Molecular Mechanisms Optimizing Photosynthesis During High Light Stress in Plants

Lan Yin

GÖTEBORGS UNIVERSITET

FACULTY OF SCIENCE
DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

Akademisk avhandling för filosofie doktorsexamen i Naturvetenskap med inriktning Biologi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras måndag den 28:a April 2014 kl. 13.00 i Hörsalen, Institutionen för biologi och miljövetenskap, Carl Skottsbergs gata 22B, Göteborg.

Examinator: Professor Adrian Clarke, Institutionen för biologi och miljövetenskap, Göteborgs Universitet

Fakultetsopponent: Professor Wolfgang Schröder, Kemiska Institutionen, Umeå Universitet

ISBN: 978-91-85529-67-4

ISBN: 978-91-85529-67-4
Tryck: Ineko AB, Göteborg

To my family

Molecular Mechanisms Optimizing Photosynthesis During High Light Stress in Plants

Lan Yin
University of Gothenburg, Department of Biological and Environmental Sciences
Box 461, SE-405 30 Gothenburg, Sweden
ISBN: 978-91-85529-67-4

ABSTRACT: Oxygenic photosynthesis is the process by which plants, algae and cyanobacteria use solar energy to convert water and carbon dioxide into molecular oxygen and carbohydrates. Photosynthesis sustains life on Earth since it provides not only energy for individual growth, but also represents the starting point of the food chain for most living organisms. Sunlight is essential for driving photosynthesis, but it is also known that in excess it can be stressful with severe consequences for plant growth. In this thesis I have used the model plant *Arabidopsis thaliana* to study molecular mechanisms optimizing photosynthesis during high light stress.

One of these mechanisms is the reversible phosphorylation of proteins in the water-oxidizing photosystem II (PSII) complex. The serine/threonine-protein kinases STN7 and STN8 are involved in the phosphorylation of the PSII light-harvesting complex (LHCII) and core proteins, respectively. In Paper II, I found variation in the phosphorylation levels of these proteins in Arabidopsis natural accessions. In high light conditions, I found a correlation between the STN8 protein abundance and the D1 protein phosphorylation level. In growth light conditions, D1 and LHCII phosphorylation correlated with longitude and in the case of LHCII phosphorylation with temperature variability as well.

Another molecular mechanism for plants to overcome high light stress is via PSII repair. STN8- mediated PSII core phosphorylation is an early and crucial step for efficient PSII repair, since it alters the folding of the thylakoid membrane in a manner facilitating lateral migration of complexes to the sites of repair. Among three laboratory Arabidopsis accessions studied, Ws-4 displayed a reduced STN8 level resulting in decreased PSII core protein phosphorylation (Paper I). Nevertheless, the downstream steps in PSII repair proceeded normal or slightly faster. This phenomenon is probably due to compensatory mechanisms involving additional lipids and carotenoids to increase membrane fluidity and thus lateral migration of complexes.

The thylakoid ATP/ADP carrier (TAAC) transports ATP into the thylakoid lumen for nucleotide-dependent reactions. In Paper III, I have found that TAAC- deficient plants displayed wild-type levels of PSII protein phosphorylation but slower disassembly of complexes and slower D1 protein degradation. I propose that ATP supplied by TAAC into the lumen is used for nucleotide-dependent reactions with roles in various steps of PSII repair. I have also found that, via its transport activity, TAAC may consume part of the proton gradient across the thylakoid membrane, which is critical for the initiation of photoprotective mechanisms.

In a proteomics study of the stroma thylakoid membrane from Arabidopsis (Paper IV), I identified 58 proteins, including previously known ones as well as new putative thylakoid proteins with roles in photosynthesis transport, translation, protein fate, metabolism, stress response and signaling. This thesis deepens our understanding of photosynthetic regulation at the molecular level and improves the biochemical overview of the chloroplast thylakoid membrane.

Keywords: high light stress, natural variation, photosynthesis, protein phosphorylation, photoprotection, photosystem II, proteomics, STN kinase, thylakoid membrane, TAAC

LIST OF PUBLICATIONS

This thesis is based on the following papers, which are referred to by their Roman numerals in the text:

I. **Yin L, Fristedt R, Herdean A, Solymosi K, Bertrand M, Andersson MX, Mamedov F, Vener AV, Schoefs B, Spetea C (2012).**
Photosystem II function and dynamics in three widely used *Arabidopsis thaliana* accessions. PLos One 7(9): e46206

II. **Flood PJ[1], Yin L[1], Herdean A, Harbinson J, Aarts MG, Spetea C (2014)**
Natural variation in phosphorylation of photosystem II proteins in *Arabidopsis thaliana* – is it caused by genetic variation in the STN kinases? Philos. Trans. R. Soc. Lond. B. Biol. Sci. 369(1640): 20130499.

III. **Yin L, Lundin B, Bertrand M, Nurmi M, Solymosi K, Kangasjärvi S, Aro EM, Schoefs B, Spetea C (2010)**
Role of the thylakoid ATP/ADP carrier in photoinhibition and photoprotection of photosystem II in Arabidopsis. Plant Physiol. 153(2): 666-677.

IV. **Yin L, Vener AV, Spetea C.**
Proteomic study of stroma thylakoid membranes from *Arabidopsis thaliana*. *Manuscript.*

[1] Shared first authorship

List of abbreviations

Chl	Chlorophyll
CID	Collision induced fragmentation
Col-0	Columbia-0
CP43	Photosystem II chlorophyll apoprotein 43
D1	Photosystem II D1 protein
ESI	Electronspray ionization
ETD	Electron transfer dissociation
FD	Ferredoxin
FQR	Ferredoxin-plastoquinone reductase
GL	Growth light
HL	High light
LC	Liquid chromatography
L*er*-0	Landsberg *erecta*-0
LHCII	Light-harvesting complex of photosystem II
MGDG	Monogalactosyl diacylglycerol
MS	Mass spectrometry
NPQ	Non-photochemical quenching
PS	Photosystem
PBCP	photosystem II core phosphatase
PsbO	Oxygen-evolving enhancer protein 1
PSI	Photosystem I
PSII	Photosystem II
STN	Serine/threonine-protein kinase
TAAC	Thylakoid ATP/ADP carrier
TAK	Thylakoid-associated kinase
TAP38/PPH1	Thylakoid phosphatase 38
Ws-4	Wassilewskija-4

TABLE OF CONTENTS

1. INTRODUCTION

1.1 Photosynthesis – a preface

Oxygenic photosynthetic organisms such as cyanobacteria, algae and plants use sunlight as the energy source to convert H_2O and atmospheric CO_2 into carbohydrates and molecular oxygen according to the following simplified equation:

(1) $$H_2O + CO_2 + \text{sunlight} \longrightarrow (CH_2O) + O_2$$

These carbohydrates provide energy directly or indirectly via food chains to living organisms on Earth, including humans. The oxygen produced is released into the atmosphere and is essential for all aerobic life forms.

Pre-historical algae sedimented and over time converted into the fossil fuels we use today. The use of fossil fuels has a limit, since the production spanned millions of years, thus alternative energy sources are needed. Artificial photosynthesis has become an attractive option. It mimics natural photosynthesis, but it uses solar energy to produce biofuels in the form of lipid-rich membranes or molecular hydrogen. The simplified equation for artificial photosynthesis can be presented as follows:

(2) $$H_2O + (CO_2) + \text{sunlight} \longrightarrow (\text{biofuel}) / H_2 + O_2$$

Thus, a deeper understanding of the basic and regulatory aspects of photosynthesis becomes an important agenda for both food and energy reasons. My thesis work contributes to the further understanding of the molecular mechanisms behind the optimization of photosynthesis during abiotic stress conditions such as high-intensity light (known as high light, HL).

1.2 Chloroplast structure, composition and function

In eukaryotic photosynthetic organisms, photosynthesis takes place in the green organelle named the chloroplast **(Figure 1)**. It has an outer and an inner envelope membrane enclosing the soluble stroma. A third membrane system called the thylakoid membrane is situated in the stroma. Further inside the thylakoid membrane is a soluble space called the thylakoid lumen. The different compartments of the chloroplast have several distinct functions. The envelope membranes are involved in the exchange of solutes and organic compounds between the stroma and the rest of the cell, and are

also the site of import for nuclear-encoded chloroplast proteins. The stroma is where carbon fixation occurs, but also biosynthesis of various compounds and the synthesis of chloroplast-encoded proteins. The thylakoid membrane harbors pigment-protein complexes involved in light harvesting, water oxidation, electron and H^+ transfer to form chemical energy (ATP, NADPH). The thylakoid lumen is involved in the regulation of the above reactions and in signaling events.

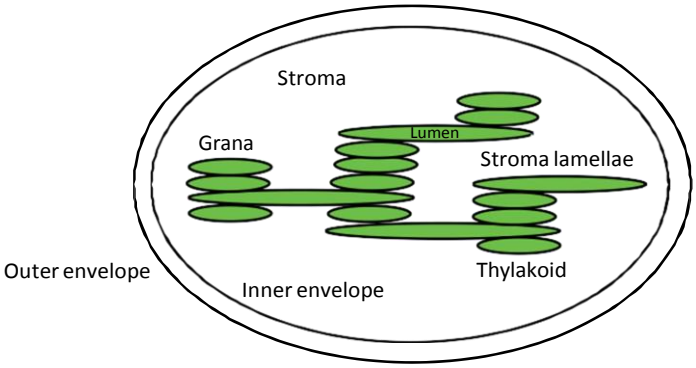

***Figure 1**. Schematic overview of chloroplast structure. Outer and inner envelope membranes enclose the soluble stroma. The thylakoid membrane is situated in the stroma and encloses the soluble lumen. The thylakoid membrane consists of stacked grana region and unstacked stroma lamellae regions.*

Thylakoid membranes contain two types of membrane regions: cylindrical stacks, comprised of multiple tightly appressed layers, called grana, and unstacked interconnecting single-membrane regions, called stroma lamellae (Anderson and Andersson 1988; Nevo et al. 2012). The stacked and unstacked regions comprise 80% and 20%, respectively, of the membrane surface (Albertsson 2001). Photosynthetic complexes are non-uniformly distributed over the thylakoid membrane. Photosystem II (PSII) and its light-harvesting complex II (LHCII) are mainly located in grana stacks, whereas most of the PSI, LHCI and H^+-translocating ATP synthases are in the unstacked membrane regions. Cytochrome b_6f complexes are distributed evenly between the two types of thylakoid membranes (Andersson and Anderson 1980; Albertsson et al. 1990). This phenomenon is known as lateral heterogeneity of the thylakoid membrane.

The dynamics in the lateral distribution of photosynthetic complexes play an important role in their biogenesis, function, regulation and repair (Kirchhoff 2014). Especially under high light conditions, fluidity of the thylakoid membrane was suggested to be crucial for the photodamaged protein complexes to be able to migrate from grana to stroma-exposed lamellae for protein degradation and subsequent replacement (Aro et al. 2005; Yamamoto et al. 2014).

1.2.1 Photosynthetic complexes

1.2.1.1 hotosystem II

Photosystem II (PSII) is considered to be 'the engine of life on Earth', due to its ability to utilize water molecules as a source of electrons. Active PSII functions in a dimeric form, where each monomer contains more than 20 protein subunits. In addition to proteins, 35 chlorophylls, two pheophytins, 11 β-carotenes, over 20 lipids, and two plastoquinones were found in the latest PSII crystal structure (Umena et al. 2011). Among the identified lipids were six monogalactosyldiacylglycerol (MGDG), five digalactosyldiacylglycerol (DGDG), four sulfoquinovosyldiacylglycerol (SQDG) and five phosphatidylglycerol molecules.

The main electron cofactors are the following: Mn_4CaO_5, a cluster which splits water into protons (H^+), electrons (e^-), and O_2; Tyr, a tyrosine molecule, which shuttles electrons to the "reaction center" of PSII; Chl P680, the reaction center pair of chlorophyll *a* molecules, which is the primary electron donor of PSII; Pheo, a pheophytin molecule, which is the primary electron acceptor of PSII; plastoquinone Q_A, which is the primary stable electron acceptor of PSII; plastoquinone Q_B, which becomes mobile after accepting two electrons and called PQ (Orr and Govindjee 2010).

The PSII core complex consists of a large number of intrinsic and extrinsic subunits (Pagliano et al. 2013; Vinyard et al. 2013). The reaction center (RC) of PSII, defined as the minimum set required to oxidize water, is composed of the core integral subunits D1 and D2 binding most electron cofactors, and the inner antenna proteins CP43 and CP47 as well as the α and β subunits (PsbE and PsbF) of cytochrome *b559* (Cyt*b559*). They are preserved in all oxygenic photosynthetic organisms, and plastid-encoded in algae and plants. Besides this, the oxygen-evolving complex (OEC) containing Mn_4CaO_5 cluster is extrinsically attached with the lumenal side of PSII and associated with four subunits in plants: PsbO (the 33 KDa manganese stabilizing protein), PsbP (23 KDa) and PsbQ (11KDa), PsbR (10 KDa). In Arabidopsis, PSII complexes harboring the PsbO1 isoform have a higher oxygen-evolving activity than those with the PsbO2 isoform (Murakami et al. 2005); PsbO2 being hypothesised to regulate dephosphorylation and turnover of the D1 protein (Lundin et al. 2007a). PsbO is thought to be a GTPase with a role in the regulation of PSII repair (Lundin et al. 2007b). PsbP plays an important role for the assembly and/or stabilization of PSII-LHCII supercomplexes (Yi et al. 2007; Ido et al. 2009). PsbR has been suggested to stabilize PSII Mn cluster also(De Las Rivas et al. 2007; Allahverdiyeva et al. 2013).

In the PSII dimeric form, the core complex interacts with LHCII proteins (Lhcb1–6). Lhcb1–3 form trimers in various compositions. Strongly bound S trimers interact with the PSII core via monomeric antenna proteins Lhcb5 (also called CP26), which form C2S2; in addition to that, two moderately bound LHCII trimers (M2) interact with the core via Lhcb4 (CP29) and Lhcb6 (CP24) to form PSII-LHCII supercomplexes (Kouril et al. 2012).

1.2.1.2 photosystem I

The PSI complex mediates light-driven electron transfer from cytochrome b_6f via plastocyanin to the ferredoxin-$NADP^+$-complex. In plants, the PSI complex consists of at least 19 protein subunits, approximately 175 chlorophyll molecules, two phylloquinones and three Fe_4S_4 clusters (Ben-Shem et al. 2003). The predominant type of PS I in peas (*Pisum sativum* var. *Alaska*) consists of 15 core subunits and four peripheral light-harvesting (LHCI) proteins organized as two heterodimers, Lhca1/Lhca4 and Lhca2/Lhca3. Only PsaA, -B, and –C, out of the 15 core proteins, are directly involved in binding the electron transport cofactors P700 (a chlorophyll dimer), A_0 (a chlorophyll *a* molecule), A_1 (a phylloquinone), F_x (a [4Fe–4S] iron–sulfur cluster), F_A and F_B (both [4Fe–4S] iron–sulfur clusters). The other core proteins perform additional functions: PsaD and PsaE provide the docking site for ferredoxin on the stromal side of the thylakoid membrane; PsaF and PsaN are essential for the interaction with the lumenal electron donor plastocyanin, and PsaF is important for the binding of the Lhca1/Lhca4-dimer (Jensen et al. 2007).

The core antenna is composed of about 100 Chl *a* and20 β-carotene molecules (Jordan et al. 2001; Ben-Shem et al. 2003). Most of the Chl and carotene molecules are bound to the main subunits PsaA and PsaB. In the case of LHCI, Lhca1-4 binds 13 Chl, but Lhca1/3 binds three carotenoid molecules per polypeptide, whereas Lhca2/4 binds two carotenoids, mainly lutein and violaxanthin.

1.2.1.3 Cytochrome b_6f

The cytochrome b_6f complex acts as the redox link between the photosynthetic reaction centers II and I and also functions in proton translocation (Cramer et al. 1996; Tikhonov 2013a). This complex functions as a dimer, with each monomer consisting of eight or nine subunits in cyanobateria and plants, respectively (Baniulis et al. 2011). These consist of four large subunits: cytochrome f with a c-type cytochrome, cytochrome b_6 with a low- and high-potential heme group, Rieske iron-sulfur protein containing a [2Fe-2S] cluster , and subunit IV. In addition to these, four small subunits (3-4 kDa) are present: PetG, PetL, PetM, and PetN, whose functions are unclear but might have critical roles in the assembly and/or stability of the complex. The plant-specific subunit is ferredoxin: $NADP^+$ reductase (FNR), with a physiological role to catalyze the final step of photosynthetic electron transport, namely the synthesis of NADPH for downstream carbon fixation (Mulo 2011).

1.2.1.4 ATP synthase

The chloroplast ATP synthase has two distinct components, CF_1 and CF_0. CF_1 is an extrinsic membrane protein subcomplex that contains the catalytic site(s) for reversible ATP synthesis. CF_0 is an integral membrane protein complex, which is responsible for

the conversion of an electrochemical proton gradient into rotational motion (Richter et al. 2005; Poetsch et al. 2007). CF_1 has a subunit stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$ (Süss and Schmidt 1982). CF_0 contains four different subunits named I, II, III_{14} and IV (Poetsch et al. 2007). The III_{14} oligomer forms the proton-driven rotor, and the rotating central stalk in the ATP synthase consists of subunits γ and ε. A second stalk, is composed of subunits I, II and δ, connecting cF_1 and cF_O at the periphery and stabilizing the rotating machinery as a stator (Poetsch et al. 2007).

1.2.2 Choroplast stroma and thylakoid lumen

1.2.2.1 Chloroplast stroma

The chloroplast stroma is the space between the inner envelope membrane and the thylakoid membrane. It contains chloroplast nucleoids, ribosomes, starch and many proteins involved in biosynthetic reactions. The most abundant protein in the chloroplast stroma is Rubisco, an enzyme that assimilates CO_2 into carbohydrates, during the called Calvin-Benson cycle. The stromal pH can reach 8.0 as compared to cytoplasmic pH in the range 7.0-7.5 during illumination (Wu and Berkowitz 1992; Hauser et al. 1995).

1.2.2.2 Thylakoid lumen

The thylakoid lumen contains not only OEC proteins, the electron carrier plastocyanin (PC) and photoprotection related violaxanthin de-epoxidase, but also up to 80 proteins in Arabidopsis (Peltier et al. 2002; Schubert et al. 2002; Kieselbach and Schroder 2003). Most of the lumenal proteins have a role in the regulation of thylakoid biogenesis, activity and turnover of PSII and of NAD(P)H dehydrogenase (NDH)-like complexes (Jarvi et al. 2013). The role of a large proportion of lumenal proteins is unknown. The luminal proteins, including PsbP, PsbR abd PsbQ, can be regulated by reversible phosphorylation using ATP imported by the thylakoid ADP/ATP carrier (TAAC) (Spetea and Lundin 2012). The redox thiol/disulfide modulation is also considered as an important regulation of lumenal protein function (Jarvi et al. 2013). Lumen size becomes large in high light conditions with pH dropping from 7.0 in darkness to 5.8-6.5 in the light (Kramer et al. 1999; Cruz et al. 2001; Tikhonov 2013b).

1.3 Photosynthetic electron transport

The photosynthetic apparatus has developed a series of adaptive mechanisms to various light intensities, as linear and cyclic electron transfer through the thylakoid membrane occurs **(Figure 2)**.

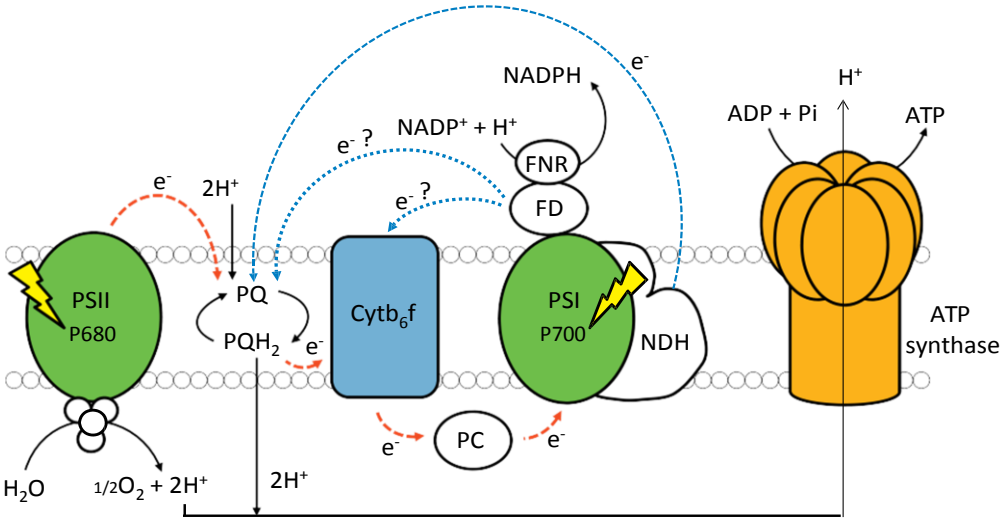

Figure 2. *Organization of photosynthetic complexes, simplified linear and cyclic electron transport chains in the thylakoid membrane. Dashed red, blue and solid black lines represent the linear, cyclic electron and proton transport, respectively.*

1.3.1 Linear electron transport

Linear electron transfer involves three major complexes, namely PSII, cytb$_6$f and PSI. Electrons are first extracted from P680 with the help of solar energy, and transferred to the primary electron acceptor pheophytin. The Mn_4CaO_5 cluster extracts electrons from water and re-oxidizes P680. Pheophytin delivers electrons to plastoquinone Q_A and then to Q_B, the later one being recycled in the PQ pool. PQ receives two H^+ from the stroma, becomes a mobile carrier that transfers electrons to Cytb$_6$f and then releases H^+ into the lumen. Some of the electrons from Cytb$_6$f are carried further on to plastocyanin (PC) through PSI and ending in $NADP^+$. The other electrons from Cytb$_6$f are recycled back to the PQ pool through several electron carriers called the 'Q cycle'. The H^+ concentration difference between the stroma and the lumen forms a proton motive force (pmf), which later on is used to produce ATP on the stromal side of thylakoids via the activity of ATP synthase.

As in the case of PSII, electrons are extracted from PSI P700 with the help of light energy. PC is a mobile electron carrier, through which electron are passed from cytb$_6$f to PSI to reoxidize P700. The electrons are further carried to ferredoxin (Fd) in the stroma, ultimately reaching $NADP^+$, with these reactions catalyzed by ferredoxin: $NADP^+$ reductase (FNR).

1.3.2 Cyclic electron transport

The electrons reaching PSI can be sent back to the linear electron transfer by cyclic electron transfer. There are several pathways: a) plastoquinone reductase (NDH) regulated pathway, when electrons are transferred back to plastoquinone via NDH (Battchikova and Aro 2007; Peng and Shikanai 2011); b) electrons are delivered from ferredoxin (FD) to PQ via a putative protein ferredoxin-plastoquinone reductase (FQR) (Cleland and Bendall 1992); c) electrons are carried from FD to $Cytb_6f$, and from there back to PQ. Cyclic electron flow does not produce O_2 nor NADPH, but pumps H^+ ions across the membrane, forming a proton motive force that can be used to power ATP synthase. ATP and NADPH are utilized in carbon fixation reactions in the stroma resulting in building blocks for carbohydrates, amino acids, lipids, etc.

1.4 Mechanisms optimizing photosynthesis during high light stress

1.4.1 PSII photoprotection

Plants have developed intricate internal defense mechanisms, also known as photoprotection, to cope with intense light conditions. In a rather broad definition, it can be considered that plants respond to prevent damage that can be caused by the accumulation of high levels of reactive oxygen species (ROS) by interactions between lipids, nucleic acids and proteins (Apel and Hirt 2004; Triantaphylides and Havaux 2009). Photoprotection can be executed to either reduce the absorption of light, by dissipating the excess light or by scavenging the ROS that are eventually produced. A photoprotective response involving the dissipation of excess light is known as non-photochemical quenching (NPQ). During this mechanism, the concentration of excited ^{1}Chl* in LHCII is decreased by the activation of a heat dissipation channel, thus limiting ^{3}Chl* formation (Müller et al. 2001; Cazzaniga et al. 2013).

NPQ has several components known as qE, qZ, qT, qI and qM. The fastest component, which is formed within 10-200 sec, is called qE and is fully dependent on thylakoid lumen acidification (Horton et al. 1996; Nilkens et al. 2010). Low lumenal pH activates the xanthophyll cycle, i.e., the conversion of violaxanthin to zeaxanthin (Yamamoto and Kamite 1972; Jahns et al. 2009). This NPQ component also involves the LHC-like subunit PsbS that senses low lumenal pH through two lumen residues that can accept protons (Li et al. 2000; Li et al. 2004). The acceptance of protons by PsbS and the binding of zeaxanthin to PSII causes conformational changes in the antennae that result in enhanced thermal dissipation of excitation energy. qZ was recently resolved strictly as a zeaxanthin-dependent NPQ component, which is formed within 10-30 min and is independent of PsbS (Nilkens et al. 2010). The photoinhibitory component (qI) takes longer (> 30min) to form, and is much less characterized than qE because of a mix of photoprotection and photodamage roles (Müller et al. 2001; Nilkens et al. 2010). The

qT component relaxes within minutes and is important for algae during state transition, but less so for photoprotection in plants (Niyogi 1999). Besides all these dissipative mechanisms/components located within the chloroplast, plants can move chloroplasts within the cell to avoid damage from over-excitation energy. The component involved in NPQ is called qM. A mutant deficient in chloroplast avoidance movement formed half of NPQ compared with wild type plants during illumination (Cazzaniga et al. 2013).

1.4.2 PSII photoinhibition: damage and repair

Abiotic and biotic stresses are the two major causes of reduction in plant growth in nature. For major crops, more than 50% reduction in the average yield is due to abiotic stresses (Navabpour et al. 2003). One abiotic stress, high-intensity/excessive light is the major cause of photosynthetic apparatus damage/inactivation (Barber and Andersson 1992; Tyystjärvi and Aro 1996; Kanervo et al. 2005). If irreversible, this process is known as photoinhibition and often leads to reduced plant growth and fitness. Plants have developed a rapid and efficient repair system to recover from the damage, named PS II repair (Aro et al. 1993; Tyystjärvi and Aro 1996; Melis 1999; Kanervo et al. 2005; Nixon et al. 2010). To measure the extent of inhibition by light (PSII inhibition), it is essential to determine the balance ratio between photodamage and repair (Murata et al. 2007).

One classical model is that PSII damage happens via a two-step process: the first step is the light-dependent destruction of the Mn_4CaO_5 cluster, which is slow and rate-limiting; the second step is the inactivation of the photochemical reaction center of PSII by light that has been absorbed by chlorophyll, which is fast (Ohnishi et al. 2005). Another classical model is that inefficiency of electron transport at the PSII acceptor side will cause formation of P680 triplet, which can interact with oxygen and form ROS. These ROS then oxidize the D1 amino acids and cause damage (Vass et al. 1992; Napiwotzki et al. 1997). A third model is one-step photodamage of the reaction center of PSII by ROS (Murata et al. 2007). ROS have also been shown to inhibit PSII repair, especially the synthesis of the D1 protein, rather than only accelerating photodamage to PSII (Nishiyama et al. 2001). Thus, ROS increase the extent of photoinhibition by inhibiting the repair of PSII.

The PSII repair cycle is a multi-step process (**Figure 3)** (Aro et al. 1993; Melis 1999; Baena-Gonzalez and Aro 2002; Nath et al. 2013a): 1. phosphorylation of PSII core proteins by STN8 kinase and monomerization of the PSII complex; 2. relocation of damaged PSII monomer towards stroma lamellae and dephosphorylation of PSII core proteins by PBCP phosphatase; 3. dissociation of CP43 and oxygen evolving complex proteins and the degradation of photo-damaged D1 protein by specific proteases (see below); 4. de novo D1 synthesis and insertion into the thylakoid membrane; 5. activation, reassembly and migration to the grana to form a newly functional PSII dimeric complex.

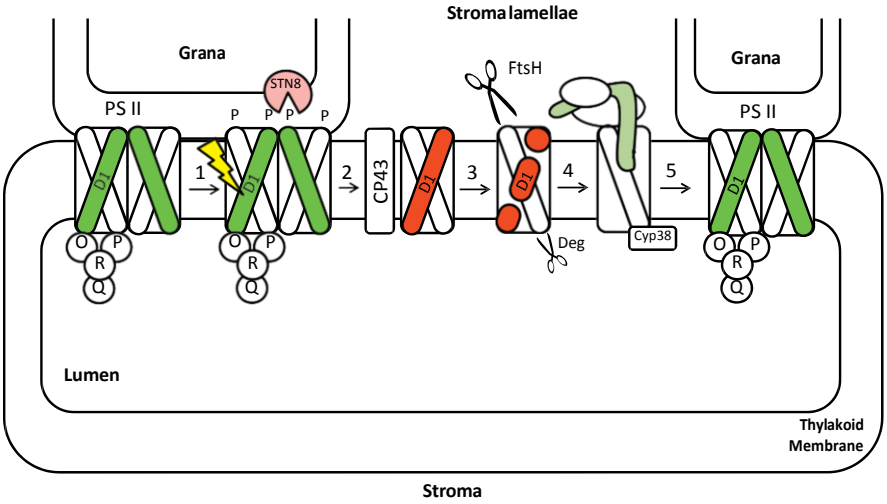

Figure 3. *Proposed model for PSII repair cycle: 1. phosphorylation of PSII core proteins and monomerization of PSII supercomplex; 2. relocation of damaged PSII monomer towards stroma lamellae and dephosphorylation of PSII core proteins; 3. dissociation of CP43 and oxygen evolving complex proteins and degradation of photo-damaged D1 protein by proteases; 4. de novo D1 synthesis and insertion into the thylakoid membrane; 5. reassembly and migration to the grana to form functional PSII dimeric complex. Figure modified from Spetea and Lundin (2012) FEBS Lett. 586: 2946-2954 with kind permission from the authors.*

It has been suggested that the rate of D1 degradation is the major rate-limiting step of the PSII repair process (Melis 1999). There are several proteases involved in the degradation of photodamaged D1 protein. Chloroplast ATP-dependent oligomeric metalloprotease FtsH has been shown to be involved in D1 protein degradation (Nixon et al. 2005; Komenda et al. 2006; Yoshioka and Yamamoto 2011). Besides FtsH, Deg1 is involved in D1 degradation, yielding C-terminal 16 kD and 5.2 kD products (Kapri-Pardes et al. 2007). Deg2 protease also can cleave the photodamaged D1 in the stroma-exposed DE-loop (Haußühl et al. 2001) yielding N-terminal 23kD and C-terminal 10kD fragments (Huesgen et al. 2009). Great efforts have recently been made to identify PSII repair cycle related kinases, phosphatases, proteases and a large array of lumenal proteins, but the PSII repair regulatory pathway is still far from completely known. (Jarvi et al. 2013; Nath et al. 2013b).

1.4.3 Chloroplast reversible protein phosphorylation

As a major post-translational modification, reversible protein phosphorylation plays an important role in the regulation of photosynthesis. The structure of a protein can be conformationally changed by the addition or removal of a phosphate (PO_4^{3-}) group to a polar R group of an amino acid residue, since the hydrophobicity of a protein is altered by this modification. As a result, phosphorylation catalyzed by kinases activates many proteins, contrary to dephosphorylation catalyzed by phosphatase, which deactivates proteins. The first evidence for chloroplast protein phosphorylation was provided by Bennett in 1977, because radioactively labelled ^{32}P was incorporated in certain amounts into chloroplast proteins (Bennett 1977). Recent large-scale mass spectrometric analyses in the chloroplast have expanded its phosphoproteome from a few proteins to around 200 (Sugiyama et al. 2008; Baginsky and Gruissem 2009; Lohrig et al. 2009; Reiland et al. 2009).

In thylakoid membranes protein phosphorylation is regulated by the redox state of photosynthetic complexes. Changes in thylakoid proteins can regulate photosynthesis in response to environmental conditions, including light, temperature and drought stress conditions (Vener et al. 1998; Aro and Ohad 2003). Recent research has been focused on two serine/threonine protein kinases, 7 and 8 (STN7 and STN8), and on the newly discovered thylakoid phosphatase 38 (TAP38/PPH1) and photosystem II core phosphatase (PBCP), because of their ability to phosphorylate/dephosphorylate thylakoid photosynthetic proteins in Arabidopsis (Bellafiore et al. 2005; Bonardi et al. 2005; Vainonen et al. 2005; Pribil et al. 2010; Shapiguzov et al. 2010; Pesaresi et al. 2011; Samol et al. 2012). The STN7 and STN8 kinases were traced from homologues Stt7 and Stl1 in green alga (Depege et al. 2003).

1.4.3.1 STN8-mediated phosphorylation

The STN8 kinase is known to phosphorylate the PSII core subunits D1, D2, CP43 and PsbH (Bonardi et al. 2005; Vainonen et al. 2005). STN8 is also involved in phosphorylation of other thylakoid proteins, such as the calcium-sensing receptor protein CaS and PGRL1-A, a protein probably involved in the modulation of cyclic electron transfer (Vainonen et al. 2008; Reiland et al. 2011).

Reversible phosphorylation of D1, D2 and CP43 proteins is suggested to play a role in the repair cycle of PSII (Aro et al. 1992; Baena-González et al. 1999). For more details, see **Figure 3**. The precise mechanism has been recently elucidated and involves control of the macroscopic structure of thylakoids (Fristedt et al. 2009b). This affirmation is based on the fact that Arabidopsis STN8-deficient plants displaying reduced PSII phosphorylation also show enhanced grana size and impairment of the PSII repair cycle (Tikkanen et al. 2008; Fristedt et al. 2009b).

1.4.3.2 STN7-mediated phosphorylation

STN7 kinase is involved in phosphorylation of proteins connected to light harvesting. It has been shown that STN7 phosphorylates subunits of LHCII, minor light-harvesting proteins CP29, CP26 and the small LHCII interacting protein TSP9 (Bellafiore et al. 2005; Tikkanen et al. 2006; Fristedt et al. 2009a). In addition, the nucleoid-associated protein pTAC16 phosphorylation is also dependent on STN7 (Ingelsson and Vener 2012).

Changes in the light environment may lead to redox changes of the PQ pool, as a part of the electron transport chain to optimize the efficiency of the photosynthetic machinery. When the PQ pool is reduced, plastoquinol (PQH_2) docks to the Q_0 site of Cyt b_6f (Vener et al. 1997), activates STN7 (Vener et al. 1995; Zito et al. 1999); when the PQ pool is oxidized (QH2—PQ), the reverse happens. Interestingly, low light can activate STN7 kinase and LHCII is phosphorylated. However, high light inactivates the STN7 kinase, although the PQ pool is greatly reduced. This is probably due to the ferredoxin-thioredoxin system on the stromal side of the thylakoid membrane (Rintamaki et al. 1997; Rintamaki et al. 2000). STN7 was suggested to be inactivated by thioredoxin-mediated reactions in the lumen as well, because STN7/Stt7contains two cysteins exposed to the lumen (Puthiyaveetil 2011). This proposal was supported by evidence that Stt7 kinase was inactivated by breaking a disulfide bridge between the two lumenal cysteins (Lemeille et al. 2009). Mutagenesis of phosphosites of STN7 itself indicates that phophorylation of STN7 may affect its turnover. However, phosphorylation of STN7 was not necessary for its activation in state transitions or for LHCII phosphorylation (Reiland et al. 2009; Willig et al. 2011). It has also been shown that STN8 is not required for the phosphorylation of STN7 (Ingelsson and Vener 2012).

1.4.3.3 Others phosphorylation-related enzymes

Except for STN7 and STN8 regulated phosphorylation, chloroplast casein kinase 2 (CK2)-dependent phosphorylation probably plays a major role in the chloroplast, as indicated by kinase target motif analysis (Reiland et al. 2009). The chloroplast sensor kinase (CSK) interacts with CK2 and controls the transcription of chloroplast genes based on the plastoquinone redox state (Puthiyaveetil et al. 2008; Puthiyaveetil et al. 2010).
In contrast to STN8 and STN7 phosphorylation activity on PSII core and LHCII proteins, PBCP (Samol et al. 2012) and PPH1/TAP38 (Pribil et al. 2010; Shapiguzov et al. 2010) dephosphorylate these proteins, respectively.

1.4.4 Photoacclimation and state transition

Unlike animals, plants cannot move, for example, to find their desired location for photosynthesis. They have to adapt themselves to different environmental conditions, such as temperature, moisture, seasonal changes and various light intensities.

Short-term acclimation happens in minutes, whereas long-term acclimation happens in hours, days, or even longer. Short-term acclimation of plants involves a dynamic reorganization of photosynthetic complexes. Long-term acclimation involves changes in chloroplast and nuclear gene expression, which in turn alters the composition of the photosynthetic machinery for optimal performance.

State transition was traditionally proposed to be a type of short-term acclimation in plants. In state 1, PSI is preferably excited and all LHCII is associated with PSII. When PSII is preferentially excited by light, a mobile pool of LHCII moves from PSII and associates with PSI, which is defined as state 2 (Allen 1992). This process is triggered by phosphorylation of LHCII by the Stn7 kinase (Bellafiore et al. 2005). In reverse, LHCII is dephosphorylated by TAP38 (Pribil et al. 2010) or PPH1 (Shapiguzov et al. 2010). Recent reports indicated that LHCII is associated with both photosystems in natural light conditions, which is in contrast to the previous knowledge that LHCII is associated with PSI only as a short-term response (Wientjes et al. 2013a; Wientjes et al. 2013b). The weakly bonded 'extra' LHCII can probably be dissociated by phosphorylation (Wientjes et al. 2013a), which is consistent with the observation that in plants only about 15-20% of LHCII are found to relocate between PSII and PSI, unlike green algae, where 80% of LHCII can undergo state transition (Delosme et al. 1996).

2. SCIENTIFIC AIMS

The general aim of this thesis work is to gain more knowledge about regulatory mechanisms of photosynthesis during light stress in plants, with a focus on PSII protein phosphorylation and regulatory protein identification and characterization.

The specific aims of each project were as follows:

Paper I: To compare PSII performance in three widely used Arabidopsis laboratory accessions: Col-0, Ws-4 and L*er*-0

Paper II: To investigate if natural variation in phosphorylation of PSII proteins is caused by genetic variation of STN kinases

Paper III: To investigate the physiological role of TAAC in Arabidopsis

Paper IV: To study of the membrane proteome of stroma thylakoids in order to find novel proteins with a putative role in photosynthetic regulation

3. METHODOLOGIES

3.1 *Arabidopsis thaliana* as a model organism

About 30 years ago, *Arabidopsis thaliana*, a member of the mustard family became a model organism for plant biology. The reason are its small size, short life cycle, efficient reproduction through self-progeny, and small genome size (Meinke et al. 1998). Since the complete sequencing of its genome (Arabidopsis Genome 2000), Arabidopsis research has developed significantly in the direction of functional annotatation of the more than 25,000 genes (Bevan and Walsh 2005; Koornneef and Meinke 2010)

3.1.1 Natural variation

Over 7,000 Arabidopsis natural accessions are distributed and collected from all over the world (Weigel 2012). Such wide range of natural accessions differ in genetic variation including allele polymorphisms, gene duplication (Bikard et al. 2009), and also gene translocation (Vlad et al. 2010). With assistance of linkage and association mapping techniques, quantitative trait loci (QTL) will allow to identify the genomic sequence location, down to genes in Arabidopsis accessions, where they control a complex phenotypic trait (Weigel and Nordborg 2005; Flood et al. 2011; Trontin et al. 2011).

Unlike mutant-based studies, which allowed advances in certain gene functions, natural variation will provide more information about plasticity and adaptation of plants to the environment, since it is a trait of multiple gene locus contributions. The goal of crop breeders is to find breeding targets among natural variants with traits in certain environments for breeding targets (Trontin et al. 2011). Furthermore, Arabidopsis natural variation will offer a great opportunity to study plant evolutionary ecology at the molecular level (Koornneef and Meinke 2010).

3.1.2 Functional genomics

Availability of large collections of mutants greatly contributed to the emergence of Arabidopsis as model organism. About 27,000 individual gene interruption lines can be purchased at the Arabidopsis Biological Resource Centre (ABRC), which covers nearly all the protein-coding genes (http://abrc.osu.edu/about-us; http://signal.salk.edu/cgi-bin/homozygotes.cgi). This great achievement is based on small-gene-intron character in Arabidopsis. Gene function can be dramatically disrupted by insertion of a piece of T-DNA during transformation with *Agrobacterium tumefaciens* (Krysan et al. 1999). This means in theory that it is possible to find out the function of every gene/protein of

interest by mutagenesis with reference inbred line-wild type. There are three commonly used laboratory wild type Arabidopsis accessions: Columbia-0 (Col-0), Landsberg *erecta-0* (L*er-0*) and Wassilewskija-4 (Ws-4). Due to mutant collections and AGI sequencing, Col-0 is generally viewed as the reference wild type (Koornneef and Meinke 2010).

3.2 Chloroplast fractionation

Procedures for isolation of chloroplasts, thylakoids, and grana and stroma thylakoids were described in the individual attached papers. In general, chloroplasts were isolated from homogenized Arabidopsis leaves by centrifugation and then purified by several washing steps. After that, chloroplasts were lysed by osmotic shock, and the thylakoids were separated by centrifugation (**Paper I & III**) or by sucrose gradient centrifugation (**Paper IV**). The grana and stroma thylakoid subfractions were obtained by digitonin solubilization and ultracentrifugation.

3.3 Protein identification and quantification by immunoblotting

First, proteins in a complex mixture were separated in acrylamide gels by electrophoresis according to their mobility. The smaller the size, the faster the protein moves. After electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane, which is then incubated with antibodies against the protein of interest. By detecting the linear signal of chemiluminescence from horseradish peroxidase (HRP) reaction with its substrate, which is directly linked to antibodies, it was possible to identify the protein and also determine its amount in a complex mixture. Phosphoprotein amounts were detected with phospho-threonine antibodies from both New England Biolabs (Cell signaling) and ZYMED.

3.4 Chlorophyll fluorescence

Chlorophyll *a* fluorescence was used to measure photosynthetic performance in our study (**Paper I & III**). Light energy absorbed by PSII chlorophyll can take three directions: first the energy can be used to drive photosynthesis, where electrons are generated from water splitting and transferred to the primary quinone acceptor Q_A; another way is to be released as heat; or to be emitted as fluorescence. These three ways compete with each other for the energy excitation. By gaining information about fluorescence emission, we can evaluate photochemistry as well as the heat dissipation (Baker 2008). Parameters named maximum quantum efficiency of PSII photochemistry Fv/Fm, the quantum yield of PSII photochemistry $\mathbf{\Phi}_{PSII}$, the excitation pressure 1-qP,

non-photochemical quenching NPQ, fast kinetics (*O-J-I-P* curves) were recorded/calculated by either PAM-210 or Dual PAM-100 in **Paper I** and **III**.

3.5 Mass spectrometry

In the last decades mass spectrometry (MS) has become invaluable method in a large-scale protein identification and characterization. In a classical MS procedure, a complex peptide mixture or other macromolecules are first separated by liquid chromatography (LC) based on reversible hydrophobic interaction with stationary phase of the chromatographic medium before subjection to ionization

There are two major ionization methods: electron spray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI). MALDI produces ions by laser excitation, the peptides are singlet charged and usually conjugated with time of flight (TOF) analyzer. Different from MALDI, ESI can produce multiply charged ions, which is usually coupled with tandem mass spectrometry (ESI-MS/MS) that can detect the amino acid sequence of the peptide fragment. The Noble Prize 2002 in Chemistry was awarded to John B. Fenn and Koichi Tanaka for development of these ionization methods (Tanaka et al. 1988; Fenn et al. 1989).

MS/MS fragments of charged ions are obtained by collision-induced dissociation (CID) and electron transfer dissociation (ETD). CID is most commonly used peptide fragmentation method for amino acid sequence determination (Mann et al. 2001). ETD is more able to preserve posttranslational modification of peptides as compared to the more crude CID technique (Syka et al. 2004). We have been using ESI followed by CID and ETD (HCTultra PTM Discovery System, Bruker Daltonics) to identify regulatory proteins in thylakoid membrane in **Paper IV**.

3.5.1 In proteomics

Proteomics, the large scale study of proteins, has become an important tool to achieve this aim by several aspects: identification and characterization of known and hypothetical proteins with different post-translation modifications in a certain subcellular compartment; comparison of protein composition in several environmental conditions, for example light-stressed and dark-adapted plants; studies of protein-protein interaction with method such as pull-down experiments and mass spectrometry (Pandey et al. 2000; Ephritikhine et al. 2004).

Chloroplast, the photosynthetic organelle, contains photosynthetic core and regulatory proteins. Proteomic study of chloroplast subfractions will provides comprehensive information about regulatory proteins. In the last ten years, several independent laboratories have conducted proteomic analysis on chloroplast envelope (Ferro et al. 2003; Froehlich et al. 2003; Rolland et al. 2003), thylakoid membrane and lumen (Peltier et al. 2002; Schubert et al. 2002; Friso et al. 2004). Most of the identified chloroplast proteins can be found in two databases of the chloroplast proteome from

Arabidopsis thaliana AT_CHLORO (Ferro et al. 2010) http://www.grenoble.prabi.fr/at_chloro/ and the Plastid Proteomics Database PPDB (Sun et al. 2009) http://ppdb.tc.cornell.edu/.

About 3600 distinct proteins are predicted in Arabidopsis chloroplasts (Abdallah et al. 2000; Kleffmann et al. 2004). However, only about 1,300 proteins were verified in AT_Chloro and 2300 are curated at PPDB. The reasons for the difference between the number of predicted and identified chloroplast proteins could be the following: low abundance; expressed only under certain conditions (for example abiotic stress); the tryptic peptides are not ionized. To be able to improve the identification rate, more sensitive and accurate mass spectrometry, more specific fractionation of chloroplasts and alternative enzyme digestion have been suggested to be employed in the investigation (van Wijk and Baginsky 2011).

4. MAJOR FINDINGS

4.1 Impact of thylakoid lipid-chlorophyll-carotenoid stoichiometry on PSII function

Few studies have been previously carried out to analyze natural variation of photosynthetic parameters in Arabidopsis, namely thermal dissipation of excess light energy (Jung and Niyogi 2009) and Rubisco small subunit diversity (Schwarte and Tiedemann 2011). In other species, for example in maize, variation in cold tolerance of photosynthesis was studied (Fracheboud et al. 2002).

Col-0, Ws-4 and Ler-0 are three widely used Arabidopsis background accessions to characterize the relevant mutant lines. In **Paper I,** I found that Ws-4 is more susceptible to high light as compared to Col-0 and L*er*-0. It displayed higher energy fluxes through PSII reaction center, and also faster closure upon both saturation pulse and continuous illumination, as indicated by fluorescence measurements such as *J* fluxes, *O-J-I-P* induction and rapid light curves of *Φ*PSII, 1-*q*P. These observations could be explained by approx. 30% higher thylakoid lipid-to-Chl ratio and 40% lower Chl/carotenoid ratio in Ws-4 as compared to Col-0 and L*er*-0. A higher thylakoid lipid-to-Chl ratio means that the Chl-protein complexes are more 'diluted' with lipids in the leaves of this accession. This could results in faster closure of RCII centers in Ws-4.

The content of two major types of lipids (MGDG and DGDG) is higher in Ws-4. Unlike DGDG, MGDG is a non-bilayer lipid that can create a lateral pressure within the hydrophobic membrane (van den Brink-van der Laan et al. 2004). How does Ws-4 solve the problem with the lateral pressure? The lower Chl-to-carotenoid ratio in Ws-4, especially due to higher violaxanthin content, means that a fraction of additional free violaxanthin may function as compensation for additional MGDG causing lateral pressure on the membrane. Another fraction of the additional violaxanthin may bind to LHCII-PSII supercomplexes to participate in RCII excitation. It has been shown that the polarity of xanthophyll bound to LHCII increases the PSII quantum efficiency because the lifetime of the Chl excited state is prolonged (Ruban and Johnson 2010).

4.2 Impact of STN8-chlorophyll-lipid stoichiometry on PSII dynamics

In **Paper I**, I also found that HL treatment caused in Ws-4 the largest extent of PSII inactivation and fastest D1 degradation. Based on further investigation using blue native gel electrophoresis and immunoblotting we demonstrated that in this accession PSII undergoes faster disassembly of PSII-LHCII supercomplexes.

It has been suggested that STN8 is essential in controlling the lateral movement of PSII-LHCII supercomplexes along the thylakoid membrane to the sites of repair since the *stn8* mutant displayed residual D1/D2 protein phosphorylation, more damaged supercomplexes, and delay in D1 protein degradation (Pesaresi et al. 2011; Tikkanen and Aro 2012). Based on this thesis work, Ws-4 doesn't show an intermediate D1 turn-over because of its intermediate phosphorylation level. Instead D1 protein is degraded faster than in the other two accessions. I observed lower STN8-Chl ratio in Ws-4 that could explain the reduced phosphorylation. This indicates that variation in STN8 levels may not be the only factor involved in the lateral movement of supercomplexes, in contrast with previous single-gene-mutation studies. In Ws-4 the dilution of protein complexes by additional lipids plays a major role in disassembly and migration of the damaged complexes to the sites of repair, thus compensating for consequence of intermediate phosphorylation levels of the PSII core proteins. Although PSII complexes may move faster in the thylakoid membrane for a faster D1 degradation, additional PSII damage occurs in Ws-4, which exceeds the D1 protein synthesis capacity, thus leading to slightly enhanced photoinhibition.

4.3 Variation of PSII protein phosphorylation in natural accessions

Enzymes named kinases catalyze phosphorylation of proteins. PSII core proteins D1, D2 PsbH and to some extent CP43 proteins can be phosphorylated by STN8 kinase under HL conditions (Vainonen et al. 2005); whereas STN7 is involved in phosphorylation of LHCII proteins under growth light (GL) conditions (Bellafiore et al. 2005). Since PSII protein phosphorylation is an important regulatory mechanism used by plants to adapt to various light intensities and qualities, our next question was to investigate if natural accessions differ in PSII protein phosphorylation and the reasons behind their variation. In **Paper II**, 16 Arabidopsis accessions from diverse geographic origins were exposed to two light regimes, and the levels of phospho-D1 and phospho-LHCII proteins were quantified by immunoblotting with anti-phosphothreonine antibodies. The levels of STN7 and STN8 kinases were also tested. D1/Lhcb2/CP43 protein levels were used to correct levels of corresponding phosphorylated protein and of STN kinases **(Figure 4)**.

4.3.1 Role of kinase activity under growth light

From our natural accessions selection, Col-0 shows the highest D1 phosphorylation in GL although the others display similar abundance of the D1 substrate and STN8 kinase. One explanation could be a poor activation of the STN8 kinase in GL, which can be related to the redox state of the PQ pool. The same goes for the STN7 kinase, i.e., it is rather STN7 activity than protein level that regulates the phosphorylation of LHCII under GL conditions.

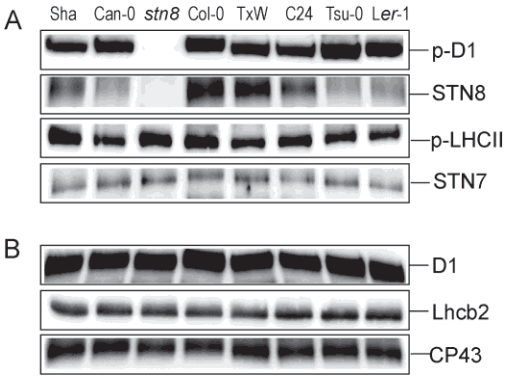

Figure 4. *Quantitative immunoblotting analysis of phospho-D1, STN8, phospho-LHCII and STN7 levels (A), and control blots with D1, Lhcb2 and CP43 level (B) in thylakoids from natural accessions, the stn8 mutant and the hybrid line Tsu-0 x Ws-4 (TxW).*

Among the accessions we examined, Ely and ELB are negative controls for kinase activity. Both of them are atrazine resistant due to deficient binding of quinones in the Q_B pocket (El-Lithy et al. 2005). Low phosphorylation of PSII core and LHCII proteins were detected in both accessions, which could be due to poor reduction of PQ pool, hence reduced activation of the STN kinase.

4.3.2 Role of kinase abundance under HL

PSII protein phosphorylation levels can be under the control of kinase amount. In paper II, figure 2 b we found that the levels of D1 phosphorylation correlated with the STN8 kinase levels in HL, supporting the initial observations in **Paper I**. This indicates that kinase abundance can be a limiting factor or play a regulatory role for phosphorylation under these conditions.

4.3.3 Role of protein phosphorylation in adaptation to diverse environments

We propose that variable phosphorylation of PSII proteins *in Arabidopsis* accessions is a result of an adaptive mechanism for survival and reproduction across the range of environmental conditions where *Arabidopsis* naturally occurs. In **Paper II**, we found a significant correlation between longitude and both D1 and LHCII phosphorylation in GL conditions (figure 4 *a, b*). This suggests that there may be some form of selective pressure that correlates with longitude.

When comparing phosphorylation levels of PSII proteins with climate data from WorldClim database (Hijmans et al. 2005) (http://www.worldclim.org/), we found positive correlation between phospho-LHCII in GL with temperature seasonality and with temperature annual range to be significant. It may be the result of similar photoprotective mechanisms as observed in evergreen trees to maintain functional

leaves in very cold conditions (Verhoeven et al. 2009). To investigate correlation between phosphorylation and the natural habitat, many more accessions should be included from a large range of environments.

4.4 Physiological roles of the thylakoid ATP/ADP carrier in Arabidopsis

Thylakoid ATP/ADP carrier (TAAC) belongs to the mitochondria carrier family. It is absent in cyanobacteria and only appeared early in the evolution of photosynthetic eukaryotes (Palmieri et al. 2011; Spetea et al. 2011). Previous study from our laboratory have shown that TAAC protein imports ATP in exchange for ADP across the cytoplasmic membrane of *Escherichia coli*, and that the *taac* mutant thylakoids have reduced ability to take up ATP and convert it to GTP as compared to the wild-type (Thuswaldner et al. 2007). **Paper III** is focused on the physiological consequence of TAAC protein deficiency in Arabidopsis.

4.4.1 Role in PSII repair

The deficiency of TAAC in the mutant has increased the susceptibility of PSII to photoinhibition under both HL conditions studied: 3h illumination and 4 weeks in the growth chamber (**Paper III**). Detailed phenotypic investigation showed that PSII repair cycle is malfunctioning in the mutant as compared with background wild type, which is the primary reason of higher susceptibility of PSII to HL. The site of malfunction was localized at the step where CP43 dissociated from damaged PSII, since a relatively low proportion of CP43-less monomer was detected by blue native gel electrophoresis together with immunoblotting in the mutant compared with the wild type.

Interestingly, previous studies of the *psbo2* mutant showed a similar phenotype as for the *taac* mutant in PSII repair aspects during HL stress. Both types of plants were smaller and less tolerant to HL conditions, displayed retarded D1 protein degradation, and blockage in PSII repair cycle step from core monomer to CP43-less monomer (Lundin et al. 2007a; Lundin et al. 2008). PsbO2 was demonstrated as a GTPase (Lundin et al. 2008), which activity is required to induce its dissociation from PSII thus also facilitating CP43 dissociation from PSII core monomer. Our hypothesis in **Paper III** was that TAAC and PsbO2 crosstalk in the CP43 dissociation step as reviewed (Spetea and Lundin 2012). In addition, ATP transported via TAAC into the lumen may induce phosphorylation of PSII lumen-exposed proteins with role in efficient turnover of the D1 protein as illustrated in **Figure 5**. Briefly a) ATP is transported by TAAC into the thylakoid lumen in exchange for ADP (Thuswaldner et al. 2007); b) ATP is then converted to GTP, which binds to the PsbO subunit of PSII complex, resulting in monomerization; c) PSII monomer moves to stroma lamellae, GTP is also used to dissociate PsbO2 from PSII monomer to CP43 less monomer (Lundin et al. 2007b); d) An unknown kinase phosphorylates CP43 lumenal-exposed loop and lumenal proteins,

leading to their dissociation from PSII/membrane (Spetea and Lundin 2012); e) The phosphorylated proteins are then dephosphorylated by a phosphatase, such as TLP18.3 (Wu et al. 2011), and the resulting phosphate is exported out to the stroma by the thylakoid phosphate transporter (PHT4;1) (Pavon et al. 2008).

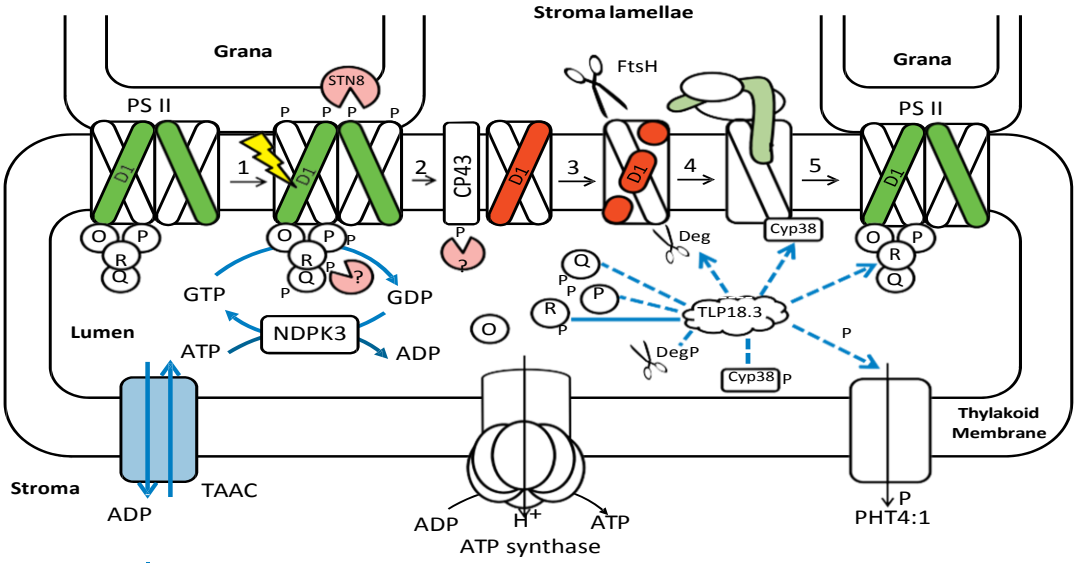

Figure 5. *Working model for TAAC involvement in PSII repair cycle. This involvement happens at several steps: a) ATP is transported into thylakoid lumen in exchange of ADP by TAAC; b) ATP is then converted to GTP, which binds to PsbO subunit of PSII complex, resulting in its dissociation; c) PSII monomer moves to stroma lamellae, GTP is also used to dissociate PsbO2 from PSII monomer to CP43 less monomer; d) An unknown kinase phosphorylates CP43 lumena- exposed loop and lumenal proteins (PsbP, PsbQ, PsbR, Deg1 and Cyp38), leading to their dissociation from PSII/membrane; e) The phosphorylated proteins are then dephosphorylated by a phosphatase, such as TLP18.3, the resulting phosphate (P) is exported out to the stroma by the thylakoid phosphate transporter (PHT4;1). Figure modified from Spetea and Lundin (2012) FEBS Lett. 586: 2946-2954 with kind permission from the authors.*

4.4.2 Role in PSII photoprotection

The *taac* mutant displayed a higher initial phase in induction of NPQ as compared to wild type, but similar steady state NPQ values. This pattern was preserved in dithiothreitol (DTT)-pretreated leaves, but not in nigericin-pretreated ones. DTT is an inhibitor of violaxanthin de-epoxidation, whereas nigericin is a common inhibitor of

trans-thylakoid pH gradient. The transiently higher NPQ in the mutant originates from higher pH gradient as compared to wild type.

A potential explanation for this result is that TAAC imports ATP^{4-} in exchange for ADP^{3-} across the thylakoid membrane. To compensate for the extra-charge, TAAC transport capacity possibly consumes a fraction of the H^+ gradient. In the mutant, this fraction can be used for energy dissipation, resulting in higher initial phase of NPQ as compared to the wild type

4.4.3 Other roles in plants

Besides a role in trans-thylakoid adenine nucleotide exchange (**Paper III**, Thuswaldner et al. 2007), a recent published paper suggested that TAAC could be located in the envelope supplying plastidic phosphoadenosine 5'-phosphosulfate to the cytosol (Gigolashvili et al. 2012). The rice homologue of TAAC was dually localized to mitochondria and envelope (Xu et al. 2013). In the same report, the protein functioned as a 3'-phosphoadenosine 5'-phosphosulfate (PAPS) carrier, having role in chloroplast retrograde signaling and chloroplast development in rice.

4.5 The grana ultrastructure limits D1 turnover rate

Previously, it was reported an increased size in Arabidopsis grana thylakoids, and retarded D1 degradation in *stn8* mutants as compared to the wild type (Tikkanen et al. 2008; Fristedt et al. 2009b). Furthermore, thylakoid membrane fluidity was suggested to facilitate access PSII complexes to stroma-exposed active protease FtsH, leading to efficient D1 turnover (Yoshioka and Yamamoto 2011). These two findings indicate there is direct connection between thylakoid ultrastructure and D1 turnover rate.

These observations were confirmed in **Paper III**. Under 3h HL illumination, TAAC deficient mutant showed increased width and height of grana thylakoids in comparison with the wild type, leading to slower PSII repair. One possible explanation is that deficiency of nucleotides in the lumen has a similar impact on stacking as the lack of PSII protein phosphorylation on the stromal side of the thylakoid membrane.

In **Paper I**, Ws-4 displayed smaller grana and more unstacked thylakoids most likely due to higher content of lipids per protein complexes that may have increased membrane fluidity. This has resulted in faster repair despite the reduced levels of PSII core protein phosphorylation.

4.6 Proteomic study explores thylakoid proteins with regulatory roles

In **Paper IV**, 58 proteins have been identified in the stroma thylakoid membrane of *Arabidopsis thaliana*. Four different methods for extraction of peptides from

hydrophobic membrane proteins have been used: in solution digestion with chymotrypsin alone and in combination with methanol extraction, in-gel digestion with trypsin and in-gel digestion with chymotrypsin. Using these methods, I was able to extract peptides from 34, 49, 5 and 6 proteins, respectively. The combination of methanol and chymotrypsin was found as the most effective way to identify membrane proteins (45 out of 58). Using a combination of prediction programs ChloroP, TargetP, and Wolf PSORT, about 50% the identified nuclear-encoded proteins were indicated as chloroplast proteins. In addition, 13 chloroplast- encoded proteins were found.

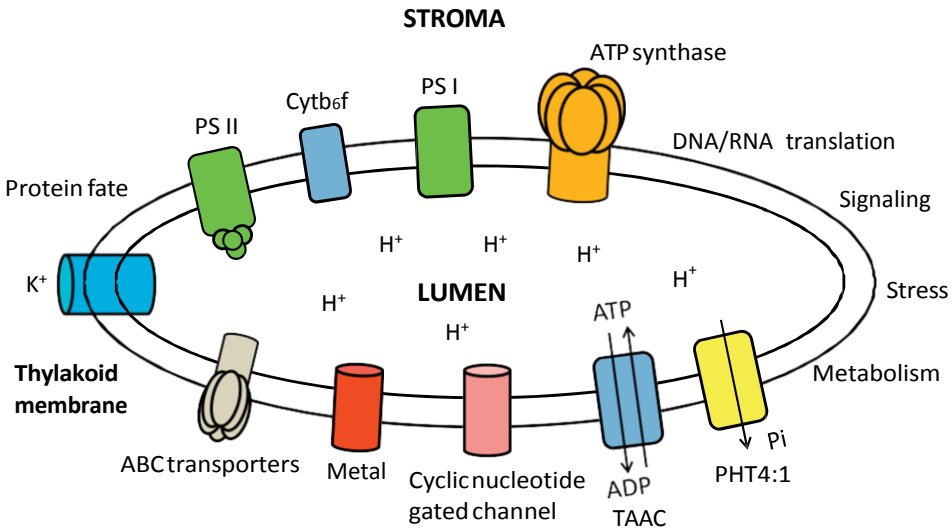

Figure 6.*Overview of the stroma thylakoid proteome. Major photosynthetic complexes PSII, Cytb$_6$f, PSI, ATP synthase were found in the thylakoid membrane. Besides this, proteins that function in transport, protein fate, translation, signaling, stress, and metabolism, were identified. In addition to putative channels and transporters identified by proteomics, the thylakoid ATP/ADP carrier (TAAC) and the thylakoid phosphate transporter PHT4;1 found by immunoblotting are shown.*

With respect to the functional classification, 25 of the identified proteins are subunits of photosynthetic complexes, nine are transporters, three have a putative role in protein fate (proteolysis, import), four in translation, two are stress-related proteins, three are involved in primary and secondary metabolism, two are related to signaling, and ten are probable contaminants. Among the 191 unique peptide sequences identified, there were 19 peptide sequences from transmembrane regions of photosynthetic proteins and transporters. This means that I have found a simple and fast strategy to extract peptides from hydrophobic low abundant membrane proteins. The obtained proteomic data require validation by individual localization studies and phenotypic characterization of mutant lines.

5. CONCLUSIONS AND PERSPECTIVES

No previous studies investigated the natural genetic variation in plant photosynthesis with focus on PSII performance and dynamics. After this thesis work, we now know that out of three widely used reference accessions Ws-4 undergoes faster PSII repair but is also more susceptible to HL, mainly due to dilution of Chl-protein complexes by lipids and carotenoids in the thylakoid membrane and reduced STN8 kinase levels **(Paper I)**. When investigating larger number of natural accessions, I found that in high light conditions phospho-D1 level is correlated with STN8 kinase amount, whereas in growth light it is correlated with longitude, and in the case of LHCII phosphorylation also with temperature variability, indicating adaptation to diverse environments for *Arabidopsis* **(Paper II).** Previously TAAC has been characterized biochemically to import ATP in exchange for ADP in *Escherichia coli* and Arabidopsis. But the physiological role of TAAC in plants was not clear. After characterization of *taac* mutants, we now postulate that TAAC plays a critical role in disassembly steps during PSII repair and in addition may balance the trans-thylakoid electrochemical H^+ gradient storage **(Paper III)**. TAAC is not the only regulatory protein for adaptation of photosynthetic process to various environments. In this thesis work, stroma thylakoid membrane proteomics in Arabidopsis has revealed a large number of low-abundant hydrophobic proteins, like transporters, which help us to better understand photosynthetic metabolism at molecular level and provides biochemical overview of chloroplast membranes **(Paper IV)**.

Photosynthesis is vital for all the living organisms on Earth, however the molecular regulatory mechanisms behind are far from being completely understood. The findings presented in this thesis add stones on the castle how plants optimize photosynthesis to tackle environmental stress. By improving the knowledge about the regulatory factors involved, we will not only be able to improve our food production, but also mimic natural photosynthesis to generate renewable energy to meet our increasing demands in the future.

6. POPULÄRVETENSKAPLIG SAMMANFATTNING

Fotosyntes är den process där växter, alger och cyanobakterier använder solenergi för att omvandla vatten och koldioxid till syre och energi i form av kolhydrater. Syre är livsnödvändigt och kolhydrater ger energi direkt eller indirekt via näringskedjan till levande organismer på jorden, inklusive människan.

Solljus är viktigt för att driva fotosyntesen, men det är också känt att alltför mycket ljus kan skada de proteiner som krävs i fotosyntesen, vilket påverkar växternas tillväxt. Växter kan inte röra sig som djur, exempelvis för att hitta sin önskade plats för fotosyntesen. De måste anpassa sig till olika miljöförhållanden, såsom temperatur, fukt, ljusintensitet och årstidsväxlingar. I denna avhandling har jag använt modellväxten *Arabidopsis thaliana* för att studera de molekylära mekanismer som reglerar fotosyntesen under hög ljusstress.

En av dessa mekanismer är proteinfosforylering i fotosystem II-komplexet (PSII). PSII finns i tylakoidmembranet, som är en avdelning i kloroplasten där fotosyntesen sker. Fosforyleringsreaktionen används för att aktivera/inaktivera proteiner och den katalyseras bl.a. av enzymet STN8-kinas. I Artikel II har jag upptäckt variationer i graden av fosforylering hos PSII-proteiner i naturliga ekotyper av Arabidopsis från olika delar av världen. Vid höga ljusförhållanden finns det en koppling mellan mängden STN8-kinas och hur mycket proteinet D1 fosforyleras. I normalt ljus korrelerar fosforyleringsgraden med geografisk longitud och säsongens temperaturvariationer. Dessa observationer tyder på en anpassning till olika miljöer för Arabidopsis.

En annan mekanism som växter använder för att övervinna hög ljusstress är PSII's reparationscykel. I denna process är fosforylering ett tidigt och avgörande steg eftersom det hjälper skadade PSII-komplex att förflytta sig till platser i tylakoidmembranet där de kan repareras. I Artikel I har jag funnit att fosforyleringsgraden hos Arabidopsis ekotyp Ws-4 är hälften så hög jämfört med andra ekotyper, såsom Col-0, vilket beror på lägre förekomst av STN8-kinas. Som kompensation för detta har Arabidopsis Ws-4 ytterligare lipider och karotenoider som kan öka rörligheten hos de skadade komplexen och på så sätt möjliggöra reparation.

I tylakoidmembranet finns ett vätskefyllt utrymme som kallas lumen. TAAC (thylakoid ATP/ADP carrier) transporterar ATP, som används för energiöverföring, in i lumen i utbyte mot ADP. I Artikel III har jag upptäckt att plantor som saknar TAAC har långsammare PSII-reparation än plantor med TAAC, trots att de uppvisar samma grad av proteinfosforylering. Vi föreslår att ATP, som transporterats in i lumen av TAAC, används för nukleotidberoende reaktioner som påverkar PSII-reparationen. En annan upptäckten är att TAAC via sin transportaktivitet kan förbruka en del av protongradienten över tylakoidmembranet, vilket är avgörande för initieringen av ljusskyddande mekanismer.

I Artikel IV har jag identifierat 58 proteiner, som inkuderar såväl tidigare kända som nya tylakoidproteiner med potentiella roller i transport, translation, stress och signalering. Denna avhandling fördjupar vår förståelse av fotosyntesen på molekylär nivå och förbättrar den biokemiska översikten av tylakoidmembranet i kloroplasten.

7. ACKNOWLEDGEMENTS

Many thanks to my coauthors without whom this thesis wouldn't be possible: *Rikard Fristedt, Katalin Solymosi, Andrei Herdean, Markus Nurmi, Saijaliisa Kangasjärvi, Eva-Mari Aro, Fikret Mamedov, Martine Bertrand, Mats X. Andersson, Pádraic J. Flood, Jeremy Harbinson, Mark G.M. Aarts, Alexander V. Vener, Benoît Schoefs, Björn Lundin,* and *Cornelia Spetea*.

Especially I would like to thank my main supervisor Prof. ***Cornelia Spetea Wiklund,*** who gave me the opportunity to become PhD student. You almost told me everything about science and your enthusiasm for research is really inspiring. And also thanks for giving example how to get a balance between family and science.

I also appreciate a lot…

My co-supervisor Prof. *Alexander V. Vener* for outstanding knowledge, help to stay in Linköping and opening the proteomic window for me. Dr. Maria Turkina for guidance in the lab and MS-analysis in Linköping.

My co-supervisor Ass Prof. *Björn Lundin* for all the friendly and critical talks in science. I still remember Jonathan's birthday when you told me about photosystem complexes.

Docent *Henrik Aronsson* for organizing the photosynthesis workshop in Göteborg 2008, being my half time opponent, introducing Göteborg and pub dance in Cornelia's promotion party, as well as career planning discussions.

Docent *Johan Edquivt* for being my half time opponent, and course leader for teaching.

Docent *Bernard Pfeil* for thesis language correction.

Docent *Mats X. Andersson* for thylakoid lipid analysis.

Prof. *Andrian Clarke* for being my examiner.

Ingela Dahllöf, BioEnv Prefect, for the assay on scientific writing.

Prof. *Benoît Schoefs* for chlorophyll fluorescence course. Prof. *Eevi Rintamäki* for scientific discussion last spring. Dr. *Katalin Solymosi* for electronmicroscopy detection and mother talk in science. Dr. *Saijaliisa Kangasjärvi* for Finnish hospitality and *Markus*

Nurmi for blue native gels and CO_2 fixation measurements. Docent *Fikret Mamedov* for EPR analysis.

Prof. *Peter Strålfors* for introduction to his Swedish research group when I first arrived to Sweden, and *Unn* for teaching me the early lab work in Sweden.

I would like to thank group members:

Lorena Ruiz Pavón for the lovely parties we had together. ☺ It's always pleasant to meet you and hear your heartly smile.
Azeez Beebo for important Friday scientific discussion and also for your always willing to help.
Patrik Karlsson for being nice conference accompany and Friday meetings.
Andrei Herdean for important and inspiring discussions in science.
Lisa Adolfsson for sharing the office and good luck with your thesis!
Jenny Carlsson for invitation to your home.
Pavan, DJ, Behrad, Ateeq, Lovisa, Somnath, *Jila* for bringing fresh blood and fun into the group.

People at BioEnv :

Anders Nilsson for the thesis format. *Cong Fei* for exploring the fruit garden and small Chinese chats in Botan. *Sazzad Karim* for discussion about thesis writing and jokes in the lunch room. *Panos* for borrowing large gel cassette and thesis discussion. All the friendly working environment created by *Göran, Oscar, Elham, Kemal, Zeynep, Frida, Per F, Amelie, Mohamed, Triranta, Feng, Ruud, Jonna, Ellen, Johan, Fernanda C, Marcela, Rosemerie, Alexander Z, Ingela L, Alexandre A, Thomas, Henrik, Mikael, Fabian, Maria, Yann, Kemal, Brigitte, Tage, Britt, Ritva, Carola* at Botan. Also thank *Sven* for outstanding problem solving and thesis printing contact. *Niclas* for computer issues. *Ylva Heed* for all the friendly talks and encouragement in thesis writing!

Also thanks the people I met at conferences and workshops. *Dieter* for discussion about the thesis.

For Alexander Vener group members in Linköping

Rikard Fristedt for all the jokes and science discovery journey, and the conference accompany.
Björn Ingelsson for all the fun and scientific discussions, still remember 'the secret phosphatase' experience!
Hanna Klang Årstrand for good discussion in the lab and also help about the babies.
Åsa Jufvas for introducing me how to use the Scaffold program.

Jacob Kuruvilla for being so friendly and nice. *Pontus, Daniel, Heiner* for bringing new faces and fun in the group.

I would like to thank all people on floor 13 and 9 people at Cell Biology in Linköping. Special thanks for *Lena, Peter, Deepti, Vivian, Christine, Sara, Susana, Eva, Anita, Chamilly, Birgitta, Håkan, Anna, Meenu, Margaretha* for friendly and inspiring working environment. *Jiang* for important scientific and life discussion. *Limin* for introducing the science world. *Wang hui, Zhao yeling* for city exchange experience and picking up my mother.

Also all people at IFM biology department in Linköping. Special thanks for *Chunxia, Davide, Per, Jordi, Matthias, Dainel N, Monika, Anders W, Dominic, Ida, Tove, Anita, Martin, Isa* for creative and easygoing atmosphere.

Even for friends outside Science:

Friends in Göteborg: *Tam, Robert, Yufang, Zou Xiang, Jiangna family, Lei family, Wang* for all the fun together.

Friends in Linköping: *Olle* and *Johan* for the first years 'Prison break'. *Dirk* and *Mieck* for visiting from time to time. *Elaine* for courses and all the meetings. *Johanna* for jogging. *Milagros* and *Marga* for nice chatting about culture and kids. *Fariba* gave the opportunity to see how the industry built up and *Tayeb* for interesting discussion and endless energies. *Lina, Liu mingna, Liujingtao* for the trips in US. *Simon, Fernando, Ramon, Ignacio, Juan, Bingfei, Olof, Johanna* for corridor accompany. *Samuel, Vinodh R, Balaji, Kahanh, Arun, Prasanna, Vivek, Vinodh S* for all the parties and danses. *Yanjuan family* for baby meetings and *Rose, Liujie* for sharing spare time. Chinese society in Linköping for organizing the meeting, make my life in Sweden more colorful.

My family and friends in China for support. My mamma's visit to Sweden (**妈妈辛苦了**); My sister's weekly talk (**真幸运有你，妹妹**). My father's endless care (**谢谢爸爸**). *Ling*s friendship (**你就像姐妹一样**). My family in Sweden *Tony* for company all the way, fully support, taking care of the little one. And his family *Sandra for all the trust*, parents for accepting me without any doubt. And my sweetheart *Caesar*!

Thank you all from the depth of my heart!

/Lan

8. REFERENCES

Abdallah F, Salamini F, Leister D (2000) A prediction of the size and evolutionary origin of the proteome of chloroplasts of Arabidopsis. Trends Plant Sci 5:141-142

Albertsson P (2001) A quantitative model of the domain structure of the photosynthetic membrane. Trends Plant Sci 6:349-358

Albertsson PA, Andreasson E, Svensson P (1990) The domain organization of the plant thylakoid membrane. FEBS Lett 273:36-40

Allahverdiyeva Y, Suorsa M, Rossi F, Pavesi A, Kater MM, Antonacci A, Tadini L, Pribil M, Schneider A, Wanner G, Leister D, Aro EM, Barbato R, Pesaresi P (2013) Arabidopsis plants lacking PsbQ and PsbR subunits of the oxygen-evolving complex show altered PSII super-complex organization and short-term adaptive mechanisms. Plant J 75:671-684

Allen JF (1992) Protein phosphorylation in regulation of photosynthesis. Biochim Biophys Acta 1098:275-335

Anderson JM, Andersson B (1988) The dynamic photosynthetic membrane and regulation of solar energy conversion. Trends Biochem Sci 13:351-355

Andersson B, Anderson JM (1980) Lateral heterogeneity in the distribution of chlorophyll-protein complexes of the thylakoid membranes of spinach chloroplasts. Biochim Biophys Acta 593:427-440

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373-399

Arabidopsis Genome I (2000) Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408:796-815

Aro EM, Kettunen R, Tyystjarvi E (1992) ATP and light regulate D1 protein modification and degradation. Role of D1* in photoinhibition. FEBS Lett 297:29-33

Aro EM, Ohad I (2003) Redox regulation of thylakoid protein phosphorylation. Antioxid Redox Signal 5:55-67

Aro EM, Suorsa M, Rokka A, Allahverdiyeva Y, Paakkarinen V, Saleem A, Battchikova N, Rintamaki E (2005) Dynamics of photosystem II: a proteomic approach to thylakoid protein complexes. J Exp Bot 56:347-356

Aro EM, Virgin I, Andersson B (1993) Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. Biochim Biophys Acta 1143:113-134

Baena-Gonzalez E, Aro EM (2002) Biogenesis, assembly and turnover of photosystem II units. Philos Trans R Soc Lond B Biol Sci 357:1451-1459; discussion 1459-1460

Baena-González E, Barbato R, Aro E-M (1999) Role of phosphorylation in the repair cycle and oligomeric structure of photosystem II. Planta 208:196-204

Baginsky S, Gruissem W (2009) The chloroplast kinase network: new insights from large-scale phosphoproteome profiling. Mol Plant 2:1141-1153

Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu Rev Plant Biol 59:89-113

Baniulis D, Zhang H, Zakharova T, Hasan SS, Cramer WA (2011) Purification and crystallization of the cyanobacterial cytochrome b6f complex. Methods Mol Biol 684:65-77

Barber J, Andersson B (1992) Too much of a good thing: light can be bad for photosynthesis. Trends Biochem Sci 17:61-66

Battchikova N, Aro EM (2007) Cyanobacterial NDH-1 complexes: multiplicity in function and subunit composition. Physiol Plant 131:22-32

Bellafiore S, Barneche F, Peltier G, Rochaix JD (2005) State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. Nature 433:892-895

Ben-Shem A, Frolow F, Nelson N (2003) Crystal structure of plant photosystem I. Nature 426:630-635

Bennett J (1977) Phosphorylation of chloroplast membrane polypeptides. Nature 269:344-346

Bevan M, Walsh S (2005) The Arabidopsis genome: a foundation for plant research. Genome Res 15:1632-1642

Bikard D, Patel D, Le Mette C, Giorgi V, Camilleri C, Bennett MJ, Loudet O (2009) Divergent evolution of duplicate genes leads to genetic incompatibilities within A. thaliana. Science 323:623-626

Bonardi V, Pesaresi P, Becker T, Schleiff E, Wagner R, Pfannschmidt T, Jahns P, Leister D (2005) Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. Nature 437:1179-1182

Cazzaniga S, Dall' Osto L, Kong S-G, Wada M, Bassi R (2013) Interaction between avoidance of photon absorption, excess energy dissipation and zeaxanthin synthesis against photooxidative stress in Arabidopsis. Plant J 76(4):568-79

Cleland RE, Bendall DS (1992) Photosystem I cyclic electron transport: Measurement of ferredoxin-plastoquinone reductase activity. Photosynth Res 34:409-418

Cramer WA, Soriano GM, Ponomarev M, Huang D, Zhang H, Martinez SE, Smith JL (1996) Some new structural aspects and old controversies concerning the cytochrome b6f complex of oxygenic photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 47:477-508

Cruz JA, Sacksteder CA, Kanazawa A, Kramer DM (2001) Contribution of electric field (Delta psi) to steady-state transthylakoid proton motive force (pmf) in vitro and in vivo. control of pmf parsing into Delta psi and Delta pH by ionic strength. Biochemistry 40:1226-1237

De Las Rivas J, Heredia P, Roman A (2007) Oxygen-evolving extrinsic proteins (PsbO,P,Q,R): Bioinformatic and functional analysis. BiochimBiophys Acta Bioenergetics 1767:575-582

Delosme R, Olive J, Wollman F-A (1996) Changes in light energy distribution upon state transitions: an in vivo photoacoustic study of the wild type and photosynthesis mutants from Chlamydomonas reinhardtii. Biochim Biophys Acta Bioenergetics 1273:150-158

Depege N, Bellafiore S, Rochaix JD (2003) Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in Chlamydomonas. Science 299:1572-1575

El-Lithy ME, Rodrigues GC, van Rensen JJ, Snel JF, Dassen HJ, Koornneef M, Jansen MA, Aarts MG, Vreugdenhil D (2005) Altered photosynthetic performance of a natural Arabidopsis accession is associated with atrazine resistance. J Exp Bot 56:1625-1634

Ephritikhine G, Ferro M, Rolland N (2004) Plant membrane proteomics. Plant Physiol Biochem 42:943-962

Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM (1989) Electrospray ionization for mass spectrometry of large biomolecules. Science 246:64-71

Ferro M, Brugiere S, Salvi D, Seigneurin-Berny D, Court M, Moyet L, Ramus C, Miras S, Mellal M, Le Gall S, Kieffer-Jaquinod S, Bruley C, Garin J, Joyard J, Masselon C, Rolland N (2010) AT_CHLORO, a comprehensive chloroplast proteome database with subplastidial localization and curated information on envelope proteins. Mol Cell Proteomics 9:1063-1084

Ferro M, Salvi D, Brugiere S, Miras S, Kowalski S, Louwagie M, Garin J, Joyard J, Rolland N (2003) Proteomics of the chloroplast envelope membranes from Arabidopsis thaliana. Mol Cell Proteomics 2:325-345

Flood PJ, Harbinson J, Aarts MG (2011) Natural genetic variation in plant photosynthesis. Trends Plant Sci 16:327-335

Fracheboud Y, Ribaut JM, Vargas M, Messmer R, Stamp P (2002) Identification of quantitative trait loci for cold-tolerance of photosynthesis in maize (Zea mays L.). J Exp Bot 53:1967-1977

Friso G, Giacomelli L, Ytterberg AJ, Peltier JB, Rudella A, Sun Q, Wijk KJ (2004) In-depth analysis of the thylakoid membrane proteome of Arabidopsis thaliana chloroplasts: new proteins, new functions, and a plastid proteome database. Plant Cell 16:478-499

Fristedt R, Carlberg I, Zygadlo A, Piippo M, Nurmi M, Aro EM, Scheller HV, Vener AV (2009a) Intrinsically unstructured phosphoprotein TSP9 regulates light harvesting in Arabidopsis thaliana. Biochemistry 48:499-509

Fristedt R, Willig A, Granath P, Crevecoeur M, Rochaix JD, Vener AV (2009b) Phosphorylation of photosystem II controls functional macroscopic folding of photosynthetic membranes in Arabidopsis. Plant Cell 21:3950-3964

Froehlich JE, Wilkerson CG, Ray WK, McAndrew RS, Osteryoung KW, Gage DA, Phinney BS (2003) Proteomic study of the Arabidopsis thaliana chloroplastic envelope membrane utilizing alternatives to traditional two-dimensional electrophoresis. J Proteome Res 2:413-425

Gigolashvili T, Geier M, Ashykhmina N, Frerigmann H, Wulfert S, Krueger S, Mugford SG, Kopriva S, Haferkamp I, Flugge UI (2012) The Arabidopsis thylakoid ADP/ATP carrier TAAC has an additional role in supplying plastidic phosphoadenosine 5'-phosphosulfate to the cytosol. Plant Cell 24:4187-4204

Hauser M, Eichelmann H, Oja V, Heber U, Laisk A (1995) Stimulation by Light of Rapid pH Regulation in the Chloroplast Stroma in Vivo as Indicated by CO2 Solubilization in Leaves. Plant Physiol 108:1059-1066

Haußühl K, Andersson B, Adamska I (2001) A chloroplast DegP2 protease performs the primary cleavage of the photodamaged D1 protein in plant photosystem II. EMBO Journal 20:713-722

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965-1978

Horton P, Ruban AV, Walters RG (1996) Regulation of Light Harvesting in Green Plants. Annu Rev Plant Physiol Plant Mol Biol 47:655-684

Huesgen PF, Schuhmann H, Adamska I (2009) Deg/HtrA proteases as components of a network for photosystem II quality control in chloroplasts and cyanobacteria. ResMicrobiol 160:726-732

Ido K, Ifuku K, Yamamoto Y, Ishihara S, Murakami A, Takabe K, Miyake C, Sato F (2009) Knockdown of the PsbP protein does not prevent assembly of the dimeric PSII core complex but impairs accumulation of photosystem II supercomplexes in tobacco. Biochim Biophys Acta 1787:873-881

Ingelsson B, Vener AV (2012) Phosphoproteomics of Arabidopsis chloroplasts reveals involvement of the STN7 kinase in phosphorylation of nucleoid protein pTAC16. FEBS Lett 586:1265-1271

Jahns P, Latowski D, Strzalka K (2009) Mechanism and regulation of the violaxanthin cycle: The role of antenna proteins and membrane lipids. Biochim Biophys Acta Bioenergetics 1787:3-14

Jarvi S, Gollan PJ, Aro EM (2013) Understanding the roles of the thylakoid lumen in photosynthesis regulation. Front Plant Sci 4:434

Jensen PE, Bassi R, Boekema EJ, Dekker JP, Jansson S, Leister D, Robinson C, Scheller HV (2007) Structure, function and regulation of plant photosystem I. Biochim Biophys Acta Bioenergetics 1767:335-352

Jordan P, Fromme P, Witt HT, Klukas O, Saenger W, Krauss N (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 A resolution. Nature 411:909-917

Jung HS, Niyogi KK (2009) Quantitative genetic analysis of thermal dissipation in Arabidopsis. Plant Physiol 150:977-986

Kanervo E, Suorsa M, Aro EM (2005) Functional flexibility and acclimation of the thylakoid membrane. Photochem Photobiol Sci 4:1072-1080

Kapri-Pardes E, Naveh L, Adam Z (2007) The Thylakoid Lumen Protease Deg1 Is Involved in the Repair of Photosystem II from Photoinhibition in Arabidopsis. Plant Cell 19:1039-1047

Kieselbach T, Schroder WP (2003) The proteome of the chloroplast lumen of higher plants. Photosynth Res 78:249-264

Kirchhoff H (2014) Structural changes of the thylakoid membrane network induced by high light stress in plant chloroplasts. Philos Trans R Soc Lond B Biol Sci 369:20130225

Kleffmann T, Russenberger D, von Zychlinski A, Christopher W, Sjolander K, Gruissem W, Baginsky S (2004) The Arabidopsis thaliana chloroplast proteome reveals pathway abundance and novel protein functions. Curr Biol 14:354-362

Komenda J, Barker M, Kuvikova S, de Vries R, Mullineaux CW, Tichy M, Nixon PJ (2006) The FtsH protease slr0228 is important for quality control of photosystem II in the thylakoid membrane of Synechocystis sp. PCC 6803. J Biol Chem 281:1145-1151

Koornneef M, Meinke D (2010) The development of Arabidopsis as a model plant. Plant J 61:909-921

Kouril R, Dekker JP, Boekema EJ (2012) Supramolecular organization of photosystem II in green plants. Biochim Biophys Acta 1817:2-12

Kramer DM, Sacksteder CA, Cruz JA (1999) How acidic is the lumen? Photosynth Res 60:151-163

Krysan PJ, Young JC, Sussman MR (1999) T-DNA as an Insertional Mutagen in Arabidopsis. Plant Cell 11:2283-2290

Lemeille S, Willig A, Depege-Fargeix N, Delessert C, Bassi R, Rochaix JD (2009) Analysis of the chloroplast protein kinase Stt7 during state transitions. PLoS Biol 7:e45

Li X-P, Bjorkman O, Shih C, Grossman AR, Rosenquist M, Jansson S, Niyogi KK (2000) A pigment-binding protein essential for regulation of photosynthetic light harvesting. Nature 403:391-395

Li XP, Gilmore AM, Caffarri S, Bassi R, Golan T, Kramer D, Niyogi KK (2004) Regulation of photosynthetic light harvesting involves intrathylakoid lumen pH sensing by the PsbS protein. J Biol Chem 279:22866-22874

Lohrig K, Muller B, Davydova J, Leister D, Wolters DA (2009) Phosphorylation site mapping of soluble proteins: bioinformatical filtering reveals potential plastidic phosphoproteins in Arabidopsis thaliana. Planta 229:1123-1134

Lundin B, Hansson M, Schoefs B, Vener AV, Spetea C (2007a) The Arabidopsis PsbO2 protein regulates dephosphorylation and turnover of the photosystem II reaction centre D1 protein. Plant J 49:528-539

Lundin B, Nurmi M, Rojas-Stuetz M, Aro EM, Adamska I, Spetea C (2008) Towards understanding the functional difference between the two PsbO isoforms in Arabidopsis thaliana--insights from phenotypic analyses of psbo knockout mutants. Photosynth Res 98:405-414

Lundin B, Thuswaldner S, Shutova T, Eshaghi S, Samuelsson G, Barber J, Andersson B, Spetea C (2007b) Subsequent events to GTP binding by the plant PsbO protein: structural changes, GTP hydrolysis and dissociation from the photosystem II complex. Biochim Biophys Acta 1767:500-508

Mann M, Hendrickson RC, Pandey A (2001) Analysis of proteins and proteomes by mass spectrometry. Annu Rev Biochem 70:437-473

Meinke DW, Cherry JM, Dean C, Rounsley SD, Koornneef M (1998) Arabidopsis thaliana: a model plant for genome analysis. Science 282:662, 679-682

Melis A (1999) Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage ? Trends Plant Sci 4:130-135

Mulo P (2011) Chloroplast-targeted ferredoxin-NADP+ oxidoreductase (FNR): Structure, function and location. Biochim Biophys Acta Bioenergetics 1807:927-934

Murakami R, Ifuku K, Takabayashi A, Shikanai T, Endo T, Sato F (2005) Functional dissection of two Arabidopsis PsbO proteins: PsbO1 and PsbO2. FEBS J 272:2165-2175

Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI (2007) Photoinhibition of photosystem II under environmental stress. Biochim Biophys Acta 1767:414-421

Müller P, Li X-P, Niyogi KK (2001) Non-Photochemical Quenching. A Response to Excess Light Energy. Plant Physiol125:1558-1566

Napiwotzki A, Bergmann A, Decker K, Legall H, Eckert H-J, Eichle H-J, Renger G (1997) Acceptor side photoinhibition in PS II: On the possible effects of the functional integrity of the PS II donor side on photoinhibition of stable charge separation. Photosynth Res 52:199-213

Nath K, Jajoo A, Poudyal RS, Timilsina R, Park YS, Aro EM, Nam HG, Lee CH (2013a) Towards a critical understanding of the photosystem II repair mechanism and its regulation during stress conditions. FEBS Lett 587(21):3372-81

Nath K, Jajoo A, Poudyal RS, Timilsina R, Park YS, Aro EM, Nam HG, Lee CH (2013b) Towards a critical understanding of the photosystem II repair mechanism and its regulation during stress conditions. FEBS Lett 587:3372-3381

Navabpour S, Morris K, Allen R, Harrison E, S AH-M, Buchanan-Wollaston V (2003) Expression of senescence-enhanced genes in response to oxidative stress. J Exp Bot 54:2285-2292

Nevo R, Charuvi D, Tsabari O, Reich Z (2012) Composition, architecture and dynamics of the photosynthetic apparatus in higher plants. Plant J 70:157-176

Nilkens M, Kress E, Lambrev P, Miloslavina Y, Muller M, Holzwarth AR, Jahns P (2010) Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated under steady-state conditions in Arabidopsis. Biochim Biophys Acta 1797:466-475

Nishiyama Y, Yamamoto H, Allakhverdiev SI, Inaba M, Yokota A, Murata N (2001) Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery. EMBO J 20:5587-5594

Nixon PJ, Barker M, Boehm M, de Vries R, Komenda J (2005) FtsH-mediated repair of the photosystem II complex in response to light stress. J Exp Bot 56:357-363

Nixon PJ, Michoux F, Yu J, Boehm M, Komenda J (2010) Recent advances in understanding the assembly and repair of photosystem II. Ann Bot 106:1-16

Niyogi KK (1999) Photoprotection revisited: Genetic and Molecular Approaches. Annu Rev Plant Physiol Plant Mol Biol 50:333-359

Ohnishi N, Allakhverdiev SI, Takahashi S, Higashi S, Watanabe M, Nishiyama Y, Murata N (2005) Two-step mechanism of photodamage to photosystem II: step 1 occurs at the oxygen-evolving complex and step 2 occurs at the photochemical reaction center. Biochemistry 44:8494-8499

Orr L, Govindjee (2010) Photosynthesis online. Photosynth Res 105:167-200

Pagliano C, Saracco G, Barber J (2013) Structural, functional and auxiliary proteins of photosystem II. Photosynth Res 116:167-188

Palmieri F, Pierri CL, De Grassi A, Nunes-Nesi A, Fernie AR (2011) Evolution, structure and function of mitochondrial carriers: a review with new insights. Plant J 66:161-181

Pandey A, Andersen JS, Mann M (2000) Use of mass spectrometry to study signaling pathways. Sci STKE 2000:pl1

Pavon LR, Lundh F, Lundin B, Mishra A, Persson BL, Spetea C (2008) Arabidopsis ANTR1 is a thylakoid Na+-dependent phosphate transporter: functional characterization in Escherichia coli. J Biol Chem 283:13520-13527

Peltier JB, Emanuelsson O, Kalume DE, Ytterberg J, Friso G, Rudella A, Liberles DA, Soderberg L, Roepstorff P, von Heijne G, van Wijk KJ (2002) Central functions of the lumenal and peripheral thylakoid proteome of Arabidopsis determined by experimentation and genome-wide prediction. Plant Cell 14:211-236

Peng L, Shikanai T (2011) Supercomplex formation with photosystem I is required for the stabilization of the chloroplast NADH dehydrogenase-like complex in Arabidopsis. Plant Physiol 155:1629-1639

Pesaresi P, Pribil M, Wunder T, Leister D (2011) Dynamics of reversible protein phosphorylation in thylakoids of flowering plants: the roles of STN7, STN8 and TAP38. Biochim Biophys Acta 1807:887-896

Poetsch A, Berzborn RJ, Heberle J, Link TA, Dencher NA, Seelert H (2007) Biophysics and bioinformatics reveal structural differences of the two peripheral stalk subunits in chloroplast ATP synthase. J Biochem 141:411-420

Pribil M, Pesaresi P, Hertle A, Barbato R, Leister D (2010) Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow. PLoS Biol 8:e1000288

Puthiyaveetil S (2011) A mechanism for regulation of chloroplast LHC II kinase by plastoquinol and thioredoxin. FEBS Lett 585:1717-1721

Puthiyaveetil S, Ibrahim IM, Jelicic B, Tomasic A, Fulgosi H, Allen JF (2010) Transcriptional control of photosynthesis genes: the evolutionarily conserved regulatory mechanism in plastid genome function. Genome Biol Evol 2:888-896

Puthiyaveetil S, Kavanagh TA, Cain P, Sullivan JA, Newell CA, Gray JC, Robinson C, van der Giezen M, Rogers MB, Allen JF (2008) The ancestral symbiont sensor kinase CSK links photosynthesis with gene expression in chloroplasts. Proc Natl Acad Sci U S A 105:10061-10066

Reiland S, Finazzi G, Endler A, Willig A, Baerenfaller K, Grossmann J, Gerrits B, Rutishauser D, Gruissem W, Rochaix JD, Baginsky S (2011) Comparative phosphoproteome profiling reveals a function of the STN8 kinase in fine-tuning of cyclic electron flow (CEF). Proc Natl Acad Sci U S A 108:12955-12960

Reiland S, Messerli G, Baerenfaller K, Gerrits B, Endler A, Grossmann J, Gruissem W, Baginsky S (2009) Large-scale Arabidopsis phosphoproteome profiling reveals novel chloroplast kinase substrates and phosphorylation networks. Plant Physiol 150:889-903

Richter M, Samra H, He F, Giessel A, Kuczera K (2005) Coupling Proton Movement to ATP Synthesis in the Chloroplast ATP Synthase. J Bioenerg Biomembr37:467-473

Rintamaki E, Martinsuo P, Pursiheimo S, Aro EM (2000) Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin-thioredoxin system in chloroplasts. Proc Natl Acad Sci U S A 97:11644-11649

Rintamaki E, Salonen M, Suoranta UM, Carlberg I, Andersson B, Aro EM (1997) Phosphorylation of light-harvesting complex II and photosystem II core proteins shows different irradiance-dependent regulation in vivo. Application of phosphothreonine antibodies to analysis of thylakoid phosphoproteins. J Biol Chem 272:30476-30482

Rolland N, Ferro M, Seigneurin-Berny D, Garin J, Douce R, Joyard J (2003) Proteomics of chloroplast envelope membranes. Photosynth Res 78:205-230

Ruban AV, Johnson MP (2010) Xanthophylls as modulators of membrane protein function. Arch Biochem Biophys 504:78-85

Samol I, Shapiguzov A, Ingelsson B, Fucile G, Crevecoeur M, Vener AV, Rochaix JD, Goldschmidt-Clermont M (2012) Identification of a photosystem II phosphatase involved in light acclimation in Arabidopsis. Plant Cell 24:2596-2609

Schubert M, Petersson UA, Haas BJ, Funk C, Schroder WP, Kieselbach T (2002) Proteome map of the chloroplast lumen of Arabidopsis thaliana. J Biol Chem 277:8354-8365

Schwarte S, Tiedemann R (2011) A gene duplication/loss event in the ribulose-1,5-bisphosphate-carboxylase/oxygenase (rubisco) small subunit gene family among accessions of Arabidopsis thaliana. Mol Biol Evol 28:1861-1876

Shapiguzov A, Ingelsson B, Samol I, Andres C, Kessler F, Rochaix JD, Vener AV, Goldschmidt-Clermont M (2010) The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in Arabidopsis. Proc Natl Acad Sci U S A 107:4782-4787

Spetea C, Lundin B (2012) Evidence for nucleotide-dependent processes in the thylakoid lumen of plant chloroplasts--an update. FEBS Lett 586:2946-2954

Spetea C, Pfeil BE, Schoefs B (2011) Phylogenetic Analysis of the Thylakoid ATP/ADP Carrier Reveals New Insights into Its Function Restricted to Green Plants. Front Plant Sci 2:110

Sugiyama N, Nakagami H, Mochida K, Daudi A, Tomita M, Shirasu K, Ishihama Y (2008) Large-scale phosphorylation mapping reveals the extent of tyrosine phosphorylation in Arabidopsis. Mol Syst Biol 4:193

Sun Q, Zybailov B, Majeran W, Friso G, Olinares PD, van Wijk KJ (2009) PPDB, the Plant Proteomics Database at Cornell. Nucleic Acids Res 37:D969-974

Syka JE, Coon JJ, Schroeder MJ, Shabanowitz J, Hunt DF (2004) Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. Proc Natl Acad Sci U S A 101:9528-9533

Süss K-H, Schmidt O (1982) Evidence for an α3, β3, γ, δ, I, II, ε, III5 subunit stoichiometry of chloroplast ATP synthetase complex (CF1−CF0). FEBS Lett 144:213-218

Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T, Matsuo T (1988) Protein and polymer analyses up to m/z 100 000 by laser ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectr2:151-153

Thuswaldner S, Lagerstedt JO, Rojas-Stutz M, Bouhidel K, Der C, Leborgne-Castel N, Mishra A, Marty F, Schoefs B, Adamska I, Persson BL, Spetea C (2007) Identification, expression, and functional analyses of a thylakoid ATP/ADP carrier from Arabidopsis. J Biol Chem 282:8848-8859

Tikhonov AN (2013a) The cytochrome bf complex at the crossroad of photosynthetic electron transport pathways. Plant Physiol Biochem http://dx.doi.org/10.1016/j.plaphy.2013.12.011Tikhonov AN (2013b) pH-dependent regulation of electron transport and ATP synthesis in chloroplasts. Photosynth Res 116:511-534

Tikkanen M, Aro EM (2012) Thylakoid protein phosphorylation in dynamic regulation of photosystem II in higher plants. Biochim Biophys Acta 1817:232-238

Tikkanen M, Nurmi M, Kangasjarvi S, Aro EM (2008) Core protein phosphorylation facilitates the repair of photodamaged photosystem II at high light. Biochim Biophys Acta 1777:1432-1437

Tikkanen M, Piippo M, Suorsa M, Sirpio S, Mulo P, Vainonen J, Vener AV, Allahverdiyeva Y, Aro EM (2006) State transitions revisited-a buffering system for dynamic low light acclimation of Arabidopsis. Plant Mol Biol 62:779-793

Triantaphylides C, Havaux M (2009) Singlet oxygen in plants: production, detoxification and signaling. Trends Plant Sci 14:219-228

Trontin C, Tisne S, Bach L, Loudet O (2011) What does Arabidopsis natural variation teach us (and does not teach us) about adaptation in plants? Curr Opin Plant Bio14:225-231

Tyystjärvi E, Aro EM (1996) The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity. Proc Nat Acad Sci USA 93:2213-2218

Umena Y, Kawakami K, Shen JR, Kamiya N (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 A. Nature 473:55-60

Vainonen JP, Hansson M, Vener AV (2005) STN8 protein kinase in Arabidopsis thaliana is specific in phosphorylation of photosystem II core proteins. J Biol Chem 280:33679-33686

Vainonen JP, Sakuragi Y, Stael S, Tikkanen M, Allahverdiyeva Y, Paakkarinen V, Aro E, Suorsa M, Scheller HV, Vener AV, Aro EM (2008) Light regulation of CaS, a novel phosphoprotein in the thylakoid membrane of Arabidopsis thaliana. FEBS J 275:1767-1777

van den Brink-van der Laan E, Killian JA, de Kruijff B (2004) Nonbilayer lipids affect peripheral and integral membrane proteins via changes in the lateral pressure profile. Biochim Biophys Acta 1666:275-288

van Wijk KJ, Baginsky S (2011) Plastid proteomics in higher plants: current state and future goals. Plant Physiol 155:1578-1588

Vass I, Styring S, Hundal T, Koivuniemi A, Aro E, Andersson B (1992) Reversible and irreversible intermediates during photoinhibition of photosystem II: stable reduced QA species promote chlorophyll triplet formation. Proc Natl Acad Sci U S A 89:1408-1412

Weigel D (2012) Natural variation in Arabidopsis: from molecular genetics to ecological genomics. Plant Physiol 158:2-22

Weigel D, Nordborg M (2005) Natural Variation in Arabidopsis. How Do We Find the Causal Genes? Plant Physiol 138:567-568

Vener AV, Ohad I, Andersson B (1998) Protein phosphorylation and redox sensing in chloroplast thylakoids. Curr Opin Plant Biol 1:217-223

Vener AV, Van Kan PJ, Gal A, Andersson B, Ohad I (1995) Activation/deactivation cycle of redox-controlled thylakoid protein phosphorylation. Role of plastoquinol bound to the reduced cytochrome bf complex. J Biol Chem 270:25225-25232

Vener AV, van Kan PJ, Rich PR, Ohad I, Andersson B (1997) Plastoquinol at the quinol oxidation site of reduced cytochrome bf mediates signal transduction between light and protein phosphorylation: thylakoid protein kinase deactivation by a single-turnover flash. Proc Natl Acad Sci U S A 94:1585-1590

Verhoeven A, Osmolak A, Morales P, Crow J (2009) Seasonal changes in abundance and phosphorylation status of photosynthetic proteins in eastern white pine and balsam fir. Tree Physiol 29:361-374

Wientjes E, Drop B, Kouril R, Boekema EJ, Croce R (2013a) During state 1 to state 2 transition in Arabidopsis thaliana the Photosystem II supercomplex gets phosphorylated but does not disassemble. J Biol Chem 288:32821-6

Wientjes E, van Amerongen H, Croce R (2013b) LHCII is an antenna of both photosystems after long-term acclimation. Biochim Biophys Acta 1827:420-426

Willig A, Shapiguzov A, Goldschmidt-Clermont M, Rochaix JD (2011) The phosphorylation status of the chloroplast protein kinase STN7 of Arabidopsis affects its turnover. Plant Physiol 157:2102-2107

Vinyard DJ, Ananyev GM, Dismukes GC (2013) Photosystem II: the reaction center of oxygenic photosynthesis. Annu Rev Biochem 82:577-606

Vlad D, Rappaport F, Simon M, Loudet O (2010) Gene transposition causing natural variation for growth in Arabidopsis thaliana. PLoS Genet 6:e1000945

WuHY, Liu MS, Lin TP, Cheng YS (2011) Structural and functional assays of AtTLP18.3 identify its novel acid phosphatase activity in thylakoid lumen. Plant Physiol 157:1015-1025

WuW, Berkowitz GA (1992) Stromal pH and Photosynthesis Are Affected by Electroneutral K and H Exchange through Chloroplast Envelope Ion Channels. Plant Physiol 98:666-672

Xu J, Yang J, Wu Z, Liu H, Huang F, Wu Y, Carrie C, Narsai R, Murcha M, Whelan J, Wu P (2013) Identification of a dual-targeted protein belonging to the mitochondrial carrier family that is required for early leaf development in rice. Plant Physiol 161:2036-2048

Yamamoto HY, Kamite L (1972) The effects of dithiothreitol on violaxanthin de-epoxidation and absorbance changes in the 500-nm region. Biochim Biophys Acta 267:538-543

Yamamoto Y, Kai S, Ohnishi A, Tsumura N, Ishikawa T, Hori H, Morita N, Ishikawa Y (2014) Quality Control of Photosystem II: Behavior of Photosystem II in the Highly Crowded Grana Thylakoids under Excessive Light. Plant Cell Physiol

Yi X, Hargett SR, Liu H, Frankel LK, Bricker TM (2007) The PsbP protein is required for photosystem II complex assembly/stability and photoautotrophy in Arabidopsis thaliana. J Biol Chem 282:24833-24841

Yoshioka M, Yamamoto Y (2011) Quality control of Photosystem II: Where and how does the degradation of the D1 protein by FtsH proteases start under light stress? – Facts and hypotheses. J Photochem Photobiol B: Biol 104:229-235

Zito F, Finazzi G, Delosme R, Nitschke W, Picot D, Wollman FA (1999) The Qo site of cytochrome b6f complexes controls the activation of the LHCII kinase. EMBO J 18:2961-2969