost in reactions that convert light energy into heat. A number of such non-photochemical quenching (NPQ) mechanisms, which dissipate excitation energy as heat, operate in parallel with photochemistry (Demmig-Adams *et al.*, 2014; Ruban, 2016; Ghosh *et al.*, 2023). As the amount of available light decreases, a proportionally larger amount of the absorbed light energy is used for photochemistry, which increases the light-use efficiency (LUE) of PET. Conversely, as the amount of light increases, more of the harvested light energy is dissipated by NPQ, leading to a decrease in the LUE of the photosynthetic apparatus. Photochemistry takes place in two macromolecular photosystem (PS) complexes (PSII and PSI), which function in series in linear electron transfer (LET) chain (Figure 1). Light energy is converted to chemical energy via light-induced charge separation in PSII and PSI reaction centre (RC) chlorophylls (Chl) P680 and P700, respectively, and in this occasion the absorbed light energy is stored as chemical energy. After charge separation, $P680^+$ has a high oxidising potential, and replaces the lost electrons by splitting water molecules with concomitant release of molecular oxygen, whereas the electrons ejected from PSII to LET eventually reduce the photo-oxidised $P700^+$. Electron acceptors from P700 have a low redox potential and when P700 is excited it is capable of reducing $NADP^+$ with electrons initially ejected to the intersystem electron transfer chain by PSII. Thus, the photosynthetic light reactions, working with highly oxidising PSII and highly reducing PSI in the presence of molecular oxygen, are subjected to production of reactive oxygen and electrophilic species (ROS and RES, respectively) that have a potential to damage the photosynthetic apparatus. To avoid extreme hazards, plants have evolved an extensive photoprotective and highly interactive regulatory network, involving rapid biophysical and various biochemical mechanisms (Tikkanen and Aro, 2014; Pinnola and Bassi, 2018). Such a network is further affected by feedback loops from carbon assimilation and other metabolic cues. Protons released to lumen during water oxidation in PSII, together with those transported to lumen not only via LET but also via cyclic electron transport (CET), contribute to forming the proton gradient across the thylakoid membrane. This gradient generates the proton motive force (pmf) that, beyond its primary role in ATP synthesis and fuelling of CO_2 fixation into carbohydrates, also plays significant regulatory roles in thylakoid embedded light reactions (Armbruster *et al.*, 2017; Davis *et al.*, 2017).

Most of the collected light energy is used in the Calvin-Benson-Bassham (CBB) cycle to convert CO_2 into sugars. During daytime, the CBB products are exported from the chloroplast as triose phosphates (TP) to the cytosol, where they are used to synthesise sucrose for further metabolism (Figure 1). A fraction of the TP in the chloroplast is directed to the synthesis of transitory starch, which is mobilised during the following night to ensure a continuous flow of energy from the chloroplast. In addition to CBB, some energy is always directed to nitrogen, sulfur and other chloroplast metabolism, and some is exported from the chloroplast as malate to support mitochondrial respiration (ATP production).

The rate of photosynthetic light reactions, which convert solar energy into chemical energy, depends on the availability of light energy. Conversely, the rate of metabolic sink reactions depends on the availability of CO_2 and the ability of metabolism to consume the products of PET (White *et al.*, 2016). The state of stomata controls the rate of gas exchange in leaves, and thus also the availability of CO_2, whereas the consumption of PET products is dependent on the rate of metabolism (Figure 1). Both the function of stomata and the rate of metabolism are strongly influenced by changes in light availability and other environmental conditions, exposing plants in our current oxygen-rich atmosphere to the production of ROS and RES, putatively leading to oxidative hazards to the photosynthetic apparatus (Engineer *et al.*, 2016). Plants, however, have evolved a robust network of protective regulatory mechanisms to constantly tune the activity of PET according to the availability of light energy and carbon dioxide, and the ability of plant to make use of the fixed energy for basic

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plant metabolism, growth and development. Indeed, to maximise the efficiency of photosynthesis, each partial reaction, from the initial capture of light energy to its consumption in metabolism should be optimally coordinated and protected (Engineer *et al.*, 2016; Davis *et al.*, 2017; Ghosh *et al.*, 2023).

In this review, we focus on regulation mechanisms that function at different structural and functional levels of the photosynthetic machinery, ranging from harvesting of light energy and its conversion to chemical energy in PSII and PSI, to the feed-back inhibition of PET from metabolism, all induced as short-term acclimation responses after expose of plants to changes in environmental and metabolic conditions (Figure 1).

Advances in understanding the regulatory mechanisms embedded in the thylakoid PET apparatus

Thylakoid membrane hosting the PET apparatus carries a number of regulatory functions

Large photosynthetic protein complexes are embedded in the thylakoid membrane, which is a dynamic structure comprising stacked grana membranes and interconnecting non-appressed stroma membranes (Figure 1). Grana membranes are packed with PSII protein complexes, whereas PSI and the ATP synthase reside in stroma-exposed thylakoid domains. Such differential distribution is called lateral heterogeneity and is thought to be a mechanism to avoid energy escaping from PSII to PSI, and thus provides the basis for smooth and efficient electron transfer in plant chloroplasts (Anderson, 2012). The 3D structure of the thylakoid membrane itself, and the location of the protein complexes in the membrane, change according to the environmental and internal conditions (Pribil *et al.*, 2014; Bussi *et al.*, 2019; Rantala *et al.*, 2020*b*; Garty *et al.*, 2024). Our understanding of the structure of thylakoids and protein complexes has become more precise in recent years, thanks to new microscopy techniques (Bos *et al.*, 2023; Streckaite *et al.*, 2024). Biochemical methods combined with proteomics have also shed new light on the composition of protein complexes, their uneven distribution along the thylakoid membrane as well as their re-localisation resulting from activation of specific regulatory mechanisms (Tikkanen *et al.*, 2008*b*; Rantala and Tikkanen, 2018). Although our knowledge has improved, it is still obvious that the importance of thylakoid structure for the function of PET is not yet fully understood. For example, it remains unclear whether LET passes from the appressed grana to non-appressed stroma-exposed membranes or whether it is functional only in the connection domains of the grana and stroma thylakoid membranes (Trotta *et al.*, 2025). Likewise, the existence of two PSI CET pathways in stroma-exposed membranes, the NDH-1 and PGR5/PGRL1 mediated pathways, remains enigmatic as different other functions have also been suggested for the PGR5/PGRL1 proteins (Buchert *et al.*, 2020; Rantala *et al.*, 2020*a*; Nikkanen *et al.*, 2025, Preprint). From PQ-pool electrons can also be transferred to plastid terminal oxidase (PTOX) and further to molecular oxygen (Rumeau *et al.*, 2007; Peltier *et al.*, 2016). Likewise, there still remains a lot to learn about the relationship between the structural and functional dynamics of the thylakoid membrane in changing environments and by internal metabolic cues.

Below we take a closer look at the components of PET from a regulatory point of view, paying attention their function in the transfer of both electrons and protons. As in the case of electrons, also all the protons eventually originate from PSII water splitting, and their regulatory roles largely depend on pumping of protons from the stroma into thylakoid lumen via PQ and Cytb$_6$f electron transfer (LET and CET) as well as via the NDH-1 in CET.

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PSII is nature's most powerful oxidant and is constantly being damaged as it works in light

Constant light-dependent PSII damage has led to an evolution of a rigorous PSII repair cycle, and the amount of active PSII is determined by the rate of damage relative to the rate of repair. The rate of damage is thought to be directly proportional to the amount of light reaching PSII (Tyystjärvi and Aro, 1996), and its repair is an extremely complex and highly regulated process (Järvi *et al.*, 2015). If PSII cannot transfer the electrons forward, for example in the absence of oxidised electron acceptors (acceptor-side limitation of PSII), it ends up in a so-called closed state, where the absorbed energy is dissipated as heat (Ivanov *et al.*, 2008). Currently, it remains unclear how much such an acceptor-side limited closed PSII increases the photodamage of PSII, but it is well known that plants have mechanisms to avoid this state by regulating the transfer of light energy from the LHC system to PSII (see below). If the damage is faster than the repair, damaged PSII centres start to accumulate. Such a decrease in the amount of active PSII centres is called PSII photoinhibition, which can be seen as a harmful reaction decreasing the photosynthetic efficiency. Nevertheless, since the inhibited PSII is an efficient dissipater of light energy as heat (Zavafer *et al.*, 2019), photoinhibition can also be seen as a regulatory mechanism preventing further damage to the photosynthetic machinery (Adams *et al.*, 2013), especially PSI (Tikkanen *et al.*, 2014).

Cyt b_6f is the regulatory hub of electron transfer in PET

Beyond its role as a key electron transfer component in PET, the cytochrome b_6f complex (Cyt b_6f) is vital for coupling electron transfer with proton translocation across the thylakoid membrane, from the chloroplast stroma to thylakoid lumen. Through the Q-cycle, Cyt b_6f significantly enhances the proton gradient necessary for ATP synthesis by pumping more protons per electron than the LET alone (Mitchell, 1975; Cramer *et al.*, 2011). Crucially, this mechanism also allows the proton gradient to regulate the electron transfer rate itself (Malone *et al.*, 2021). Consequently, as the proton gradient increases, the electron transfer from the PQ pool via Cyt b_6f to PC molecules slows down, resulting in a scarcity of electrons to be transferred to PSI with respect to the capacity of PSI to accept electrons (donor-side limitation of PSI). This regulation, called photosynthetic control, is also dependent on the PGR5 protein (Munekage *et al.*, 2002), but the mechanism by which PGR5 controls Cyt b_6f electron flow and proton pumping according to the capacity of PSI electron acceptors remains unknown and will be discussed later. Establishment of the canonical rapidly relaxing NPQ is very likely to be heavily dependent on the thylakoid proton translocation activity. Nevertheless, the precise mechanistic contribution of Cyt b_6f to NPQ regulation remains largely unresolved, likely because most attempts to inactivate the Q-cycle have failed, and the introduced mutations have led to dramatic decreases in the accumulation of the entire Cyt b6f complex (Malnoë *et al.*, 2011).

PSI is prone to damage by excess electrons but is protected from excess light energy when P700 is oxidised ($P700^+$)

PSI is the strongest reductant in nature and is easily damaged when it receives more electrons than can be handled by available oxidised PSI electron acceptors (acceptor side limitation of PSI). Despite the extreme sensitivity of PSI to photodamage under acceptor side limitation, PSI is not equipped with an efficient repair mechanism (Kudoh and Sonoike, 2002). For this reason, the electron transfer to PSI is strictly controlled by

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1 various mechanisms, especially by the photosynthetic control generated in Cyt b_6f. Oxidised PSI ($P700^+$)
2 possesses an inherent capacity to safely dissipate excess excitation energy as heat and this dissipative
3 capability remains also in photoinhibited PSI (Tiwari *et al.*, 2016). It currently remains poorly understood how
4 the efficient excitation energy quenching in the oxidised and inhibited PSI reaction centres functions as part of
5 the photosynthetic regulatory network. Nevertheless, it is clear that excess electron flow towards $P700^+$ is
6 dangerous to PSI only when combined with sufficient excitation energy, whereas the role of regulation of
7 excitation energy dissipation in protecting PSI remains unknown. It should also be noted that PSI inhibition
8 may be more common than previously thought, as it is often monitored solely by a decrease in the P700
9 oxidation signal. However, such a decrease in the P700 signal only occurs when all three iron-sulphur centres
10 F_A, F_B and F_X of PSI are damaged (Tiwari *et al.*, 2024). Crucially, the damage of PSI FeS clusters under fluctuating
11 light starts from the F_A and F_B clusters, which, however, cannot be detected by simple P700 redox
12 measurement (Tiwari *et al.*, 2024). Furthermore, the Fv/Fm measurement, typically employed to assess PSII
13 inhibition, cannot entirely distinguish between PSII and PSI inhibition (Tikkanen *et al.*, 2017). Consequently, a
14 decrease in Fv/Fm that is generally interpreted as PSII inhibition based on the measurement could, in fact,
15 indicate PSI inhibition.

16

17 **Small PET components also carry important roles in regulation of photosynthesis**

18 As already discussed above, plastoquinone (PQ) transfers electrons from PSII to Cyt b_6f and concomitantly
19 pumps protons from the stroma to thylakoid lumen, allowing electron transfer to be regulated by the proton
20 gradient. The redox state of the PQ pool, as such, also carries various regulatory roles (Havaux, 2020). In short
21 term (mins to hours), it regulates the activity of the STN7 and STN8 kinases (Rintamäki *et al.*, 1997), thereby
22 playing an important role in regulation of the excitation energy flow from the LHCII antenna system to PSII and
23 PSI. As to the acceptor side of PSI, reduced Fd transfer electrons forward from PSI, mainly to FNR and reduction
24 of $NADP^+$ for carbon, nitrite and sulfite reduction pathways, but also to TRX and reduction of various
25 thioredoxin molecules in chloroplasts. Fd exists in several forms and can be membrane-bound or free, similar
26 to FNR, which certainly reflects the many roles of Fd and FNR in regulation, still, after a thorough review by
27 (Hanke and Mulo, 2013), being under active research

28

29 Dynamics and regulation of capture of the light energy and its transfer to 30 photosystems or dissipation as heat (NPQ) – achieving the excitation balance of PSII 31 and PSI

32 In most of current literature, the regulation of the excitation energy distribution to PSII and PSI, based on
33 thylakoid protein phosphorylation, has been investigated separately from regulation of excitation energy
34 dissipation as heat (NPQ). However, these two mechanisms are inextricably linked. Below, we focus on
35 dynamics of plant light harvesting system (LHC) and elaborate how redox-regulated phosphorylation
36 mechanisms interact with pH-regulated NPQ and photosynthetic control.

37 PSII and PSI core complexes have their own fixed light-harvesting antennas, comprised of CP43 and CP47 in PSII
38 and of a large ChlA antenna bound the PSI RC proteins PSAA and PSAB. In addition, there is a light-harvesting
39 LHCI antenna bound to the PSI core, and a large light-harvesting antenna called LHCII comprised of the inner
40 (CP29, CP26 and CP24) and the peripheral (LHCB1,2 and 3) ChlA and ChlB binding proteins. The inner LHCII

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1 serves only PSII but the peripheral LHCII serves both PSII and PSI (Rantala *et al.*, 2020*b*). The light-harvesting
2 efficiency and how the peripheral LHCII is distributed to serve the transfer of harvested light energy between
3 PSII and PSI are dynamically controlled depending on external cues (Horton *et al.*, 1994; Rochaix, 2014;
4 Vetoshkina *et al.*, 2023). The control of light collection preferably for PSII or PSI is linked to regulation of the
5 rate of PET, as the light-harvesting efficiency is also controlled by trans-thylakoid proton gradient. The
6 distribution of energy between PSII and PSI, on the other hand, is controlled by the redox state of the PQ pool,
7 which is determined by the relative rates of PSII and PSI (Figure 2).

8 The light energy collected by Chls of the LHCII complexes is, under optimal condition, mainly used in
9 photochemistry (charge separation) of PSII and PSI (Figure 2A). In cases of imbalanced excitation of PSII and PSI
10 or the availability of more energy than can be used for photochemistry, the excess energy will be quenched
11 non-photochemically (NPQ) by dissipation as heat. Light energy dissipation mechanisms provide flexibility,
12 from one hand, to increase the efficiency of light harvesting when the amount of available light energy
13 decreases (Figure 2B) and, from the other hand, to protect the photosynthetic apparatus when the amount of
14 light increases (Figure 2C) (Demmig-Adams *et al.*, 2018; Ghosh *et al.*, 2023). It is also well known that in cases
15 of excessive light energy, with respect to available oxidised PSII electron acceptors, the excess electrons easily
16 find the way to react with molecular oxygen to form ROS, for example highly reactive singlet oxygen in PSII,
17 which can only be prevented by keeping the lifetimes of excited PSII RC Chls short enough (Triantaphylidès *et*
18 *al.*, 2008; Triantaphylidès and Havaux, 2009). Instead, PSI cannot avoid the formation of superoxide and
19 hydrogen peroxide in cases of excessive electron flow to PSI RC, due to the scarcity of oxidised PSI electron
20 acceptors (Kudoh and Sonoike, 2002; Sonoike, 2011). It is important to emphasise here that there is always a
21 trade-off between maximising photosynthetic LUE (Figure 2B) and protecting the photosynthetic apparatus
22 from oxidative damage (Figure 2C). Consequently, enhancing photosynthesis by altering its regulation
23 essentially minimises the safety margin between efficient photosynthesis and damage. Therefore, attempts to
24 enhance photosynthesis must be based on a holistic understanding of interaction within the photosynthetic
25 regulatory network.

26

27 **Balancing of excitation energy distribution from LHCII to PSII and PSI**

28 In order to avoid unnecessary losses in the efficiency with which absorbed light energy is used for
29 photochemistry, excitation energy must be distributed properly to PSII and PSI (Bonaventura and Myers, 1969;
30 Murata, 1969; Bellafiore *et al.*, 2005). In the long term (e.g. during the growth), a functional balance between
31 PSII and PSI is ensured by a proper PSII-to-PSI ratio and the size of the peripheral LHCII antenna (Walters,
32 2005). In the short term, excitation energy distribution from LHCII to PSII and PSI is regulated by thylakoid
33 protein phosphorylation (Figure 2). The main contributor to LHCII phosphorylation is STN7 kinase, but
34 regulation also involves interaction with STN8 kinase-dependent phosphorylation of PSII core proteins D1, D2,
35 CP43, and possibly PSBH too (Tikkanen and Aro, 2014).

36 It is generally thought that the role of LHCII phosphorylation is to adjust the relative excitation of PSII and PSI in
37 response to changes in light quality, given that PSII and PSI have slightly different absorption maxima (Haldrup
38 *et al.*, 2001; Allen, 2003; Rochaix, 2007). This "state transition" theory remains the dominant explanation for
39 the physiological role of LHCII phosphorylation. However, this explanation does not consider that the extent of
40 changes in light quality required to induce “state transitions” does not occur in nature, as discussed in the next
41 chapter. Furthermore, the state transition theory fails to explain the peculiarities in the light intensity-
42 dependent regulation of LHCII phosphorylation (Rintamäki *et al.*, 1997). Indeed, in plants grown under constant

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white light, irrespective of the growth light intensity, some LHCII is always phosphorylated. When light intensity decreases, the amount of LHCII phosphorylation increases; conversely, LHCII phosphorylation decreases as light intensity increases (Figure 2). However, these changes in LHCII phosphorylation do not affect the energy distribution from LHCII to PSII and PSI in WT plants (Tikkanen *et al.*, 2010; Mekala *et al.*, 2015).

LHCII phosphorylation at low light maintains proper PSI excitation

Comparative studies of Arabidopsis WT and the *stn7* mutant have shown that continuous LHCII phosphorylation is necessary under steady-state light conditions to support PSI excitation and to maintain efficient electron transfer from PSII to PSI without over-reduction of the PET (Figure 2B) (Tikkanen *et al.*, 2010; Grieco *et al.*, 2012). However, the underlying factor causing the functional imbalance between PSII and PSI, and which requires correction by LHCII phosphorylation, is not properly understood. PSI inhibition experiments (Lima-Melo *et al.*, 2019; Lempiäinen *et al.*, 2022) suggest that higher light intensities require a lower amount of active PSI to maintain balanced electron transfer. Conversely, in low light, even a slight decrease in PSI abundance begins to restrict the rate of PET (Lima-Melo *et al.*, 2019; Lempiäinen *et al.*, 2022). This also affects the way the plant regulates LHCII phosphorylation. PSI inhibition/damage increases LHCII phosphorylation in low light and shifts the light intensity threshold at which LHCII dephosphorylation begins to higher levels (Mekala *et al.*, 2015; Lempiäinen *et al.*, 2022). Therefore, PSI centres are required in excess to maintain efficient electron transfer in low light conditions. Based on these results, it is reasonable to suggest that the phosphorylation of LHCII has evolved to minimise the requirement for additional PSI centres in low light conditions (Figure 2A and B). Indeed, as light levels decrease, PSI is the first to limit the rate of PET. This leads to availability of PQH2, which activates the STN7 kinase and increases LHCII phosphorylation (Figure 2C). This enhances the excitation of PSI relative to PSII, correcting the imbalance between the two. This system is likely to function in moderate and low light intensities, when LHCII is efficient in harvesting light and transferring electrons to PSI is not limited by pmf. This regulation would only be based on sensing the redox state of the PQ pool.

Dephosphorylation of LHCII under high light maximises photosynthetic LUE in fluctuating light

LHCII dephosphorylation, which occurs when light intensities exceed those experienced by plants during the growth (Rintamäki *et al.*, 2000), results from the inhibition of the STN7 kinase due to increased stromal thiol reduction state, as well as from the activity of the TAP38/PPH1 phosphatase (Pribil *et al.*, 2010; Shapiguzov *et al.*, 2010). However, the underlying reason for the need to correct the imbalance between PSII and PSI excitation by LHCII dephosphorylation remains elusive and is affected by phosphorylation of other PSII proteins. Under high light conditions, in addition to LHCII dephosphorylation, the phosphorylation of PSII core proteins, D1, D2, CP43 and PSBH, substantially increases (Figure 2C). This STN8 kinase-dependent phosphorylation loosens the structure of PSII supercomplexes (Tikkanen *et al.*, 2008*a*), affecting thylakoid ultrastructure (Fristedt *et al.*, 2009). Both processes contribute to the efficient repair of damaged PSII centres (Tikkanen and Aro, 2012; Kirchhoff, 2014). However, these changes, together with LHCII phosphorylation, cause an increasing fraction of the LHCII excitation to end up in PSI (Mekala *et al.*, 2015). To prevent this, the STN7 kinase becomes inhibited and LHCII is dephosphorylated by the TAP38/PPH1 phosphatase (Figure 2C).

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1 LHCII dephosphorylation under high light conditions coincides with an increase in photosynthetic control. This
2 slows down the electron transfer from PSII to PSI, protecting PSI from photoinhibition, and generates a strong
3 NPQ (qE) from LHCII as heat (Figure 2C). Under these conditions PSI is not harmed even in the absence of LHCII
4 dephosphorylation (Mekala *et al.*, 2015), making it difficult to understand the physiological role of LHCII
5 dephosphorylation in high light. However, experiments in which plants were abruptly exposed to low light after
6 high light conditions could suggest the importance of LHCII dephosphorylation under high light conditions. This
7 leads to rapid relaxation of NPQ and photosynthetic control (Tikkanen *et al.*, 2010; Grieco *et al.*, 2012; Suorsa
8 *et al.*, 2012). In these conditions, PSII benefits from dephosphorylated LHCII, which collects light energy to
9 enable PSII to function at its maximum rate. Therefore, it is highly likely that dephosphorylated LHCII
10 guarantees proper excitation of PSII when transferring from high light to low light, thus ensuring maximal light
11 use efficiency (LUE) in fluctuating light conditions.

12

13 Dephosphorylation of LHCII and PSII core protein by far red light does not increase the relative 14 excitation of PSII

15 Exposure of plants grown under white light to far-red light, results in the dephosphorylation of the LHCII and
16 PSII core proteins. However, this does not result in any notable changes to relative excitation of PSII and PSI
17 compared to white light, which contradicts the traditional state transition theory (Mekala *et al.*, 2015).
18 Nevertheless, this apparent contradiction becomes understandable when we consider the evolutionary
19 purpose of PSI's far-red absorption. PSI likely evolved to protect itself during rapid shifts between direct
20 sunlight and the far-red light filtered through upper leaf layers (Kono *et al.*, 2017). If dephosphorylation were
21 to strongly enhance PSII excitation in far-red light, it would prevent far-red-induced oxidation of PET, which
22 could potentially endanger PSI during sudden bursts of direct sunlight. This raises a crucial question: Why do
23 thylakoid proteins undergo dephosphorylation in far-red light? Since PET oxidation indicates light-limited
24 photosynthesis, it is possible that dephosphorylation improves photosynthetic LUE by subtly modifying the
25 thylakoid structure and enhancing energy transfer between pigment-protein complexes. Further investigation
26 is required to confirm this intriguing possibility.

27

28 Feed-back regulation of light reactions - sensing the stromal metabolic state

29

30 In addition to the regulatory mechanisms that originate directly from the interaction between the thylakoid-
31 embedded photosynthetic apparatus and environmental cues, as discussed above, the operation of PET must
32 also be balanced with the CBB and other metabolic reactions in the chloroplast stroma (Foyer *et al.*, 1990). The
33 operation of these reactions depends on the products of PET: Fd_{RED} and ATP. The capacity of the CBB cycle is
34 strongly influenced by stomatal regulation, as discussed above, while the rate of other metabolic reactions
35 depends on the overall metabolic state of the entire cell, which in turn reflects the metabolic state of the tissue
36 and the entire plant. Although the regulatory mechanisms of CBB, chloroplast sugar metabolism and stomata
37 have been extensively studied (Weber *et al.*, 2005; Smith and Zeeman, 2020; Cejudo *et al.*, 2021; Gurrieri *et al.*,
38 2021; Zhang *et al.*, 2024), the feedback regulatory mechanisms from the sink metabolism to PET reactions
39 remain largely unknown. It has been shown that the proton antiporter KEA3 senses the pH and ATP status of
40 the stroma and by that it enables fast transient increase in lumenal pH when light intensity decrease and by
41 that quick recovery of LUE (Uflewski *et al.*, 2024). This however do not explain how LUE is decreased when the

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1 stromal capacity to accept electrons becomes saturated. Indeed, without such regulation, PSI is destroyed in
2 naturally fluctuating light and the plant eventually dies (Suorsa *et al.*, 2012). Currently, we can only hypothesise
3 about the molecular mechanism underlining this fundamental process (Figure 3), which is a matter of life and
4 death for the plant.
5 Strict regulation of the stromal redox state depends on PGR5, a protein that controls the proton gradient
6 across the thylakoid membrane, and thus the photosynthetic control at Cyt b_6f (Munekage *et al.*, 2002).
7 However, the mechanism by which PGR5 senses the imbalance between light reactions (PET) and stromal
8 metabolism, which occurs on a second timescale and induces photosynthetic control to prevent the flow of
9 excess electrons from Cyt b_6f to PSI, remains unknown (Nawrocki *et al.*, 2019). Since the stromal metabolic
10 reactions utilise the energy of both the NADPH, reduced via LET, and the protons pumped to thylakoid lumen
11 to drive ATP synthesis, it is conceivable that stromal pH would coincide with the reduction state of the stroma.
12 Thus, sensing stomal pH would provide a perfect feedback mechanism between LET and the sink metabolism
13 (Figure 3). This would be analogous to the regulation of the mitochondrial electron transport chain, which is
14 known to rely on sensing matrix pH and leading to control of proton conductivity via ATP synthase. This
15 mitochondrial regulation is carried out by a small IF1 protein, which acts as a master regulator of mitochondrial
16 energy metabolism (Green and Grover, 2000; Gledhill *et al.*, 2007; García-Bermúdez and Cuezva, 2016). When
17 the mitochondria are actively producing ATP, the pH of the mitochondrial matrix is alkaline. At this pH, IF1
18 exists as an inactive tetramer or higher-order oligomer. This oligomerization masks the inhibitory region of the
19 protein, preventing it from binding to and inhibiting ATP synthase. When the electron transfer slows down, the
20 proton pumping decreases and the pH of the mitochondrial matrix becomes more acidic. This change in pH
21 triggers a conformational change in IF1, causing the inactive tetramers to dissociate into active dimers. The
22 active, dimeric form of IF1 is now able to bind to and inhibit the ATP synthase. The active IF1 dimer binds to the
23 F1 catalytic domain of the ATP synthase, specifically at the interface between the α and β subunits.

24 IF1 is not found in chloroplasts, but the chloroplast PGR5 protein is composed of similar rod-like alpha-helices
25 and unfolded domains as IF1 (Uniprot Q9SL05). Such proteins cannot transfer electrons or pump protons. In
26 addition, the putative ferredoxin quinone reductase (FQR) that would be required for PGR5-dependent CET has
27 not been characterised despite decades of in-depth analysis of the thylakoid proteome. Furthermore, it is
28 difficult to explain how Cyt b_6f could simultaneously accelerate CET and slow down LET; this would require
29 separate electron transfer chains for CET and LET, with CET-specific Cyt b_6f being tolerant to photosynthetic
30 control. Rather than CET (Munekage *et al.*, 2002), we hypothesise that the PGR5 protein functions in the
31 chloroplast in a manner analogous to mitochondrial IF1 protein (Gledhill *et al.*, 2007). Figure 3 illustrates a
32 putative mechanism for the role of PGR5 in the feedback regulation of PET via control of the proton
33 conductance of the ATP synthase in the chloroplast (see the figure legend for details); however, further
34 investigation is needed to substantiate this hypothesis. It is important to acknowledge that both the canonical
35 PGR5 cyclic electron transfer theory and the theory of PGR5 interaction with Cyt b_6f in photosynthetic control
36 currently lack definitive molecular evidence.

37

38 Interaction of different PET regulation mechanisms in fluctuating light conditions

39 There is growing concern among plant and photosynthesis researchers about the physiological relevance and
40 practical usability of available post-genomic data in plant breeding approaches, due to the fact that such data
41 has mostly been generated on plants grown under stable artificial light and constant other conditions, too
42 (Annunziata *et al.*, 2017; Matsubara, 2018; Chiang *et al.*, 2020). As photosynthesis readily responses to

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environmental changes, also regulatory mechanisms are subject to changes which, however, are not directly predictable. Accordingly, we next describe how fluctuating light and other environmental conditions affect the balance, from one hand, between excitation of PSII and PSI and, from the other hand, between the production of reducing power in PET (LET + CET) and its utilization in CBB and stromal metabolism, and also outline the critical points that need to be taken into consideration in future research toward agricultural applications.

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It is well known that the amount of light in nature varies with the time of the day, season, cloud cover, and general shading. In addition to the amount of light, shading from overhead vegetation has a major impact on the quality of light, increasing the relative excitation of PSI under the canopy. In addition to relatively rapid movements of clouds, the wind causes very rapid changes in the amount and quality of light reaching the shaded vegetation. In the case of light changes due to shading by upper leaves, the proportion of far-red light in shade light is strongly pronounced compared to direct sun light, whereas shading by stems and other light-impermeable objects only reduces the quantity of light but does not much affect the quality of light (Sellaro *et al.*, 2025).

Based on traditional experimental setups, in which plants grown under stable conditions are exposed to fluctuating light conditions, the main regulatory mechanisms (both short-term high-light and low-light acclimation mechanisms, see Figure 2), can be expected to work together to protect PSI from damage. In contrast, the damage of PSII is generally less fatal (discussed later). To prevent PSI photoinhibition under short-term exposure to high light, the electron transfer to PSI via the intersystem electron transfer chain should be limited by photosynthetic control according to the availability of oxidised PSI electron acceptors on stromal side of PSI. This is achieved through the PGR5-dependent regulation of the Cyt b_6f complex, which prevents excess electron transfer from the PQ pool to PC and subsequently to PSI (Figure 3C). In addition, PSBS-dependent canonical NPQ reduces PSII function, thereby preventing over-reduction of the PQ pool (Tikkanen *et al.*, 2015) (Figure 2C). In low light, on the other hand, the accumulation of excess electrons in LET is prevented by proper relative excitation of PSII and PSI (Grieco *et al.*, 2012) (Figure 2A and B). Under canopy shade, the far-red absorbing chlorophylls of PSI cause PSI to dominate in electron transfer, thus preventing the accumulation of excess electrons in the PQ pool (Kono *et al.*, 2017). However, in neutral shading, the far-red absorption of PSI does not help, and therefore the optimal oxidation of LET should be ensured by other means. Here, the maintenance of equal relative PSII and PSI excitation is achieved by steady state phosphorylation of the LHCII proteins (Figure 2A and B), thereby increasing the relative excitation of PSI and preventing the accumulation of excess electrons in the PQ pool (Tikkanen *et al.*, 2010).

As described above, the regulated electron transfer and proper excitation energy distribution between PSII and PSI are necessary to protect PSI from damage in fluctuating light. On the contrary, the role of regulated NPQ in fluctuating light conditions has remained unclear. NPQ prevents the excessive electron flow to the PQ pool when light intensity increases (Figure 2 C). However, PSII is not exceptionally sensitive to fluctuating light (Tikkanen *et al.*, 2010) or to photoinhibition (Sarvikas *et al.*, 2006) in mutants lacking NPQ, probably because the excess energy just closes the PSII centres, which are able to quench the excess energy by reaction centre quenching (qRC) (Ivanov *et al.*, 2008). In the presence of PGR5, photosynthetic control seems to be able to prevent excess electrons from moving from PQ to PC and PSI (Figure 3). However, there is a strong possibility that in some conditions, the photosynthetic control is not sufficient to prevent excess electrons from flowing to PSI, and NPQ is needed to support photosynthetic control by decreasing the PSII activity and reduction of PQ-pool. Indeed, if both the PSBS-dependent NPQ and STN7-dependent excitation energy distribution to PSI are missing (*stn7 npq4*), the mutant plants cannot properly oxidise P700 despite the functional PGR5-depedent

1 photosynthetic control (Tikkanen *et al.*, 2015), which indicates that the photosynthetic control has its limit to
2 resist electron flow from PSII to PSI.

3 Furthermore, after exposure of plants to high light illumination that induces a strong reduction state in the PQ
4 pool, a subsequent decrease in light intensity and consequent relaxation of the photosynthetic control at Cyt
5 b_6f is likely to result in excess electrons flowing to PC. If, at this point, the light intensity increases again, the
6 highly reduced PC pool can maintain several charge separations in PSI RC (P700), exceeding the capacity of Fd
7 to forward electrons at the acceptor side of PSI and leading to PSI inhibition. It is also possible that a rather
8 slow relaxation of NPQ (xanthophyll cycle dependent NPQ, qZ) has evolved to protect PSI from inhibition.
9 Indeed, when LHCII is dephosphorylated, the relaxation of NPQ leads to high reduction state of PET, sensitising
10 PSI for high light flashes (Grieco *et al.*, 2012). Accordingly, the interaction of various regulatory mechanisms
11 under fluctuating light conditions should be thoroughly considered when designing plants with modified light
12 harvesting properties to enhance photosynthetic efficiency. In case the intended changes affect both the
13 regulation of NPQ and the means how it is synchronised with the regulation of excitation energy distribution
14 and development of photosynthetic control in LET, it is highly likely that the intended change (e.g. transgene or
15 targeted mutation) will hamper PSI under a given change in light conditions.

16

17 Acclimation to photoinhibition, another layer of photosynthetic regulation

18 Despite a number of different cooperative regulation mechanisms, plants cannot completely prevent the
19 photoinhibition and photodamage of PSII and PSI, and therefore the system has also evolved mechanisms to
20 acclimate to such damages (Figure 4). So far, the regulation of photosynthesis has mainly been investigated
21 from the viewpoint of short-term response of plants and their photosynthesis to changes in light intensity.
22 However, the reported mechanisms do not provide sufficient protection against stresses caused by light
23 fluctuations, inducing both PSII and PSI damage, depending on conditions as described above. Photoinhibition
24 reduces PSII and PSI core functions in the same way as a decrease in the amount of light (Figure 2B) or a
25 change in the relative excitation of PSII and PSI, causing the need for acclimation to keep the electron transfer
26 rate as optimal as possible (Figure 4A). Indeed, the phosphorylation of LHCII is also highly sensitive to changes
27 in the amounts of active PSI and PSII centres (Lempiäinen *et al.*, 2022; Gunell *et al.*, 2023). PSII is easily
28 inhibited under high light stress, which reduces the amount of functional PSII (Tyystjärvi and Aro, 1996). PSI, on
29 the other hand, is inhibited in fluctuating light and under chilling stress, though such a sensitivity is strongly
30 species-dependent (Sonoike, 2011; Allahverdiyeva *et al.*, 2015; Armbruster *et al.*, 2017). When the amount of
31 functional PSII is sufficiently low, it starts to limit PET instead of PSI, leading to PET oxidation. This leads to
32 dephosphorylation of LHCII and to an increase in the relative PSII excitation by LHCII, thus correcting the
33 excitation balance between PSII and PSI and mitigating the consequences of PSII inhibition (Figure 4B).
34 Conversely, a decrease in PSI leads to an increased reduction state of PET, which in turn increases the
35 phosphorylation of LHCII and the excitation of PSI (Figure 4 C).

36 For reasons described above, it is highly conceivable that reversible phosphorylation in LHCII plays an
37 important role in maintaining the functional balance between PSII and PSI also upon photoinhibition
38 conditions. Moreover, common for the inhibition of both photosystems is that when the regulated NPQ
39 decreases, increasing the LUE of the light harvesting system serving the still functional photosystems (PSII:
40 (Kornyeyev and Hendrickson, 2007; Gunell *et al.*, 2023) PSI: (Lempiäinen *et al.*, 2022)) (Figure 4B and C). It was
41 also shown that at least PSI inhibition changes the energy distribution from PSI to stromal metabolism,

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1 increasing the CBB in relation to other pathways (Lima-Melo *et al.*, 2019; Lempiäinen *et al.*, 2022). The ability of
2 various mutants lacking specific photosynthetic regulatory mechanisms to acclimate to photoinhibition has not
3 yet been tested experimentally. It is possible that the physiological role of regulated NPQ and the regulation of
4 excitation energy distribution between PSII and PSI are closely linked to acclimation to photoinhibition.

5

6 New tools for understanding the complexity and vivid cooperation of PET regulatory 7 mechanisms to facilitate knowledge transfer from laboratories to plant breeding

8

9 PET regulatory mutants and their phenotyping

10 Much of our knowledge of the regulation of photosynthetic energy conversion in plant chloroplasts comes
11 from ever-increasing annotation of plant genomes. This is followed by reverse or forward genetics to identify
12 new proteins involved in PET regulation. While these results are extremely important for our research, they do
13 not directly reveal the exact physiological role of the protein in question and often do not reveal any new
14 phenotypes that differ from those of the wild-type (WT) plants. This is because the mutant plants, usually
15 grown under constant laboratory conditions, often acclimatise to the imbalances in photosynthetic
16 performance induced by the mutation. This occurs via modulating, enhancing or inhibiting, other PET
17 regulatory mechanisms or by altering the stoichiometry of functional photosynthetic complexes (for example,
18 the *stn7* mutant increases the number of PSI (Grieco *et al.*, 2012), resulting in the maintenance of
19 photosynthesis and growth at WT level. For these reasons, it is often difficult to determine whether we are
20 studying the actual physiological significance of the mutated gene or merely the plasticity of the mutant plants
21 to acclimatise to the mutation during growth under laboratory settings. Furthermore, under standard
22 laboratory growth conditions, the photosynthetic regulation mechanisms are only required when the lights are
23 turned on in the morning, and during the daytime if it is necessary to adjust the photosynthetic efficiency
24 according to changes in sink metabolism. This requires much less regulatory capacity than needed under
25 natural environments. It is therefore highly likely that the laboratory-grown plants have only a fraction of the
26 regulatory capacity directly available to be used when light conditions abruptly change, compared to plants
27 from natural environments. In addition, the light spectrum also matters, affecting the relative excitation of PSII
28 and PSI. In response to long-term uneven excitation of photosystems during growth, plants change the relative
29 amounts of PSII to PSI (Chow *et al.*, 1990; Kim *et al.*, 1993). Indeed, the differences in the spectrum of the
30 growth light lead to development of very different photosynthetic machineries, both under laboratory
31 conditions and in natural environments (Flannery *et al.*, 2021). For these reasons, our artificial laboratory
32 setups are prone to lead to misinterpretations of the physiological roles of different regulatory proteins and
33 pathways, and can mislead the design of "better plants" through genome editing approaches.

34 Currently, it is of the utmost importance to develop better experimental practices that reveal the true
35 physiological significances and cooperation of the different proteins and regulatory mechanisms of light energy
36 conversion in PET.

37 We need modern, flexible plant growth systems to properly phenotype various PET regulatory mutants. The
38 light environment should follow the circadian rhythm, but also include simulated bright and cloudy days, as
39 well as short-term light fluctuations of different lengths (FL). Additionally, the PSII and PSI excitation effects of
40 the light spectrum should closely resemble the excitation effects of sunlight. Also, the low-light phases under

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1 FL should be both neutral and mimic the light conditions in the canopy preferred by PSI. Apart from the light
2 conditions, photosynthetic light and carbon reduction reactions are differentially susceptible to changes in
3 other environmental conditions. For example, the metabolic reactions are more susceptible to changes in
4 temperature or the CO_2 levels than the light reactions, which further challenges the design of photosynthesis
5 regulation experiments and their interpretation towards engineering more efficient PET.

6

7 **All available and emerging technologies should to be fully utilised**

8 Improving our understanding of the complex regulatory network of PET, and moving towards the highest
9 physiological and practical relevance, requires exploiting all the possibilities offered by latest technologies.
10 These include the CRISPR-Cas9 gene editing, which enables the targeted generation of single and multigene
11 mutants (Cardi *et al.*, 2023), and systems level multiomics analyses of these mutants for revealing interactive
12 regulation pathways (Sarfraz *et al. 2025*). New LED, sensor and camera technologies, which together with new
13 data systems, allow the development of research platforms for AI-based phenotyping (Harfouche *et al.*, 2023).
14 For example, in order to fully understand how the phosphorylation of thylakoid proteins modulates the
15 function of the photosynthetic machinery, it is not enough only to generate and characterise the knock-out
16 mutants of kinases and phosphatases, but also to eliminate all the phosphorylation sites of phosphoproteins
17 one by one.

18 The adoption of new technologies would also facilitate the functional elucidation of still elusive regulation
19 mechanisms, such as those of the PGR5 protein, by systematically modifying the amino acid sequence - and
20 thus the structure - of the protein while searching for changes in regulation of PET. AI-based programs for
21 predicting the structure and protein-protein interactions (Abramson *et al.*, 2024) would then enable the
22 determination of the type and location of amino acid substitutions to be made by CRISPR-Cas for optimal
23 regulatory properties under the planned growth conditions.

24 Author Contribution

25 MT and EMA co-wrote the manuscript, with MT taking the lead on the initial draft and figures

26 Conflict of Interest statement

27 The authors have no conflicts to declare

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31

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16 ***Figure 1: Framework for the regulation of photosynthetic electron transfer (PET) reactions in plant***
17 ***chloroplasts.*** PET reactions occur in the thylakoid membrane, producing sufficient amounts and proportions of
18 NADPH and ATP, which drive the Calvin-Benson-Bassham (CBB) cycle and other chloroplast metabolic
19 processes in the stroma. In nature, these reactions occur under conditions where the amount and the quality
20 of light, affecting the relative excitation of PSII and PSI, as well as the sink's ability to use the transduced light
21 energy are constantly changing. Therefore, it is necessary for plants to adjust their light-harvesting efficiency
22 according to both the amount of light available and the capacity of the metabolic sinks. Additionally, the
23 photosynthetic machinery must also ensure equal relative excitation of PSII and PSI, all this for maximal
24 efficiency while preventing the oxidative damage of the photosystems, in particular that of PSI. PSII is
25 constantly damaged in light, but it undergoes a rapid and regulated repair cycle that is integrated into the
26 regulatory network of PET and protection of PSI. Sink capacity is affected by the CBB rate and the rate at which
27 CBB products are transported and used in metabolism. The CBB rate depends on CO_2 availability, which is
28 largely regulated by stomata. The sugar molecules produced by CBB are either transported out of the
29 chloroplast or stored in starch granules within the chloroplast for use at night. Starch granules store the
30 transduced excess energy in chloroplasts when other sinks are saturated. All of these processes are highly
31 integrated, requiring exceptionally dynamic regulation of PET to avoid oxidative hazards in chloroplasts.
32 Abbreviations: CBB, Calvin–Benson–Bassham cycle; FNR, Ferredoxin—NADP(+) reductase; MHD, malate
33 dehydrogenase; NA, nitrogen assimilation; PMF, proton motive force; PQ, Plastoquinone; PQH_2, plastoquinol;
34 PTOX, plastid terminal oxidase; STN7, LHCII kinase; STN8, PSII core protein kinase; TP, triose phosphate; Trx,
35 thioredoxin

36

37 ***Figure 2: Regulatory interactions between transfer of excitation energy and electron transfer in PET.*** In
38 nature, there is no stable light intesity similar to that in the laboratory. Instead, light conditions constantly

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change and plants synthesize photosynthetic machinery that can safely and efficiently utilize the average daily light dose. Photosynthetic light reactions enhance their light use efficiency (LUE) when light intensity decreases, and decrease LUE when light intensity increases, while maintaining the correct level of excitation of PSII and PSI. The regulation of harnessing excitation energy and the rate of electron transfer are interlinked. **A.** Under average growth light conditions, the regulatory mechanisms of PET fucntion at low efficiency. A small amount of the collected harnessed excitation energy is dissipated as heat through non-photochemical quenching (NPQ), and electron transfer is only slightly limited by photosynthetic control at Cyt b_6f. Steady-state phosphorylation of LHCII supports PSI excitation, keeping the PET chain optimally oxidized and preventing sunflex-induced damage to PSI. **B.** As light intensity decreases, both NPQ and photosynthetic control relax, thereby maximizing the efficiency of light harvesting and electron transfer. LHCII phosphorylation increases further to promote PSI excitation. This maximizes the LUE of PSI and protects it from the damaging effects of sunflexes. **C.** An increase in light strengthens both the photosynthetic control at Cyt b_6f and NPQ, downregulating PET to match the capacity of CBB and thus preventing excess electron flow to PSI. Photosynthetic control is essential for maintaining the integrity of PSI under high light conditions, whereas NPQ helps to maintain an optimally oxidized PQ pool under these conditions, thereby decreasing the electron pressure towards PSI. When high light conditions persist, the PSII core proteins D1 and D2 as well as CP43 undergo heavy phosphorylation. This alters the thylakoid ultrastructure to facilitate PSII turnover. Meanwhile, the LHCII proteins undergo dephosphorylation to compesate for the effect of strong PSII core protein phosphorylation on the excitation balance between PSII and PSI. For abbreviations in the figure, see the legend for Figure 1.

Figure 3 shows the proposed and partially hypothetical functions of the PGRL1-PGR5 proteins, the NDH-1 complex and the PTOX protein in the regulation of redox and pH homeostasis in chloroplasts. From this perspective, PGR5 primarily acts as the master regulator of PET by controlling the proton conductivity of ATP synthase in response to stroma pH. Furthermore, the quantity of PGR5 that can interact with ATP synthase is regulated by thioredoxin (Trx) via redox processes. **A.** Under steady-state illumination conditions, the function of PSII water oxidation and the rest of the linear electron transfer (LET, represented by the bold black arrows) are in balance with the sink metabolism. Most of the electrons that are excited sequentially in PSII and PSI are directed towards NADPH production. Additionally, some of electrons from the PSI electron acceptor ferredoxin (Fd) are returned back to the PQ pool via the NDH-1 complex in a process known as cyclic electron transfer (CET, thin black arrows). NDH-1 CET is coupled with efficient proton translocation (dotted lines) from the stroma to the thylakoid lumen. In addition to LET and CET, electrons can be directed also to PTOX, which uses them to reduce O_2 to H_2O in a reaction that consumes protons from the stroma. This further strengthens the ΔpH generated by LET and CET. A fraction of PGR5 is released from PGRL1 by thioredoxin making it ready to bind to ATP synthase. **B.** An abrupt increase in light intensity accelerates PSII function, thereby injecting more electrons into the LET. This results in an increased accumulation of protons in the lumen, and accelerates the rate at which protons are translocated to the stroma. **C.** Under high-light conditions, the PGR5 protein senses the decrease in pH and blocks some of the ATP synthases. This increases the concentration of protons in the lumen, triggering photosynthetic control at Cytb_6f. This process is faster than electron transfer via Cytb_6f, enabling PGR5 to prevent excess electrons from entering PSI and causing damage. The excess electrons that accumulate in the PQ pool are consumed by PTOX, strengthening the ΔpH further by consuming protons from the stroma. Prolonged exposure to high light leads to stronger reductions in the stroma and the Trx system.

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This increases the amount of free PGR5 that can bind to ATP synthase. For abbreviations in the figure, see the legend for Figure 1.

Figure 4: Acclimation of PET to photoinhibition represents an another layer of regulation. The regulation of phosyntesis has generally been understood as acclimation to changing light and other environmental conditions. However, such mechanisms do not fully protect PSII and PSI, as both photosystems are susceptible to inhibion under certain conditions. This reduces photosynthetic efficiency, but plants also mitigate the effects of photoinhibition. **A.** In average light conditions, when photoinhibition is not present, moderate photosynthetic control and NPQ maintain balance in the entire PET. **B.** PSII inhibition leads to limitation of electron transfer by PSII and oxidation of the entire PET chain, including the PQ pool. This results in the inactivation of the two kinases, which leads to the dephosphorylation of LHCII and PSII core proteins, and cosequently increases the excitation of the remaining active PSII centers. **C.** Conversely, when PSI is inhibited, electron transfer is limited by PSI, resulting in increased reduction of the PQ pool. This leads to the strong activation of the STN7 and STN8 kinases, resulting in strong phosphorylation of the LHCII and PSII core proteins. This, in turn, increases the excitation of the remaining active PSI centers. Inhibiting both PSI and PSII results in a decrease in NPQ and photosynthetic control, which increses the efficiency of LET, helping to offset the effects of inhibition on PET. After recovery from photoinhibition, it is also noteworthy that plants can reprogram stromal metabolism to favor the Calvin-Benson-Bassham (CBB) cycle over other metabolic processes and malate export. For abbreviations in the figure, see the legend for Figure 1.

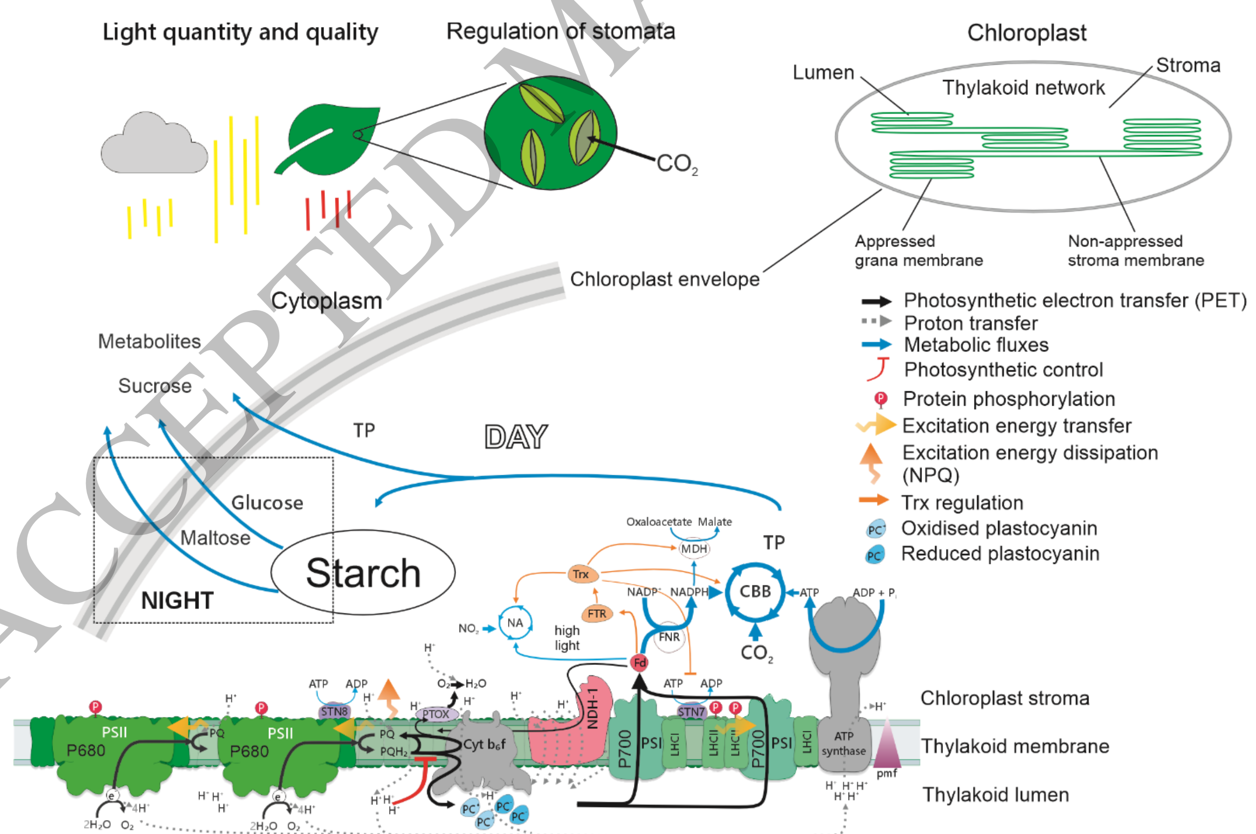

Figure 1

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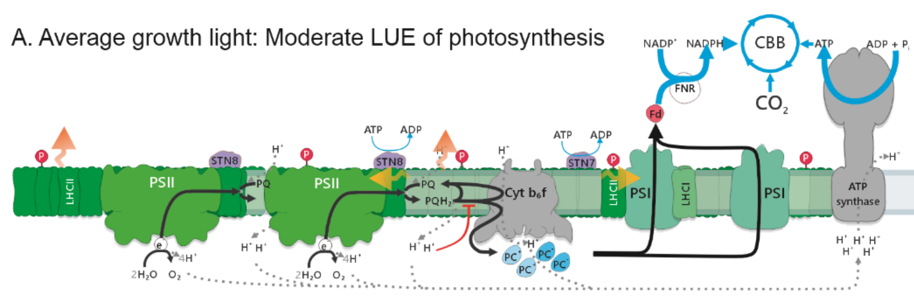

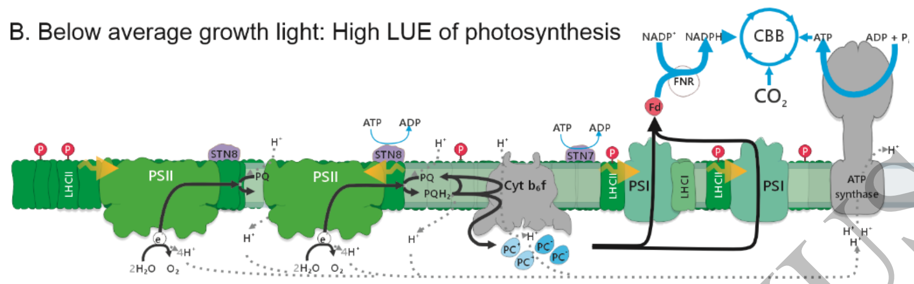

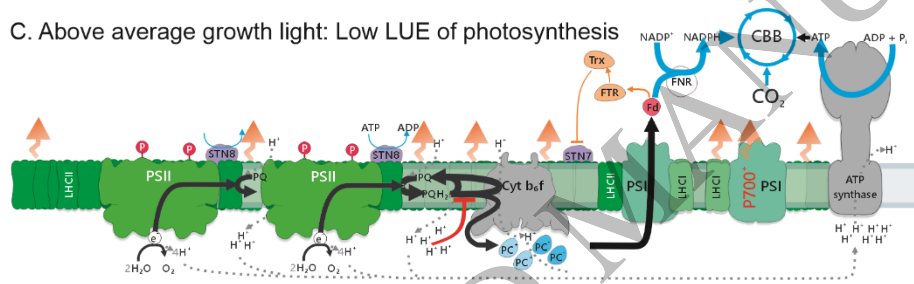

Figure 2
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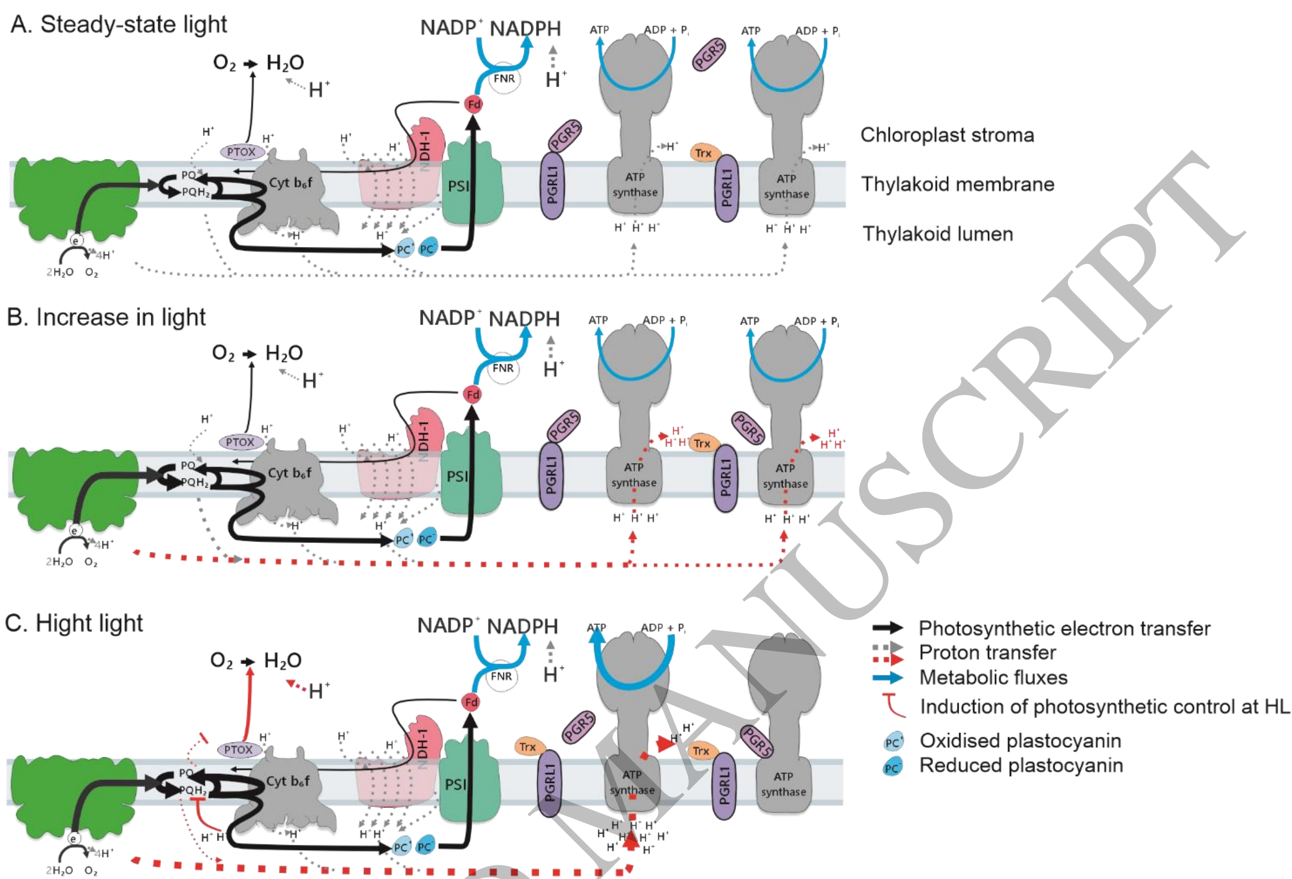

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2 *Figure 3*
3 *170x121 mm (x DPI)*

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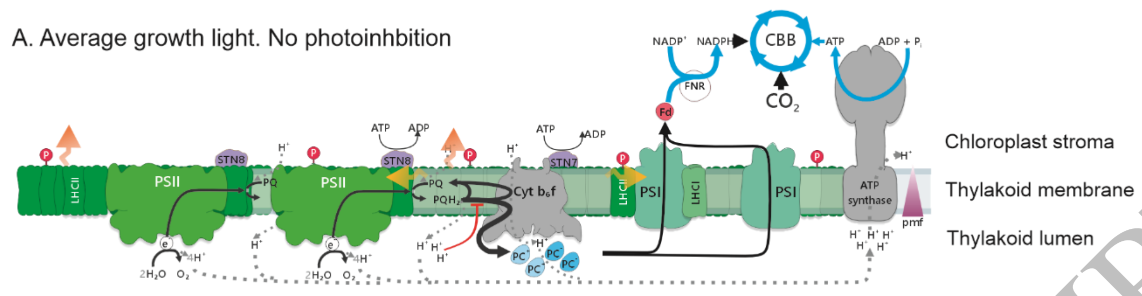

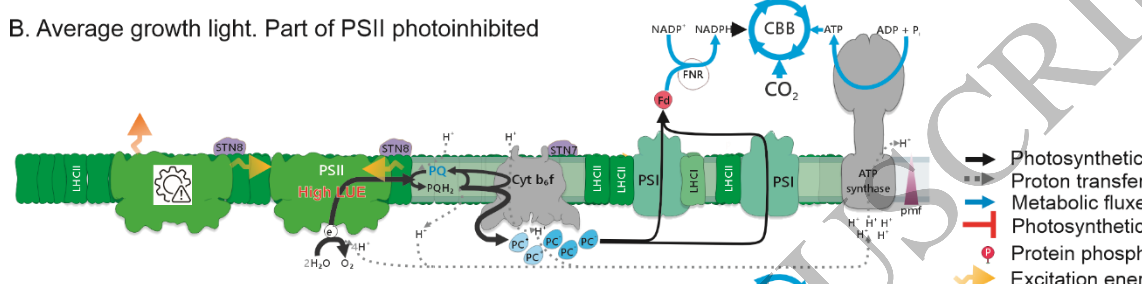

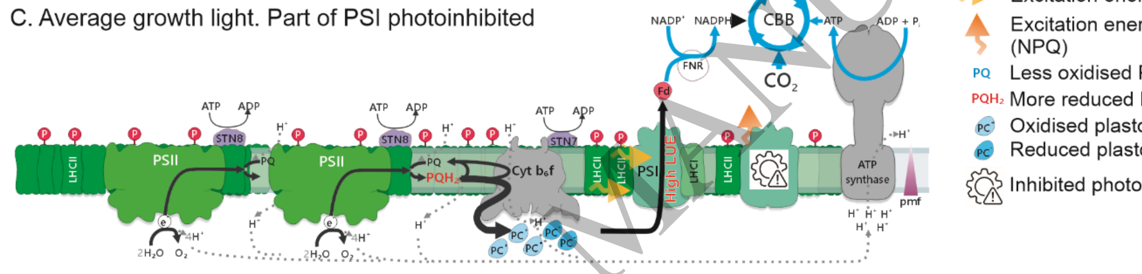

Photosynthetic electron transfer
Proton transfer
Metabolic fluxes
Photosynthetic control
Protein phosphorylation
Excitation energy transfer
Excitation energy dissipation (NPQ)
PQ Less oxidised PQ
PQH2 More reduced PQ
Oxidised plastocyanin
Reduced plastocyanin
Inhibited photosystem

1

2 *Figure 4*
3 *170x117 mm (x DPI)*

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