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REGULATION OF PHOTOSYNTHETIC ELECTRON TRANSFER IN PLANT CHLOROPLASTS

by

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ABBREVIATIONS

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
CET	Cyclic electron transfer around PSI
Chl	Chlorophyll
CP	Chlorophyll-protein
Cyt b_6f	Cytochrome b_6f complex
ΔpH	transthylakoid proton gradient
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
ETC	Photosynthetic electron transfer chain
F'	Chlorophyll fluorescence under actinic light illumination
Fd	Ferredoxin
F_m	Maximal chlorophyll fluorescence in dark adapted leaf
F_m'	Maximal chlorophyll fluorescence under actinic light illumination
FNR	Ferredoxin-$NADP^+$ reductase
F_s'	Steady-state chlorophyll fluorescence under actinic light illumination
HL	High light intensity
K	Kelvin degrees
LET	Linear electron transfer
LHCI	Light harvesting complex I
LHCII	Light harvesting complex II
LL	Low light intensity
ML	Moderate light intensity
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced $NADP^+$
NDH	NAD(P)H dehydrogenase-like complex
NPQ	Non-photochemical quenching of excitation energy
npq1	Knock-out mutant with no violaxanthin de-epoxidase

npq4	Knock-out mutant with no PsbS protein
P700	Reaction center Chl of PSI
PC	Plastocyanin
pgr5	Mutant with knock-down *PGR5* gene
Pi	Inorganic phosphate
P-LHCII	LHCII trimer with phosphorylated Lhcb 1 and/or Lhcb2
P_m	Maximal absorbance change at 830 nm induced by full oxidation of P700
PQ	Plastoquinone
PQH_2	Plastoquinol
PSI	Photosystem I
PSII	Photosystem II
qE	Energy-dependent quenching
stn7	Mutant with knock-out *STN7* gene
STN7	STATE TRANSITION7 kinase
stn8	Mutant with knock-out *STN8* gene
STN8	STATE TRANSITION8 kinase
WT	Wild-type

TABLE OF CONTENTS

ABSTRACT

This thesis focuses on the molecular mechanisms regulating the photosynthetic electron transfer reactions upon changes in light intensity. To investigate these mechanisms, I used mutants of the model plant *Arabidopsis thaliana* impaired in various aspects of regulation of the photosynthetic light reactions. These included mutants of photosystem II (PSII) and light harvesting complex II (LHCII) phosphorylation (*stn7* and *stn8*), mutants of energy-dependent non-photochemical quenching (NPQ) (*npq1* and *npq4*) and of regulation of photosynthetic electron transfer (*pgr5*). All of these processes have been extensively investigated during the past decades, mainly on plants growing under steady-state conditions, and therefore many aspects of acclimation processes may have been neglected. In this study, plants were grown under fluctuating light, i.e. the alternation of low and high intensities of light, in order to maximally challenge the photosynthetic regulatory mechanisms. In *pgr5* and *stn7* mutants, the growth in fluctuating light condition mainly damaged PSI while PSII was rather unaffected. It is shown that the PGR5 protein regulates the linear electron transfer: it is essential for the induction of transthylakoid ΔpH that, in turn, activates energy-dependent NPQ and downregulates the activity of cytochrome b_6f. This regulation was shown to be essential for the photoprotection of PSI under fluctuations in light intensity. The *stn7* mutants were able to acclimate under constant growth light conditions by modulating the PSII/PSI ratio, while under fluctuating growth light they failed in implementing this acclimation strategy. LHCII phosphorylation ensures the balance of the excitation energy distribution between PSII and PSI by increasing the probability for excitons to be trapped by PSI. LHCII can be phosphorylated over all of the thylakoid membrane (grana cores as well as stroma lamellae) and when phosphorylated it constitutes a common antenna for PSII and PSI. Moreover, LHCII was shown to work as a functional bridge that allows the energy transfer between PSII units in grana cores and between PSII and PSI centers in grana margins. Consequently, PSI can function as a quencher of excitation energy.

Eventually, the LHCII phosphorylation, NPQ and the photosynthetic control of linear electron transfer via cytochrome b_6f work in concert to maintain the redox poise of the electron transfer chain. This is a prerequisite for successful plant growth upon changing natural light conditions, both in short- and long-term.

1. INTRODUCTION

1.1 Photosynthesis for sustainability

Humankind has a new challenge: the expanding global population and concomitant consumption of natural resources have never reached the threatening levels and rates of change that we are experiencing nowadays.

Before the industrial revolution the world was very large. The global population was so small and technologies so simple, that human activity had little to no impact on the global biophysical system.

Today the world is very small. Already thirty percent of Earth's land is used for crops and pasture, and there is little space for expanding it (FAO Statistical Yearbook 2013, World Food and Agriculture). The fishing industry has reached so large a scale that the amount of fish in the oceans is decreasing, and yet fishing activity is still growing (The State of World Fisheries and Aquaculture, FAO, 2012). The atmospheric concentration of carbon dioxide is growing at a rate never registered before (about 2 ppm per year; NOAA, US Depart. of Commerce), provoking a climate change that is clearly dependent on human activities (America's Climate Choices: Panel on Advancing the Science of Climate Change, 2010). These are only a few examples of many interconnected problems that are making the current system unsustainable, and are leading to a probable collapse of global civilization (Ehrlich and Ehrlich, 2013).

Everything is growing, except our global wisdom and awareness that an infinite growth is not possible. Many educated people have a strong resistance to accepting the idea that a short history of exponential growth does not imply that such growth is sustainable. Politicians in democratic countries are dependent on the short-term effects of their decisions, while developing countries now stake their claim for the exploitation of their share in global resources. Economists could greatly contribute to solving the problem by designing steady-state financial and economic systems. On the contrary, they look like disciples of a new religion, believing in the dogma of an infinite growth of consumption in a finite world. In the meanwhile, the human socio-economic system is expanding in the biosphere with no control, like a cancer in an organism.

In this context, researchers can fulfill mainly two tasks. The first one is to be scientifically informed, to effectively communicate the current situation to the public and politicians. The second one is to provide knowledge and technical tools that, in conjunction with reduced population growth rates and resource consumption, can contribute to reaching global sustainability. To this end, research on photosynthesis can play an important role by contributing to improvements in the yields of crops and the development of non-food bioenergy production from agricultural wastes, algae and cyanobacteria.

1.2 Photosynthetic machinery: structure

Photosynthesis occurs in two chloroplast compartments. In the thylakoid membrane, biophysical processes, called light reactions, allow the synthesis of ATP and NADPH. In the stroma, the Calvin-Benson cycle utilizes ATP and NADPH to assimilate the atmospheric carbon dioxide for synthesis of carbohydrates. The present thesis focuses on the study of light reactions, and therefore the term 'photosynthetic machinery' refers here mainly to the thylakoid structure, function and regulation.

1.2.1 The chloroplast in plants

In plants, photosynthesis takes place in specific organelles called chloroplasts. Chloroplasts derive from cyanobacteria that were engulfed into a eukaryotic cell more than one billion years ago by endocytosis (Dyall et al, 2004). This event was followed by a conspicuous translocation of genetic material from the chloroplast to the host cell nucleus. The chloroplast is delimited by a double layer of membranes that constitutes the envelope and is one of the lines of evidence of the ancient endocytosis. Inside the organelle, a very complex system of membranes forms the thylakoids. The thylakoid membrane forms sacs that are partially appressed and piled as coins forming *grana*. Grana, in turn, are connected by single non-appressed thylakoids (*stroma lamellae*). The chloroplast thylakoid network constitutes a membrane-surrounded internal continuum, called *lumen*. The liquid space between thylakoids and the envelope is denoted as *stroma*. The membrane curvatures at the external part of granum are called *margins*; the upper and lower external membranes of every granum are called *ends*. How lamellae are connected to grana is still under debate. Many models have been developed in the last decades. One of the most popular is the fork model (Arvidsson and Sundby, 1999), where folding of a single continuous thylakoid forms a fork, so that grana are formed by invaginations of lamellae. The helical model instead proposes the stroma lamellae to wind around the granum, connecting obliquely many appressed thylakoids (Paolillo, 1970; Mustárdy and Garab, 2003; Austin et al, 2011).

Chloroplasts are connected with each other by long and narrow tubules, called *stromules* (Hongladarom et al, 1964), which allow the exchange of low molecular weight molecules and small proteins (Köhler et al, 1997).

1.2.2 Thylakoid protein complexes

The basic function of photosynthetic light reactions is carried out mainly by the protein complexes that are embedded in the thylakoid membranes: photosystem II (PSII) and photosystem I (PSI), their respective light harvesting complexes (LHCII and LHCI), cytochrome b_6f (Cyt b_6f) and ATP synthase.

PSII in the functional form is a dimer, in which every monomer includes 20 protein subunits, bound to 35 chlorophylls (Chl) *a*, two pheophytins, 11 β-carotenes, more than

20 lipids, two plastoquinones, and the Mn_4CaO_5 cluster (Umena et al, 2011). The main polypeptides of the PSII core complex are D2, D1, CP47 and CP43. CP47 and CP43 are the internal antennae of PSII. The main electron transport cofactors of PSII are the following: a special Chl pair, denoted as P680; pheophytin (Pheo), a Chl molecule lacking the central Mg^{2+}; quinone A (Q_A), which is the first stable electron acceptor in PSII; and quinone B (Q_B), which is converted into a mobile electron carrier after accepting two electrons. PSII and LHCII trimers form the so-called 'PSII supercomplex'.

LHCII proteins consist of six polypeptides in *Arabidopsis thaliana* (hereafter Arabidopsis) that share high homology and are encoded by Lhcb1-6 genes. Lhcb4-6 proteins, also called CP29, CP26 and CP24, respectively, are monomeric and bound directly to the PSII core. Lhcb1-3 proteins form trimers in different compositions, bound to PSII through Lhcb4-6 in an ordered and hierarchical way: Lhcb1-2 trimers are strongly bound to D1 and CP43 through CP26 (S-trimers); trimers that also include Lhcb3 are moderately bound to D2 and CP47 through CP24 and CP29 (M-trimers). PSII core dimer bound to two S-trimers is the minimal structure and is called C_2S_2 supercomplex. The addition of one or two M-trimers to PSII is indicated as C_2S_2M and $C_2S_2M_2$, respectively (Boekema et al, 1999). Lhcb proteins bind Chls (*a* and *b*) and carotenoids. The LHCII trimer, in particular, binds 24 Chl *a* and 18 Chl *b* (Chl a/b ratio equal to 1.3), six lutein, three neoxanthin and three violaxanthin (Jansson, 1994).

The PSI-LHCI supercomplex binds 173 chlorophylls and 15 carotenoids (Amunts et al, 2010; Schöttler et al, 2011). The PSI reaction center is formed by two large subunits, PsaA and PsaB, which bind 80 chlorophylls *a* and the majority of the redox-active cofactors. Among 15 core proteins constituting the PSI core, only PsaA, -B, and -C directly bind the electron transport cofactors P700 (a chlorophyll dimer), A_0 (a chlorophyll *a*), A_1 (a phylloquinone), F_x (a [4Fe–4S] iron–sulfur cluster), F_A and F_B ([4Fe–4S] iron–sulfur clusters). The other PSI protein subunits accomplish other functions: PsaD and PsaE provide the docking site for ferredoxin on the stromal side of the thylakoid membrane, PsaF and PsaN interact with plastocyanin and PsaF is also needed for binding of the Lhca1/Lhca4-dimer (Jensen et al, 2007).

LHCI is organized in the two adjacent heterodimers Lhca1/4 and Lhca2/3, which are bound together like a belt at one side of PSI. Since LHCI is tightly bound to PSI, its precise pigment composition is difficult to determine. Lhca proteins bind about 58 Chl in total (Schöttler et al, 2011), both Chl *a* and *b* are bound, but there is a lower affinity for Chl b. Chl *a*/*b* ratios have been estimated to be 4.0, 1.9, 5.9 and 2.4 in Lhca1, Lhca2, Lhca3 and Lhca4, respectively (Castelletti et al, 2003). LHCI polypeptides belong to the same superfamily of proteins of LHCII (Dreyfuss and Thornber, 1994) and, like LHCII, every Lhca protein contains three transmembrane helices (Kuhlbrandt at al, 1994)

Cyt b_6f is a dimeric complex, composed of nine subunits per monomer: four large subunits (cytochrome b_6, cytochrome f, Rieske iron sulfur protein, subunit IV) that

bind the redox prosthetic groups (four hemes, one [2Fe-2S] cluster and a plastoquinone); four small subunits PetG, -L, -M, and –N, forming hydrophobic sticks that span the membrane; ferredoxin:$NADP^+$ reductase (FNR), which is relevant for the cyclic electron transfer linked to PSI (Whitelegge et al, 2002).

ATP synthase consists of a membrane integral portion CF_o (composed of subunits I, II, III_{14}, IV), embedded in the thylakoid membrane, and a stroma-exposed portion CF_1 (subunits: α_3, β_3, γ, δ, ε). The III_{14} oligomer forms the proton-driven rotor, while the rotating central stalk is composed of subunits γ and ε. A second stalk, comprising of subunits I, II and δ, constitutes a stator that connects CF_o and CF_1 at the periphery and stabilizes the rotating machinery (Poetsch et al 2007).

With the exception of Cyt b_6f complex, the photosynthetic protein complexes are not homogeneously distributed along the thylakoid membrane. Grana cores are enriched in PSII and LHCII, while PSI-LHCI and ATP synthases are mostly located at grana margins, stroma lamellae and grana end membranes (Anderson and Andersson, 1980; Albertsson 2001). The density of PSI is highest at grana margins (Kaftan et al, 2002). Such a configuration is called *lateral heterogeneity*.

1.3 Photosynthetic machinery: function

1.3.1 Light absorption and connectivity

The inner and peripheral antennae of PSII and PSI absorb the incident light by their pigments. The absorbed energy packet, called exciton, can be transferred between pigments by *Förster resonance energy transfer* (FRET) (Förster, 1948). FRET is strongly dependent on the distance between pigments (within a 1-10 nm range) and therefore occurs only between pigment-protein complexes that are in close proximity to each other. Moreover, due to differences in the electronic energetic levels, the exciton preferentially moves from carotenoids to chlorophyll *b* and then to chlorophyll *a*. Consequently, the absorbed light energy is funneled into the photosystem reaction center that, in this way, functions as an energy trap. According to the puddle model, the PSII photosynthetic units are not connected with each other. This is contrasted in the lake model, where PSII photosynthetic units can exchange excitons (Joliot and Joliot, 1964; Lavergne and Trissl, 1995) and, if an exciton reaches a closed PSII, it can return to the antenna system and reach another PSII. This phenomenon is denoted as 'antenna connectivity'. In this respect, LHCII can function as a bridge for the energy flow between PSII centers.

1.3.2 Linear and cyclic electron transfer

Once harvested energy has reached the PSII or PSI reaction center, it can be used to oxidize specific substrates. PSII can split water and use it as a source of electrons that in turn reduce plastoquinone (PQ) (Figure 1). PQ, in the oxidized form, is bound to

PSII. When a single molecule of PQ accepts two electrons from water and two protons from the stroma, it is converted to plastoquinol (PQH_2) and becomes a mobile carrier that can easily move in the membrane to reach the Cyt b_6f complex. Cyt b_6f oxidizes PQH_2 and reduces plastocyanin (PC). Since PQH_2 carries two electrons and PC carries only one, this redox reaction needs to be coordinated. This is achieved by the so-called 'Q cycle', a series of redox steps in the Cyt b_6f complex that redirect one electron from PQH_2 back to the PQ pool through several electron carriers. This electron flow is associated with proton translocation from stroma to lumen, so that Cyt b_6f works also as a proton pump. PC is a mobile electron carrier that reduces P700, the reaction center of PSI. Through several intermediates, PSI reduces ferredoxin (Fd) in the stroma. Eventually, Fd can donate electrons to nicotinammide dinucleotide ($NADP^+$) that turns to NADPH, in a reaction catalyzed by ferredoxin:$NADP^+$ reductase (FNR). The entire chain of reactions that starts from the splitting of water and ends with the production of NADPH is called *linear electron transfer* (LET). As a result of this chain of redox reactions, protons are pumped from the stroma into the lumen, producing a transmembrane electrochemical gradient that constitutes the proton motive force to synthetize ATP in the stroma through ATP synthase. Ultimately, the final products of the photosynthetic light reactions are NADPH and ATP.

Besides linear electron flow, other electron routes have been discovered. Particularly important are the routes that redirect part of the reducing power of the PSI electron acceptors back to the photosynthetic electron transfer chain and back to the PSI itself, constituting the so-called *cyclic electron transfer around PSI* (CET).

Two main routes for CET have been identified. One route is dependent on the thylakoid NAD(P)H dehydrogenase (NDH)-like complex related to cyanobacterial NDH-1L, which is involved in respiration and PSI cyclic electron transport (Battchikova and Aro, 2007). Eleven subunits of the chloroplast NDH complex are homologs to subunits in the eubacterial NADH dehydrogenase and the mitochondrial complex I (Matsubayashi et al, 1987; Peng et al, 2011). NDH carries out CET by redirecting electrons from the stroma and reducing the PQ pool (NDH-dependent CET) (Peng et al, 2011). The role of electron donor to NDH was previously assigned to NADPH. However, according to recent reports, Fd reduces NDH that is bound to PSI to form the NDH-PSI supercomplex (Yamamoto et al., 2011; Ifuku et al, 2011; Figure 1). The NDH activity has a significant role in the electron transfer in the bundle sheath cells of C4 plants (Majeran et al, 2008). Since the *ndh* mutants of *Arabidopsis thaliana* show no drastic growth phenotype (Shikanai et al, 2007), it has been proposed the NDH-mediated pathway is not the main component of CET in C3 plants (Nandha et al, 2007; Shikanai et al, 2007).

An alternative CET pathway has been identified for its sensitivity to antimycin A (AA-sensitive CET) and involves the reinjection of electrons from Fd to LET. However, it is not yet clear at which point electrons should return to LET and the functionality and interactions of the molecular components at the basis of this

mechanism are still under debate (reviewed in Bendall and Manasse, 1995; Johnson, 2011). Two thylakoid proteins named PROTON GRADIENT REGULATION 5 (PGR5) (Munekage et al, 2002) and PGR5-LIKE PHOTOSYNTHETIC PHENOTYPE1 (PGRL1) (DalCorso et al, 2008) have been proposed to be involved in AA-sensitive CET, but their roles are not clear (Hald et al, 2008; Nandha et al, 2007). PGRL1 is an integral thylakoid protein, expressed in Arabidopsis as two isoforms (PGRL1A and PGRL1B) (DalCorso et al, 2008). In the green alga *Chlamydomonas reinhardtii* PGRL1 carries out a redox-induced conformational change and forms a supercomplex including PSI-LHCI-LHCII-FNR-Cyt*bf*-PGRL1, which mediates CET (Iwai et al, 2010). PGR5 is an extrinsic thylakoid protein that has been proposed to be bound to PGRL1 at the stroma-exposed surface of thylakoids and to mediate AA-sensitive Fd-dependent CET via the formation of a PGRL1-PGR5 complex transiently interacting with PSI and Cyt b_6f to allow efficient CET around PSI (DalCorso et al, 2008). However, following observations of the *pgr5* mutant performing CET, the role of PGR5 protein to mediate the switch from LET to CET was proposed (Nandha et al, 2007; Joliot and Johnson, 2011). Most recently, it was proposed that PGRL1 could play the role of ferredoxin-plastoquinone reductase (FQR) (Hertle et al, 2013), a putative enzyme whose existence was postulated many years ago (Moss and Bendall, 1984). Moreover, the phosphorylation of PGRL1 by the STN8 kinase is important for an optimal electron transfer rate at the onset of illumination (Reiland et al, 2011).

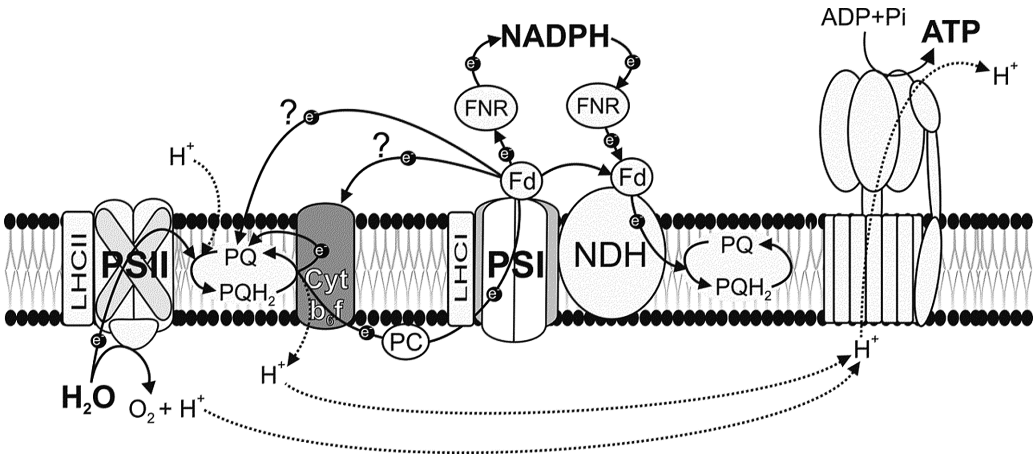

Figure 1. Simplified scheme of the linear and cyclic pathways of the photosynthetic electron transfer. The route of the antimycin A-sensitive cyclic electron transfer is still unclear (marked with ?). LHCII = light harvesting complex II; PSII = photosystem II; PQ = plastoquinone; PQH_2 = plastoquinol; Cyt b_6f = cytochrome b_6f complex; PC = plastocyanin; LHCI = light harvesting complex I; PSI = photosystem I; Fd = ferredoxin; FNR = ferredoxin-$NADP^+$ reductase; NADPH = reduced nicotinamide adenine dinucleotide phosphate; ADP = adenosine diphosphate; Pi = inorganic phosphate; ATP = adenosine triphosphate; NDH = NAD(P)H dehydrogenase-like complex.

1.3.3 Carbon assimilation

The products of light reactions of photosynthesis, ATP and NADPH, are utilized in the chloroplast stroma to assimilate atmospheric CO_2. The carbon assimilation process, i.e. the reduction of CO_2 to carbohydrates, is a metabolic pathway that can be schematically divided into three phases:

1. *Carboxylation* of ribulose-1,5-bisphosphate, forming two molecules of 3-phosphoglycerate
2. *Reduction* of 3-phosphoglycerate to glyceraldehyde-3-phosphate, at the expense of ATP and NADPH
3. *Regeneration* of ribulose-1,5-bisphosphate from glyceraldehyde-3-phosphate by utilizing ATP

Part of glyceraldehyde-3-phosphate is used for the synthesis of starch (for energy storage), and sucrose (for energy transportation through phloem to the sinks).

1.4 Photosynthetic machinery: regulation

1.4.1 Short-term regulation of photosynthetic electron transfer chain

1.4.1.1 Protein phosphorylation

Protein phosphorylation is a post-translational modification that plays a major role in adjusting the photosynthetic electron transfer chain activity upon short-term changes of environmental and metabolic conditions. In thylakoids, phosphorylation of threonine residues in the PSII core and LHCII proteins is mainly under the control of two homologous Ser-Thr kinases, named STN8 and STN7 in Arabidopsis. Respective orthologues are called Stl1 and Stt7 in the green alga *Chlamydomonas reinhardtii* (Bonardi et al, 2005; Bellafiore et al, 2005).

STN8 kinase catalyzes the light-dependent phosphorylation of the D1, D2, CP43 and PsbH proteins of the PSII core complex, and of the calcium-sensing receptor CaS in the thylakoid membrane (Vainonen et al, 2005). The level of PSII phosphorylation adjusts the folding of the thylakoid membrane. In the Arabidopsis *stn8* mutant (lacking STN8), it has been observed that an increased membrane stacking hampers the lateral migration of membrane proteins, and consequently the turnover of damaged D1 in the plants exposed to high light (Tikkanen et al, 2008; Fristedt et al, 2009). PHOTOSYSTEM II CORE PHOSPHATASE (PBCP) is required for dephosphorylation of PSII core subunits D1, D2, CP43 and PsbH (Samol et al, 2012). Due to the similar substrate specificity, PBCP and STN8 are therefore seen as an antagonistic pair for the reversible PSII core phosphorylation/dephosphorylation.

STN7 is required for phosphorylation of Lhcb1, Lhcb2 and Lhcb4 (CP29) polypeptides and of TSP9, a soluble protein involved in regulation of light harvesting

(Tikkanen et al, 2006; Depege et al, 2003; Bellafiore et al, 2005; Fristedt et al, 2009). STN7 also has activity in phosphorylation of PSII core proteins (Bonardi et al, 2005; Fristedt et al, 2011). A lack of STN7 does not affect thylakoid stacking (Fristedt et al, 2009), but it is crucial for 'state transitions', a short-term acclimation process discovered more than 40 years ago. This acclimation process was initially attributed to stacking/unstacking of grana and energy spillover between photosystems, which is a mechanism that involves the direct transfer of excitation energy from PSII to PSI through FRET (Bonaventura and Myers, 1969; Murata 1969a, b; Arntzen and Dito, 1976; Barber, 1980). Later, the spillover explanation was abandoned on the basis of a strict lateral heterogeneity model of the thylakoid membrane, according to which PSII and PSI cannot be in direct contact (Anderson and Andersson, 1980). The state transitions phenomenon was then related to the phosphorylation of LHCII (Bennet et al, 1979; Allen et al, 1981; Horton and Black, 1981). According to this up-dated state transitions model, the physiological function of LHCII phosphorylation is to promote rapid acclimation upon changes of light quality in order to maintain the redox balance in the ETC. The term 'redox balance' refers to the equal excitation of PSII and PSI and to the consequent capacity of photosynthetic apparatus to keep the ETC at a low reduction level (fluent electron transfer). In the state transition theory, when the incident light is enriched in red wavelengths, it preferentially excites PSII causing redox imbalance in the ETC. To reestablish balance, both the PSII core and LHCII are phosphorylated and, consequently, the mobile part of LHCII moves from PSII to PSI in order to adjust the absorption cross sections of the two photosystems, a condition called 'state 2'. Conversely, the enrichment of far-red wavelengths leads to preferential excitation of PSI, dephosphorylation of both PSII and LHCII and return of mobile LHCII to PSII (state 1).

The activity of STN7 depends on the redox state of both the electron transfer chain and stroma. The binding and release of plastoquinone to the Qo site of Cyt b_6f respectively activates and deactivates the kinase (Vener et al, 1997; Lemeille et al, 2009). Upon increased irradiance, reductants in the stroma inhibit LHCII kinase (Rintamäki et al, 2000). As a consequence of this dual regulation, LHCII is dephosphorylated in darkness, maximally phosphorylated when the light intensity decreases, and dephosphorylated after exposure to high light (Rintamäki et al, 1997). The protein phosphatase PPH1/TAP38 is responsible for the dephosphorylation of Lhcb1 and Lhcb2 (Shapiguzov et al, 2010; Pribil et al, 2010). Thus, the reversible LHCII phosphorylation controlled by STN7 and PPH1/TAP38 is essential for state transitions in plants. In Chlamydomonas, however, additional phosphorylation of the CP29 protein is required for state transitions to occur (Tokutsu et al, 2009).

The scheme of the antagonistic pairs STN7/PPH1 and STN8/PBCP is an oversimplification of complex regulation, and the exact interplay between STN7 and STN8 dependent pathways remains elusive. It is worth noting that the PSII core can be significantly phosphorylated in *stn8* and that only the *stn7 stn8* double mutant showed

full abolishment of both PSII core and LHCII phosphorylation (Tikkanen et al, 2008; Fristedt et al, 2009). Table 1 shows the most abundant STN7- and STN8- dependent phosphorylation sites of PSII core and LHCII.

Table 1. The most abundant PSII core and LHCII phosphorylation sites in *Arabidopsis thaliana* are the targets of the STN7 and STN8 kinases (from Fristedt et al, 2011).

	protein	phospho-site(s)	kinase(s) involved
PSII core	CP43	Thr1	STN8, STN7
	D2	Thr1	STN8
	D1	Thr1	STN8, STN7
	PsbH	Thr2, Thr4	STN8
LHCII trimer	Lhcb1	Thr3	STN7
	Lhcb2	Thr3	STN7
CP29	Lhcb4.1	Thr72, Thr74	STN7
	Lhcb4.2	Thr6	STN8, STN7
	Lhcb4.2	Thr78, Thr80	STN7

1.4.1.2 Non-photochemical quenching (NPQ)

The terminology 'non-photochemical quenching' (NPQ) initially defined the fraction of Chl *a* fluorescence quenching that was not related to the photochemical activity of photosynthesis (Bilger and Björkman, 1990). However, NPQ was subsequently associated to the fraction of absorbed light energy that cannot be used by photochemistry and has to be dissipated as heat. Therefore, the term NPQ is often used to describe the 'quenching mechanism of excess excitation energy'.

NPQ has been divided into three main components. The major component is known as either *energy dependent quenching*, or, *feedback de-excitation,* and is denoted *qE*. This terminology refers to the dependence of the NPQ process on a decrease in lumenal pH. The drop in lumenal pH is a result of photosynthetic activity, which in turn triggers two mechanisms: protonation of the PsbS protein, and reversible conversion of carotenoid violaxanthin to zeaxanthin via de-epoxidation (*xanthophyll cycle*). However, the precise molecular mechanism underlying qE and the identity of the quencher molecules are still under strong debate. Another component of NPQ (qT) is attributed to state transitions, whereby the phosphorylated fraction of LHCII would leave PSII and, due to decreased energy input to PSII, the fluorescence yield would decrease (Bennet et al, 1979; Horton and Black, 1980). A third NPQ component depends on PSII photoinhibition (qI). In this case, fluorescence yield decreases because part of PSII units are damaged under excess light and then subjected to a repair cycle (Krause and Weis, 1991).

1.4.1.3 Photosynthetic control via Cyt b_6f

The term 'photosynthetic control' generally refers to short- and long-term mechanisms that coordinate the photosynthetic production of ATP and NADPH in relation to their

utilization by metabolism. In this meaning, the 'photosynthetic control' term can be extended to also include the regulation of nuclear gene expression in response to environmental and metabolic conditions. Strictly speaking, however, photosynthetic control can be limited to the regulation of photosynthetic electron transfer through the acidification of thylakoid lumen and development of a transmembrane electrochemical gradient. In this respect, NPQ can be regarded as a mechanism of photosynthetic control, since its major component (qE) is triggered by the increase in transthylakoid ΔpH, as described above. While NPQ has been (and still is) an object of great scientific attention, another form of photosynthetic control that operates via Cyt b_6f has not received such attention, despite its decades-old discovery.

In 1969, Rumberg and Siggel used the reduction kinetics of P700, upon increasing illumination, to monitor transmembrane ΔpH and demonstrated a correlation between the two. They proposed that the mechanism responsible is located between the two photosystems (Rumberg and Siggel, 1969; confirmed later by Tikhonov et al, 1981). Later, the downregulation of Cyt b_6f was identified as abottleneck to linear electron flow, when Nishio and Whitmarsh (1993) correlated the changes of lumenal pH to the Cyt f reduction kinetics.

From these studies, we can understand that the downregulation of the Cyt b_6f activity is responsible for the oxidation of PSI normally observed upon increases in light intensity. It is surprising that despite the significant physiological implications of this finding, the importance of photosynthetic control via Cyt b_6f has been largely underestimated, particularly when compared to the attention focused on NPQ and state transitions mechanisms.

The dependence of Cyt b_6f activity on lumenal pH has been attributed to the reaction between PQH_2 and the Rieske Fe-S subunit of Cyt b_6f. The oxidation of PQH_2 takes place after the formation of an H-bond between a hydroxyl group of PQH_2 and the lone electron pair of a nitrogen in a histidine residue adjacent to the binding pocket for PQH_2 (Crofts et al, 1999). Decrease of lumenal pH leads to protonation of the lone pair, thus blocking the formation of the H-bond and subsequent PQH_2 oxidation. The pH optimum for the Cyt f reduction is in the range 6.5-7.2 (Tikhonov et al, 1984; Nishio and Whitmatsh, 1993; Hope et al, 1994; Finazzi, 2002).

1.4.2 Long-term regulation of photosynthetic electron transfer chain

If changes in the light conditions are more permanent, lasting from hours to several days, the acclimation processes take place at the gene level. The PSII/PSI ratio changes according to changes in both the quantity and quality of incident light. PSII/PSI increases by increasing the irradiance (Chow et al, 1988; Bailey et al 2001; Fan et al, 2007). Its value is species-dependent and has been found to range between 1.5 and 1.9 in sun plants (Melis, 1989; Fan et al, 2007). Adjustments of the PSII/PSI ratio according to changes in light quality have mainly focused on the so called 'state 1' and

'state 2' light regimes. Under far-red enriched light (PSI light) the PSII/PSI ratio increases, while it decreases under illumination favoring PSII excitation (PSII light) (Melis, 1991; Chow et al., 1991).

The regulation of the light-harvesting antenna size involves only LHCII, since the PSI-LHCI ratio has been shown to remain constant (Ballottari et al, 2007). The PSII antenna size increases upon exposure to low light intensity while it decreases under high light (Leong and Anderson, 1984; Yang et al., 1998). Moreover, when plants have been grown in low light conditions, an addition of one or two loosely bound LHCII trimers (L-trimers) to the $C_2S_2M_2$ PSII complexes has been observed, resulting in $C_2S_2M_2L$ and $C_2S_2M_2L_2$ complexes (Dekker et al, 1999; Boekema et al, 1999b).

1.4.3 Dynamics of thylakoid structure

The complex thylakoid structure responds to changing environmental conditions, above all to light intensity, in both short- and long- term. Grana stacks diameters usually range between 300 and 600 nm, while the extent of grana membrane stacking is highly variable. Upon long-term acclimation, shade plants exhibit broader grana and more stacked thylakoids per granum comparative to sun plants (Lichtenthaler et al, 1981). In short-term, similar structural modifications happen: plants shifted from standard growth light to shade have exhibited increases in both grana diameter and height of stacks, together with a decrease in the number of grana per chloroplast (Rozac et al, 2002). In the same study, a switch to high light intensity caused opposite effects. Such changes were visible after 10 minutes of light treatment and were shown to be reversible in the same time scale. Herbstová et al. (2012) showed that high light stress induces lateral shrinkage of grana diameter and increased protein mobility in grana thylakoids. These structural changes work synergistically to improve the accessibility between damaged PSII in grana and its repair machinery in stroma lamellae. This process involves changes in the phosphorylation of PSII and LHCII proteins.

How such a complex structure as a granum can react so rapidly and be so flexible in response to environmental cues is not yet fully understood. Formation and flexibility of grana are possible due to the interplay of attractive and repulsive forces between adjacent appressed thylakoids (for a review see Anderson, 2008). Attractive forces are: (1) van der Waals interactions; (2) Mg^{2+}-dependent electrostatic interactions; (3) maximization of stroma entropy that should favor the formation of grana (Chow 1999); and (4) the attraction between opposing LHCII trimers through the complementarity of the positive and negative charges on their stroma-exposed surfaces (Standfuss et al, 2005), and between the extrinsic proteins of opposing PSII units in the lumen (de Las Rivas et al. 2007). Regarding the last point, it is noteworthy that in spinach and Arabidopsis the PSII-LHCII arrays in opposing membranes were found to intersect each other at preferential angles that optimize the overlap of opposing LHCII trimers (Yakushevska et al, 2001).

The repulsive forces in grana are: (1) electrostatic repulsions between negative charges on the stroma-exposed surface, strongly regulated by protein phosphorylation; and (2) steric hindrance due to the stroma-protruding moieties of ATP synthase and PSI, determinant in maintaining the lateral heterogeneity of PSII and PSI. The dynamics of grana response is correlated to the exchange of ions between the lumen and stroma. The lumenal pH is equal to 7.5 in darkness and decreases to 5.7 under light saturation (Takizawa et al, 2007). The transthylakoid *proton motive force* (pmf) induces Cl^- influx into the lumen through voltage-gated Cl^- thylakoid membrane channels (Spetea and Schoefs, 2010), Ca^{2+} influx by a Ca^{2+}/H^+ antiporter (Ettinger et al, 1999), and Mg^{2+} efflux from the lumen (Hind et al, 1974). These ions fluxes are responsible for the light-dependent osmotic swelling of the lumen that can facilitate PC movement and consequently, photosynthetic activity (Kirchhoff et al, 2011).

2. AIMS OF THE STUDY

My thesis research focused on the molecular mechanisms regulating the photosynthetic electron transfer upon changes of light intensity. In order to challenge such regulatory mechanisms, I used a fluctuating growth light condition, i.e. the alternation of low and high intensities of light.

As plant material I used wild-type and mutants of the model plant *Arabidopsis thaliana* which are impaired in: PSII core and LHCII phosphorylation; in energy-dependent NPQ; or in regulation of photosynthetic electron transfer.

Although all of these processes have been extensively studied during the last few decades, many aspects remain elusive, and controversial results are still published. Moreover, the interaction of these regulatory mechanisms has remained almost unexplored. The specific objectives of my thesis were:

1. To revisit the role of LHCII phosphorylation in the frame of physiological relevant conditions, and its relationship with other acclimation processes.

2. To investigate the mechanism of the regulation of excitation energy distribution by LHCII phosphorylation on the basis of the recent knowledge of thylakoid structure and protein distribution.

3. To investigate the physiological role of PSI oxidation upon exposure to high light and its implications in the regulation of electron transfer and photoprotection.

4. To elucidate the cooperation between the LHCII phosphorylation, NPQ and the photosynthetic control of LET via Cyt b_6f in maintaining the redox poise of ETC under changes of light intensity.

3. MATERIALS AND METHODS

3.1 Plant material and growth conditions

Wild-type Arabidopsis (*Arabidopsis thaliana*) ecotype Columbia and the *stn7* (Bellafiore et al., 2005), *stn8* (Bonardi et al., 2005), *stn7 stn8* (Bonardi et al., 2005), *npq4* (Niyogi et al., 2005), *npq4 stn7* (Frenkel et al., 2007), *npq1* (Niyogi et al., 1998) and *pgr5* (Munekage et al., 2002) mutant plants (Table 2) were grown in a phytotron at 23°C, relative humidity of 60%, and 8-h photoperiod under moderate light (ML, 120-130 µmol photons m^{-2} s^{-1}). In Papers II and III, plants were grown also under low (LL, 50 µmol photons m^{-2} s^{-1}) and high (HL, 500 µmol photons m^{-2} s^{-1}) light intensities. In Papers I, II and III, plants were also grown in fluctuating light (FL) conditions, which consisted of alternations of 5 min of low and 1 min of high light, provided by an automatic shading system, during the 8-h photoperiod. The values of light intensities during the low and high light phases of FL are specified in the papers. Light was provided by OSRAM PowerStar HQIT 400/D Metal Halide Lamps.

Table 2. Thylakoid regulatory mutants used in the experiments

Mutant	Defective protein product	Function	References
stn7	STN7 kinase	LHCII phosphorylation	Bellafiore et al. 2005; Bonardi et al. 2005;
stn8	STN8 kinase	PSII core phosphorylation	Bonardi et al. 2005; Vainonen et al., 2005
stn7 stn8	STN7 and STN8 kinases	LHCII and PSII core phosphorylation	Bonardi et al., 2005
npq4	PsbS	Feedback de-excitation (NPQ)	Niyogi et al., 2005
npq4 stn7	STN7 kinase and PsbS	LHCII phosphorylation and NPQ	Frenkel et al., 2007
npq1	violaxanthin de-epoxidase (VDE)	Zeaxanthin-dependent NPQ	Niyogi et al., 1998
pgr5	PGR5	Regulation of photosynthetic electron transfer	Munekage et al., 2002

3.2 Thylakoid isolation and solubilization

Thylakoid membranes were isolated from light-acclimated leaves (leaves were harvested four hours after the beginning of the light period) as described in Suorsa et al. (2004). The Chl content was determined according to Porra et al. (1989).

Different thylakoid solubilization methods were used in the thesis. In Paper III, the thylakoids were solubilized for 2D lpCN electrophoresis according to Järvi et al (2011). In Paper IV the same procedure was used with some modifications. The

samples were solubilized with digitonin by gentle agitation for 8 minutes in darkness at room temperature. Subsequently, thylakoids were centrifuged (18 000 g at 4°C, 25 min) and the supernatant, enriched in solubilized grana margins and stroma lamellae, was collected. The pellet, enriched in unsolubilized grana cores, was resuspended into 25BTH20G buffer and further solubilized with 1% n-dodecyl β-D-maltoside (DM) by keeping the sample 2 min on ice. The insoluble material was removed by centrifugation at 18 000 g at 4°C for 15 min, and equal volumes of the WT and *stn7* supernatants were loaded in the wells.

In paper IV, for sequential solubilization of thylakoid protein complexes by digitonin and DM, the isolated WT and *stn7* thylakoids were first resuspended into the storage buffer (50 mM Hepes/KOH pH 7.0, 100 mM sorbitol, 10 mM $MgCl_2$, 10 mM NaF). Detergents were then added to different aliquots to reach final concentrations of 0.2 %, 0.5 %, 1.0 % and 1.5 % (w/v). The digitonin solubilization was carried out as described above, the DM solubilization according to Järvi et al (2011).

3.3 Gel electrophoresis, immunoblotting and staining

The lpCN and lpBN gels as well as 1D- and 2D -SDS-PAGE were carried out as described in Järvi et al. (2011). In paper IV, the acrylamide gradient in lpCN and lpBN gels was modified to 3.5-12.5%. After electrophoresis, the proteins were electroblotted to a polyvinylidene difluoride membrane (Millipore), blocked with 5% milk (non fat dry milk; Bio-Rad) and immunodetected with antibodies.

In Papers III and IV, SYPRO® Ruby and ProQ® Diamond gel staining were carried out following the Invitrogen Molecular ProbesTM instructions. The Geliance 1000 Imaging System (Perkin-Elmer) was used to acquire the ProQ and Sypro-stained electophoresis images. The ProFinder 2D software, version 2005 (Nonlinear Dynamics), was then used for the spot densitometry. The intensity values of ProQ- and SYPRO-stained spots were normalized based on the total intensity of all spots in the sample.

3.4 *In vivo* measurements of PSII and PSI functional parameters

In Paper I, the *in vivo* PSII activity was monitored using a JTS-10 spectrofluorometer (Bio-Logic SAS), by applying green actinic light (520 nm wavelength).

In Papers II and III, the simultaneous measurements of PSI and PSII photosynthetic parameters were performed by using Dual-PAM-100 (Heinz Walz), based on P700 redox signal (depending on the absorbance at 830 nm; Klughammer and Schreiber 2008) and chlorophyll *a* fluorescence, respectively. The measuring light (460 nm wavelength) intensity for fluorescence measurements was 5 µmol photons m^{-2} s^{-1}. The red actinic light (635 nm wavelength) used to mimic the low and high light phases of FL had the intensities of 56 and 632 µmol photons m^{-2} s^{-1}. NPQ was measured as (Fm-

Fm´)/Fm´ (Bilger and Björkman, 1990), where Fm´ is the maximal fluorescence yield from illuminated leaf during a saturating pulse (SP, 6000 µmol photons m^{-2} s^{-1}, 300 msec, at 30 s frequency) and Fm is the maximal fluorescence from a dark-adapted leaf during a SP. The PSI acceptor side limitation [Y(NA)] and the P700 oxidation level were determined according to Klughammer and Schreiber (1994 and 2008).

In Paper I, the reduction level of the electron transfer chain (ETC) was measured as F´, i.e. the fluorescence yield under actinic light. In Papers II and III, the F´ values were normalized to Fm, resulting in F´/Fm. This parameter was considered suitable to estimate the relative changes of the Q_A redox state under fluctuating (non-steady-state) actinic light. In Paper II, the reduction level of ETC was determined also by the 1-q_L parameter (Kramer et al., 2004) under the steady-state phases of illumination.

In Paper II, the amounts of functional PSI and PSII reaction centers were estimated by the Pm and Fm parameters, respectively. The leaves were incubated in darkness for 20 min prior to measurements. Pm was determined according to Klughammer and Schreiber (2008). Both the Pm and Fm values were normalized according to the Chl content (Pm and Fm per microgram of chlorophyll). The high light (1200 µmol photons m^{-2} s^{-1}) treatments were performed on leaf discs (9-mm diameter). The DCMU solution (80 µM in 330 mM sorbitol) was infiltrated by syringe.

3.5 Chlorophyll fluorescence measurements from thylakoids

In Papers I and III, the measurements of 77 K Chl *a* fluorescence emission spectra were performed using a diode array spectrophotometer (S2000; Ocean Optics) equipped with a reflectance probe. In Paper I, to obtain the 77 K Chl *a* fluorescence excitation spectra, the samples were excited with wavelengths from 400 to 540 nm with 5-nm steps using an f/3.4 Monochromator (Applied Photophysics).

In paper IV a specific solubilization of thylakoids was employed before the fluorescence measurements. Isolated thylakoids were first suspended in a storage buffer and then solubilized mildly by detergents in order to detach the most exposed pigment-protein complexes from the intact thylakoid membrane. Note that the solubilized and unsolubilized thylakoid fractions were not separated from each other but, instead, every sample containing both the detached (solubilized) and membrane-associated (unsolubilized) protein complexes, was homogenized and frozen in liquid nitrogen at the final concentration of 40 µg Chl/ml (50 µl volume). Fluorescence emission spectra were then measured from frozen suspension at 77 K by using an Ocean Optics S2000 spectrometer. The samples were excited with blue light (480 nm wavelength). Spectra were normalized based on PSI emission at 733 nm.

The flash fluorescence induction measurements shown in Paper IV were carried out at room temperature on the same samples used for 77K spectra, after dilution to 1.6 µg Chl/ml with storage buffer. Before measurements, samples were incubated in darkness

for two minutes in the presence of 20 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). The fluorescence was detected beyond 700 nm (Corion LG 697 filter) by using a FL3500 dual-modulation fluorometer (Photon System Instruments, Czech Republic).

4. MAIN FINDINGS

4.1 The growth of *pgr5* and *stn7* mutants is affected under fluctuating light

It was first demonstrated in Paper I, that the growth of *pgr5* and *stn7* mutants of Arabidopsis was compromised under fluctuating light conditions, but not under constant light, as compared to the wild type (WT). A more comprehensive study with a number of different mutants (*stn7*, *stn8, stn7 stn8, npq4, stn7 npq4, npq1* and *pgr5*) and different growth conditions was then undertaken (Papers II and III). When WT and mutant plants grew under constant low (LL), moderate (ML), and high (HL) light intensities, no difference in the visual phenotype was observed (Figure 2). In contrast, under fluctuating light condition (FL), the *stn7*, *stn7 stn8* and *stn7 npq4* mutant plants showed a stunted growth (Figure 2). This indicated that the LHCII phosphorylation is needed under fluctuating light, whilst in constant light conditions specific mechanisms could compensate the lack of the STN7 kinase (Paper III). Also, the *pgr5* mutant plants appeared healthy if grown under constant light, whereas in fluctuating light conditions they died after the formation of the first true leaves (Paper II).

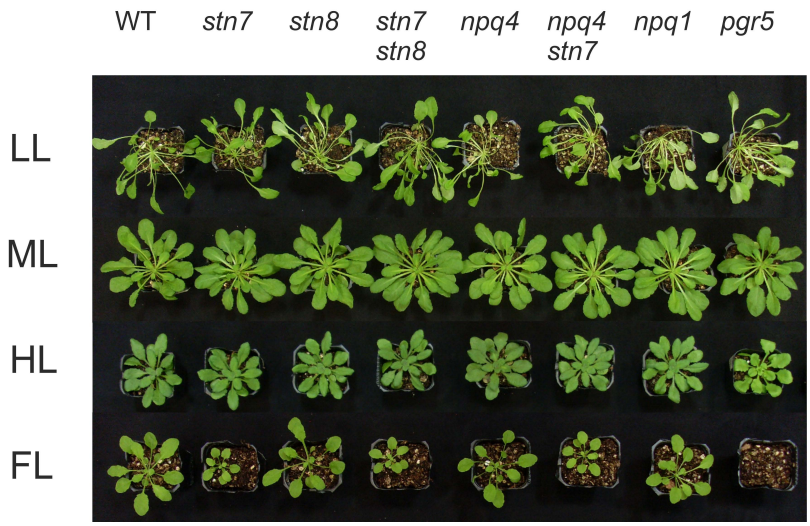

Figure 2. Phenotypes of Arabidopsis wild-type (WT) and mutant plants grown under constant light and FL. Visual phenotypes of plants grown under constant LL, ML, and HL white light intensity (50, 130, and 500 µmol photons m^{-2} s^{-1}, respectively) and under FL (i.e. alternation of 5 min of low-intensity white light [60 µmol photons m^{-2} s^{-1}] and 1 min of high-intensity white light [600 µmol photons m^{-2} s^{-1}]) during the 8-h photoperiod. Plants were photographed 8 weeks (LL), 4 weeks (ML), 3 weeks (HL), and 6 weeks (FL) after sowing the seeds.

4.2 PSI in *pgr5* mutant is damaged under fluctuating light

The functionality and protein stability of both photosystems were estimated in WT and *pgr5* plants grown under constant moderate light (ML), and then shifted to fluctuating

light growth condition (FL) for 9 days (Paper II). WT was able to maintain the same functionality and amount of both PSII and PSI in FL as in ML condition (Figure 3 A-D). Accordingly, the intersystem redox balance was also maintained (Figure 3 E). In *pgr5*, PSII was as robust as in WT while PSI functionality had already fallen to approximately 40% of the initial value by the end of the first day under exposure to FL. Concomitantly, the reduction level of electron transfer chain increased dramatically (Figure 3 E). The amount of PSI appeared to decrease after 4-5 days after the shift to FL (Figure 3 B).

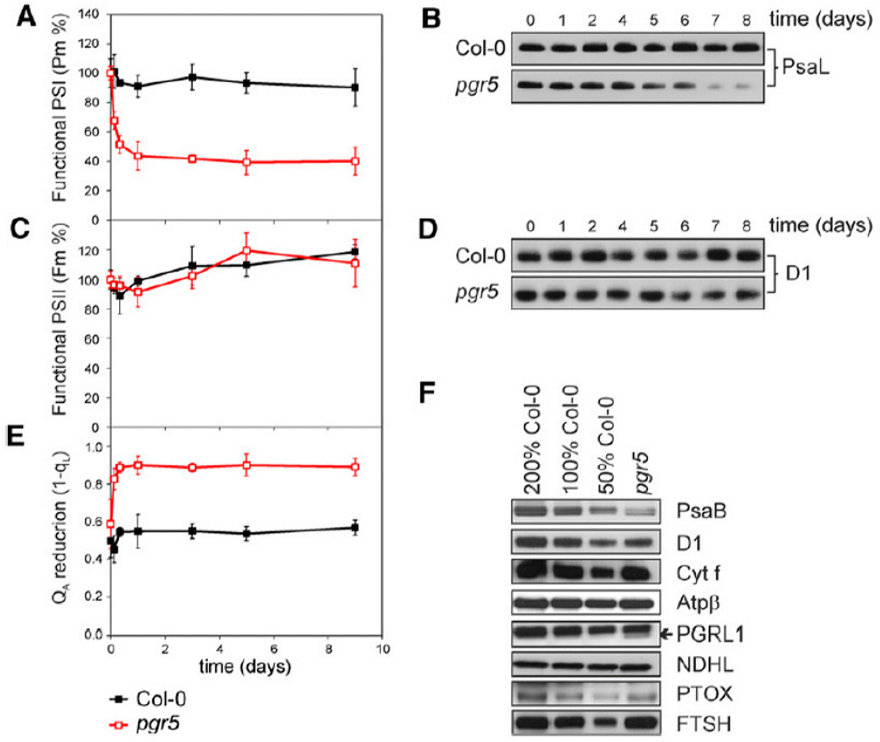

Figure 3. Structural and functional changes in the photosynthetic machinery of WT (Col-0, Columbia-0) and *pgr5* plants upon shift from constant light to fluctuating light for 9 days. WT and pgr5 plants were grown under constant light (120 µmol photons m^{-2} s^{-1}) for 5 weeks (time point 0) and then transferred to fluctuating light during the photoperiod with 5 min of low light (50 µmol photons m^{-2} s^{-1}) and 1 min of high light (500 µmol photons m^{-2} s^{-1}) for 9 d. (A and B) The relative changes in the functionality (A) and the amount of PSI detected by immunoblots of the PsaL protein (B). The values represent means ± SD, n = 3. (C to E) Changes in functionality of PSII (C), the amount of PSII based on the amount of the D1 protein (D), and in the Q_A reduction level (1-q_L) during 9-d growth of mature leaves under fluctuating light (E). The fluorescence measurements were performed using intact leaves after 20 min dark incubation. The values represent means ± SD, n = 3. (F) Immunoblots of thylakoid membrane proteins 9 d after the transfer to fluctuating light. Representative examples from at least three biological replications are shown with the dilution series from WT leaf extracts (50 to 200%). (Figure from Paper II)

4.3 Photosynthetic electron transfer in *pgr5* mutant plant under fluctuating light

In order to reveal the mechanism causing damage to PSI in *pgr5*, photosynthetic parameters were measured *in vivo* on leaves from plants grown in FL condition (Paper II). Measurements were performed by using actinic light that mimicked the fluctuating growth light (AFL). In the first low light phase of AFL, the ML-grown *pgr5* plants showed the same reduction level of electron transfer chain (ETC) and PSII yield as WT (Figure 4 A and B) but, upon application of one minute-high light pulses, the reduction level of ETC suddenly increased. During three cycles of low and high light phases, the reduction of ETC gradually increased and the PSII yield decreased. The FL-grown *pgr5* mutant showed the same trends, however the ETC redox poise was already affected at the beginning of measurement. During the high light phases, all *pgr5* mutants, developed both in ML and FL, were unable to induce NPQ and to oxidize PSI (Figure 4 C and D). The results indicated that upon increase of light intensity the *pgr5* plants intensely accumulate reducing power in the ETC and are not able to reestablish the redox poise, even after return to low light intensities.

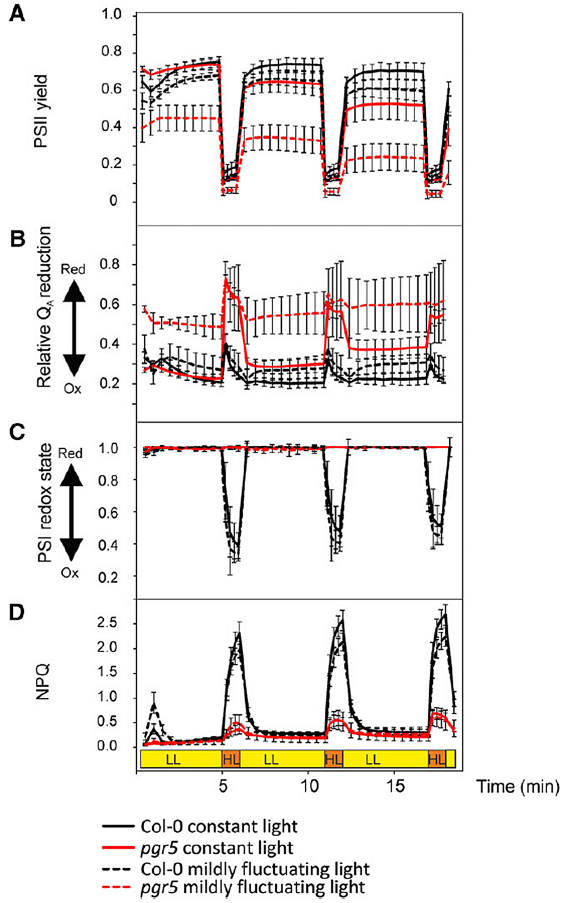

Figure 4. Photosynthetic parameters of WT (Col-0) and *pgr5* plants measured under illumination mimicking the fluctuating growth light condition. The plants were grown either under constant moderate light (120 µmol photons m^{-2} s^{-1}) or under mildly fluctuating light with 5 min of low light (50 µmol photons m^{-2} s^{-1}) and 1 min of moderate high light (350 µmol photons m^{-2} s^{-1}) repeating for the entire photoperiod. (A) to (D) Actinic light that mimics the fluctuating light (5 min of low, 58 µmol photons m^{-2} s^{-1} light, and 1 min of high, 533 µmol photons m^{-2} s^{-1} light) was applied on detached leaves. The values represent means ± SD, n = 4 to 6. (A) Photochemical quantum yield of PSII. (B) The relative Q_A reduction level, measured by the chlorophyll *a* fluorescence parameter F′/Fm. (C) The PSI redox state. (D) Induction of NPQ. HL, high light; LL, low light. (Figure from Paper II)

4.4 The *pgr5* mutant is impaired in the control of linear electron flow upon switch to high light

Photoinhibition of PSI and PSII was measured from leaf discs of WT (Col-0) and *pgr5* plants, grown under constant moderate light, after high light treatment (1200 μmol photons m^{-2} s^{-1} for 15 min) (Figure 5; Paper II). The functionality of PSI and PSII reaction centers was determined by the Pm and Fm parameters respectively, measured from the treated leaf discs after 20 min dark incubation and expressed as percentage of the initial values. In WT, the functionality of PSII decreased by almost 40%, while functionality of PSI was not affected at all. In contrast, PSI of the *pgr5* mutant was highly damaged (with approximately 70% of functional reaction centers lost), and PSII was affected even more than in WT. The same experiment was repeated after introduction of DCMU (80 μM in 330 mM sorbitol) into leaf discs to block the electron transfer from PSII. In this condition, WT showed the same result as without DCMU. In *pgr5* mutant, PSII was damaged to the same extent as without DCMU, while PSI was almost unaffected. These results indicated that the electron flow generated by PSII is responsible for the PSI damage observed under high light in *pgr5* mutant plants, and that the PGR5 protein is therefore essential for the control of LET upon increase of light intensity.

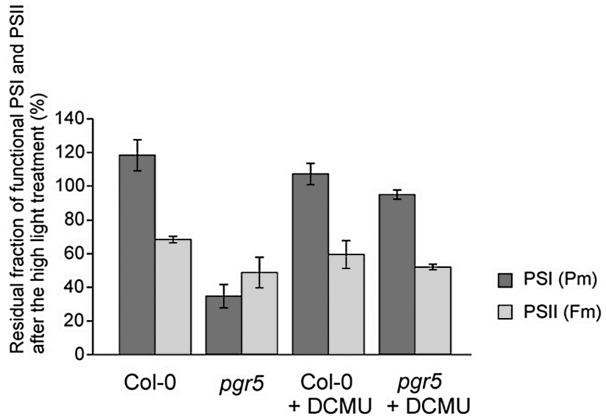

Figure 5. Photoinhibition of PSI and PSII in WT and *pgr5* plants by a high light treatment in the presence and absence of DCMU. Following the high light treatment of 1200 μmol photons m^{-2} s^{-1} for 15 min, the functionality of PSI and PSII reaction centers was determined by the Pm and Fm parameters, respectively, measured from leaf discs (n = 3) after 20 min dark incubation and expressed as percentage of the initial values. Plants were grown under constant moderate light. DCMU (80 μM in 330 mM sorbitol) was infiltrated by a syringe. The values represent means ± SD from three independent measurements. (Figure from Paper II)

4.5 Acclimation strategy of *stn7* mutant plants under constant and fluctuating growth light

To study the role of thylakoid protein phosphorylation in light acclimation, a detailed analysis of the *stn7* mutant was performed in Paper III. The relative differences of

photosystems stoichiometry between WT and *stn7* plants grown in constant and FL conditions were estimated by densitometry of two-dimensional blue-native electrophoretic gels (2D-BN-PAGE) (Figure 6 A) and by immunoblotting (Figure 6 B). Under constant low, moderate and high growth light, the *stn7* mutants were able to acclimate by decreasing the PSII/PSI ratio in comparison to the WT grown in the same condition: in *stn7* the higher amount of PSI enables drainage of the excess of electrons "pumped" by PSII into ETC, thus compensating for the unbalanced energy distribution resulting from the lack of LHCII phosphorylation. In contrast, *stn7* plants failed in this FL acclimation strategy, showing a PSII/PSI ratio even higher than in WT (Figure 6 A and B).

Despite these adjustments of the photosynthetic apparatus, the PSII and LHCII phosphorylation patterns in WT and *stn7* plants were surprisingly similar between the four growth conditions (Figure 6C). Even upon fluctuations of light intensity, the LHCII phosphorylation level was stable and maintained a steady-state level (Paper III). This indicated that the applied fluctuations of light were too fast to trigger the LHCII phosphorylation/dephosphorylation processes.

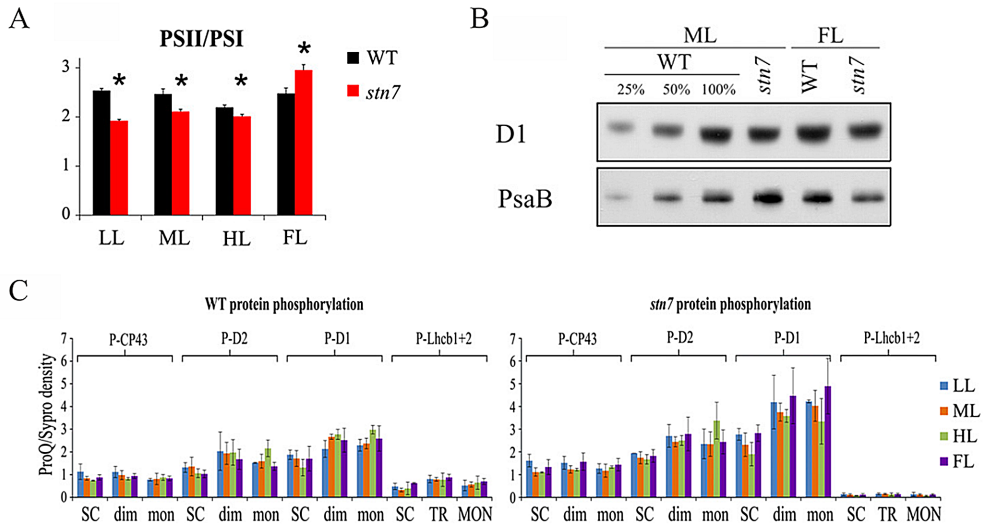

Figure 6. (A) Relative amounts of photosystems in plants grown under LL, ML, HL, and FL. PSII/PSI, PSII/PSI ratio. Data were obtained by spot densitometry of 2-D lpBN gels stained by SYPRO Ruby dye. Asterisks indicate statistically significant differences between WT and *stn7* plants at $P<0.05$. Values are means of three independent experiments ± SD. (B) Immunoblotting of D1 (PSII) and PsaB (PSI) proteins in WT and *stn7* mutant plants grown under ML and FL. Gels were loaded based on chlorophyll content. (C) Phosphorylation patterns of PSII (P-CP43, P-D2, P-D1) and LHCII (P-Lhcb1+2) in plants grown under LL, ML, HL, and FL. The protein phosphorylation level was calculated as a ProQ/SYPRO densitometric ratio of 2-D lpBN-PAGE spots. Gels were loaded based on chlorophyll content. SC, Supercomplex; dim, PSII dimer; mon, PSII monomer; TRIM, LHCII trimer; MON, LHCII monomer. Values are means of three independent experiments ± SD. (Modified Figure from Paper III)

4.6 Photosynthetic electron transfer in *stn7* mutant plants under fluctuating light

Photosynthetic parameters were measured from WT and *stn7* plants grown in ML and FL conditions under actinic light mimicking the fluctuating growth light (Paper III), in the same way as for the *pgr5* mutant plants (Section 4.3). During the low light phases of AFL, all plants lacking STN7 kinase and grown under constant ML condition showed a similar reduction level of ETC compared to WT and other mutants (Paper III). If instead grown under FL, the ETC reduction was much higher in all *stn7* mutants (Figure 7 A). Upon the switch to high light phases of AFL, all other plants were able to keep the ETC relatively oxidized, except for those plants impaired in NPQ. At the same time, all *stn7* mutants oxidized PSI to a lesser extent than WT (Figure 7 B), and showed a peak of limitation of the PSI electron acceptors (Figure 7 D). These results showed that the LHCII phosphorylation plays a crucial role in both the low and high light phases of AFL: it is necessary to maintain the redox poise of ETC when NPQ is not induced, and to prevent an electron burst against PSI when the light intensity suddenly increases.

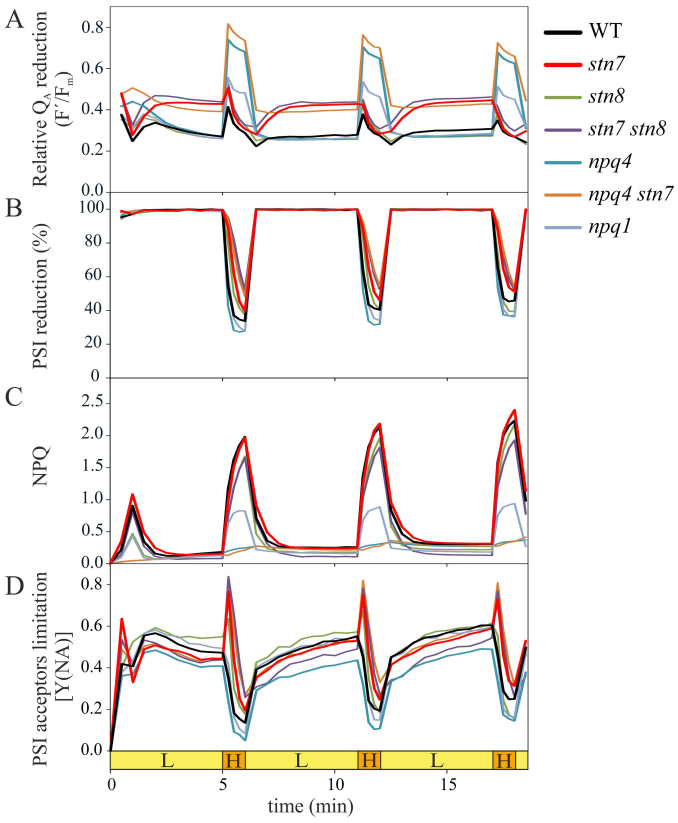

Figure 7. Photosynthetic parameters of WT and *stn7*, *stn8*, *stn7 stn8*, *npq4*, *npq4 stn7*, and *npq1* mutant plants grown in FL conditions. Measurements were performed under actinic light that mimicked the FL growth condition (AFL). (A) Relative Q_A reduction measured by the chlorophyll fluorescence parameter F´/Fm. (B) PSI reduction monitored as a percentage of reduced P700 reaction centers. (C) NPQ. (D) PSI acceptors limitation [Y(NA)]. Detached leaves were incubated in darkness for 15 min before measurements. Values are means from three independent experiments. L, LL phase of AFL; H, HL phase of AFL. (Figure from Paper III)

4.7 LHCII phosphorylation and energy spillover

The study described in Papers I and III focused on the role of LHCII phosphorylation in ensuring a balanced energy distribution between PSII and PSI and thus maintaining the redox poise of ETC. In Paper IV, the mechanism that allows the phosphorylated LHCII to regulate the distribution of excitation energy in the antenna system was further investigated. Thylakoids were isolated from WT and *stn7* mutant plants and solubilized with increasing concentrations of digitonin (from 0 to 1.5%, w/v) in order to detach protein complexes from the stroma lamellae. Importantly, it has been demonstrated that digitonin does not solubilize protein complexes from the grana core (refer to Anderson et al (1966) and Figure 1 of Paper IV). The solubilized (i.e. complexes detached from the thylakoid membrane) and unsolubilized (mainly the grana core membranes) fractions were not separated from each other but instead they were thoroughly mixed again, frozen in liquid nitrogen and the 77K chlorophyll fluorescence emission spectra were recorded. Spectra were decomposed into their components (Gaussian peaks), the areas of which were integrated and normalized to the total fluorescence emission. Digitonin treatment provoked an increase of fluorescence from free and aggregated LHCII fractions and, at the same time, an increase of emission from PSII compared to that from PSI (Figure 8 A-C). Particularly, the *stn7* mutant showed a systematic over-excitation of PSII compared to WT over the different digitonin concentrations (Figure 8 C). The enhanced fluorescence emission from PSII upon digitonin solubilization (i.e. upon detachment of PSI and some LHCII from the thylakoids) was confirmed by fluorescence induction measurements. These were recorded at room temperature from the same samples used for 77K spectra, in the presence of 20 μM DCMU. Both in WT and *stn7*, the 1.5% digitonin treatment induced a significant increase of fluorescence yield compared with non-treated thylakoids (Figure 8 D). These results revealed that PSI is energetically connected to PSII at grana margins, and that LHCII phosphorylation contributes to regulation of PSI-PSII connectivity.

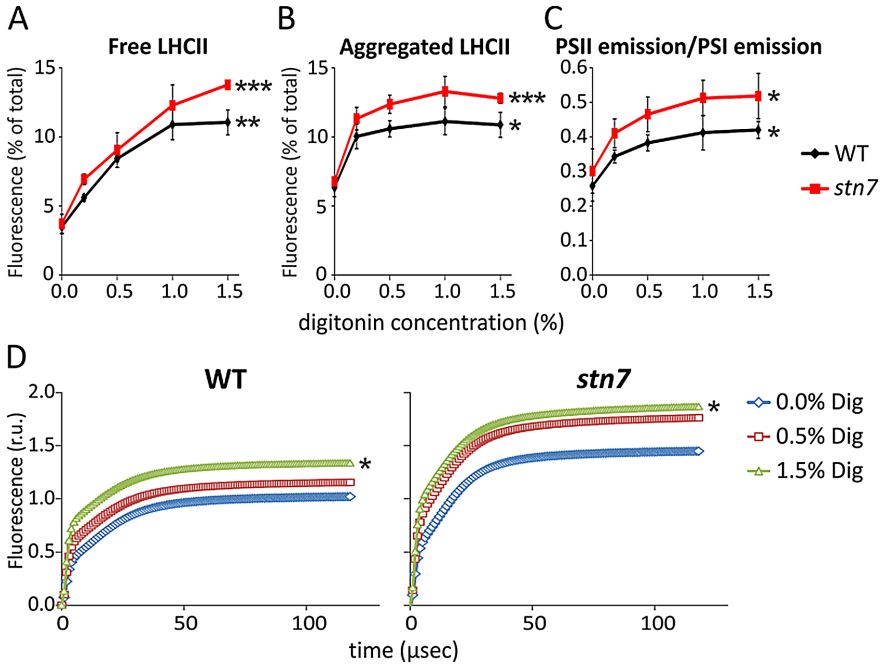

Figure 8. Effect of digitonin treatment on distribution of excitation energy in the thylakoid membrane of WT and *stn7* plants. Digitonin in different concentrations (%, w/v) was allowed to solubilize the thylakoid membrane, subsequently the suspension was mixed and subjected to recording of the chlorophyll fluorescence emission spectra at 77K (A-C) and flash fluorescence induction at room temperature (D). (A-C) Emission spectra were decomposed into their components and the areas of single peaks were quantified and plotted against digitonin concentration: free LHCII (A), aggregated LHCII (B), ratio of PSII emission over PSI emission (C). Data are means ± SD, n=3. Asteriscs (*) indicate that the value of 1.5% digitonin-treated sample was significantly higher than the value of the same sample that was not treated by digitonin: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ (Student t test). (D) Fluorescence induction was monitored in the presence of 20 µM DCMU. The fluorescence yield at 1.5% digitonin was significantly higher compared to the yield at 0% digitonin both for WT and stn7 ($*P < 0.05$, n=3, Student t test). Dig = digitonin. (Figure from Paper IV)

4.8 Response of LHCII phosphorylation to slow changes in light intensity

As shown above, the fluctuating growth light conditions applied (1min HL pulse every 5 min at low growth light conditions) did not modify the phosphorylation level of the thylakoid proteins. In order to study the role of PSII and LHCII phosphorylation in the light acclimation process, the WT and *stn7*, *stn8*, *stn7 stn8* plants (grown in ML) were exposed to fluctuations of light intensity, which were long enough to induce changes in the phosphorylation pattern of the thylakoid membrane (Paper I). After 30 minutes of low light illumination, WT showed low phosphorylation of PSII and high phosphorylation of LHCII (Figure 9 A). After subsequent 30 min of exposure to high light, the pattern was inverted: PSII was highly phosphorylated while the LHCII phosphorylation was low. This pattern, confirmed upon repeated cycles of low and high light, was different compared to the pattern obtained by state 1 light treatment that

induced the concomitant dephosphorylation of PSII core and LHCII, and state 2 light, inducing the concomitant PSII and LHCII phosphorylation (Figure 9 B). Upon fluctuations of white light intensity in 30 min cycles, the *stn8* plants showed a similar trend as WT except for the PSII core phosphorylation level, which was generally lower. In *stn7* mutants, instead, the PSII phosphorylation was higher than in WT under low light, while the LHCII phosphorylation was not detectable by immunoblotting. In *stn7 stn8* plants neither PSII nor LHCII phosphorylation was detectable. These experiments showed how the PSII core and LHCII phosphorylation patterns change according to light intensity and how they differ from the patterns obtained by the traditionally used state 1 and state 2 conditions.

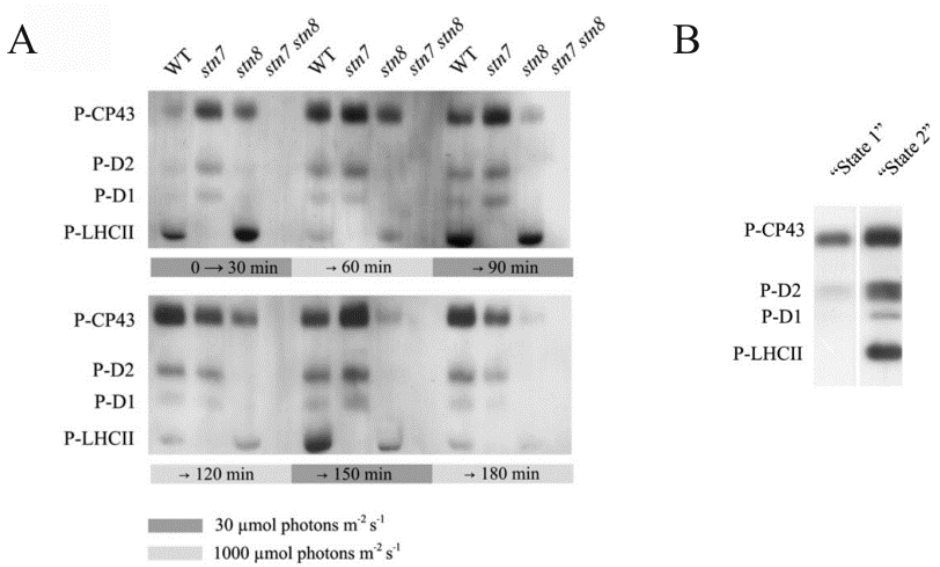

Figure 9. Regulation of thylakoid protein phosphorylation by the STN7 and STN8 kinase pathways. (A) Phosphorylation of thylakoid proteins in WT and the *stn7*, *stn8*, and *stn7 stn8* mutant plants exposed to 30 and 1000 µmol photons m^{-2} s^{-1} in 30 min intervals. (B) Phosphorylation of thylakoid proteins in the wild type after 1 h of treatment with far-red light (state 1) and red light (state 2). Phosphorylation of thylakoid proteins was determined by immunoblotting with P-Thr antibody, and 0.2 mg of chl was loaded in each well. P-CP43, P-D2, P-D1, and P-LHCII represent phosphorylated forms of the PSII core proteins CP43, D2, and D1 and the LHCII proteins Lhcb1 and Lhcb2. Representative data from three independent experiments are shown. (Modified Figure from Paper I)

5. DISCUSSION

5.1 Natural fluctuations of light quality and quantity

Plants are sessile organisms and therefore have been forced to develop sophisticated and efficient mechanisms to cope with changing environmental conditions that are potentially harmful. In this respect, light is particularly difficult to handle by plants as a substrate of photosynthesis and concomitantly as a dangerous inducer for production of reactive and toxic side products.

Incident light can change in its spectral composition (light quality) or in its amount (light quantity or intensity per area and time). Fluctuations of light quantity occur much more frequently than variations of light quality (Smith 1982; Endler 1993). Clouds do not significantly alter the spectral features of incident solar light but rather behave as neutral filters. Consequently, in land environments, the red:far-red ratio (660/730 nm) is very stable along the day (around 1.2). This is despite consistent fluctuations of light amount due to cloudiness (until 90% decrease of full sunlight intensity). There are substantial changes in the red:far-red ratio only under a few environmental conditions. Changes mainly occur under canopy shade (~0.2) and at sunrise and sunset (~0.8) (Smith 1982; Endler 1993). It is worth noting that such variations of natural spectral composition are always in favor of enriching far-red wavelengths. Therefore, in natural land environments, there are no conditions in which light is highly enriched in the red region of the sunlight spectrum.

Photosynthesis as a basic function is not sufficient to ensure survival in a highly dynamic environment. For this reason, higher plants evolved many regulatory mechanisms to adjust the photosynthetic apparatus to both fast and slow environmental variations, and to coordinate this with the metabolic necessities and physiology of plant development.

5.2 Short-term light acclimation mechanisms

In the last decades many molecular mechanisms have been identified as crucial for plant acclimation to environmental light changes. These include: NPQ, state transitions, CET and the photosynthetic control via $Cytb_6f$ (Nishio and Whitmarsh, 1993; Niyogi, 1999; Allen and Forsberg, 2001; Horton and Ruban, 2005; Rochaix, 2007; Joliot and Johnson, 2012). Despite numerous studies, the understanding of these mechanisms is still emerging and, above all, the interplay between these mechanisms upon short-term acclimation is not yet clear and some aspects are even controversial.

NPQ is known to be important for heat dissipation of excess energy under high light intensity (Ruban et al, 2012). There is no consensus in the literature about the location

of the quenching sites, or on the mechanistic relationship between the PsbS- and xanthophyll- dependent NPQ components (both induced by thylakoid lumen acidification). Moreover, the relationship between NPQ and LHCII phosphorylation upon changes in light intensity has been poorly studied.

The roles assigned to CET are mainly: (1) the adjustment of the stromal ATP/NADPH concentration ratio; (2) directing electrons from stroma to the intersystem ETC under exposure to high light intensities, thus inducing the lumenal acidification, and consequently NPQ, and decreasing the electron flow pressure to the stroma (Johnson, 2005). The latter process, however, looks controversial. Upon increasing light intensity, PSI is normally highly oxidized. For several decades, the kinetics of PSI oxidation has been correlated to the downregulation of the Cyt b_6f activity, the so-called photosynthetic control via Cyt_6f (Rumberg and Siggel, 1969; Tikhonov et al, 1981; Nishio and Whitmarsh, 1993). The proposed CET activity under high light seems to work in the opposite direction, because it implies the redirection of part of the electron flow from the stroma back to PSI, and the activation of the Q cycle of $Cytb_6f$.

The state transitions are believed to induce changes in the antenna absorption cross section of PSII and PSI, according to LHCII phosphorylation (Bennet et al, 1980; Allen et al, 1981; Horton and Black, 1981). This process is commonly measured by comparing the chlorophyll fluorescence emission (emission spectra at 77K and induction curves) under red and far-red light illumination. LHCII phosphorylation also changes upon short-term modification of light quantity: the phosphorylation level is low at extremely low light intensities (5-20 μmol photons m^{-2} s^{-1} in Arabidopsis); maximized at low intensities (below the medium growth light intensity); and decreases by increasing the amount of light (Rintamäki et al, 2000). During such fluctuations the PSII core and LHCII show inverse phosphorylation patterns: the PSII core phosphorylation decreases after exposure to low light, while it increases under high light (Paper I). It is noteworthy that while low, high or far-red light treatments resemble natural light changes, the state 2 condition (induced in the lab by using almost monochromatic red light (Tullberg et al, 2000)), does not resemble any condition that occurs naturally in terrestrial environments (Smith, 1982; Endler, 1993). On the contrary, strong fluctuations in the intensity of light take place frequently due to cloud movement and shading. Moreover, it is only in state 2 that the concomitant increase of both PSII core and LHCII phosphorylation occurs. This condition induces strong electrostatic repulsions that have remarkable consequences on the PSII-LHCII complexes and could even provoke the opening of the grana structure (Chuartzman et al, 2008).

In the present work, the interplay between NPQ, state transitions and the photosynthetic control via Cyt b_6f was studied under fluctuation of light intensity.

5.2.1 Role of PGR5 protein, photosynthetic control via Cyt b_6f and CET

In the literature, the role of PGR5 protein has generally been related to the Fd dependent CET around PSI (Munekage et al, 2002; Nandha et al, 2007; DalCorso et al, 2008; Joliot and Johnson, 2011). In the present work, however, an alternative mechanistic model is presented.

In Paper II it was demonstrated that the PGR5 protein is necessary for the formation of the transthylakoid ΔpH which, in turn, induces the activation of PsbS- and zeaxanthin-dependent NPQ and the downregulation of Cyt b_6f. The downregulation of Cyt b_6f upon switching to high light intensity leads to oxidation of PSI. This mechanism is not dependent on the downregulation of PSII activity by NPQ, since both *npq4* and *npq1* mutant plants can oxidize PSI to a similar extent as the WT, upon the shift to high light conditions (Paper III). Oxidation of PSI is not even dependent on CET. Indeed, in Paper II we demonstrate that the electrons responsible for the damage of PSI in the *pgr5* mutant during the high light peaks originate from PSII. The PGR5-dependent mechanism allows for the avoidance of an electron burst from PSII against PSI upon increasing light intensity. The selective photoinhibition of PSI has been considered to take place very seldom in vivo (Sonoike, 2011). Here it is shown that in the absence of PGR5, PSI is the primary target of the damage caused by fluctuating light, presumably because of damage to the iron-sulfur cluster at the acceptor side of PSI (Tsuyama and Kobayashi, 2009).

I therefore conclude that the primary role of PGR5 is to regulate LET and that this regulation is necessary for photoprotection of PSI under fluctuating light.

5.2.2 Role of LHCII phosphorylation and antenna connectivity

The research group in which I carried out my thesis work has investigated for several years the role of reversible LHCII phosphorylation in maintaining the redox poise of the photosynthetic electron transfer chain upon changing light intensity (Rintamäki et al, 2000; Paper I).

My thesis work focused on the role of LHCII phosphorylation upon fast fluctuations of light intensity, which on one hand resemble the natural conditions, and on the other hand, are too fast to allow the dephosphorylation/phosphorylation process to occur. In Paper III, it was shown that under these dynamic conditions, the level of LHCII phosphorylation does not change significantly but remains at a steady-state level. Under constant growth light conditions, the *stn7* mutants acquire chloroplast redox homeostasis by increasing the relative amount of PSI: the lack of LHCII phosphorylation can potentially induce the over-excitation of PSII that in turn provokes the over-reduction of ETC; the excess of electrons is however counterbalanced by the increased amount of PSI that can “drain” the ETC. This acclimation strategy was not achieved by *stn7* plants grown under fluctuating light: the relative amount of PSI was even lower than the amount found in WT (Paper III).

As a consequence, under the low light phases of FL, in all plants lacking the STN7 kinase the ETC was excessively reduced. Moreover, upon the switch to the high light phase of FL, an electron burst at the acceptor side of PSI occurs, potentially leading to damage. The PSI damage itself could be the reason of low amount of PSI in *stn7*. An alternative explanation could be based on the redox imbalance between ETC and stroma and on the consequent disturbance of acclimation signals. Thus, in plant chloroplasts, steady-state LHCII phosphorylation plays a major role in regulating the photosynthetic electron transfer and preserving PSI upon rapid fluctuations in white light intensity (Paper III).

Analysis of the consequences of chemical detachment of LHCII and PSI from the WT and *stn7* thylakoid membranes revealed connectivity between the PSII and PSI centers (Figure 8; Paper IV). These results provided evidence that the entire LHCII antenna works as a functional bridge that allows energy transfer between PSII and PSI in grana margins (LHCII connectivity) (Figure 10). I conclude that both PSII and PSI can function as energy traps embedded in the same "antenna lake" and compete for excitation energy. LHCII phosphorylation is not limited to the "migrating" LHCII pool as supposed in the state transitions model but all LHCII fractions in the grana core, margins and stroma lamellae show similar redox dependent reversible phosphorylation. It seems evident that LHCII protein phosphorylation tunes the distribution of excitation energy between PSII and PSI by increasing the probability for excitons to be trapped by PSI. This new comprehensive model contradicts with the canonical strict lateral heterogeneity (Anderson and Andersson, 1980) and state transition (Fork and Satoh, 1986; Williams and Allen, 1987) models.

My observations on the connectivity of the PSII and PSI antenna system and those on regulation of the electron transfer are in fact two different aspects of the same mechanism. Since PSII and PSI function in series, the intersystem excitation energy distribution at the antennae level and the photosynthetic electron transfer are strictly interdependent: the energy collected by PSII centers is used to "pump" electrons into the electron transfer chain; the energy trapped by PSI is used in turn to "drain" the electron flow from PSII. Ultimately, the LHCII phosphorylation contributes to coordinate the energy distribution in the antennae system with the photosynthetic electron transfer by regulating the LHCII connectivity between PSI and PSII. It is conceivable that the extent of excitation energy spillover from PSII to PSI is highly regulated by environmental light conditions in accordance with LHCII phosphorylation.

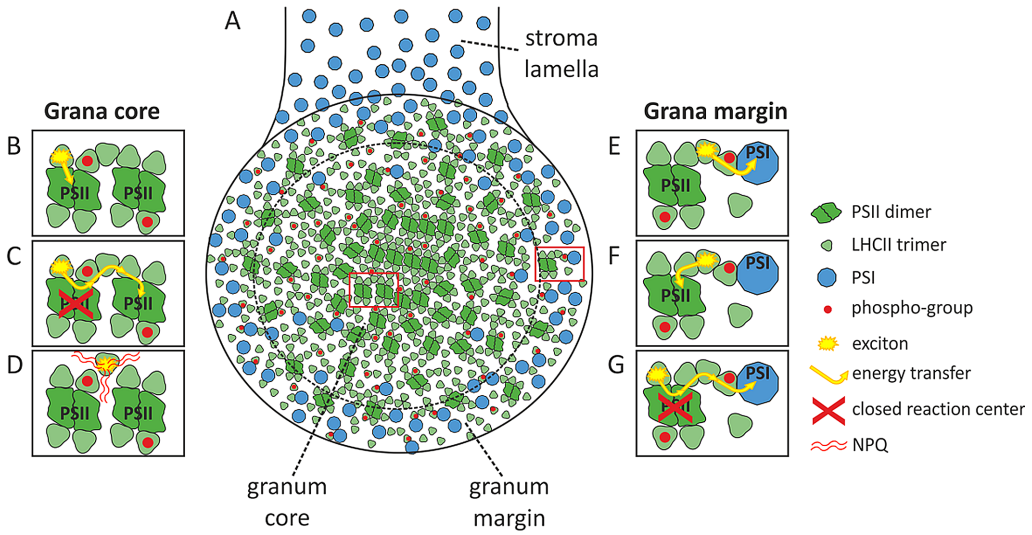

Figure 10. Schematic representation of the distribution of photosystems and their antennae in the thylakoid membrane (A) and the possibilities of excitation energy flow in the grana core (B-D) and the grana margins (E-G). (A) PSII-LHCII complexes and the PSI complexes are enriched in the grana core and non-appressed membranes (the grana margins as well as stroma lamellae), respectively. In light-adapted plants the lateral heterogeneity is not strict and the PSII and PSI populations are partially mixed, particularly in grana margin regions. A considerable amount of LHCII trimers is interposed between the photosystems and all LHCII trimer populations are partially phosphorylated (for clarity, the phosphorylation of PSII is not shown). (B) Light is absorbed by an LHCII trimer, thus forming an excitation energy packet (exciton) that is transferred to an open PSII center. (C) Exciton moves from LHCII to a closed PSII and subsequently reaches an open PSII through LHCII connectivity. (D) Light energy is absorbed and quenched in LHCII by non-photochemical mechanisms (PsbS- and xanthophylls-dependent). In grana margins, the exciton can move through LHCII connectivity toward PSI (E) or PSII (F) or it can reach a closed PSII center, return to the antenna system and be trapped by PSI (G). The excitation energy trapped by PSI can be quenched photochemically or non-photochemically, depending on the reduction state of the photosynthetic electron transfer chain. (Figure from Paper IV)

5.3 Interplay between the acclimation processes upon short- and long-term exposure to changed light environment

Under steady-state illumination both the PSII core and LHCII are phosphorylated at a certain extent. Such configuration ensures excitation energy balance between PSII and PSI and therefore maintains fluent photosynthetic electron flow, i.e. low reduction level of ETC (Paper I). In case of changes in light intensity, several different regulatory mechanisms are simultaneously either induced or down-regulated, including NPQ, photosynthetic control via Cyt b_6f, and LHCII phosphorylation. Strict cooperation of these mechanisms is a prerequisite to keep the electron transfer chain relatively oxidized, thereby preventing harmful damages to the photosynthetic apparatus.

In Figure 11, I have depicted how these regulatory mechanisms operate synergistically in a hypothetical switch from steady state to high light conditions of either short (from seconds to minutes and hours) or longer duration (from hours to days).

(1) Under any constant growth light, LHCII is phosphorylated and works as a functional bridge in light absorption between the two photosystems: the excitation energy transfer from the LHCII antenna system to PSII and PSI is efficient and balanced, because of NPQ inactivation and LHCII phosphorylation.

(2) Upon sudden increase of light intensity, the PGR5-dependent decrease of lumenal pH triggers two important mechanisms: the PsbS-dependent NPQ and the down-regulation of Cyt b_6f activity (photosynthetic control via Cyt b_6f). The former protects PSII by reducing the excitation pressure; the latter protects PSI by leading to downregulation of LET and to oxidation of P700. At the antenna level, the LHCII phosphorylation is not required any longer and at the same time PSI possibly acts as a quencher of excess excitation energy.

(3) If the exposure to high light continues, in the time scale of several minutes to a few hours, the xanthophyll cycle-dependent NPQ further downregulates PSII activity by changing the carotenoid composition of the antenna (from violaxanthin to zeaxanthin). The consequent potential imbalance in the excitation energy distribution between PSII and PSI is prevented by LHCII dephosphorylation, which decreases the efficiency of excitation energy transfer to PSI. At the same time, PSII core phosphorylation increases, thus facilitating the repair of damaged PSII units (Tikkanen et al, 2008).

(4) Upon a decrease of the light intensity to the normal growth light condition, NPQ becomes relaxed and concomitantly the LHCII antenna efficiency induces a transient excess of excitation pressure on PSII. Such imbalance is then promptly counterbalanced by the activation of the STN7 kinase and subsequent redistribution of excitation energy to PSI by LHCII phosphorylation (condition 1).

(5) If, instead, the exposure to high light is further prolonged, a new steady-state phosphorylation level is reached and long-term acclimation processes are triggered, mainly in the adjustment of the antenna size and the PSII/PSI ratio. Once the long-term acclimation adjustments are implemented, the LHCII phosphorylation returns to the initial steady-state level.

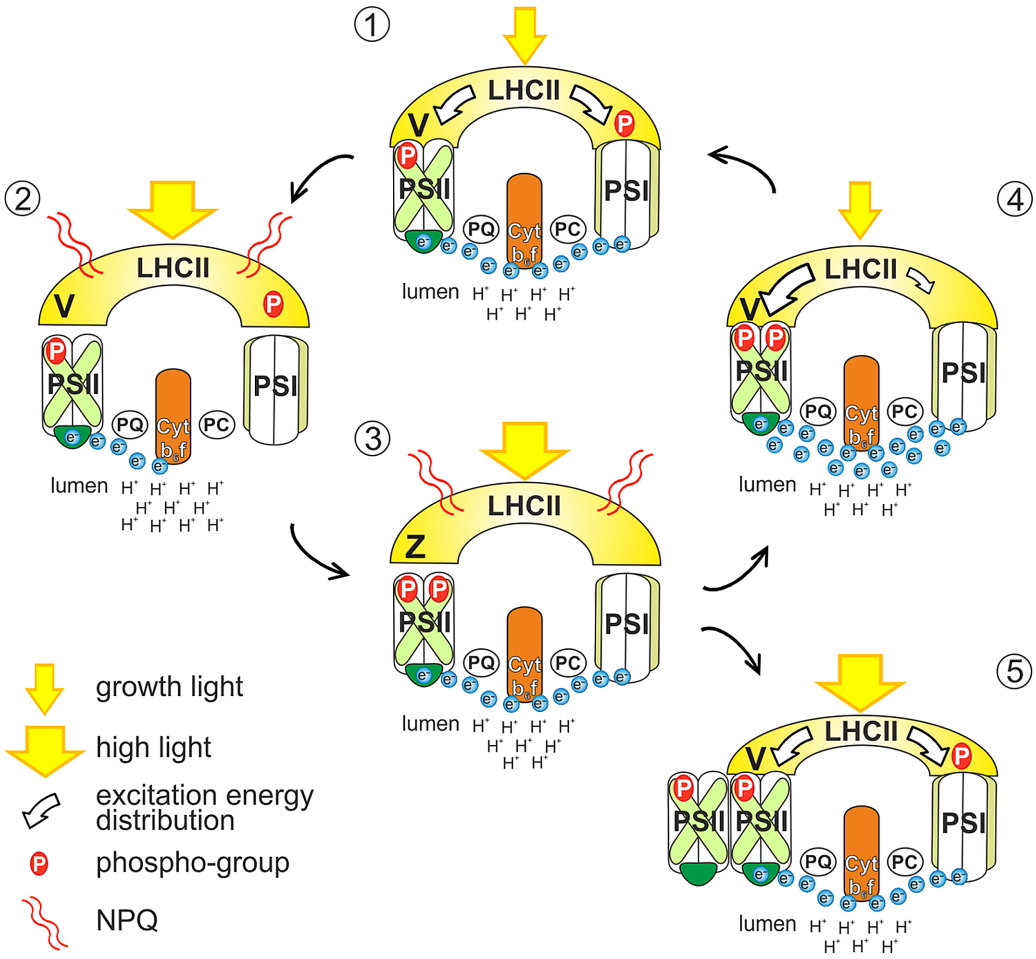

Figure 11. Model on the interplay between light acclimation mechanisms upon change of light intensity. Phases from 1 to 4 represent short-term changes (from seconds to hours) and phase 5 is a long term change (hours to days). V = violaxanthin; Z = zeaxanthin. For details, see explanation in the text.

According to the scheme presented in Figure 11, the interplay between LHCII connectivity and NPQ (including PsbS-dependent, xanthophyll cycle and PSI quenching) allows the maintenance of the excitation and redox balance of the thylakoid membrane under any light condition. Upon sudden exposure of plants to high light intensity, the PsbS-dependent NPQ is induced and LHCII phosphorylation-enhanced spillover is no longer needed to enhance PSI excitation and thereby the redox balance between PSII and PSI (Paper III). These two processes likely cooperate to functionally disconnect part of the LHCII antenna system from photosystems in a high light intensity regime. In addition to the xanthophyll cycle, the PSII core protein phosphorylation is regulated in similar time scales as LHCII phosphorylation, yet with opposite light-intensity-dependent kinetics (Tikkanen et al, 2010; Fristedt et al. 2010). Increased PSII core protein phosphorylation at high light allows the PSII-LHCII

supercomplexes to disassemble in the grana core, thus increasing the thylakoid membrane fluidity and protein mobility (Tikkanen et al 2008). This likely pushes more LHCII trimers to the margin regions of the thylakoid network. Here, LHCII phosphorylation increases the efficiency of energy transfer from LHCII to PSI. Indeed, in grana margins of the *stn7* plants devoid of LHCII protein phosphorylation, the PSII core protein phosphorylation and the LHCII/PSII ratio are clearly higher than in WT (Paper IV). This situation likely represents a partial compensation mechanism for the lack of LHCII phosphorylation-dependent excitation of PSI. Such protection of PSI results from LHCII phosphorylation-dependent equal distribution of excitation energy to both PSII and PSI from the shared LHCII antenna and occurs in cooperation with non-photochemical quenching and the PGR5-dependent control of LET, which are likewise strictly regulated by light intensity (Papers II and III).

6. CONCLUSIONS

My thesis work has provided following new information to the field of plant light acclimation:

- The fluctuating growth light condition induces damages to PSI, rather than to PSII.
- The PGR5 protein regulates LET in the following manner: it is essential for the induction of transthylakoid ΔpH that, in turn, activates the energy-dependent NPQ and downregulates the activity of Cyt b_6f. This regulation is essential for photoprotection of PSI under fluctuations of light intensity.
- LHCII can function as a common antenna for PSII and PSI.
- The LHCII connectivity allows energy transfer between PSII units as well as between the PSII and PSI centers in grana margins.
- LHCII phosphorylation ensures the balance of the excitation energy distribution between PSII and PSI by increasing the probability for excitons to be trapped by PSI.
- PSI is a quencher of excitation energy. The PSI quenching could possibly play an important role in photoprotection.
- LHCII phosphorylation, NPQ, and photosynthetic control of LET via Cyt b_6f function in concert to maintain the redox poise of ETC upon changes of light intensity.

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Michele Grieco

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