Turun yliopisto
University of Turku

MOLECULAR MECHANISMS AND INTERACTIONS IN REGULATION OF PHOTOSYNTHETIC REACTIONS

Markus Nurmi

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. AI osa - tom. 493 | Astronomica - Chemica - Physica - Mathematica | Turku 2014

University of Turku

Faculty of Mathematics and Natural Sciences
Department of Biochemistry
From the Laboratory of Molecular Plant Biology

Supervised by

Professor Eva-Mari Aro
Dr. Saijaliisa Kangasjärvi
Laboratory of Molecular Plant Biology
Department of Biochemistry
University of Turku
FI-20014 Turku, Finland

Reviewed by

Professor Wolfgang Schröder
Department of Chemistry
University of Umeå
SE-90187 Umeå, Sweden

Dr. Julia Vainonen
Department of Biosciences
University of Helsinki
FI-00014 Helsinki, Finland

Opponent

Professor Anja Krieger-Liszkay
Laboratory of Fundamental Mechanisms
in Bioenergetics
Institute of Biology and Technology Saclay
F-91191 Gif-sur-Yvette Cedex, France

ISBN 978-951-29-5855-9 (PRINT)
ISBN 978-951-29-5856-6 (PDF)
ISSN 0082-7002
Painosalama Oy - Turku, Finland 2014

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred in the text by their Roman numerals.

I 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. Photosynthesis Research, 98:405-414

II Allahverdiyeva Y, Mamedov F, Holmström M, Nurmi M, Lundin B, Styring S, Spetea C, Aro EM (2009) Comparison of the electron transport properties of the *psbo1* and *psbo2* mutants of *Arabidopsis thaliana*. Biochimica et Biophysica Acta, 1787:1230-1237

III Suorsa M, Järvi S, Grieco M, Nurmi M, Pietrzykowska M, Rantala M, Kangasjärvi S, Paakkarinen V, Tikkanen M, Jansson S, Aro EM. (2012) PROTON GRADIENT REGULATION5 is essential for proper acclimation of Arabidopsis photosystem I to naturally and artificially fluctuating light conditions. The Plant Cell 24:2934-2948

IV Nurmi M, Kangasjärvi S, Hüdig M, Järvi S, Suorsa M, Grouneva I, Tikkanen M, Weinl S and Aro EM (2014) Calcium sensing receptor CaS – a regulatory link between the light and carbon reduction reactions? Manuscript.

*Equal contributions

ABBREVIATIONS

2D	Two-dimensional
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ATPase	ATP synthase
BiFC	Bimolecular fluorescence complementation
BN	Blue Native
CaS	Calcium sensing receptor
CBB	Calvin-Benson-Bassham
CET	Cyclic electron transfer
CL	Constant light
Col	Columbia ecotype
CP	Chlorophyll binding protein
Cyt b_6f	Cytochrome b_6f complex
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
ΔpH	Transthylakoid proton gradient
DIGE	Differential in gel electrophoresis
ECS	Electrochromic shift
FD	Ferredoxin
FL	Fluctuating light
F_m	Maximal chlorophyll fluorescence in dark adapted leaf
FNR	Ferredoxin-$NADP^+$ reductase
F_v	Variable fluorescence
GAPA/GAPB	Glyceraldehyde 3-phosphate dehydrogenase subunit A/B
GDP	Guanosine diphosphate
GL	Growth light
GTP	Guanosine triphosphate
IEF	Isoelectric focusing
HL	High light intensity

LET	Linear electron transfer
LHC	Light harvesting complex
MS	Mass spectrometry
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced $NADP^+$
NDH	NAD(P)H dehydrogenase-like complex
NO	Nitric oxide
NPQ	Non-photochemical quenching of excitation energy
OEC	Oxygen evolving complex
P680	Reaction center chlorophyll of PSII
P700	Reaction center chlorophyll of PSI
PAGE	Polyacrylamide gel electrophoresis
PAM	Pulse amplitude modulation
PC	Plastocyanin
PGR5	Proton gradient regulation 5
PGRL1	PGR5-like photosynthetic phenotype 1
pI	Isoelectric point
pmf	Proton motive force
PQ	Plastoquinone
PQH_2	Plastoquinol
PRK	Phosphoribulokinase
PS	Photosystem
PsbO	Photosystem II subunit O
P-Thr	Phosphothreonine
PTOX	plastid terminal oxidase
ROS	Reactive oxygen species
Rubisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
STN7	State transition 7 kinase
STN8	State transition 8 kinase
T-DNA	Transferred DNA
WT	Wild-type

ABSTRACT

In photosynthesis, light energy is converted to chemical energy, which is consumed for carbon assimilation in the Calvin-Benson-Bassham (CBB) cycle. Intensive research has significantly advanced the understanding of how photosynthesis can survive in the ever-changing light conditions. However, precise details concerning the dynamic regulation of photosynthetic processes have remained elusive. The aim of my thesis was to specify some molecular mechanisms and interactions behind the regulation of photosynthetic reactions under environmental fluctuations.

A genetic approach was employed, whereby *Arabidopsis thaliana* mutants deficient in specific photosynthetic protein components were subjected to adverse light conditions and assessed for functional deficiencies in the photosynthetic machinery. I examined three interconnected mechanisms: (i) auxiliary functions of PsbO1 and PsbO2 isoforms in the oxygen evolving complex of photosystem II (PSII), (ii) the regulatory function of PGR5 in photosynthetic electron transfer and (iii) the involvement of the Calcium Sensing Receptor CaS in photosynthetic performance.

Analysis of photosynthetic properties in *psbo1* and *psbo2* mutants demonstrated that PSII is sensitive to light induced damage when PsbO2, rather than PsbO1, is present in the oxygen evolving complex. PsbO1 stabilizes PSII more efficiently compared to PsbO2 under light stress. However, PsbO2 shows a higher GTPase activity compared to PsbO1, and plants may partially compensate the lack of PsbO1 by increasing the rate of the PSII repair cycle.

PGR5 proved vital in the protection of photosystem I (PSI) under fluctuating light conditions. Biophysical characterization of photosynthetic electron transfer reactions revealed that PGR5 regulates linear electron transfer by controlling proton motive force, which is crucial for the induction of the photoprotective non-photochemical quenching and the control of electron flow from PSII to PSI. I conclude that PGR5 controls linear electron transfer to protect PSI against light induced oxidative damage.

I also found that PGR5 physically interacts with CaS, which is not needed for photoprotection of PSII or PSI in higher plants. Rather, transcript profiling and quantitative proteomic analysis suggested that CaS is functionally connected with the CBB cycle. This conclusion was supported by lowered amounts of specific calcium-regulated CBB enzymes in *cas* mutant chloroplasts and by slow electron flow to PSI electron acceptors when leaves were reilluminated after an extended dark period. I propose that CaS is required for calcium regulation of the CBB cycle during periods of darkness. Moreover, CaS may also have a regulatory role in the activation of chloroplast ATPase.

Through their diverse interactions, components of the photosynthetic machinery ensure optimization of light-driven electron transport and efficient basic production, while minimizing the harm caused by light induced photodamage.

TIIVISTELMÄ

Fotosynteesissä valon energia muunnetaan kemialliseksi energiaksi, jota käytetään hiilensidontaan Calvin-Benson-Bassham (CBB) kierron reaktioissa. Viimeaikainen tutkimus on kasvattanut merkittävästi tietämystä siitä, miten fotosynteesi kykenee vastaamaan vaihtelevien olosuhteiden asettamiin haasteisiin. Kuitenkin yksityiskohtainen tietämys fotosynteesin säätelystä on suurilta osin ratkaisematta. Väitöskirjatyöni tavoitteena on täsmentää molekulaarisia mekanismeja vaihtelevissa ympäristöolosuhteissa tapahtuvan fotosynteesin taustalla.

Lähestyin ongelmaa käyttäen *Arabidopsis thaliana* mutantteja, joilta puuttuvat tietyt fotosynteesissä tärkeät proteiinit ja altistin nämä mutantit haastaviin valo-olosuhteisiin. Tutkimuksessani keskityin kolmeen toisiinsa yhteydessä olevaan mekanismiin: (i) Valoreaktio kahden (PSII) happea vapauttavan kompleksin isoformien PsbO1:n ja PsbO2:n avustaviin tehtäviin, (ii) PGR5:n tehtävään fotosynteesin elektroninsiirtoketjun säätelyssä ja (iii) kalsiumia tunnistavan reseptorin (CaS) rooliin fotosynteesissä.

psbo1 ja *psbo2* mutanttien fotosynteettisten ominaisuuksien analyysi paljasti PSII:n valoherkkyyden kasvavan, mikäli PSII sisälsi vain PsbO2 isoformia. Havaitsin PsbO1:n vakauttavan PSII:n rakennetta tehokkaammin verrattuna PsbO2:en kun kasvit altistettiin voimakkaalle valolle. Kuitenkin, PsbO2:lla on suurempi GTPaasi aktiivisuus verrattuna PsbO1:en ja kasvit voivat osittain korvata PsbO1:n puuttumisen lisäämällä PSII:n korjauskierron tehokkuutta.

PGR5 osoittautui elintärkeäksi Valoreaktio yhden (PSI) suojelemisessa vaihtelevissa valo-olosuhteissa. Biofysikaalisten mittausten perusteella voitiin todeta PGR5:n säätelevän lineaarista elektroninsiirtoa kontrolloimalla protonigradienttia tylakoidin yli, mikä on tärkeää ei-fotokemiallisen energiavaimennuksen aktivoimisessa sekä elektronivirran säätelyssä PSII:lta PSI:lle. Täten päättelin PGR5:n säätelevän lineaarista elektroninsiirtoa ja olevan tärkeä PSI:n suojaamisessa valon aiheuttamaa oksidatiivista vahinkoa vastaan.

Lisäksi osoitin PGR5:n olevan fysikaalisessa vuorovaikutuksessa CaS- proteiinin kanssa, joka levistä poiketen on kasveilla tarpeeton PSII:n ja PSI:n suojelussa. Transkriptien profilointi ja kvantitatiivinen proteiinien analysointi paljastivat CaS:n liittyvän toiminnallisesti CBB -syklin toimintaan. Ajatusta vahvisti kalsium-säädeltyjen CBB –entsyymien vähäisempi määrä *cas* mutantin viherhiukkasissa sekä hitaampi elektronien siirtyminen PSI:n elektroninvastaanottajille kun kasvien lehdet olivat ennen valotusta sopeutuneet pimeään. Täten ehdotan CaS:n toimivan kalsium välitteisessä CBB –syklin säätelyssä. Tämän lisäksi, CaS saattaa osallistua ATP syntaasin aktivaation säätelyyn.

Fotosynteesikoneiston komponenttien monipuolinen yhteistyö varmistaa ihanteellisen elektronisiirron sekä tehokkaan perustuotannon samalla vähentäen valon aiheuttamia vahinkoja fotosynteettiselle koneistolle.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 Photosynthesis

Evolution made a huge leap forward 2.5 to 3.5 billion years ago, when oxygenic photosynthesis first began. At this time, ancient cyanobacteria changed the earth's atmosphere by producing oxygen that later formed an ozone layer in the stratosphere, thus preventing harmful UV-radiation from reaching the surface of the earth. This made it possible for life to spread from water to land. By increasing the oxygen concentration in air, photosynthesis enabled the development of modern oxygen consuming organisms. Nowadays, photosynthesis produces nearly all energy needed by living organisms (Hohmann-Marriott and Blankenship, 2011).

In photosynthesis, light energy is captured with light absorbing pigments and is subsequently converted to chemical energy in the form of adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH). These high energy molecules, together with CO_2, are further used to produce carbohydrates for energy storage and building molecules. In higher plants, photosynthetic reactions take place in an organelle known as the chloroplast. According to the theory of endosymbiosis, chloroplasts originate from ancient cyanobacteria. Throughout evolution, most of the cyanobacterial genome has been transferred to the plant nucleus, with only a minor part still remaining in chloroplast (Dorrell and Howe, 2012). The chloroplast has three membranes that enclose soluble compartments. The outer and inner membranes form the chloroplast envelope, which separates the chloroplast from the surrounding cell (Inoue, 2011). The thylakoid membrane forms a flexible membrane system where the photosynthetic light reactions take place. The soluble compartment surrounded by the inner membrane is called the chloroplast stroma, whereas the thylakoid membrane encloses a soluble lumen compartment (Vothknecht and Westhoff, 2001).

1.2 Thylakoid protein complexes

The thylakoid membrane harbors five multiprotein complexes involved in photosynthetic light reactions: Photosystem II (PSII); Cytochrome b_6f (Cyt b_6f); Photosystem I (PSI); ATP synthase (ATPase); and NAD(P)H dehydrogenase (NDH) – like complex. These complexes form the site of the light driven reactions of photosynthesis. Structural and functional analysis of photosynthetic light reactions has produced a wealth of high-resolution information concerning the structural organization, function and regulation of the photosynthetic complexes (Nevo, et al., 2012; Kouril, et al., 2012; Horton, 2012). Although these studies have revealed key mechanisms underlying the dynamic regulation of photosynthetic apparatus, a comprehensive understanding of photosynthetic regulation has yet to be realized (Kirchhoff, 2014; Järvi, et al., 2013; Rochaix, et al., 2012; Tikkanen, et al., 2012).

PSII is a multi-subunit complex that uses the energy gained from light to split water to protons and oxygen. PSII releases protons to the lumen to drive the production of ATP and feeds electrons to electron transfer chain. The PSII core contains seven proteins: D1, D2, CP43, CP47, PsbE, PsbD and PsbI. The first four of these proteins bind chlorophyll and form the inner antenna system of PSII. D1 binds the important electron acceptors pheophytin and the plastoquinone Q_B, whilst D2 binds the electron acceptor plastoquinone Q_A. The reaction center chlorophylls of D1 and D2 are both called P680, in reference to their absorption maxima. CP43 and CP47 proteins are required for binding the oxygen evolving complex (OEC) on the lumenal side of PSII. OEC contains the Mn_4Ca cluster and three OEC stabilizing proteins PsbO, PsbP and PsbQ in higher plants. In cyanobacteria, PsbP and PsbQ are substituted by PsbU and PsbV, which differ in protein structure compared to their plant-based counterparts (Ifuku, et al., 2010). The PSII supercomplex is surrounded by an outer antenna formed by six different LHCB proteins. The main proteins at the PSII outer antenna include LHCB1, LHCB2 and LHCB3, which form trimers called light harvesting complex II (LHCII). Three minor chlorophyll binding (CP) antenna proteins, CP24, CP26 and CP29, are located between LHCII and PSII. In addition, PSII contains over 20 other subunits, which are mainly involved in the assembly, stabilization, dimerization and photoprotection of PSII (Kouril, et al., 2012; Barber, 2012; Vinyard, et al., 2013).

Cyt b_6f mediates electron flow from PSII to PSI, along with the concomitant transfer of protons from the stroma to the lumen in a process called the Q-cycle. Together with PSII, Cyt b_6f forms a proton gradient across the thylakoid membrane. This proton gradient is used to drive the formation of ATP. Cyt b_6f is present as a dimer in the thylakoid membrane. It is built up from four major and four minor subunits. The major subunits are Cytochrome b_6 (Cyt b_6) retaining b-type heme groups, Cytochrome *f* (Cyt *f*) retaining c-type heme-groups, Rieske retaining a 2Fe-2S cluster and subunit IV. The four minor subunits are PetG, PetL, PetM and PetN (Baniulis, et al., 2008).

PSI is the second photosystem in the thylakoid membrane. Energy from photons harvested by pigment molecules excites the reaction center chlorophyll pair called P700. An electron from $P700^+$ is then transferred to quinone, and finally to the electron acceptor protein ferredoxin on stromal side of PSI. $P700^+$ is then reduced by the electrons from reduced plastocyanin, which is an electron donor of PSI. Later, the energy captured by PSI is used to build NADPH. Plant PSI contains 19 protein subunits. Two main proteins, PsaA and PsaB, contain the reaction center chlorophyll pair P700. PsaC contains two iron-sulfur clusters and together with PsaD and PsaE, forms a docking site for ferredoxin. Plastocyanin binds to the PsaF protein in the lumenal side of the thylakoid membrane. Four proteins: PsaH; PsaI; PsaL; and PsaO are essential for the docking of LHCII to PSI in a phenomena called state transitions (state 2), where part of phosphorylated LHCII is connected to PSI (Lunde, et al., 2000). As for PSII, PSI is surrounded by chlorophyll binding proteins, known as LHCI and comprised of four proteins: LHCA1, LHCA2, LHCA3 and LHCA4 (Kargul, et al., 2012).

ATP synthase converts the energy stored in proton electrochemical potential to the form of ATP and contains two main units: CF_0, the membrane embedded unit that

forms rotary torque during proton translocation; and CF_1, the water-soluble unit that works as an ATP generating motor to bond phosphorus to ADP molecules. CF_0 and CF_1 are connected via a stalk protein that keeps the structure together. Aside from these main components, ATP synthase also contains smaller proteins that regulate its functional status (Okuno, et al., 2011).

In higher plant thylakoids, the NDH -like complex shuttles electrons from ferredoxin to the plastoquinone (PQ) and thus functions in cyclic electron transfer (CET) around PSI (see 1.4.2.) and in chlororespiration (Kofer, et al., 1998; Sazanov, et al., 1998) and is shown to be important for protecting photosynthetic machinery, especially under stress conditions (Li, et al., 2004; Wang, et al., 2006). The NDH-like complex also plays a significant role in electron transfer in the bundle sheath cells of C4 plants (Takabayashi, et al., 2005). The higher plant NDH-like complex is structurally similar to mitochondrial complex I and cyanobacterial NDH-1 complex (Friedrich and Weiss, 1997). The chloroplast NDH-like complex consists of four distinct subcomplexes: the A; B; membrane localized; and lumen-localized subcomplexes (Ifuku, et al., 2011). Subcomplex A and the membrane localized subcomplex form a typical L-shaped backbone of the NDH-like complex. In cyanobacteria, the membrane localized complex acts to transfer protons over the thylakoid membrane and couples this with electron transfer in subcomplex A (Ifuku, et al., 2011). Subcomplex B and the lumenal subcomplex are unique to higher plants. Interestingly, the lumenal subcomplex contains several homologs of proteins belonging to the PSII oxygen evolving complex (Ifuku, et al., 2010). In addition to these, the existence of a fifth subcomplex, the electron donor subcomplex, has recently been proposed (Yamamoto, et al., 2011). This electron donor subcomplex interacts with subunit A and serves as a binding site for ferredoxin (Yamamoto, et al., 2011). The chloroplast NDH-like complex interacts with PSI through two minor PSI antenna proteins, Lhca5 and Lhca6 (Peng, et al., 2008; Peng, et al., 2009). Formation of the NDH-PSI supercomplex in higher plants is required for the stability of NDH, especially in high light conditions (Peng and Shikanai, 2011). However, the reason for the instability of the NDH-like complex without PSI remains unknown.

1.3 Lateral heterogeneity of the thylakoid membrane

Higher plant thylakoid membranes can be separated into three functionally distinct regions: the grana core; grana margins (edges of grana stacks); and stroma thylakoids, all of which exhibit different compositions of photosynthetic protein complexes. ATP synthase, NDH-like complex and PSI are enriched in stroma thylakoids, whereas the grana core contains predominantly PSII-LHCII supercomplexes and higher order megacomplexes. In grana margins, both PSI-LHCI and PSII-LHCII are located near each other (Danielsson, et al., 2004; Danielsson, et al., 2006). Over the past few decades, segregation of the thylakoid membrane into grana and stroma structures and the functional implications of doing so, have been matters of scientific debate. Several theories have been introduced to explain the need for lateral heterogeneity: (*i*) to control the excitation balance between the reaction centers and to avoid energy spill-

over to PSI (Trissl and Wilhelm, 1993); (*ii*) to separate linear and cyclic electron flow (Albertsson, 2001; Anderson and Aro, 1994); (*iii*) to avoid damage due to ROS produced by PSI and degradation of PSII during high light (Anderson and Aro, 1994; Tjus, et al., 2001); and (*iv*) to maintain the proper PSII repair cycle (Zhang, et al., 1999; Tikkanen, et al., 2012). However, no final evidence supporting any single theory has yet been provided. Furthermore, it has been recently shown that the grana structure is highly dynamic and shows both lateral and vertical rearrangements according to the prevailing light conditions (Herbstova, et al., 2012; Kirchhoff, 2013).

1.4 Photosynthetic electron transfer routes

Photosynthesis is functionally separated into two parts. Light driven electron transfer uses energy from photons to liberate electrons by splitting water molecules and releases oxygen as a byproduct. The reducing power of these electrons is used to form high energy molecules that are further used to fix inorganic carbon to organic carbohydrates in a series of reactions which form the Calvin-Benson-Bassham (CBB) cycle, the second part of photosynthesis. Photosynthetic electron transfer can be separated into two energy storing routes: the linear electron transfer (LET) route; and the cyclic electron transfer (CET) route. LET produces adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH), whereas CET produces only ATP.

1.4.1 Linear electron transfer

LET is the main electron transfer route in higher plant chloroplasts. LET begins when the energy of a photon is collected and transferred from the outer antenna to the reaction center chlorophyll *a*. In P680, this leads to charge separation whereby an electron from P680 is moved to pheophytin, the first electron acceptor of the LET route. From pheophytin, the electron is rapidly transferred to plastoquinone (Q_A), the first stable electron acceptor. From Q_A, a relatively slow transfer is made to Q_B, the second quinone electron acceptor. After Q_B has received two electrons from Q_A, it binds two protons from the stroma, forming plastohydroquinol (PQH_2), and leaves the D1 protein to dissolve in the lipid bilayer of the thylakoid membrane. P680 becomes a strong oxidant ($P680^+$), which gains an electron from water. Four electrons from the Mn_4Ca cluster, located in oxygen evolving complex (OEC), are sequentially extracted by $P680^+$. This is followed by the splitting of two water molecules into protons, oxygen and electrons. Electrons from the water molecules replace those primarily donated to $P680^+$ from the manganese cluster. The water splitting occurs in a process called the S-cycle (S_0-S_4), whereby four electrons are donated to $P680^+$ and four protons are released to the lumen.

The PQH_2 formed in the first part of the LET process moves out from PSII, binds to the lumenal side of Cyt b_6f and releases protons to the lumen. At the same time, PQH_2 donates one electron to cytochrome *f* through the Rieske iron-sulfur cluster and another one to cytochrome b_6. The electron from Cyt b_6 is transferred to another plastoquinone located in the stromal side of Cyt b_6f. After receiving two electrons, plastoquinone is protonated, moves to the lumenal side of Cyt b_6f and releases protons to the lumen.

Protons transferred from the stroma to the lumen create a proton motive force (pmf) that drives ATP formation in ATP synthase. Electrons donated by PQH_2 to Cyt *f* moves to a soluble electron carrier, plastocyanin (PC), which further donates electrons to PSI, where light has caused charge separation between excited P700 and chlorophyll A_0. The final step of the process is the transfer of electrons to ferredoxin, which reduces nicotinamide adenine dinucleotide phosphate ($NADP^+$) to NADPH, used later in the CBB cycle as reducing power (Figure 1).

1.4.2 Cyclic electron transfer

In addition to LET, CET around PSI also takes place in plant chloroplasts. CET shares common components with LET, including PSI, Cyt b_6f and NDH-like complexes, as well as the electron carriers, plastocyanin and ferredoxin. In higher plants, two distinct CET routes have been described, the NDH-dependent (or NDH-like) route and the proton gradient regulation 5 (PGR5)-mediated route (also known as ferredoxin-dependent or antimycin-sensitive CET). In NDH-dependent CET, it has been proposed that electrons are transferred from NADPH to the plastoquinone pool and further to PSI via Cyt b_6f (Shikanai, 2007). However, recent findings regarding the possible docking site of ferredoxin in the NDH-like complex suggest that electrons are actually transferred directly from ferredoxin to the NDH-like complex (Yamamoto, et al., 2011). In the PGR5-mediated CET route, electrons are moved directly from ferredoxin to the plastoquinone pool (Figure 1). Whilst the exact mechanism of this route is still unknown, two proteins that are involved have been characterized as PGR5 and PGR5-like photosynthetic phenotype 1 (PGRL1) (Munekage, et al., 2002; DalCorso, et al., 2008). These proteins are capable of forming a complex and are possibly involved in partitioning electron transfer between LET and CET (DalCorso, et al., 2008).

The exact role and importance of CET in C3 plants has yet to be convincingly characterized. However, CET seems to support at least two different functions: (*i*) balancing of the NADPH/ATP ratio to meet metabolic demands in chloroplasts under changing environments; and (*ii*) the induction of the transthylakoid proton gradient necessary for NPQ (see 1.7).

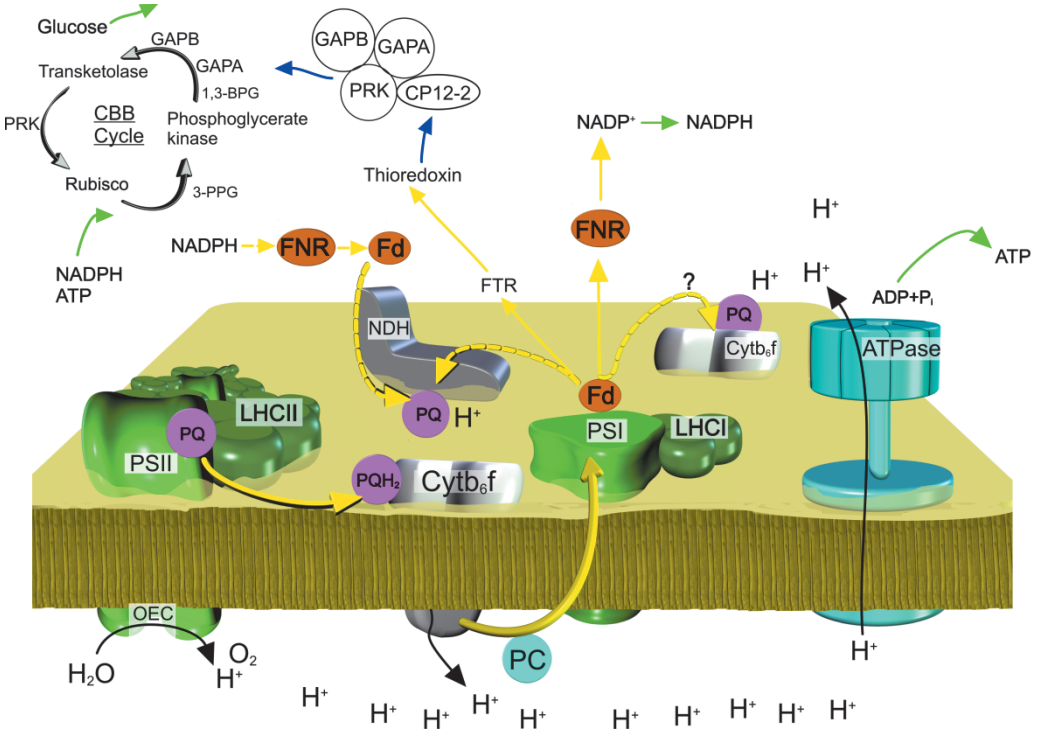

Figure 1. Linear and cyclic routes of the photosynthetic electron transfer. Linear electron transfer is indicated by yellow arrows, cyclic electron transfer by yellow dashline arrows and proton movement by black arrows. Blue lines represent the activation of proteins/enzymatic reactions. Green lines represent the intake or output of molecules. The electron route in Antimycin A-sensitive cyclic electron transfer is still unclear (marked with "?"). PSII=photosystem II; OEC=oxygen evolving complex; LHCII=light harvesting complex II; PQ=plastoquinone; PQH_2=plastoquinol; Cyt b_6f=cytochrome b_6f; PSI=photosystem I; LHCI=light harvesting complex I; FD=ferredoxin; ATPase=thylakoid ATP synthase; ADP=adenosine diphosphate; P_i=inorganic phosphate; ATP=adenosine triphosphate; FTR=ferredoxin-thioredoxin reductase; FNR=ferredoxin-$NADP^+$ reductase; $NADP^+$=nicotinamide adenine dinucleotide phosphate; NADPH=reduced nicotinamide adenine dinucleotide phosphate; NDH=NAD(P)H dehydrogenase-like complex; GAPA/GAPB=glyceraldehyde 3-phosphate dehydrogenase subunit A/B; PRK=phosphoribulokinase.

1.5 Assembly of PSII and OEC

The synthesis and assembly of the photosynthetic protein complex PSII is a multistep process that involves at least 20 different subunits as well as the incorporation of a variety of inorganic and organic cofactors. Thus, PSII assembly is a highly organized process which requires large numbers of auxiliary proteins for each step (Nickelsen and Rengstl, 2013). The first step of PSII assembly in developing leaves (so called *de*

novo assembly) involves formation of the PSII reaction center (RC) core complex. Of the PSII RC proteins, the α (PsbE) and β (PsbF) subunits of the Cyt b_{559}, together with the low molecular mass protein PsbI, form an assembly unit to receive the D1 protein (Muller and Eichacker, 1999; Tsiotis, et al., 1999; Zhang, et al., 1999). After the assembly of the RC core complex, the inner antenna protein CP47 becomes attached to it (Rokka, et al., 2005). Subsequently, four low molecular mass proteins, PsbH, PsbM, PsbR and PsbT are assembled to PSII RC to form a so called RC47 complex, also known as CP43-less PSII monomer (Nickelsen and Rengstl, 2013). Assembly of CP43 and PsbK proteins to the RC47 complex enables the assembly of the first OEC protein PsbO, followed by the assembly of the low molecular mass proteins, PsbW and PsbZ, and other OEC proteins, PsbP and PsbQ (Suorsa and Aro, 2007).

PsbR and PsbJ are required for the proper assembly of PsbP to the OEC and the docking of PsbQ is dependent on the proper assembly of PsbP (Suorsa, et al., 2004; Suorsa, et al., 2006; Allahverdiyeva, et al., 2013). A lack of PsbQ and PsbP in higher plants has been shown to weaken the interaction between PSII core and light harvesting complex II (LHCII) (Boekema, et al., 2000) and to cause reduction in the amount of PSII-LHCII supercomplexes that are usually present in non-stressed WT plants (Ido, et al., 2009; Allahverdiyeva, et al., 2013). After successful assembly of the OEC, PSII monomers are dimerized and peripheral antenna proteins are assembled, resulting in the formation of a functional PSII-LHCII supercomplex. The Arabidopsis PSII-LHCII supercomplex contains two cores (C) and four LHCII trimers. These trimers can be divided into two that are strongly bound LHCII (S), and two that are moderately bound LHCII (M). In addition to this $C_2S_2M_2$ configuration, two weakly bound LHCII (so called extra LHCII) could bind to supercomplexes, depending on the prevailing light conditions (Peter and Thornber, 1991; Dekker and Boekema, 2005; Kouril, et al., 2013). Several auxiliary proteins, such as HC136, LPA1, LPA2, LPA19 and PAM68, have been shown to be essential for correct PSII assembly (Meurer, et al., 1998; Peng, et al., 2006; Ma, et al., 2007; Wei, et al., 2010; Armbruster, et al., 2010; Mulo, et al., 2008; Chi, et al., 2012).

PSII re-assembly under the PSII repair cycle differs from the *de novo* PSII assembly. The repair cycle involves only partial disassembly of PSII, as only the OEC, CP43 and the damaged D1 protein are detached from the complex (for details, see 1.7.).

1.6 Manganese-stabilizing protein PsbO and its two isoforms in *Arabidopisis thaliana*.

The 33 kDa protein PsbO functions as a stabilizer of the OEC in PSII and is present in all oxygenic phototrophs (De Las Rivas, et al., 2004). The importance of this protein in water splitting has been demonstrated by drastic reductions in oxygen produced by mutants lacking the PsbO protein (Bricker, 1992). The conformation of PsbO changes in response to pH and to calcium and/or manganese concentrations (Shutova, et al., 2005). At an approximately neutral pH, which represents the physiological pH of the lumen in darkness, hydrophobic amino acid residues located inside the PsbO protein are in a “closed” formation and do not bind manganese or calcium. Acidification of the

lumen upon illumination leads to the first conformational change in PsbO, revealing the hydrophobic amino acid residues and making them accessible for ions of the surrounding medium to bind to. Binding of calcium ions leads to a second conformational change that enables water oxidation and the removal of protons out from the OEC. However, under light conditions, the interaction between PsbO and the PSII reaction center weakens as compared to the situation occurring in darkness (Shutova, et al., 2005). It has also been shown that formation of a redox sensitive disulphide bridge regulates the folding, assembly and degradation of PsbO (Hall, et al., 2010; Roberts, et al., 2012) and that the enzyme called lumen thiol oxidoreductase 1 (LTO1) likely catalyzes the formation of the disulfide bond (Karamoko, et al., 2011)

PsbO has been suggested to possess GTP binding ability and to function as a GTPase (Lundin, et al., 2007b). GTPase activity domains, called G domains, are conserved only in higher plant PsbOs (Lundin, et al., 2007b). Together with either Mg^{2+} or Ca^{2+}, hydrolysis of GTP also induces a conformational change in PsbO. Interestingly, this conformational change may decrease the area of interconnection between the PSII monomers and also affect their dimerization. Soluble PsbO binds GTP with a higher affinity compared to PsbO bound to PSII (Lundin, et al., 2007b). PsbO dissociates from PSII during photoinactivation (Hundal, et al., 1990). GTP is not needed for the release of PsbO. However, when added to isolated PSII-LHCII supercomplexes *in vitro*, GTP causes the removal of PsbO from PSII, which leads to a loss of photosynthetic activity (Lundin, et al., 2007b).

Arabidopsis thaliana contains two PsbO isoforms, PsbO1 and PsbO2 (Murakami, et al., 2002). The amount of PsbO isoforms vary within the plant kingdom and based on phylogenetic sequences, no conserved “PsbO1-type” or “PsbO2-type” isoforms can be recognized. In Arabidopsis, PsbO1 isoforms make up approximately 90 % of the total pool of PsbO proteins. The lack of PsbO1 in *psbo1* mutants induces upregulation of the PsbO2 isoform to reach protein amounts equal to that of PsbO1 in the WT plant (Murakami, et al., 2005). The mechanism that leads to the increased expression of PsbO2 in *psbo1* is unknown (Liu, et al., 2007). The phenotype of *psbo1* is one of slower growth and reduced oxygen evolution compared to the WT (Murakami, et al., 2002). This indicates that PsbO2 cannot fully substitute PsbO1, even though sequence similarity is high between these two isoforms. Functional differences between PsbO1 and PsbO2 are caused by amino acid changes at the C-terminus: Val186 in PsbO1 for Ser in PsbO2; Val204 for Ile; and Leu246 for Ile (Murakami, et al., 2005). Although PsbO2 is weaker in supporting oxygen evolution, other functions for this isoform have been suggested. A lack of PsbO2 causes accumulation of D1 protein under HL conditions. Further, when only PsbO2 is present, dephosphorylation of D1 and D2 has been demonstrated to occur significantly faster. This indicates that PsbO2 assists the removal of damaged D1 from PSII, possibly by allowing access of phosphatases to D1 (Lundin, et al., 2007a).

1.7 Regulation of energy distribution to PSII and PSI

Light intensity and quality change continuously in nature. These variations are caused by the time of the day and by the shading of clouds and leaf canopy. Because of different pigment compositions, the absorption maxima of PSI and PSII are different to each other. This causes an imbalance in the energy collected by PSI and PSII. In order to maintain a fluent linear electron transfer, this difference must be re-balanced. While the relatively higher energy capture of PSII will cause reduction of the PQ pool; the relatively higher illumination of PSI will cause oxidation of the PQ pool. The redox state of the PQ pool is sensed by specific proteins that subsequently regulate the processes that balance this uneven distribution of light energy (Puthiyaveetil, et al., 2012).

1.7.1 Thylakoid protein phosphorylation and State transitions

State transitions describe a phenomenon where energy distribution between PSI and PSII is balanced via regulating energy delivery from LHCII. LHCII can bind and transfer energy to both PSI and PSII. Under light conditions that favor PSI, LHCII is connected to PSII (State 1) (Puthiyaveetil, et al., 2012). When light preferably excites PSII, the PQ pool becomes reduced, thus activating the serine/threonine-protein kinase STN7 (Bellafiore, et al., 2005), named STT7 in Chlamydomonas (Fleischmann, et al., 1999). STN7 phosphorylates LHCII causing LHCII to bind PSI, increasing the absorption cross-section of PSI and thus increasing the amount of light captured by PSI at the expense of PSII. This situation is referred to as “State 2”. However, only part of the phosphorylated LHCII is moved to PSI, while a pool of phosphorylated LHCII remains attached to PSII. LHCII trimer that is found to move in State 2 is so called extra LHCII, whereas strongly and moderately bound LHCII trimers stay in PSII even in phosphorylated form. This clearly indicates that phosphorylation is not sufficient to disconnect LHCII from PSII (Wientjes, et al., 2013).

When the PQ pool becomes oxidized, STN7 is inactive and LHCII is dephosphorylated by the 38 kDa thylakoid-associated phosphatase TAP38 (also named as PP2C-type protein phosphatase; PPH1 (Shapiguzov, et al., 2010)). This leads to the dissociation of LHCII from PSI and to its binding to PSII (Pribil, et al., 2010). As the phosphorylation of LHCII is at least seven-fold faster than dephosphorylation, the main regulator of state transitions is likely to be STN7. In this case, STN7 kinase and TAP38 phosphatase can be active at the same time, but when inactivation of STN7 takes place, the phosphatase will predominate, leading to the dephosphorylation of LHCII (Puthiyaveetil, et al., 2012).

Under laboratory conditions, state transitions are traditionally triggered by using State 1 or State 2 lights favoring either PSI or PSII. However, the use of State 1 and State 2 lights is an artificial condition that does not exist in nature. The use of laboratory conditions that better mimic nature has revealed that, under high light conditions, STN7 is inhibited by the ferredoxin-thioredoxin system (Rintamäki, et al., 2000). This indicates that state transitions are a low light phenomenon, rather than a regulatory mechanism for high light conditions. Indeed, the use of artificially fluctuating light has revealed that STN7 is needed for a proper energy distribution between photosystems in

low light conditions. *stn7* mutants have shown slower growth under fluctuating light compared to WT plants. This is caused by an uneven distribution of energy and the overexcitation of PSII under the low light phase, but not during the high light phase (Tikkanen, et al., 2010).

1.7.2 Regulation at the level of protein expression

State transitions represent fast and short term acclimation to changing light conditions, whereas other mechanisms have developed to adapt photosynthetic machinery for long term changes in light quality. To balance the energy distribution between photosystems, photosynthetic organisms can change the amount of photosystems and the size of their antennas. For example, chloroplast sensor kinase (CSK) is able to perform autophosphorylation and this phosphorylation event is activated upon oxidation of the PQ pool (Puthiyaveetil, et al., 2012). CSK then phosphorylates chloroplast sigma factor 1 (SIG1) that, in its phosphorylated form, suppresses the transcription of PSI genes (Puthiyaveetil, et al., 2013; Shimizu, et al., 2010). This leads to a relatively higher production of PSII proteins compared to PSI proteins.

In addition to the regulation of chloroplast genes, acclimation of photosynthesis is regulated by retrograde signaling between chloroplasts and the nucleus. Three main signals are: (i) the redox state of components related to electron transfer reaction; (ii) ROS molecules and their foreplay with ROS scavenging enzymes; and (iii) metabolites mainly produced by the CBB cycle. In addition to these, other molecules such as plant hormones regulate photosynthetic genes. Multiple signaling molecules undertake retrograde signaling between plastids and the nucleus, forming a complex network of signaling cascades that partially overlap each other (Szechynska-Hebda and Karpinski, 2013).

1.8 Light susceptibility, photoprotection and repair cycle of PSII

Photoinhibition of PSII results in a loss of oxygen evolution and electron transport activity and is dependent on light intensity (Tyystjärvi and Aro, 1996). Plants have evolved several photoprotective mechanisms to prevent the light induced damage of the photosynthetic machinery (for review see Li, et al., 2009 and Tyystjärvi, 2013). One of the most important mechanisms is called non-photochemical quenching (NPQ), where excess light energy is quenched as heat (de Bianchi, et al., 2010). NPQ is dependent on the lumenal pH. In high light, acidification of the thylakoid lumen activates the so called xanthophyll cycle, where violaxanthin becomes de-epoksidized to zeaxanthin via anteraxanthin (Jahns and Holzwarth, 2012). A low lumenal pH triggers the activation of the PsbS protein that is needed for induction of NPQ (Li, et al., 2000; Niyogi, et al., 2005). However, exact molecular mechanisms behind the action of this protein are not known. Mutant plants lacking NPQ have shown an approximately 25 % higher rate constant of photoinhibition, as compared to the WT (Tyystjärvi, et al., 2005). Even though NPQ is a relatively weak protector against the harmful reactions of photoinhibition, it can be an important protector against the formation of ROS under high light conditions. As ROS have been shown to inhibit the PSII repair cycle, NPQ could act as important protector against photodamage by

securing the correct function of the PSII repair cycle (Nishiyama, et al., 2011; Tyystjärvi, 2013).

In spite of protective mechanisms, photoinhibition of PSII occurs under all light intensities. The main target for photodamage is the D1 protein in the PSII reaction center. The most important ROS causing photodamage is considered to be singlet oxygen ($^1O_2$). Singlet oxygen is usually formed by reaction between oxygen and triplet-state chlorophyll (Schweitzer and Schmidt, 2003). Triplet-state chlorophyll is formed in two ways, by a spontaneous spin change of excited chlorophyll, or by the charge recombination reaction of P_{680} (Hoff, 1981; Tyystjärvi, 2013). Three different mechanisms for photoinhibition have been proposed: (i) according to the acceptor side photoinhibition model, the reduction of electron acceptors in PSII leads to double reduction of Q_A. This leads to formation of tripled chlorophyll (Krieger-Liszkay, 2005), from which an electron can move to molecular oxygen forming singlet oxygen (Aro, et al., 1993); (ii) according to the donor side photoinhibition model, oxidized $P680^+$ cannot oxidize the manganese complex in OEC, but instead it oxidizes other components in PSII, leading to photoinhibition; and (iii) in the manganese hypothesis, PSII inactivation occurs through the release of manganese ions from the OEC to the lumen. This results in oxidation of other PSII components (Hakala, et al., 2005). Singlet oxygen reacts with surrounding proteins and lipids causing damage to PSII. Depending on the mechanism producing singlet oxygen, it could also inhibit the repair cycle of damaged PSII instead of causing direct damage to PSII (Hakala-Yatkin, et al., 2011).

To maintain functionality of PSII, damaged D1 is rapidly degraded and replaced with new, functional D1 in a process called the PSII repair cycle (Aro, et al., 1993; Aro, et al., 2005). PSII core protein phosphorylation plays a crucial role in the PSII repair cycle and thus participates in the regulation of PSII protective mechanisms. PSII core proteins are phosphorylated by STN8 kinase and dephosphorylated by PSII core phosphatase (PBCP) (Vainonen, et al., 2005; Bonardi, et al., 2005). STN8 is the main kinase involved in this process, but STN7 also phosphorylates PSII core proteins and thus partially overlaps with STN8 (Tikkanen, et al., 2008). The PSII repair cycle starts with core protein phosphorylation triggered disassembly of the PSII-LHCII supercomplexes and monomerization of the damaged PSII (Tikkanen, et al., 2008; Fristedt, et al., 2009). The PSII monomer migrates to the stroma membrane, which is followed by the removal of OEC and CP43. The phosphorylation of PSII core proteins modulates the thylakoid structure that is required for the efficient lateral movement of the PSII complex from the grana to the stroma membrane (Fristedt, et al., 2009; Goral, et al., 2010). Unstacking of the thylakoid helps in the PSII repair cycle by increasing the probability of proteases recognizing and digesting D1. Unstacking also lowers the ROS production that inhibits the repair cycle (Kouril, et al., 2013). However, the trafficking of PSII complexes between grana and stroma thylakoids during the PSII repair cycle has been recently challenged, as it has been shown that light stress induces lateral shrinkage of grana diameter and also triggers protein mobility in the grana thylakoids (Herbstova, et al., 2012; Kirchhoff, 2013). These dynamic rearrangements are likely to play a crucial role in facilitation of a fluent PSII repair cycle.

In stromal thylakoids, dephosphorylation triggers the removal and degradation of the damaged D1 protein. D1 is degraded by two protease families: the ATP-dependent zinc metalloprotease filament temperature sensitive H (FtsH) and the ATP independent serine endoprotease Deg proteases (Lindahl, et al., 2000; Haussuhl, et al., 2001). In Arabidopsis, four FtsH proteases are found to be located in the chloroplast and form a hexamer crucial to D1 degradation (Yu, et al., 2004). FtsH1 and FtsH5 represent "Type A" and FtsH2 and FtsH5 represent "Type B" subunits in the complex, and mutants lacking FtsH2 and FtsH5 have been shown to be more sensitive to high light (Yu, et al., 2004; Sakamoto, et al., 2002; Bailey, et al., 2002). Four Deg proteases are found in the chloroplast (Deg1, Deg2, Deg5 and Deg8) (Nath, et al., 2013) and all four of these are linked to D1 degradation (Sun, et al., 2007; Kapri-Pardes, et al., 2007). However, D1 has been found to be degraded in mutants lacking Deg2 (Huesgen, et al., 2006). Some of the FtsH and Deg proteases are separated by the thylakoid membrane, thus they are likely being synchronized in a functional manner (Kapri-Pardes, et al., 2007). Once the damaged D1 protein is removed, new D1 protein is synthesized and co-translationally inserted into PSII, which is followed by the reassembly of CP43 and the OEC. Finally, PSII becomes dimerized and migrates back to grana thylakoids (Baena-González, et al., 1999).

Taken together, the cooperation of NPQ and the repair cycle of PSII play a crucial role in protecting PSII against harmful light radiation and in maintaining efficient photosynthesis.

1.9 Regulatory role of calcium in the chloroplasts

Calcium is a universal second messenger in eukaryotic cells (Berridge, et al., 2000). In plants, environmental changes cause fluctuations in nuclear and cytosolic calcium levels. Changes in calcium level are sensed by a diverse group of calcium sensors. Calcium signals oscillate in both amplitude and frequency, enabling a specific allocation of the signal (Dodd, et al., 2010). Calcium signaling in a plant cell is not only restricted to cytosol and nucleus, but appears to also be intimately connected with organelles (Rocha and Vothknecht, 2012).

Chloroplasts accumulate high amounts of calcium; however calcium precipitates with phosphate causing a shortage of phosphate and, consequently, problems in ATP production. Therefore, most calcium must be associated either to membranes and/or to macromolecules (Brand and Becker, 1984). Free stromal calcium concentrations vary from the nanomolar up to micromolar scale (Johnson, et al., 1995). So far, details concerning the mechanism of calcium transport into the chloroplast remain poorly understood. However, evidence supporting the existence of membrane potential activated uniport-type calcium transporters has been provided (Roh, et al., 1998). Photosynthetic water splitting requires calcium to be transported to the lumenal side of the thylakoid membrane. Calcium is transferred over the thylakoid membrane through a Ca^{2+}/H^{+} antiporter in a light-dependent manner (Ettinger, et al., 1999). It has been shown that even though calcium transport to the lumen requires a proton gradient, calcium can also be transferred to the lumen in darkness, with the ΔpH formed through

ATP hydrolysis by ATPase (Ettinger, et al., 1999). After a shift from light to dark, a strong calcium burst occurs in chloroplast stroma. The origin of calcium is not yet known, but it has been suggested that the calcium is released either from the lumen, or from a thus far unidentified stromal calcium store. Calcium is subsequently released out from the chloroplast and into the cytosol (Sai and Johnson, 2002).

1.9.1 Calcium regulation in CO_2 fixation

The significance of calcium in the control of CO_2 fixation is well established, while calcium dependent regulation of photosynthetic light reactions remains poorly understood. Several CBB cycle enzymes, including Fructose-1,6-biphosphatase (FBPase), Sedoheptulose-1,7-biphosphatase (SBPase) and CP12, are regulated by calcium. FBPase and SBPase are thioredoxin regulated CBB enzymes whose activities increase when the calcium concentration increases to a certain level (Chardot and Meunier, 1991). High calcium concentrations, in contrast, inhibit the activity of these enzymes (Charles and Halliwell, 1980). Furthermore, two key enzymes of CBB, phosphoribulokinase (PRK) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) have been shown to form a complex with CP12, a protein responsible for the reversible assembly of the complex. The activity of CP12 is regulated by thioredoxin (Trost, et al., 2006). Recently, CP12 was found also to bind calcium with high affinity, but the physiological significance of this remains unresolved (Rocha, 2013). Ferredoxin, the electron carrier between PSI and Ferredoxin-$NADP^+$ reductase (FNR), has also been shown to bind calcium. The calcium binding capacity is higher when ferredoxin is present in its reduced state (Surek, et al., 1987). Ferredoxin also participates in the CBB-cycle by reducing thioredoxins via ferredoxin-thioredoxin-reductase (Buchanan, et al., 2002). Together, these findings demonstrate the dynamic interplay between redox and calcium-dependent regulation of photosynthesis.

1.9.2 Calcium regulation in photosynthetic light reactions

The requirement of calcium in the redox reaction that oxidizes water to oxygen and hydrogen has long been known. However, very little is known about the regulatory role of calcium in photosynthetic light reactions. Thylakoid ATP synthase subunit III of CF_0 forms a calcium binding pocket on the lumenal side of the thylakoid membrane. Calcium binding to subunit III is pH dependent and the release of calcium takes place in acidic pH (Zakharov, et al., 1993). Consequently, when the lumenal pH rises above 5.5, the movement of protons through the ATPase complex becomes blocked by subunit III in its calcium-bound form. However, proton translocation can be rapidly reactivated when lumenal pH decreases below 5.5 and causes the release of calcium from subunit III. Without calcium binding, the pH threshold value of 5.5 disappears and the movement of protons across thylakoid takes place whenever the transthylakoid ΔpH is sufficient to support the formation of ATP (Van Walraven, et al., 2002). It has also been shown that the phosphorylation of two thylakoid ATPase subunits is regulated by calcium. These are the gamma subunit, important for the regulation of ATPase activity (Imashimizu, et al., 2011), and subunit F (Stael, et al., 2011).

Little is known about calcium regulated proteins in PSII and PSI complexes. In PSII, PsbO has been shown to bind calcium (Murray and Barber, 2006) and the

phosphorylation of the PsbQ2 protein has been suggested to depend on calcium (Stael, et al., 2011). In addition, the FtsH2 and FtsH5 proteases, crucial for the PSII repair cycle (Kato, et al., 2009), are targets of calcium dependent phosphorylation (Stael, et al., 2011). However, the biological significance for FtsH2 and FtsH5 phosphorylation is not yet known. In PSI, targets for the calcium regulated phosphorylation are PsaN and PsaH (Stael, et al., 2011). PsaN locates on the lumenal side of thylakoid membrane and is proposed to participate in the binding of plastocyanin (Haldrup, et al., 1999; Haldrup, et al., 2000), whereas PsaH is needed for the docking of the phosphorylated LHCII to PSI during state transitions (Lunde, et al., 2000).

1.9.3 Calcium sensing receptor (CaS)

CaS is a 40 kDa thylakoid bound phosphoprotein found in unicellular algae and higher plants. It is mainly located in the stroma membrane, although it is also found in grana stacks (Vainonen, et al., 2008). The CaS domain structure contains a rhodanese-like domain, a 14-3-3 binding domain, and a forkhead-associated domain that is commonly linked to signal transduction, especially under stress conditions (Li, et al., 2000; Denison, et al., 2011; Bordo and Bork, 2002). The phosphorylation site, Thr380, is located in the middle of the 14-3-3 binding and the forkhead-associated domains on the stromal side of the thylakoid membrane. CaS is phosphorylated by STN8 kinase and its phosphorylation is light and calcium dependent. However, it is not yet known if the same threonine residue is a target for both light induced and calcium induced phosphorylation (Vainonen, et al., 2008). The lumenal exposed region of CaS binds calcium with low affinity, but with high capacity (Han, et al., 2003). The CaS protein is needed for transduction of extracellular calcium signal to cytosolic calcium oscillation (Nomura, et al., 2008; Tang, et al., 2007) and a lack of CaS leads to a lower magnitude of calcium oscillation in the chloroplast compared to the WT (Nomura, et al., 2012).

CaS has been shown to participate in stomatal closure during drought stress and thus affects the transpiration rate in plants (Wang, et al., 2014; Sun, et al., 2010). In Arabidopsis, elevating external calcium induces stomatal closure (Allen, et al., 1999). In Arabidopsis mutants lacking CaS (*cas*), stomatal guard cells are not responsive to elevated concentrations of external calcium and stomata remain open (Nomura, et al., 2008). CaS acts in a signaling cascade where an increase in calcium concentration triggers H_2O_2 production in chloroplasts, which further triggers nitric oxide (NO) production in the cell, leading to stomatal closure (Wang, et al., 2011). The CaS-dependent signaling pathway is also responsible for stomatal closure induced by flg22, a conserved peptide of bacterial flagellum (Nomura, et al., 2012). Further, flg22-induced chloroplast to nucleus retrograde signaling does not show a similar response in *cas* compared to the WT. Gene expression related to salicylic acid (SA) biosynthesis in WT plants is increased as a response to flg22, whilst *cas* plants lack the activation of SA related gene expression, and show reduced production of SA during pathogen attack compared to the high SA burst in the WT. This indicates an important role for CaS in the induction of plant defense responses against flagellated bacteria (Nomura, et al., 2012).

De-etiolation of leaves is promoted by high concentrations of calcium. CaS has been shown to act in this signaling cascade, as CaS antisense plants showed delayed de-

etiolation in both low and high calcium concentration when compared to WT plants. Further, the expression level of CaS was higher in etiolated seedlings grown in high calcium concentration compared to the medium and low calcium concentration grown seedlings (Huang, et al., 2011). As CaS triggers calcium mediated NO production in cells and NO is known to be a signal molecule for triggering de-etiolation (Beligni and Lamattina, 2000), it is possible that the role of CaS in the de-etiolation process is via NO signaling.

In the green alga *Chlamydomonas reinhardtii*, CaS plays a critical role in adaptation under high light conditions. The *C.reinhardtii* mutant line with reduced CaS expression (RNAi-*cas*) is unable to properly induce the expression of LHCSR3, a protein crucial for induction of NPQ, similar to PsbS in higher plants (Petroutsos, et al., 2011). Further, in RNAi-*cas* CET is downregulated under anaerobic conditions. CaS interacts with the anaerobic response 1 (ANR1), protein important under anaerobic conditions, which could explain the importance of CaS under this condition (Terashima, et al., 2012). Both the high light induced and the anaerobiosis induced phenotypes of RNAi-*cas* have been rescued by providing a high external calcium concentration. This indicates that calcium and CaS are directly involved in the adaptation and survival of *C.reinhardtii* under high light stress and under anaerobic stress conditions (Petroutsos, et al., 2011; Terashima, et al., 2012).

Taken together, calcium plays an important role in signaling cascades in cells. Currently, little is known about the regulatory role of calcium in chloroplasts. The importance of calcium in the activation of the CBB cycle and ATPase has been demonstrated and a number of chloroplast calcium binding proteins have been identified. Among the signaling components, CaS has been found to be vital in signaling cascades that integrate cytosolic calcium signals to chloroplastic functions. For this reason, the *cas* mutant provides a powerful tool to study the effects of calcium fluxes in the chloroplast.

2 AIMS OF THE STUDY

Studies on the structural and functional aspects of photosynthetic membrane protein complexes have provided a wealth of information concerning photosynthetic electron transport in higher plant chloroplasts. However, precise details concerning the physiological significance of protein isoforms and their impact on the dynamic regulation of photosynthetic processes still remain elusive. The aim of my thesis was to specify the molecular mechanisms and their interactions in the regulation of photosynthetic light reactions under environmental fluctuations. To this end, I examined the auxiliary functions of *Arabidopsis thaliana* PsbO isoforms in PSII, the regulatory function of PGR5 in photosynthetic electron transfer and the involvement of CaS on photosynthetic performance. A genetic approach was employed, whereby Arabidopsis mutants deficient in specific photosynthetic protein components were subjected to adverse environmental conditions and assessed for functional deficiencies in the photosynthetic machinery.

More specifically, my thesis examined:

1. The auxiliary roles of PsbO1 and PsbO2 isoforms of the oxygen evolving complex in the electron transfer and repair of PSII.

2. The role and interactions of PGR5 in the control of linear and cyclic electron transfer.

3. The linkage of calcium signaling and CaS in photosynthetic regulation.

3 METHODOLOGICAL ASPECTS

3.1 Plant material and growth conditions

Arabidopsis (*Arabidopsis thaliana*) ecotype Columbia, tobacco (*Nicotiana tabacum*) and spinach (*Spinacia oleracea*) were used as experimental material. The following Arabidopsis mutant lines were used: *cas* (SALK_070416 and GABI_ 665G12), *pgr5* (AT2G05620), *pgrl1a* (SAIL_443_E10), *psbo1* (SALK_093396), *psbo2* (SALK_024720 and SALK_024386), *ndh-O* (SALK_068922.46.65.x) and *stn8* (SALK_060869). Unless otherwise stated, the plants were grown under 150 µmol photons $m^{-2}s^{-1}$, 23 C^{o}, 50% air humidity or as described in respective papers.

Field experiments (Paper III) were done at the experimental garden of Umeå University in Northern Sweden. Field experiments were performed essentially as described in (Kulheim, et al., 2002; Frenkel, et al., 2008). The *pgr5* and wild-type plants were first pre-grown for two weeks in a growth chamber under a low light intensity of 50 µmol photons m^{-2} s^{-1} and then transferred outside to the fields. The experiment was set between the 22nd of July and the 4th of August, and between 8 – 25th of August 2011.

3.2 Isolation and separation of proteins and protein complexes

Intact chloroplasts for Differential In Gel Electrophoresis (DIGE) quantitation analysis were isolated using Percoll gradient as described in paper IV. Proteomes of the chloroplasts were first separated with isoelectric focusing (IEF) (Paper IV). For other experiments thylakoid membranes were isolated as in Järvi, et al., 2011. Protease inhibitor cocktail (Complete, Roche, Germany) was added to all buffers to avoid degradation of proteins during the isolation process. Chlorophyll concentrations were determined according to Porra, et al., 1989 and protein concentration was determined as described in Lowry, et al., 1951. For the separation of thylakoid protein complexes, intact thylakoid membranes or mechanically fractionated thylakoid membranes (Danielsson, et al., 2004) were used. The protein complexes were solubilized with n-dodecyl β-D-maltoside. Blue Native polyacrylamide gel electrophoresis (BN-PAGE) and SDS-PAGE were carried out as described in Järvi, et al., 2011.

3.3 Detection and identification of proteins

The chloroplast proteome was detected by labeling proteins from the wild type (WT) and *cas* mutant plants with different CyDyes. Mixed protein samples were separated and visualized with Geliance 1000 imaging system (Perkin Elmer). For visualization of all of the proteins in a gel, silver staining (Blum, et al., 1987) was used. For the identification of single proteins from stained gels, LC-MS/MS-analysis was performed as described in paper IV. For quantitative analysis of single proteins, immunoblotting with specific antibodies was used.

3.4 Chlorophyll fluorescence and absorption measurements

The redox state of P700 was measured with a PAM 101/103 chlorophyll fluorometer (Walz) connected to ED-P700DW emitter-detector unit. Leaves were illuminated either with far-red light (60 µmol photons m^{-2} s^{-1}, 720 nm) (Paper IV), or white light (Paper III) and the absorbance was recorded at 820 nm and 860 nm. Fluorescence measurements were performed using PAM 101/103-, Dual PAM or Plant efficiency analyzer -fluorometers (Walz). The flash-induced increase and relaxation of chlorophyll fluorescence were recorded with FL3300 dual-modulation fluorometer (Photon System Instruments, Brno, Czech Republic) as described in Allahverdiyeva, et al., 2004. The electrochromatic shift (ECS) of carotenoids was measured with a Joliot type spectrophotometer (JTS-10, Biologic science instruments) using an external white light source to follow the changes in pmf (as described in Paper IV).

3.5 Bimolecular fluorescence complementation

All constructs for BiFC analyses were introduced into pGPTVII backbone vectors. The complete protein encoding regions of all proteins analyzed were amplified by PCR or subcloned and either fused to the C-terminal fragment of YFP (SPYCE), or the N-terminal fragment (SPYNE), under the control of the constitutive MAS-promoter. All BiFC analysis were performed in *Nicotiana bentamiana* and visualized with a Leica DMI6000 confocal microscope equipped with a Leica TCS SP5 II laser scanning device. Confocal microscopy was performed according to (Batistic, et al., 2008) and (Waadt and Kudla, 2008).

3.6 Transcript profiling

For microarray analysis, total RNA was extracted from both WT and *cas* leaves using Trizol reagent. RNA was spotted to Arabidopsis 24 k oligonucleotide arrays and scanned with an Agilent scanner, followed by quantification with a ScanArray Express Microarray Analysis system 2.0 (PerkinElmer Life Sciences, USA). The Bioconductor packages were used for data analysis. Northern blot analysis was performed as described in Paper I.

3.7 Histochemical staining and visualization of H_2O_2, O_2^-, $^1O_2$ and GTP hydrolysis assay

The accumulation of the H_2O_2 was studied by the 3,3'-diaminobenzidine (DAB) staining method (Thordal-Christensen, et al., 1997). Leaves and seedlings were allowed to uptake DAB for time periods of 2 hours to overnight, which was followed by different light treatments (Paper III). O_2^- staining was performed in a similar manner, but using 0.1 % NBT instead of DAB. Leaves and seedlings were cleared with 96 % ethanol and images were acquired with stereomicroscope/camera combination (Zeiss Lumar V12). For detecting $^1O_2$, seedlings were treated with singlet oxygen

sensor green reagent (Molecular Probes) and visualized with confocal laser scanning microscopy (Zeiss LSM510 META). Argon diode lasers were used to excite the label at 488 nm and fluorescent detection was measured between 535- to 590-nm.

For analysis of GTPase activity, PSII membranes were dark incubated in solution containing radioactive labeled GTP for 15 minutes. After incubation, radioactive GTP and GDP were separated by thin-layer chromatography and detected using a phosphorimager (FLA-5100) (Lundin, et al., 2007b).

4 OVERVIEW OF THE RESULTS

4.1 Functional difference between two PsbO isoforms in *Arabidopsis thaliana*

Arabidopsis has two PsbO isoforms, referred to as PsbO1 and PsbO2, in the OEC complex of PSII. Although the differences between amino acid sequences of these isoforms are small, a mutant lacking the main isoform, PsbO1, demonstrated a drastic phenotypic difference compared to the WT plant, or to the mutant lacking PsbO2 (Lundin, et al., 2007a). The functional difference between the two PsbO isoforms was studied in Arabidopsis to explain the roles of the isoforms in the mutant phenotypes (Papers I and II).

4.1.1 Comparison of electron transfer properties of PsbO1 and PsbO2

Electron transfer properties of PSII were measured using flash fluorescence method on WT, *psbo1* and *psbo2* thylakoids. Flash fluorescence relaxation kinetics comprise three phases: (1) the fast phase, originating from electron transfer from Q_A^- to Q_B in the state of an occupied Q_B pocket; (2) the middle phase, arising from the electron transfer from Q_A^- to Q_B in the state of an empty Q_B pocket; and (3) the slow phase, originating from $S_2Q_B^-$ recombination. Comparison between the WT (fast phase 0.51 ms, middle phase 17.8 ms) and *psbo2* (fast phase 0.49 ms, middle phase 24.7 ms) did not show any significant difference. However, *psbo1* demonstrated a slower time constant in both the fast (0.78 ms) and the middle phase (59.5 s) relaxation kinetics (Paper II). This indicates disrupted electron transfer from Q_A^- to Q_B and possibly, a more reduced state of the PQ-pool. Measurement of the intersystem electron chain evidenced a larger electron pool in *psbo1* compared to the WT, supporting the possibility of a larger and/or more reduced PQ-pool.

To rule out the effect of electron transfer from Q_A^- to Q_B and further to the PQ-pool, differences in the donor side of PSII were studied by using the photosynthesis inhibitor DCMU ((3-(3,4-dichlorophenyl)-1,1-dimethylurea). DCMU binds to the plastoquinone binding site of PSII, preventing electron transfer from Q_A^- to Q_B. Thus, in the presence of DCMU, FF-relaxation kinetics are mainly due to the recombination of Q_A^- with the donor side components. This methodological approach was employed to measure FF-relaxation kinetics from the WT, *psbo1* and *psbo2* thylakoids. Again, no significant difference was observed between the WT and *psbo2*. In this case, *psbo1* demonstrated a slower time constant of fast phase kinetics (27.9 ms) compared to the WT (0.32 ms). Additionally, *psbo1* showed a fast exponential component that was not detectable in the WT or *psbo2* (Paper II).

To further study the role of PsbO1 and PsbO2 in photosynthesis, other properties of PSII were examined. NPQ is an important mechanism that protects photosynthesis against high light excitation. Dark adapted leaves from WT, *psbo1* and *psbo2* were exposed to actinic light (800 μmol photons $m^{-2}s^{-1}$) for 15 minutes and NPQ was calculated from the recorded fluorescence parameters. Compared to the WT leaves,

psbo1 showed a faster induction, but a lower steady-state level of NPQ. No differences were observed between the WT and *psbo2* (Paper II).

To examine cyclic electron flow around PSI, re-reduction kinetics of $P700^+$ were measured in darkness, after far-red induction. $P700^+$ re-reduction was significantly faster in *psbo1* compared to the WT and *psbo2*. In addition, western blot analysis of *psbo1* plants revealed a higher amount of the NdhH protein, which is a subunit of the NDH complex responsible for cyclic electron flow. This was particularly evident in plants that were GL grown, but was also observed in HL adapted plants. Interestingly, the amount of PTOX (plastid terminal oxidase), a protein capable of oxidizing the PQ-pool and functioning as a safety valve in photosynthesis, was higher in *psbo1* compared to the WT in GL conditions. However, in HL adapted plants, the amount of PTOX decreased in *psbo1* (Paper II).

Taken together, these results indicate that electron transfer inside PSII was significantly altered in *psbo1*, leading to the requirement of increased activity of alternative electron routes to compensate for the problems in PSII function.

4.1.2 PsbO proteins in stabilization of the PSII complex

PsbO proteins have previously been shown to have auxiliary functions besides the stabilization of the calcium-manganese-cluster. One such function is the stabilization of the PSII dimeric structure and assistance in removal of the D1 protein during the PSII repair cycle (Lundin, et al., 2007a; Lundin, et al., 2007b). Paper I demonstrated the importance of PsbO isoforms in the maintenance of normal PSII structure both in growth light and high light conditions. Using BN-PAGE, different photosynthetic complexes from DM solubilized thylakoid membranes were separated and immunoblotted with D1 and CP43 antibodies to visualize PSII complexes. GL and HL adapted leaves from WT, *psbo1* and *psbo2* plants were used to compare differences in the function of the two PsbO isoforms in Arabidopsis. In growth light conditions, *psbo1* showed reduced amount of PSII supercomplexes and dimers compared to WT and *psbo2*. The amount of monomers lacking CP43 was much greater in *psbo1*, indicating a fast ongoing PSII repair cycle. Under HL conditions, the reduction of the amount of supercomplexes and dimers was even more drastic in *psbo1* compared to the WT. Also, *psbo2* showed a higher monomer/dimer –ratio and a higher proportion of monomers lacking CP43 than the WT (Paper I).

The role of PsbO1 and PsbO2 in PSII photochemical efficiency was investigated in GL and HL treated plants. The WT, *psbo1* and *psbo2* mutant plants were exposed to 3 hours high light treatment before the Fv/Fm value was measured. *psbo1* showed a lower overall photochemical efficiency compared to the WT and *psbo2* in both GL and HL conditions. However, the relative decrease in Fv/Fm upon HL treatment, as compared to GL, was similar in the WT and both mutants. After HL treatment, plants were transferred back to normal GL conditions and photochemical efficiency was recorded, which showed no significant differences between the WT and mutants. A similar tolerance of *psbo* mutants against HL was found with HL acclimated plants. Three week old plants were transferred to HL for a further three weeks and Fv/Fm values were recorded from the WT (0.72), *psbo1* (0.70) and *psbo2* (0.68). The values

obtained were surprising, because in GL conditions *psbo1* demonstrated a dramatically lower Fv/Fm value (0.47) compared to the WT (0.82) and *psbo2* (0.81). To test the hypothesis of a faster ongoing repair cycle in *psbo1*, leaves were treated with lincomycin, which inhibits protein translation in the chloroplast and consequently halts the PSII repair cycle. After two hours of HL, Fv/Fm values were measured from the WT and both *psbo* mutants. An 80 % loss in activity of PSII centers was shown in *psbo1* compared to a 44 % and 48 % loss in WT and *psbo2* respectively, demonstrating an enhanced repair cycle in *psbo1* and the importance of the PsbO1 protein in stabilizing PSII (Paper II).

4.1.3 Differential GTPase activity of PsbO proteins

Earlier studies have shown the importance of PsbOs to the PSII repair cycle and particularly to GTP-dependent D1 degradation (Suorsa and Aro, 2007; Lundin, et al., 2007a; Spetea, et al., 1999). It has also been shown that PsbO could bind GTP, leading to a conformational change of PsbO. It has been proposed that this conformational change could be important for the removal of damaged D1 protein from the PSII complex (Lundin, et al., 2007b).

To compare the GTPase activity of the two PsbO isoforms, isolated Arabidopsis PSII containing membranes were incubated with [α-^{32}P]GTP followed by nucleotide separation with thin-layer chromatography. GTPase activity was calculated against both chlorophyll content and the amount of PsbO protein. Using the WT, *psbo1* and *psbo2* plants, it was shown that the PsbO2 isoform has a higher GTPase activity rate than the PsbO1 isoform (Paper I). GTPase activity in the WT was somewhat higher than in the *psbo2* mutant (containing the PsbO1 isoform only) and much lower in activity compared to the *psbo1* mutant (PsbO2 isoform only). This result fit well with observations from the thylakoid preparation of the WT, which contained only a small proportion of PsbO2 compared to PsbO1 isoform.

To study the effect of auxiliary and other OEC proteins on PsbO GTPase activity, a NaCl wash was used to remove these proteins from the membrane. This had a huge effect on the GTPase activity of *psbo1*, which dropped to the level of the WT and *psbo2*. NaCl treatment did not have any effect of PsbO1 GTPase activity (Paper I). To conclude, PsbO2 showed higher GTPase activity compared to PsbO1 and the GTPase activity of PsbO2 is dependent on auxiliary proteins.

4.1.4 Expression levels of PsbO proteins during development and in different stress conditions

To determine whether either of the PsbO isoforms plays a specific role in tolerance against stress conditions, WT, *psbo1* and *psbo2* plants were placed under various abiotic stress conditions and the amount of both isoforms was monitored on transcript and on protein levels. Further, the amounts of both PsbO isoforms were measured during leaf greening and during various developmental stages to understand the possible specificity of the individual isoforms in Arabidopsis development.

The expression levels of *PsbO1* and *PsbO2* were measured from dark grown seedlings, which were then exposed to illumination with 50 photons m^{-2} s^{-1}. When the seedlings

were first exposed to light for 6 hours, expression levels of both genes lowered, but increased again after 8 hours under the same light condition. After 12 hours of light exposure, the expression levels of *PsbO* genes lowered again and remained stable for up to 20 hours. On the protein level, the amount of both isoforms followed transcript abundance. The amount of PsbO protein remained mostly stable during the greening process, except after 8-10 hours after exposure to light, when a clear increase in the amount of protein was observed. The amount of PsbO was also stable during leaf development, except in senescent leaves where PsbO abundance was dramatically lowered. No differences were found between the relative expression patterns of PsbO isoforms in WT plants (Paper I).

To study whether either of the PsbO isoform has a specific function under particular stress conditions, the expression levels of *PsbO* were measured after two hours of high light, desiccation, wounding, oxidative stress, salt stress, heat shock and cold treatment. *PsbO1* and *PsbO2* transcript levels were lowered under stress conditions, except under extreme temperatures, when compared to non-treated control samples. On the protein level, the amount of PsbO stayed mostly stable during stress treatments. Further, the ratios between PsbO isoforms remained static. A drastic reduce was seen in the amount of PsbO2 in the *psbo1* mutant during oxidative stress, salt stress and heat stress. Such a change in the amount of PsbO1 in *psbo2* was not found (Paper I).

Overall, the ratio between PsbO1 and PsbO2 remained fairly stable during leaf development and also during short term stress. This indicates that the PsbO isoforms do not have specific roles during leaf development.

4.2 Role of thylakoid protein PGR5 in electron flow in chloroplasts

Photosynthetic complexes must tightly control the electron flow in thylakoids to avoid the transfer of excess electrons to molecular oxygen and the subsequent production of ROS. Under sudden changes in light intensity, NPQ is the main mechanism responsible for the dissipation of excess excitation energy. NPQ is controlled by the pH gradient between stroma and the lumen. An Arabidopsis mutant lacking the PGR5 protein (*pgr5*) is documented to fail in generating a ΔpH over the thylakoid membrane (Munekage, et al., 2002). As a consequence, *pgr5* fails in the formation of NPQ during fast increases in light intensity. PGR5 has been proposed to participate in PGR5-mediated CET and consequently, to control the transfer of protons to the lumen. This proposal, however, has two problems: (i) enhancing CET leads to lumenal protonation that slows down electron transfer via Cyt b_6f, resulting in these two redox reactions to pushing down each other (Tikkanen, et al., 2012); and (ii) the total lack of ΔpH in *pgr5* under a rapid change to high light indicates that PGR5-mediated CET would be responsible for total protonation of lumen in high light. However, this is not likely the case in C3 plants (Laisk, et al., 2005; Laisk, et al., 2007). Biophysical and biochemical methods were employed to identify the mechanism behind PGR5 regulated protonation of the lumen, and the overall physiological significance of PGR5 in natural conditions (Paper III).

4.2.1 PGR5 is essential for acclimation to changing light conditions

To investigate the role of PGR5 in dynamic light acclimation, WT and *pgr5* mutant plants were grown in growth chambers under constant light (CL) and under fluctuating light (FL) (mimicking natural light conditions). As shown in paper III, under CL, the *pgr5* mutant did not show any clear growth phenotype compared to the WT plants. In FL conditions, *pgr5* seedlings developed normally until the cotyledon stage when a strong growth defect appeared. Cell size was smaller in *pgr5* after 9 days growth under FL, whereas no differences were found in plants grown under CL conditions. Under FL, *pgr5* remained at the two- or four-leaf state and finally started to wilt, while the WT continued normal growth. Growth was also tested by sowing the seeds on Murashige and Skoog plates supplemented with 0, 1 or 2% sucrose. The *pgr5* phenotype was rescued only in 2 % sucrose, however, if the *pgr5* seedlings were first grown for two weeks under CL and then transferred to FL, even 2 % sucrose failed to rescue the *pgr5* phenotype.

In nature, plants must face constantly changing light conditions. Therefore, the fitness of *pgr5* was tested by conducting field experiments in Northern Sweden. These experiments were undertaken to confirm the results that arose from using FL simulating chambers. Indeed, the *pgr5* mutant had a mortality rate two to three times higher than the WT plants grown in field conditions (Paper III). Even though the plants had to cope with several different environmental stresses when grown in the outdoor field, the agreement of the artificial FL conditions with those obtained in the field suggest that the inability of *pgr5* to adapt to FL conditions was the likely reason for the low survival rate.

4.2.2 In pgr5, linear electron transfer is disturbed and PSI is damaged under fluctuating light condition

In order to identify the physiological cause of the *pgr5* phenotype in fluctuating light conditions, nine days old seedlings were collected from both CL and FL conditions. From these seedlings, chloroplast protein content was compared between the WT and *pgr5* mutant by Western blotting. In CL and in FL conditions, no differences were found in the levels of D1 protein, representing PSII, and Cyt *f* protein, representing Cyt b_6f. Instead, the amount of PSI core protein PsaB was found to decrease by nearly 50 % compared to the WT in CL. Under FL, the amount of PsaB was only 36 % of that of the WT. In the WT, the amount of the PsaB increased 149 % in an acclimation strategy to FL conditions. The amount of ATPβ protein, representing thylakoid ATP synthase, was elevated in *pgr5* (142 %) but only under FL. To confirm these results, protein complexes were separated using large pore Blue Native –PAGE (Järvi, et al., 2011). Thylakoid membranes were solubilized with digitonin to avoid overlapping of PSII and PSI. In *pgr5*, the amount of PSI and the state transition complex, composed of PSI, LHCI and LHCII (Pesaresi, et al., 2009), were clearly reduced compared to the WT (Paper III).

Proteins involved in alternative electron transport and state transitions were also studied. For these experiments, four proteins were included: PGRL1, representing PGR5-mediated CET; NDHL, representing NDH-mediated CET; plastid terminal oxidase (PTOX); and STN7 kinase for state transition. No differences were found in

the amounts of NDHL or STN7. The amount of PGRL1 in *pgr5* was slightly reduced (86 % of the wild type level) especially under FL. The level of PTOX was slightly higher in *pgr5* in both light conditions (~110 % of the wild type level) (Paper III).

Sensitivity of PSI and PSII to photoinhibition was studied by measuring the maximal oxidation of P700 (Pm) and maximal fluorescence (Fm) of the WT and *pgr5*. Leaves from GL conditions were kept in darkness for 20 minutes to relax NPQ and Pm and Fm were measured. Leaves were then illuminated with HL for 15 minutes, again followed by measurements of Pm and Fm. After HL treatment, *pgr5* showed a drastic decrease in Pm value (35 % of the control value) compared to measurements from GL, indicating high photoinhibition of PSI. When linear electron flow was halted by DCMU, PSI functionality was rescued in *pgr5* and no differences between the WT and *pgr5* were found regarding PSI photodamage (Paper III). These results demonstrated that damage of PSI was caused by electrons which originated from PSII and that PGR5 was essential for the control of LET.

4.2.3 pgr5 does not accumulate reactive oxygen species

To study the role of ROS in photodamage of PSI in FL, histochemical staining of ROS was used. Nitroblue tetrazolium (NBT) was used for recognition of superoxide (O_2^-), 3,3-diaminobenzidine (DAB) for hydrogen peroxide (H_2O_2) recognition and Singlet Oxygen Green Sensor for the recognition of singlet oxygen ($^1O_2$). No significant differences were found between WT and *pgr5* seedlings grown under CL. Interestingly, 9 day old seedlings of *pgr5* grown under FL accumulate less superoxide and hydrogen peroxide compared to the WT grown either under FL or CL, as well as *pgr5* grown under CL. The reaction of Singlet Oxygen Green Sensor, however, did not differ between the WT and *pgr5*.

Amounts of ROS scavengers were measured using immunoanalysis. The *pgr5* mutant contained higher amounts of Cu/Zn superoxide dismutase (SOD) compared to the WT grown under both CL (170%) and FL (177%) conditions. The amount of thylakoid-bound ascorbate peroxidase (tAPX) increased in *pgr5* under CL (129%) and FL (152%). Similarly, the amount of cytosolic ascorbate peroxidase (cAPX) increased in *pgr5* under CL (143%) and FL (143%) conditions. Despite higher ROS scavenging capacity and lower amounts of ROS in *pgr5* compared to the WT, a stronger oxidation of proteins was observed, particularly under FL conditions, as demonstrated by the OxyBlot protein oxidation detection kit (Paper III). Collectively, these results indicated higher ROS scavenging capacity in *pgr5*.

4.2.4 Role of PGR5 for redox balance and formation of proton motive force under high light conditions

To study the role of PGR5 in the control of LET more closely, PSI redox state and NPQ were measured from WT and *pgr5* by using FL conditions mimicking actinic light (Paper III). In addition to plants grown under CL, mildly fluctuating light (5 min 50 µmol photons $m^{-2}s^{-1}$, 1 min 350 µmol photons $m^{-2}s^{-1}$) conditions were used to gain the sufficient size of the leaves required for measurements. PSI redox behavior differed strongly between WT and *pgr5*. In HL, P700 was strongly oxidized in WT leaves and reduced during the subsequent low light phase. In *pgr5*, P700 remained reduced, even

during the HL phase. P700 redox state behavior was not dependent on the light condition under which plants were grown.

Lumenal pH regulates the electron transfer through Cyt b_6f and is responsible for the induction of NPQ. In contrast to the WT, which induced high NPQ levels, *pgr5* lacked the ability to form NPQ under the HL phase. For this reason, measurements were undertaken of the proton motive force (pmf) across the thylakoid membrane in both the WT and *pgr5* under FL. Pmf was measured spectrophotometrically using the phenomena known as the electrochromic shift (ECS) of carotenoids. In the WT, pmf increased dramatically during the HL phase. In *pgr5*, HL caused a relaxation of pmf, whereas the shift back to the low light phase returned pmf kinetics to a level similar to the WT. Together, these results showed that PGR5 is essential for the control of electron transfer to PSI and for maintaining ΔpH over the thylakoid membrane under short term exposure to HL.

4.3 Functional role of the thylakoid calcium sensing receptor (CaS) in chloroplasts

Thylakoid phosphoprotein CaS has been shown to play an important role in cellular signaling regulating calcium levels (Petroutsos, et al., 2011; Nomura, et al., 2008; Vainonen, et al., 2008; Weinl, et al., 2008). In higher plants, CaS participates in translating calcium signals from outside of the chloroplast into a calcium burst in the chloroplast stroma. As a physiological function, CaS participates in the greening process of leaves (Huang, et al., 2011) and contributes to the regulation of stomatal movements in response to changes in hydrogen peroxide, nitric oxide or calcium concentrations (Han, et al., 2003; Nomura, et al., 2008; Weinl, et al., 2008; Wang, et al., 2011). It is likely that, through calcium signaling, CaS has a role in triggering an immune responses in plant-pathogen interactions (Nomura, et al., 2012). In the green algae *Chlamydomonas reinhardtii*, CaS has been reported to be vital in the photoprotection of PSII (Petroutsos, et al., 2011). Thus far, no specific role for CaS in the regulation of higher plant photosynthesis has been reported. Furthermore, there has yet to be any reports of specific proteins interacting with CaS in higher plants.

4.3.1 Deletion of CaS has no effect on the light tolerance of photosystem II in Arabidopsis thaliana

To examine the role of CaS in the photoprotection of PSII, a 24 hour HL experiment was undertaken for both the WT and *cas* mutant plant, which fully lacks the CaS protein. The functionality of PSII was monitored by measuring the photosynthetic efficiency of PSII (Fv/Fm) throughout the experiment. In addition to the efficiency measurements, the amount of PsbS, a protein responsible for induction of NPQ, was determined after 12 hours of HL. As shown in paper IV, even after 24 hours, no significant differences in photosynthetic efficiency were found between the WT and *cas*. Overall, amount of PsbS were 20 % lower in *cas* compared to the WT after HL treatment. The PSII complex composition was also analyzed from the dark and 12 h HL treated samples. HL treatment did not differentially affect the PSII complexes in

cas compared to the WT. However, in samples taken from the darkness, *cas* contained slightly more PSII monomers and less PSII dimers compared to the WT.

To rule out the possibility that *cas* could compensate its sensitive PSII by increasing the repair cycle of PSII, a similar HL experiment was performed using lincomycin, an inhibitor of protein synthesis in the chloroplast. This experiment was performed with only a 3 hour time period and with lower light intensity compared to the experiment performed without lincomycin. No difference in photosynthetic efficiency was found between the WT and *cas*, demonstrating that neither the light tolerance of PSII, nor the PSII repair cycle in *cas* differs from the WT.

To test if CaS has a role in photoprotection of PSI, *cas* mutant plants were grown under FL conditions, which are known to lead to PSI photoinhibition in *pgr5* (Paper III). *pgr5* and *pgrl1* were grown at the same time to serve as control plants. No difference was found in viability between the WT and *cas* in conditions that led to the death of both *pgr5* and *pgrl1* (Paper IV).

4.3.2 Deletion of CaS has a highly specific effect on transcript and protein profile

To gain insights into the physiological role of CaS, microarray and 2D differential fluorescence gel electrophoresis (2D-DIGE) were performed from *cas* and WT plants (Paper IV). When transcript levels between the WT and *cas* leaves grown under normal growth conditions were compared, seven genes relating to photosynthesis showed higher transcript abundance in *cas*. Those genes encoded proteins PsbO2, Lhcb1, Lhcb4, Lhca2, PsaD2, PsaH-1 and PGRL1. Interestingly, PGRL1 has been implicated to function in PGR5-dependent cyclic electron flow (DalCorso, et al., 2008) and to be phosphorylated by STN8 (Reiland, et al., 2011), which is the same kinase that phosphorylates CaS (Vainonen, et al., 2008). A second group of gene transcripts higher in abundance in *cas* included those encoding enzymes and regulatory proteins of the CBB cycle. Especially noticeable, were the transcripts encoding components of the chloroplast glyceraldehyde 3-phosphate dehydrogenase (GAPDH) complex (GAPA, GAPC2 and CP12-2), which were highly abundant in *cas* compared to the WT.

Contrasting these increased transcript levels, the GAPDH complex proteins were of lower abundance in *cas*. The CBB cycle related enzymes rubisco, rubisco activase, phosphoglycerate kinase and transketolase, were also low in abundance in *cas*. On the protein level, elevated levels of ATP synthase gamma chain, plastid transcriptionally active 16 (PTAC16) and NDH-dependent cyclic electron flow 1 (NDF1) were observed in *cas* compared to the WT (Paper IV).

4.3.3 CaS interacts with PGR proteins and with itself and modulates electron flow around PSI

An interaction of CaS with PGRL was recently shown for *C. reinhardtii* (Terashima, et al., 2012). This, together with results from our microarray experiment (Paper IV) led us to test the possible physical interaction between CaS and PGR-proteins by using the bimolecular fluorescence complementation (BiFC) technique. To this end, PGR5, PGRL1A and PGRL1B proteins were fused to the C-terminal fragment of yellow fluorescent protein (YFP) and CaS to the N-terminal fragment. In all cases, YFP-

fluorescence was detected with confocal microscopy showing the interaction of CaS with all tested PGR-proteins. Moreover, homodimers of CaS were observed when CaS was fused to both N- and C-terminal fragments of YFP.

In addition to the BiFC-experiment, the amount of both PGR proteins and CaS were compared in plants grown under different light conditions. Tight correlation between the amounts of CaS and PGRL1 were found. Moreover, a lack of CaS protein caused slight increases in PGRL1 and PGR5, while the amount of CaS was reduced in the *pgr5* mutant (Paper IV). These results suggest the possible existence of a CaS/PGRL1/PGR5 –complex.

Since PGR5 and PGRL1A/B have been suggested to play regulative roles in CET (DalCorso, et al., 2008), I tested the possible role of CaS in the regulation of electron transfer. *cas* showed a slightly higher level in fluorescence F0 rise, which originates from NDH-mediated CET (Shikanai, et al., 1998), compared to the WT. In the same measurements, *pgr5* revealed a lower level of fluorescence rise (Paper IV).

The kinetics of P700 oxidation was subsequently measured from leaves adapted to darkness or light. Dark adapted leaves were also treated with methyl viologen, an artificial electron acceptor of PSI. In dark-adapted leaves, *cas* showed slower P700 oxidation compared to the WT and *pgr5*, which actually demonstrated the opposite behavior to the *cas* mutant. Light adapted or methyl viologen treated leaves did not show any differences between *cas*, *pgr5* and WT plants (Paper IV). This indicates that there is a scarcity of electron acceptors after PSI in *cas*.

4.3.4 CaS associates with ATP synthase in a phosphorylation dependent manner

To find other candidate interaction partners for CaS in plant thylakoids, another approach was used. Using Blue-Native gel electrophoresis, thylakoid protein complexes were separated, which was followed by the solubilization and separation of individual proteins in each complex by SDS-PAGE. The location of CaS and all main thylakoid complexes were revealed by immunoblotting using a CaS specific antibody or antibodies against other representative proteins of thylakoid protein complexes. CaS was found to co-migrate with ATP synthase in Arabidopsis thylakoids. However, because some complexes were overlapping with each other in BN-PAGE, the experiments were repeated with spinach thylakoids, mechanically fractionated to stroma lamella and whole grana (including grana margins). In spinach thylakoids, CaS co-migrated with three different ATPase complexes in grana and with one ATPase complex in the stroma lamellae.

Since the phosphorylation site of CaS locates in the middle of a possible interaction domain, it was tested if CaS co-migrates with ATPase in its phosphorylated or non-phosphorylated state. To this end, Arabidopsis thylakoids were extracted and separated with 2D-BN-SDS-PAGE and immunoprobed with the P-Thr antibody followed by immunoprobing with the CaS antibody. No phosphorylated CaS was found to co-migrate with ATPase. To clarify that both non-phosphorylated and phosphorylated forms of CaS were present in the sample, 1D-SDS-PAGE was performed from the same thylakoid sample. Thylakoids were found to contain both non-phosphorylated

and phosphorylated forms of CaS (Paper IV). This shows that the interaction of CaS with ATPase is regulated by the phosphorylation state of CaS.

5 DISCUSSION

Tight regulation of the photosynthetic machinery allows optimization of light-driven electron transport and efficient basic production of biomass, while minimizing the harm caused by light induced photodamage. Discoveries on alternative electron transfer routes and the multitude of photoprotective mechanisms have significantly increased the understanding of how photosynthesis is protected in different environmental conditions. Regulation and co-operation of different processes in photosynthesis is especially important under fluctuating light conditions. In this thesis, an insight to the function of protective mechanisms operating under fluctuating light is presented. Also, the role of calcium in the regulation and synchronization of these mechanisms is discussed.

5.1 Differential roles and characteristics of PsbO1 and PsbO2 in *Arabidopsis thaliana*

Several components of the photosynthetic light reactions occurring in plant chloroplasts are found as different isoforms, which may have specific functions under different environmental cues. In Arabidopsis, PsbO is present in two isoforms that show high similarity of amino acid sequences. Even so, mutants lacking one of either isoforms show clear differences in their phenotypes (Lundin, et al., 2007a). Not much is known about the unique roles and functions of these isoforms. PsbO1 dominates in WT thylakoids and constitutes as much as 90 % of the total amount of PsbO. In the *psbo1* mutant, the amount of PsbO2 increases to the level of total PsbO in WT plants (Murakami, et al., 2005). Similarly, the abundance of PsbO1 increases in *psbo2* plants (Lundin, et al., 2007a). This makes the *psbo1* and *psbo2* mutants effective tools to study the functional differences between the two PsbO isoforms. In this work, I provide new information concerning the effects of PsbO isoforms in the electron transfer properties of PSII and reveal a new auxiliary role for PsbO2.

5.1.1 PsbO1 stabilizes PSII under high light conditions

Under normal growth conditions, I observed that the *psbo1* mutant plant malfunctioned on the donor and acceptor side of PSII, whereas the *psbo2* mutant displayed no differences compared to the WT (Paper II). These results are in line with previous reports showing that PsbO2 cannot functionally substitute for a lack of PsbO1 (Murakami, et al., 2002; Murakami, et al., 2005). Further, in agreement with previous observations (Liu, et al., 2007), I found that plants containing only PsbO2 display a longer S_2-state of OEC compared to WT plants (Paper II). In theory, longer S_2-state could lead to a higher probability of singlet oxygen formation, and thus a higher rate of photodamage in PSII. This theory is supported by my observation that *psbo1* contained more PSII monomers and less PSII supercomplexes, indicating a higher rate of PSII photodamage when compared to the WT plant under GL conditions (Paper I). It has previously been assumed that the differential electron transfer properties of the PsbO

isoforms stem from the inability of PsbO2 to stabilize calcium and magnesium ions in the OEC (Bricker and Frankel, 2008). This view is supported by the demonstrated rescue of oxygen evolution in *psbo1* by increasing the calcium concentration (Bricker and Frankel, 2008). The results provided in my study, however, do not support this idea, because the incubation of *psbo1* thylakoids in buffer containing a high calcium concentration does not reactivate flash-induced oxygen evolution. Moreover, the EPR signals measured from *psbo1*, *psbo2* and WT indicate the integrity of the OEC (Paper II). Thus, the exact explanation for the poor performance of PsbO2 in evolving oxygen remains an open question.

5.1.2 Light susceptibility of psbo1 is compensated by fast PSII repair cycle and alternative electron sinks.

In the *psbo1* mutant, I observed PSII hypersensitivity to light induced damage (Paper I and II). To compensate for this, excess light energy must be dissipated by modulating alternative electron transfer routes. In the chloroplast, PTOX acts as a safety valve for the electron transfer chain by oxidizing the over-reduced plastoquinone pool (McDonald, et al., 2011). PTOX can work together with the NDH-like complex in a process called chlororespiration, where the NDH-like complex reduces the quinone pool and PTOX subsequently oxidizes it (Rumeau, et al., 2007). A strong upregulation of the amount of PTOX and NDH-like complexes, indicative of a high rate of chlororespiration, was found in *psbo1* under GL conditions (Paper II). Additionally, the *psbo1* mutant showed higher CET around PSI and a larger plastoquinone pool (e^-/P700) than WT plants. CET protects PSII against photodamage by promoting NPQ through lowering ΔpH across the thylakoid membrane (Yamamoto, et al., 2006). PSII complexes containing PsbO2 are more sensitive to photodamage, and increased amounts of PTOX and a higher rate of CET therefore seem logical adjustments in compensating for this sensitivity and protecting PSII in *psbo1*. These regulatory adjustments illustrate the high sensitivity of the sensory systems that allow the photosynthetic machinery to signal and respond to any imbalances in its function.

Since *psbo1* shows poor photosynthetic performance in GL conditions, it could be expected that an even greater disruption of photosynthetic capacity would be detected under HL conditions. It was therefore surprising that, under HL conditions, the relative loss of photosynthetic efficiency did not differ between *psbo1* and either the WT, or *psbo2* (Paper II). A relatively higher amount of PSII monomers and PSII monomer-CP43 less complexes was found in *psbo1* (Paper I), indicating the higher rate of the PSII repair cycle as compared to the wild type. This was corroborated by the observation that a major decline in photosynthetic efficiency could be observed in *psbo1* leaves when chloroplast translation, and thus PSII repair, was inhibited by lincomycin. An accelerated PSII repair cycle in *psbo1* thus explains the similar photosynthetic efficiencies observed for *psbo1*, WT and *psbo2* under HL. Two alternative hypotheses can be drawn based on these results. Firstly, PsbO1 may be able to stabilize the structure of PSII better than PsbO2. Secondly, PsbO2 may be more effective in "opening" the PSII structure for triggering the PSII repair cycle. One possible explanation behind the effective PSII repair cycle in *psbo1* could be the higher GTPase activity of PsbO2 compared to that of PsbO1, thus supporting the latter

hypothesis (Paper I). GTPase activity has earlier been shown to induce PSII monomerization and to be important for the PSII repair cycle (Lundin, et al., 2007b).

5.1.3 Calcium, possible regulator of PSII repair cycle

Several pieces of evidence suggest that the PSII repair cycle may be controlled through calcium-dependent mechanisms. Firstly, PsbO2 shows a high GTPase activity (Paper I) that is linked to the PSII repair cycle (Spetea, et al., 1999; Lundin, et al., 2007b) and PsbO is capable of binding calcium (Murray and Barber, 2006). Interestingly, calcium binding to GTPases, such as mammalian calexcitin, has been shown to increase their GTPase activity (Nelson, et al., 2003). Although the effect of calcium on the GTPase activity of PsbO has not been studied, it is plausible to speculate that calcium may trigger the GTPase activity of PsbO and subsequently induce the PSII repair cycle. Secondly, the FtsH2 and FtsH5 proteases responsible for degradation of the D1 protein were recently shown to be phosphorylated in a calcium dependent manner (Stael, et al., 2011). Thirdly, CaS is a known calcium flux regulator in chloroplast and analysis by the publicly available Atted-II tool (http://atted.jp/) indicates that the *CAS* gene is co-expressed with *PsbO2* (Figure 2). Moreover, a lack of CaS causes specific upregulation of *PsbO2* but not *PsbO1* (Paper IV) and, specifically, PsbO2 has been shown to be needed for D1 dephosphorylation and the turnover of PSII (Lundin, et al., 2007a). Collectively, these pieces of evidence suggest that CaS and PsbO2 are functionally closely connected.

In summary, PSII complexes containing only PsbO2 are more sensitive to light induced photodamage than PSII containing only PsbO1. However, a faster ongoing PSII repair cycle can, at least partially, compensate for this problem. PsbO2 also shows high GTPase activity, which is likely related to the PSII repair cycle and D1 degradation. One important question remains to be answered, this is: why during evolution has it been beneficial for Arabidopsis, and several other plant species, to maintain and actively express the PsbO2 isoform, which is not as efficient in photosynthesis and stabilization of PSII compared to PsbO1. In WT plants, the ratio of PsbO1 to PsbO2 appears to be quite stable under different stress conditions (Paper I), indicating the importance of a strictly balanced ratio of these isoforms.

5.1.4 What is the benefit of having two PsbO isoforms?

The thylakoid lumen in grana stacks is a highly crowded environment filled with many OECs and other proteins (Kirchhoff, et al., 2011). A lack of PsbP protein in the OEC causes major changes in thylakoid arrangement (Yi, et al., 2009). It is also speculated that granal structure is at least partly built with the interacting forces of OECs (De Las Rivas, et al., 2007; Anderson, et al., 2008; Dekker and Boekema, 2005). If the two PsbO isoforms have different features in stabilizing thylakoid granal structure, it could have an effect on the PSII repair cycle via the different migration capabilities of PSII on the membrane. Indeed, both *psbo1* and *psbo2* mutants accumulate more PSII monomers in HL conditions than the WT. In the case of *psbo1*, this phenomena is particularly obvious because of the faster degradation of PSII complexes, but it doesn't explain why PSII monomers accumulate in *psbo2* (Paper I). PsbO2 could possibly affect the thylakoid ultrastructure during HL conditions by opening the granal

structure, thus aiding the migration of damaged PSII for repair. This theoretical possibility, however, remains to be confirmed experimentally. A second explanation could be that PsbO2 protects PSI against HL induced damage. A pool of more light sensitive PSII complexes could act as a safety buffer against sudden increases in light intensity, in order to avoid over-reduction of the LET and consequent damage to PSI (Paper III). When light intensity decreases again, this pool of PSII can be quickly repaired. Even though the ratios of PsbO isoforms remain stable after two hours exposure to cold (Paper I), the amount of PsbO1 protein decreased and PsbO2 increased after long term cold treatment. Consequently, the ratio of PsbO1 to PsbO2 decreases under prolonged exposure at low temperature (Goulas, et al., 2006). PSI has been shown to be vulnerable to photodamage especially under conditions where cold temperatures are combined with weak light intensities (Sonoike, et al., 1995). As shown in Paper III, PGR5 by itself cannot fully protect PSI under fluctuating light. This suggests that sensitive PSII complexes containing PsbO2 could support the PGR5-mediated protective mechanism of PSI.

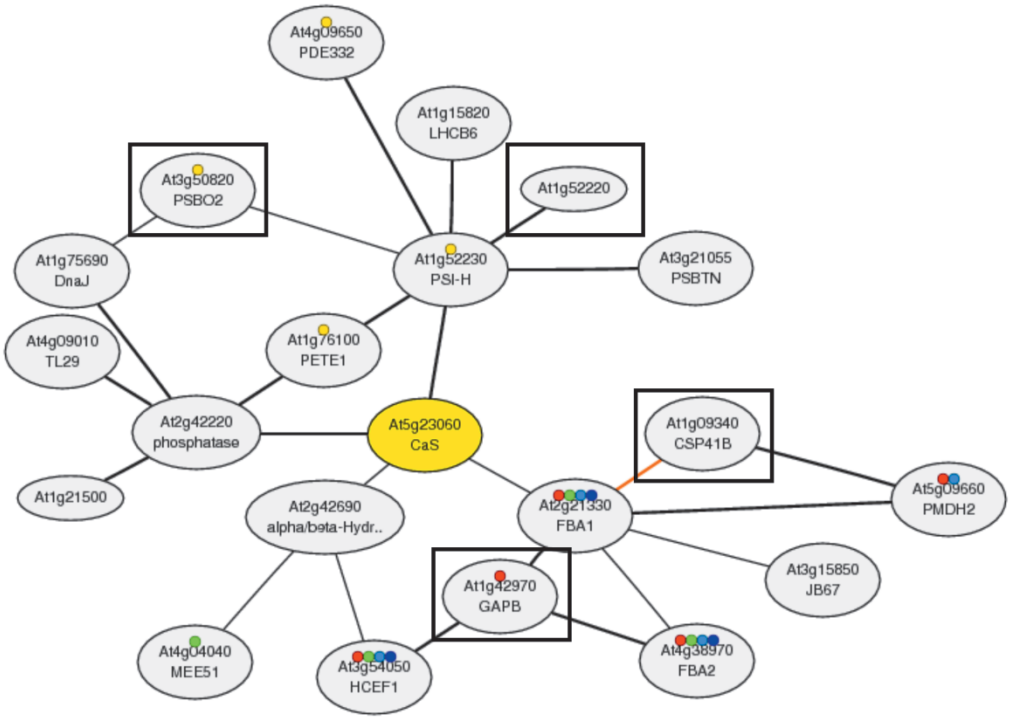

Figure 2: Atted-II analysis and visualization of genes co-expressed with *CaS* (Obayashi, et al., 2014). Genes with increased transcript abundance in *cas* compared to WT include *PSBO2* and *CURT1C* (At1g52220) (Paper IV). Proteins downregulated on DIGE in *cas* compared to WT include GAPB and CSP41B (Paper IV).

5.2 Functional role of PGR5 under artificially and naturally fluctuating light conditions

In nature, plants have to continuously adjust their photosynthetic machinery to cope with rapidly changing light intensities that are caused by clouds, wind and by the canopy of the leaves. In the laboratory, plants are usually grown in a stable and well controlled environment where important signaling and acclimation mechanisms are not found. In this study, PGR5 protein was found to be crucial for plants to survive under artificially fluctuating light, as well in natural conditions, and also to play a critical role in photosynthetic control (Paper III).

PGR5 was first found to be involved in NPQ induction under HL conditions (Munekage, et al., 2002). PGR5 is commonly linked to ferredoxin-dependent CET and, via this mechanism, to increased ΔpH and subsequent induction of NPQ (Munekage, et al., 2002). PGR5 is also thought to protect PSI against photodamage by limiting over-reduction of the acceptor side of the PSI (Munekage, et al., 2002). The importance of PGR5 became evident when *pgr5* was crossed with the *crr2-2* mutant impaired in NDH-mediated CET (Munekage, et al., 2004). This double mutant showed impaired photosynthesis and protracted growth. However, the results presented in paper III strongly suggest that PGR5 preferentially controls LET between PSII and PSI, rather than being a component of CET.

5.2.1 PGR5 controls linear electron transfer to protect PSI against light induced damage

PSI has been reported to be a relatively stable complex and photodamage of PSI has been shown to occur under special conditions, for example during cold stress of cold intolerant species (Sonoike, et al., 1995). While the PSII repair cycle is fast, repair of PSI is slow and, in practice, photodamage of PSI is irreversible. For this reason, plants have to protect PSI from damage. PSI photodamage requires electrons supplied by PSII (Sonoike, 2011). These electrons may ultimately reduce oxygen at the acceptor side of PSI, leading to the formation of ROS and to damage of adjacent photosynthetic components. For that reason, PSI photodamage can be avoided by treating isolated thylakoids with DCMU (Sonoike, 2011). Growth of plants under fluctuating light conditions revealed the vulnerability of PSI complexes in *pgr5* and demonstrated that the phenotype of *pgr5* under fluctuating light is caused by PSI photodamage (Paper III). This conclusion is further supported by absorbance measurements of P700, which showed a clear decrease in the Pm-value, indicative of PSI degradation in *pgr5* leaves under HL conditions. Moreover, under fluctuating light, thylakoid proteins of *pgr5* are more oxidized compared to the WT (Paper III), indicating elevated ROS production. Thus, the lower amount of ROS in *pgr5* as detected by ROS staining methods (Paper III) could be explained by the increased amount of ROS scavenging enzymes in *pgr5* and the quenching of ROS upon oxidation of proteins.

5.2.2 PGR5-mediated regulation of linear electron transfer occurs by controlling the proton motive force

P700 can be oxidized experimentally in two different ways, either illuminating with far-red light that favors PSI, or by shifting plants to high light. White light excites both

photosystems equally (Tikkanen, et al., 2010) and can still oxidize PSI. This means that electron flow from PSII to PSI must be slowed down and controlled in WT plants. In *pgr5*, PSI stays reduced during the high light shift, providing evidence that PGR5 is needed for controlling LET. Further, the functionality of PSI under fluctuating light in *pgr5* could be rescued to the level of the WT by limiting electron transfer between PSII and PSI with DCMU (Paper III). This indicates that damage of PSI in *pgr5* is due to unlimited electron flow from PSII to PSI. The role of PGR5 in the control of LET is actually more logical than the assumed role in increasing CET under high light. This is because CET transfers electrons back to the PQ-pool with a consequent reduction of the electron transfer chain. In line with this finding, the *ndho* mutant lacking NDH-mediated CET grows normally under fluctuating light (Paper III). Similarly, *crr2-2*, which is deficient in the NDH complex, showed no difference in phenotype when compared to the WT under fluctuating light (Kono, et al., 2014). This indicates that NDH-mediated CET does not play a major role as a buffering mechanism under fluctuating light conditions. In *pgr5*, no changes in the amount of NDH-like complex were found (Paper III), demonstrating that a lack of PGR5 is not compensated for by an increase in NDH-mediated CET. These results show that PGR5 is an important regulator when light intensity is rapidly increasing.

A shift from low light to high light decreases the level of pmf in *pgr5,* whereas a strong increase in pmf level takes place in the WT (Paper III). If PGR5 would function in CET, it would mean that during HL, PGR5-mediated CET would be the main mechanism to building a proton gradient over the thylakoid membrane. However, CET is considered to be a minor mechanism in building up pmf over thylakoids in C3 plants (Avenson, et al., 2005). LET is controlled by Cyt b_6f in a manner that depends on the lumenal pH, so that increased acidification of the lumen limits electrons transported through Cyt b_6f. This regulatory action is referred to as photosynthetic control (Rumberg and Siggel, 1969; Harbinson, et al., 1990; Tikhonov, 2012; Tikhonov, 2013). In *pgr5*, the lumenal proton concentration does not increase during HL, leading to unlimited electron flow through Cyt b_6f to PSI. The proton gradient over the thylakoid membrane is released through ATPase, which is able to limit proton flow through the thylakoid and, in doing so, affect electron transfer through Cyt b_6f. When PGR5 is present, ATPase is able to limit proton movement from the lumen to the stroma, and to subsequently lower the electron flow through Cyt b_6f to PSI. The *pgr5* mutant accumulates more ATPβ, representing ATPase, than the WT (Paper III), which is perhaps an attempt to compensate for its leaky ATPase. In fact, *pgr5* behaves in a manner opposite to that of the tobacco ATPase mutant, which contains only a small fraction of ATPase and demonstrates increased NPQ and pmf, with a consequent inhibition of LET (Rott, et al., 2011). The exact location of PGR5 in the thylakoid membrane is not yet known. In paper IV, I provided evidence that PGR5 interacts with CaS, which in turn may impact on the function of the ATPase. However, PGR5 has also been found to interact with Cyt b_6f (DalCorso, et al., 2008). For this reason, specification of the exact location of PGR5 requires further study.

5.3 Role of CaS in chloroplasts

CaS has been shown to participate in the regulation of calcium homeostasis in plants and algae (Nomura, et al., 2008; Weinl, et al., 2008; Petroutsos, et al., 2011). Transient increases in cytosolic calcium concentrations trigger calcium fluxes in the chloroplast stroma. In the *cas* mutant, transient cytosolic calcium fluxes are not followed by calcium spiking in chloroplasts (Nomura, et al., 2012), which indicates that Cas is required for signaling of chloroplast calcium levels. However, the exact mechanism through which CaS participates in this signaling is not known. Despite this, *cas* is a useful tool for studying the role and effect of calcium in processes that take place in the chloroplast. Although CaS has several important roles in plant physiology, such as the regulation of stomatal closure and defence signaling (Nomura, et al., 2008; Nomura, et al., 2012), its role in higher plant photosynthesis currently remains poorly understood.

5.3.1 CaS is replaceable in photoprotection in higher plants

In the unicellular alga *Chlamydomonas reinhardtii*, CaS plays an important role in photoprotection of PSII and is needed for accumulation of the PsbS homolog LHCSR3 (Petroutsos, et al., 2011). In Arabidopsis, there was no difference observed in the efficiency of photosynthesis between *cas* and the WT, even during prolonged high light (Paper IV). This demonstrates that CaS in plants does not function in photoprotection as it does in green algae. Further, the amount of PsbS was determined to be similar in *cas* and the WT (Paper IV). Previously, Nomura and co-workers have shown only minor reductions of NPQ in *cas* compared to the WT (Nomura, et al., 2008). I studied the role of CaS in the photoprotection of PSI by growing *cas* under fluctuating light. Light conditions leading to the death of *pgr5* and *pgrl1* mutants did not induce similar effects in *cas* (Paper IV). This strongly indicates that CaS is not involved in the photoprotection of PSI by limiting LET from PSII to PSI. Similarities between algal and plant CaS protein sequences are mainly located in the rhodonase homology domain, whereas the overall sequence similarities are low. This could explain the differential function of CaS in Arabidopsis and Chlamydomonas. From those results, I concluded that CaS belongs to the group of proteins whose function has changed during evolution. However, I cannot rule out the possibility that other proteins may be able to compensate for the lack of CaS in higher plants.

5.3.2 CaS interacts with PGR5, PGRL1 and ATPase and may contribute to the regulation of ATPase activity

To gain insights into the regulatory roles of CaS, its interaction partners were explored. PGRL1 was selected as a candidate protein because it was recently shown to be phosphorylated by STN8 (Reiland, et al., 2011). STN8 is also the kinase responsible for phosphorylating CaS (Vainonen, et al., 2008). PGR5 was included in my study since PGRL1 and PGR5 have earlier been shown to interact with each other (DalCorso, et al., 2008). Physical interaction between CaS, PGR5 and PGRL1 was detected using the BiFC technique (Paper IV). In addition to this, expression and/or stability of Cas and PGRL1 strictly correlates in various light conditions. This underlines the possible existence of a CaS/PGRL1/PGR5 complex. The interaction of Cas with PGR5 and PGRL1 is in agreement with results from Chlamydomonas, where PGRL1 and CaS

interact with each other and with ANR1, forming a CET regulatory complex (Terashima, et al., 2012). In Arabidopsis, the PGR5/PGRL1 complex is hypothesized to regulate the transition between LET and CET in a similar manner to that observed in Chlamydomonas (Iwai, et al., 2010; DalCorso, et al., 2008). However, as shown in paper III, PGR5 is more likely to be involved in the regulation of LET rather than CET.

To further identify possible interaction partners of CaS, and subsequently, to validate if CaS is located with any of the known photosynthetic thylakoid protein complexes, I separated these complexes with BN-PAGE and identified those containing the CaS protein. CaS was found to co-migrate with ATPase in both intact Arabidopsis thylakoids, as well as in fractionated thylakoids from spinach (Paper IV). Unexpectedly, CaS did not co-migrate with Cyt b_6f, which was expected based on previous findings from Chlamydomonas (Terashima, et al., 2012). In addition to co-migration with ATPase, CaS co-migrates together with complexes that were not identified in this study. It is possible that one or more of the novel CaS containing complexes are in fact homo-oligomers of CaS, since BiFC analysis also suggested that CaS could interact with itself (Paper IV). CaS co-localizes with the ATPase complex only in its non-phosphorylated form (Paper IV). A phosphorylated Thr-residue in CaS locates in the middle of the 14-3-3 and forkhead-associated interaction domain (Vainonen, et al., 2008), which could be the site of interaction. However, this was not confirmed in my study. Phosphorylation of CaS is dependent on calcium level and light intensity (Stael, et al., 2011; Vainonen, et al., 2008). Hence, increased calcium concentration, as well as high light, cause phosphorylation and consequent disengagement of CaS from the ATPase. CaS could participate in the regulation of ATPase activity during the shift from darkness to light and vice versa. In line with this idea, the ATPase gamma subunit is upregulated in *cas* compared to the WT (Paper IV). The ATPase gamma subunit is responsible for the activation of ATPase during the shift from darkness to light via thioredoxin regulation (Ketcham SR, et al.; Nalin and McCarty, 1984; Kohzuma, et al., 2013), further supporting the possibility of a regulatory role for CaS in the activation of ATPase.

5.3.3 CaS is functionally connected to specific Calvin-Benson-Bassham cycle enzymes

Considering that CaS is involved in several functions in plants, it is surprising how exclusive the set of differentially expressed genes are in *cas* compared to the WT. The major group of upregulated genes in *cas* code for proteins involved in the CBB cycle. This could be a compensatory adjustment, since, on protein level, the specific CBB cycle enzymes are present in lower levels in *cas* chloroplasts. The CBB cycle activity is dually controlled by redox state and calcium concentration (Chardot and Meunier, 1991; Charles and Halliwell, 1980; Trost, et al., 2006). Shifting plants from light to dark conditions results in an abrupt increase in stromal calcium concentration, and this calcium peak is assumed to inhibit the CBB cycle during periods of darkness (Sai and Johnson, 2002). In *cas*, stromal calcium concentration during a light to dark peak is significantly lowered to 25 % of WT concentrations (Nomura, et al., 2012). It is therefore possible that CaS effects the inactivation of the CBB cycle in an indirect manner, by modulating calcium oscillation. In *cas* plants, lowered levels of calcium in

the stroma could thus slow down the inactivation of CBB cycle. This could lead to a required balancing of the amounts of the CBB enzymes as a response to the disturbed regulation. Lowered amounts of CBB enzymes in *cas* would also slow down the flow of electrons to PSI electron acceptors. This is in fact visible, as slower oxidation of P700, in *cas* when leaves are transferred to light after an extended dark period (Paper IV). However, no difference was found on P700 oxidation kinetics in light adapted leaves, or in dark adapted leaves when treated with the artificial electron acceptor methylviologen (Paper IV). Further, *cas* contains less rubisco activase compared to the WT (Paper IV) and the activity of rubisco activase is dependent on the ATP/ADP ratio (Zhang and Portis, 1999). Hypothetical model of the effect of ATP/ADP ratio to the function of CBB cycle in WT and *cas* is represented in Figure 3. ATP/ADP ratio is in turn partly dependent on ATPase activity, whereas ATPase activity is regulated both by the binding of calcium to the F0 c-subunit (Zakharov, et al., 1993; Van Walraven, et al., 2002) and by ATPase gamma subunit (Imashimizu, et al., 2011). I therefore conclude that in *cas*, the slow phase of P700 oxidation, occurring at the beginning of illumination with far red light, is caused by a lower capacity and slower activation of the CBB cycle. Consequently, CaS may also play an indirect role in the fine tuning of the CBB cycle activity by participating in stromal calcium oscillations and affecting the amount of CBB enzymes.

Taken together, it is conceivable that CaS functions as a generic calcium sensor that participates in fine tuning of the photosynthetic machinery. In Arabidopsis, CaS participates in activation of the CBB cycle, possibly via regulating calcium fluxes in the chloroplast stroma. CaS could also participate in the fine tuning of ATPase activity during shifts from darkness to light. Calcium signaling could therefore be an important link between controlling photosynthetic electron transfer and carbon fixation and will be an interesting topic for future study.

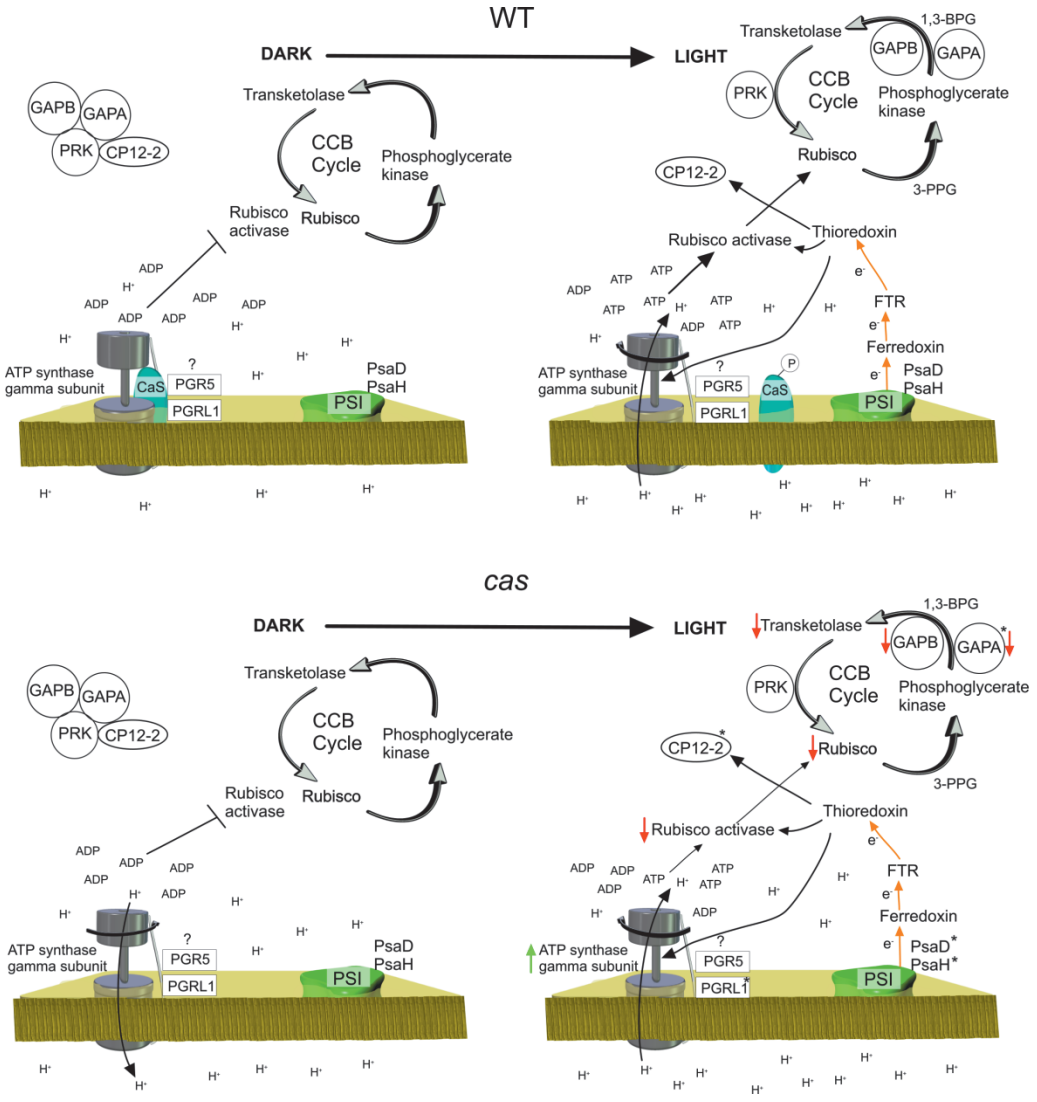
WT
DARK
LIGHT
GAPB
GAPA
PRK
CP12-2
Transketolase
CCB Cycle
Phosphoglycerate kinase
Rubisco activase
Rubisco
1,3-BPG
3-PPG
Thioredoxin
FTR
Ferredoxin
ATP synthase gamma subunit
CaS
PGR5
PGRL1
PSI
PsaD
PsaH
ADP
ATP
H+
e-
P
cas
DARK
LIGHT
GAPB
GAPA
PRK
CP12-2
Transketolase
CCB Cycle
Phosphoglycerate kinase
Rubisco activase
Rubisco
1,3-BPG
3-PPG
Thioredoxin
FTR
Ferredoxin
ATP synthase gamma subunit
PGR5
PGRL1
PSI
PsaD*
PsaH*
ADP
ATP
H+
e-

Figure 3. A hypothetical scheme for the function of CaS in coordination of the photosynthetic light and carbon fixation reactions. Upper picture shows the activation of the CBB cycle during a shift from darkness to light in WT plants. Lower picture depicts a possible malfunction of the ATP synthase in the absence of the CaS protein and its effect on the activation of the Calvin-Benson-Bassham (CBB) cycle upon shift from darkness to light. Black arrows indicate the activation of target protein and orange arrows the reduction of proteins by PSI. Small arrows in front of the protein names show an increase (green arrow pointing up) or decrease (red arrow pointing down) of the protein amount in the *cas* mutant compared to WT. Asterisk following the protein name indicates a change in the transcript level of the gene coding for the protein in *cas* compared to WT. During the dark period, ATP synthase is inactive to avoid proton translocation from stroma to lumen and hydrolysis of ATP to ADP. Activity of Rubisco is controlled via Rubisco activase that is activated by ATP and reduced thioredoxin. Other CBB cycle enzymes GAPA, GAPB and PRK are regulated by CP12 that is controlled via thioredoxin. When exposed to light, activation of the photosynthetic machinery and ATP synthase change the ATP/ADP-ratio in stroma and together with other regulatory pathways activate the CBB cycle. Shift to light also phosphorylates CaS causing its release from the ATP synthase. Lack of CaS in darkness is postulated to reverse the action of the ATP synthase i.e. to hydrolyze ATP, leading to a change in the ATP/ADP –ratio. CBB=Calvin-Benson-Bassham; PSI=photosystem I; CaS=calcium sensing receptor; FTR=ferredoxin-thioredoxin reductase; PGR5=proton gradient regulation 5; PGRL1=PGR5-like photosynthetic phenotype 1; PRK=Phosphoribulokinase; GAPA/GAPB= Glyceraldehyde-3-phosphate dehydrogenases; 3-PPG= 3-phosphoglycerate; 1,3-BPG=1,3-biphosphoglycerate.

5.4 Does calcium synchronize photoprotective mechanisms and photosynthetic reactions in the chloroplast?

Photoprotection of PSI and PSII requires multiple levels of regulation. Photodamage to PSI is irreversible, whereas light-induced damage to PSII is reversible via a repair cycle mechanism, and the photoinhibition of PSII can therefore be controlled. PSI photodamage requires excess electron flow originating from PSII (Sonoike, 2011). It is therefore reasonable to state that protection of PSI is mainly achieved through the control of activity in PSII and the transfer of electrons through Cyt b_6f to PSI. In this thesis, I present evidence demonstrating the importance of PGR5 in the protection of PSI under fluctuating light conditions (Paper III). By controlling the flow of protons through ATPase, PGR5 is necessary for a proper increase in ΔpH over the thylakoid membrane. A high ΔpH in turn decreases electron transfer via Cyt b_6f, thus preventing over-reduction of LET and any consequent damage to PSI. In addition to lowering LET, an increased lumenal proton concentration triggers NPQ, which dissipates excess excitation energy as heat in PSII, thereby protecting both PSII and PSI from light induced damage. The functional significance of a PGR5-CaS interaction in these regulatory circuits, however, remains unresolved. Even so, it is notable that different observations suggest that CaS may adjust the flow of protons through ATPase (Paper IV). Firstly, my own work showed that CaS co-localizes with ATPase (Paper IV), which is known to be controlled by calcium binding (Zakharov, et al., 1993). Further, the regulatory ATPase gamma subunit (Imashimizu, et al., 2011) is phosphorylated in a calcium dependent manner (Stael, et al., 2011). It is therefore interesting that I found the ATPase gamma subunit to be upregulated in *cas* (Paper IV). Together, these findings suggest that CaS and ATPase are functionally connected.

I also demonstrated that PSII is sensitive to photodamage when PsbO2, rather than PsbO1, is present in the OEC (Papers I and II). As PGR5 cannot fully protect PSI against photodamage (Paper III), it is possible that the pool of PsbO2 containing PSII centers is present in the thylakoid membrane in order to protect PSI. When sudden increases in the light intensity take place, this photosensitive pool of PSII could rapidly become inhibited, thus lowering the electron pressure on the LET route. When light intensity decreases, this pool of PSII can be rapidly repaired. The importance of such a controlled PSII repair cycle in the protection of PSI has recently been demonstrated (Tikkanen, et al., 2014).

In Chlamydomonas, calcium and CaS play important roles in the photoprotection of PSII (Petroutsos, et al., 2011). However, in higher plants, CaS is not needed for photoprotection of PSII or PSI (Paper IV). This does not rule out the possibility that calcium has a role in the regulation of photoprotection and repair cycle of PSII, particularly since Nomura and co-workers have recently demonstrated that calcium fluxes in the chloroplast stroma are not completely missing in *cas*, but are instead only lowered in comparison to the WT (Nomura, et al., 2012). It is possible that in higher plant chloroplasts, another protein that is yet unknown is partially substituting the function of CaS. It is also tempting to connect calcium flux based regulation and the repair cycle of PSII. In this thesis, I showed a high GTPase activity of PsbO2 (Paper I) that is linked to D1 degradation (Lundin, et al., 2007a). Calcium binding to GTPases

has earlier been shown to increase GTPase activity (Nelson, et al., 2003). However, calcium induced enhancement of GTPase activity in PsbO2 is not shown, even though PsbO2 is known to bind calcium that causes a conformational change to its protein structure (Shutova, et al., 2005). Further, CaS and PsbO2 are transcriptionally co-regulated (Figure 2) and a lack of CaS results in upregulation of *PsbO2* (Paper IV). Unfortunately, it is not known how the calcium concentration in chloroplasts responds to high light. If high light induces an increase in calcium concentrations, it could enhance D1 degradation from PSII during the repair cycle. The D1 protease FtsH2 has previously been shown to be phosphorylated in response to high calcium concentrations (Stael, et al., 2011). Even though the effect of phosphorylation on the activity of FtsH2 is not known, it forms another link between calcium signaling and the PSII repair cycle. Furthermore, the PSII repair cycle and "opening" of thylakoid grana stacks are linked together (Nath, et al., 2013) and CaS is also co-regulated with Curvature thylakoid 1C (CURT1C;AT1G52220) (Figure 2), which controls grana stacking (Armbruster, et al., 2013). *CURT1C* was also upregulated in *cas* (Paper IV), again supporting the linkage between calcium and the PSII repair cycle.

Calcium is an interesting secondary messenger that is known to participate in various cell functions. In this thesis, I have provided evidence that calcium demonstrates high potential for participation in the regulation and synchronization of various photosynthetic reactions, such as the PSII repair cycle, photoprotection of PSI and PSII, as well as the CBB cycle. This relationship between calcium signaling and photosynthesis presents great potential for exploration in future.

6 CONCLUDING REMARKS

Results gained from this study lead to following conclusions:

Presence of only PsbO2 in PSII causes a clear malfunction in both the donor and acceptor sides of PSII. PsbO1 stabilizes PSII more efficiently compared to PsbO2 under light stress. However, PsbO2 increases the rate of PSII repair cycle and thus is capable of partially compensating the lack of PsbO1.

PsbO2 shows higher GTPase activity compared to PsbO1 and possibly assists in D1 degradation.

PGR5 is needed for limiting linear electron transfer upon a shift to high light and to protect PSI against photodamage under fluctuating light conditions.

CaS interacts with PGR5, PGRL1 and co-migrates together with ATPase. Through its diverse interactions, CaS may delicately regulate photosynthetic functions under environmental challenges.

CaS participates in the regulation of Calvin-Benson-Bassham cycle and effects its activation during a dark to light shift.

ACKNOWLEDGEMENTS

This work was carried out in the Laboratory of Molecular Plant Physiology at the University of Turku. Financial support from the Academy of Finland, Finnish Doctoral School of Plant Science and University of Turku Graduate School is gratefully acknowledged.

I am very grateful to my supervisors Prof. Eva-Mari Aro and Dr. Saijaliisa Kangasjärvi for the possibility to work in a highly motivating environment and for all the help and guidance provided. I also want to thank my "unofficial" supervisor Dos. (Fos.) Marjaana Suorsa, who has spent a lot of time helping me with all kind of problems. I'm also grateful for advice provided by my follow up group members Prof. Eevi Rintamäki and Prof. Jaakko Kangasjärvi.

Prof. Wolfgang Schröder and Dr. Julia Vainonen are acknowledged for critically reviewing the thesis. Thanks to Fiona Lynch for revising the language.

I thank Mika, Vipu, Kurt, Ulla-Maija, Eve and Anniina for technical assistance.

Many thanks to Mikko, Ninni, Pikku-Marjaana, Moona, Tuomas, Lauri, Minna, Pikku-Minna and all other present and former co-workers for interesting discussions and all the help you have provided. You are more than co-workers, you are friends. Special thanks to Emeritus Prof. Marjatta Raudaskoski for all the advice and help you provided, not forgetting our nice discussions.

I also want to thank my family and friends for all the support that you have provided to me.

Turku, August 2014

Markus

REFERENCES

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

Allahverdiyeva Y, Deak Z, Szilard A, Diner BA, Nixon PJ, Vass I (2004) The function of D1-H332 in Photosystem II electron transport studied by thermoluminescence and chlorophyll fluorescence in site-directed mutants of Synechocystis 6803. Eur J Biochem **271:** 3523-3532

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 GJ, Kuchitsu K, Chu SP, Murata Y, Schroeder JI (1999) Arabidopsis abi1-1 and abi2-1 phosphatase mutations reduce abscisic acid-induced cytoplasmic calcium rises in guard cells. Plant Cell **11:** 1785-1798

Anderson J,M, Aro E,M (1994) Grana stacking and protection of photosystem II in thylakoid membranes of higher-plant leaves under sustained high irradiance- an hypothesis. Photosynthesis Research **41:** 315-326

Anderson JM, Chow WS, De Las Rivas J (2008) Dynamic flexibility in the structure and function of photosystem II in higher plant thylakoid membranes: the grana enigma. Photosynth Res **98:** 575-587

Armbruster U, Labs M, Pribil M, Viola S, Xu W, Scharfenberg M, Hertle AP, Rojahn U, Jensen PE, Rappaport F, Joliot P, Dormann P, Wanner G, Leister D (2013) Arabidopsis CURVATURE THYLAKOID1 proteins modify thylakoid architecture by inducing membrane curvature. Plant Cell **25:** 2661-2678

Armbruster U, Zuhlke J, Rengstl B, Kreller R, Makarenko E, Ruhle T, Schunemann D, Jahns P, Weisshaar B, Nickelsen J, Leister D (2010) The Arabidopsis thylakoid protein PAM68 is required for efficient D1 biogenesis and photosystem II assembly. Plant Cell **22:** 3439-3460

Aro EM, Suorsa M, Rokka A, Allahverdiyeva Y, Paakkarinen V, Saleem A, Battchikova N, Rintamäki 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

Avenson TJ, Cruz JA, Kanazawa A, Kramer DM (2005) Regulating the proton budget of higher plant photosynthesis. Proc Natl Acad Sci U S A **102:** 9709-9713

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

Bailey S, Thompson E, Nixon PJ, Horton P, Mullineaux CW, Robinson C, Mann NH (2002) A critical role for the Var2 FtsH homologue of Arabidopsis thaliana in the photosystem II repair cycle in vivo. J Biol Chem **277:** 2006-2011

Baniulis D, Yamashita E, Zhang H, Hasan SS, Cramer WA (2008) Structure-function of the cytochrome b_6f complex. Photochem Photobiol **84:** 1349-1358

Barber J (2012) Photosystem II: the water-splitting enzyme of photosynthesis. Cold Spring Harb Symp Quant Biol **77:** 295-307

Batistic O, Sorek N, Schultke S, Yalovsky S, Kudla J (2008) Dual fatty acyl modification determines the localization and plasma membrane targeting of CBL/CIPK Ca^{2+} signaling complexes in Arabidopsis. Plant Cell **20:** 1346-1362

Beligni MV, Lamattina L (2000) Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. Planta **210:** 215-221

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

Berridge MJ, Lipp P, Bootman MD (2000) The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol **1:** 11-21

Blum,H:Beier,H:Gross,H (1987) Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. **8:** 93-99

Boekema EJ, van Breemen JF, van Roon H, Dekker JP (2000) Conformational changes in photosystem II supercomplexes upon removal of extrinsic subunits. Biochemistry **39:** 12907-12915

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

Bordo D, Bork P (2002) The rhodanese/Cdc25 phosphatase superfamily. Sequence-structure-function relations. EMBO Rep **3:** 741-746

Brand JJ, Becker DW (1984) Evidence for direct roles of calcium in photosynthesis. J Bioenerg Biomembr **16:** 239-249

Bricker TM (1992) Oxygen evolution in the absence of the 33-kilodalton manganese-stabilizing protein. Biochemistry **31:** 4623-4628

Bricker TM, Frankel LK (2008) The *psbo1* mutant of Arabidopsis cannot efficiently use calcium in support of oxygen evolution by photosystem II. J Biol Chem **283:** 29022-29027

Buchanan BB, Schurmann P, Wolosiuk RA, Jacquot JP (2002) The ferredoxin/thioredoxin system: from discovery to molecular structures and beyond. Photosynth Res **73:** 215-222

Chardot T, Meunier JC (1991) Properties of oxidized and reduced spinach (*Spinacia oleracea*) chloroplast fructose-1,6-bisphosphatase activated by various agents. Biochem J **278 (Pt 3):** 787-791

Charles SA, Halliwell B (1980) Action of calcium ions on spinach (*Spinacia oleracea*) chloroplast fructose bisphosphatase and other enzymes of the Calvin cycle. Biochem J **188:** 775-779

Chi W, Sun X, Zhang L (2012) The roles of chloroplast proteases in the biogenesis and maintenance of photosystem II. Biochim Biophys Acta **1817:** 239-246

DalCorso G, Pesaresi P, Masiero S, Aseeva E, Schunemann D, Finazzi G, Joliot P, Barbato R, Leister D (2008) A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in Arabidopsis. Cell **132:** 273-285

Danielsson R, Albertsson PA, Mamedov F, Styring S (2004) Quantification of photosystem I and II in different parts of the thylakoid membrane from spinach. Biochim Biophys Acta **1608:** 53-61

Danielsson R, Suorsa M, Paakkarinen V, Albertsson PA, Styring S, Aro EM, Mamedov F (2006) Dimeric and monomeric organization of photosystem II. Distribution of five distinct complexes in the different domains of the thylakoid membrane. J Biol Chem **281:** 14241-14249

de Bianchi S, Ballottari M, Dall'osto L, Bassi R (2010) Regulation of plant light harvesting by thermal dissipation of excess energy. Biochem Soc Trans **38:** 651-660

De Las Rivas J, Balsera M, Barber J (2004) Evolution of oxygenic photosynthesis: genome-wide analysis of the OEC extrinsic proteins. Trends Plant Sci **9:** 18-25

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

Dekker JP, Boekema EJ (2005) Supramolecular organization of thylakoid membrane proteins in green plants. Biochim Biophys Acta **1706:** 12-39

Denison FC, Paul AL, Zupanska AK, Ferl RJ (2011) 14-3-3 Proteins in Plant Physiology. Semin Cell Dev Biol **22:** 720-727

Dodd AN, Kudla J, Sanders D (2010) The language of calcium signaling. Annu Rev Plant Biol **61:** 593-620

Dorrell RG, Howe CJ (2012) What makes a chloroplast? Reconstructing the establishment of photosynthetic symbioses. J Cell Sci **125:** 1865-1875

Ettinger WF, Clear AM, Fanning KJ, Peck ML (1999) Identification of a Ca^{2+}/H^{+} antiport in the plant chloroplast thylakoid membrane. Plant Physiol **119:** 1379-1386

Fleischmann MM, Ravanel S, Delosme R, Olive J, Zito F, Wollman FA, Rochaix JD (1999) Isolation and characterization of photoautotrophic mutants of Chlamydomonas reinhardtii deficient in state transition. J Biol Chem **274:** 30987-30994

Frenkel M, Jänkänpää HJ, Moen J, Jansson S (2008) An illustrated gardener's guide to transgenic Arabidopsis field experiments. New Phytol **180:** 545-555

Friedrich T, Weiss H (1997) Modular evolution of the respiratory NADH:ubiquinone oxidoreductase and the origin of its modules. J Theor Biol **187:** 529-540

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

Goral TK, Johnson MP, Brain AP, Kirchhoff H, Ruban AV, Mullineaux CW (2010) Visualizing the mobility and distribution of chlorophyll proteins in higher plant thylakoid membranes: effects of photoinhibition and protein phosphorylation. Plant J **62:** 948-959

Goulas E, Schubert M, Kieselbach T, Kleczkowski LA, Gardestrom P, Schroder W, Hurry V (2006) The chloroplast lumen and stromal proteomes of *Arabidopsis thaliana* show differential sensitivity to short- and long-term exposure to low temperature. Plant J **47:** 720-734

Hakala M, Tuominen I, Keränen M, Tyystjärvi T, Tyystjärvi E (2005) Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of Photosystem II. Biochim Biophys Acta **1706:** 68-80

Hakala-Yatkin M, Sarvikas P, Paturi P, Mäntysaari M, Mattila H, Tyystjärvi T, Nedbal L, Tyystjärvi E (2011) Magnetic field protects plants against high light by slowing down production of singlet oxygen. Physiol Plant **142:** 26-34

Haldrup A, Naver H, Scheller HV (1999) The interaction between plastocyanin and photosystem I is inefficient in transgenic Arabidopsis plants lacking the PSI-N subunit of photosystem I. Plant J **17:** 689-698

Haldrup A, Simpson DJ, Scheller HV (2000) Down-regulation of the PSI-F subunit of photosystem I (PSI) in Arabidopsis thaliana. The PSI-F subunit is essential for photoautotrophic growth and contributes to antenna function. J Biol Chem **275:** 31211-31218

Hall M, Mata-Cabana A, Akerlund HE, Florencio FJ, Schroder WP, Lindahl M, Kieselbach T (2010) Thioredoxin targets of the plant chloroplast lumen and their implications for plastid function. Proteomics **10:** 987-1001

Han S, Tang R, Anderson LK, Woerner TE, Pei ZM (2003) A cell surface receptor mediates extracellular $Ca^{(2+)}$ sensing in guard cells. Nature **425:** 196-200

Harbinson J, Genty B, Foyer CH (1990) Relationship between Photosynthetic Electron Transport and Stromal Enzyme Activity in Pea Leaves : Toward an Understanding of the Nature of Photosynthetic Control. Plant Physiol **94:** 545-553

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

Herbstova M, Tietz S, Kinzel C, Turkina MV, Kirchhoff H (2012) Architectural switch in plant photosynthetic membranes induced by light stress. Proc Natl Acad Sci U S A **109:** 20130-20135

Hoff AJ (1981) Magnetic field effects on photosynthetic reactions. Q Rev Biophys **14:** 599-665

Hohmann-Marriott MF, Blankenship RE (2011) Evolution of photosynthesis. Annu Rev Plant Biol **62:** 515-548

Horton P (2012) Optimization of light harvesting and photoprotection: molecular mechanisms and physiological consequences. Philos Trans R Soc Lond B Biol Sci **367:** 3455-3465

Huang SS, Chen J, Dong XJ, Patton J, Pei ZM, Zheng HL (2011) Calcium and calcium receptor CAS promote Arabidopsis thaliana de-etiolation. Physiol Plant

Huesgen PF, Schuhmann H, Adamska I (2006) Photodamaged D1 protein is degraded in Arabidopsis mutants lacking the Deg2 protease. FEBS Lett **580:** 6929-6932

Hundal,T:Virgin,I:Styring,S:Andersson,B (1990) Changes in the organization of photosystem II following light-induced D1 protein degredation. Biochim. Biophys. Acta **1017:** 235-241

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

Ifuku K, Endo T, Shikanai T, Aro EM (2011) Structure of the chloroplast NADH dehydrogenase-like complex: nomenclature for nuclear-encoded subunits. Plant Cell Physiol **52:** 1560-1568

Ifuku K, Ishihara S, Sato F (2010) Molecular functions of oxygen-evolving complex family proteins in photosynthetic electron flow. J Integr Plant Biol **52:** 723-734

Imashimizu M, Bernat G, Sunamura E, Broekmans M, Konno H, Isato K, Rogner M, Hisabori T (2011) Regulation of F0F1-ATPase from Synechocystis sp. PCC 6803 by gamma and epsilon subunits is significant for light/dark adaptation. J Biol Chem **286:** 26595-26602

Inoue K (2011) Emerging roles of the chloroplast outer envelope membrane. Trends Plant Sci **16:** 550-557

Iwai M, Takizawa K, Tokutsu R, Okamuro A, Takahashi Y, Minagawa J (2010) Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis. Nature **464:** 1210-1213

Jahns P, Holzwarth AR (2012) The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. Biochim Biophys Acta **1817:** 182-193

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

Järvi S, Suorsa M, Paakkarinen V, Aro EM (2011) Optimized native gel systems for separation of thylakoid protein complexes: novel super- and mega-complexes. Biochem J **439:** 207-214

Johnson CH, Knight MR, Kondo T, Masson P, Sedbrook J, Haley A, Trewavas A (1995) Circadian oscillations of cytosolic and chloroplastic free calcium in plants. Science **269:** 1863-1865

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

Karamoko M, Cline S, Redding K, Ruiz N, Hamel PP (2011) Lumen Thiol Oxidoreductase1, a disulfide bond-forming catalyst, is required for the assembly of photosystem II in Arabidopsis. Plant Cell **23:** 4462-4475

Kargul J, Janna Olmos JD, Krupnik T (2012) Structure and function of photosystem I and its application in biomimetic solar-to-fuel systems. J Plant Physiol **169:** 1639-1653

Kato Y, Miura E, Ido K, Ifuku K, Sakamoto W (2009) The variegated mutants lacking chloroplastic FtsHs are defective in D1 degradation and accumulate reactive oxygen species. Plant Physiol **151:** 1790-1801

Ketcham SR, FAU - Davenport JW, Davenport JW, FAU - Warncke K, Warncke K, FAU - McCarty RE, McCarty RE (1984) Role of the gamma subunit of chloroplast coupling factor 1 in the light-dependent activation of photophosphorylation and ATPase activity by dithiothreitol. - J Biol Chem. **259:** 7286-93.

Kirchhoff H (2014) Diffusion of molecules and macromolecules in thylakoid membranes. Biochim Biophys Acta **1837:** 495-502

Kirchhoff H (2013) Architectural switches in plant thylakoid membranes. Photosynth Res **116:** 481-487

Kirchhoff H (2013) Structural constraints for protein repair in plant photosynthetic membranes. Plant Signal Behav **8:** e23634

Kirchhoff H, Hall C, Wood M, Herbstova M, Tsabari O, Nevo R, Charuvi D, Shimoni E, Reich Z (2011) Dynamic control of protein diffusion within the granal thylakoid lumen. Proc Natl Acad Sci U S A **108:** 20248-20253

Kofer W, Koop HU, Wanner G, Steinmuller K (1998) Mutagenesis of the genes encoding subunits A, C, H, I, J and K of the plastid NAD(P)H-plastoquinone-oxidoreductase in tobacco by polyethylene glycol-mediated plastome transformation. Mol Gen Genet **258:** 166-173

Kohzuma K, Dal Bosco C, Meurer J, Kramer DM (2013) Light- and metabolism-related regulation of the chloroplast ATP synthase has distinct mechanisms and functions. J Biol Chem **288:** 13156-13163

Kono M, Noguchi K, Terashima I (2014) Roles of the Cyclic Electron Flow Around PSI (CEF-PSI) and O_2-Dependent Alternative Pathways in Regulation of the Photosynthetic Electron Flow in Short-Term Fluctuating Light in Arabidopsis thaliana. Plant Cell Physiol **55:** 990-1004

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

Kouril R, Wientjes E, Bultema JB, Croce R, Boekema EJ (2013) High-light vs. low-light: effect of light acclimation on photosystem II composition and organization in *Arabidopsis thaliana*. Biochim Biophys Acta **1827:** 411-419

Krieger-Liszkay A (2005) Singlet oxygen production in photosynthesis. J Exp Bot **56:** 337-346

Kulheim C, Agren J, Jansson S (2002) Rapid regulation of light harvesting and plant fitness in the field. Science **297:** 91-93

Laisk A, Eichelmann H, Oja V, Peterson RB (2005) Control of cytochrome b_6f at low and high light intensity and cyclic electron transport in leaves. Biochim Biophys Acta **1708:** 79-90

Laisk A, Eichelmann H, Oja V, Talts E, Scheibe R (2007) Rates and roles of cyclic and alternative electron flow in potato leaves. Plant Cell Physiol **48:** 1575-1588

Li J, Lee GI, Van Doren SR, Walker JC (2000) The FHA domain mediates phosphoprotein interactions. J Cell Sci **113 Pt 23:** 4143-4149

Li XG, Duan W, Meng QW, Zou Q, Zhao SJ (2004) The function of chloroplastic NAD(P)H dehydrogenase in tobacco during chilling stress under low irradiance. Plant Cell Physiol **45:** 103-108

Li XP, 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 Z, Wakao S, Fischer BB, Niyogi KK (2009) Sensing and responding to excess light. Annu Rev Plant Biol **60:** 239-260

Lindahl M, Spetea C, Hundal T, Oppenheim AB, Adam Z, Andersson B (2000) The thylakoid FtsH protease plays a role in the light-induced turnover of the photosystem II D1 protein. Plant Cell **12:** 419-431

Liu H, Frankel LK, Bricker TM (2007) Functional analysis of photosystem II in a PsbO-1-deficient mutant in *Arabidopsis thaliana*. Biochemistry **46:** 7607-7613

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem **193:** 265-275

Lunde C, Jensen PE, Haldrup A, Knoetzel J, Scheller HV (2000) The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. Nature **408:** 613-615

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, 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

Ma J, Peng L, Guo J, Lu Q, Lu C, Zhang L (2007) LPA2 is required for efficient assembly of photosystem II in Arabidopsis thaliana. Plant Cell **19:** 1980-1993

McDonald AE, Ivanov AG, Bode R, Maxwell DP, Rodermel SR, Huner NP (2011) Flexibility in photosynthetic electron transport: the physiological role of plastoquinol terminal oxidase (PTOX). Biochim Biophys Acta **1807:** 954-967

Meurer J, Plucken H, Kowallik KV, Westhoff P (1998) A nuclear-encoded protein of prokaryotic origin is essential for the stability of photosystem II in *Arabidopsis thaliana*. EMBO J **17:** 5286-5297

Muller B, Eichacker LA (1999) Assembly of the D1 precursor in monomeric photosystem II reaction center precomplexes precedes chlorophyll a-triggered accumulation of reaction center II in barley etioplasts. Plant Cell **11:** 2365-2377

Mulo P, Sirpiö S, Suorsa M, Aro EM (2008) Auxiliary proteins involved in the assembly and sustenance of photosystem II. Photosynth Res **98:** 489-501

Munekage Y, Hashimoto M, Miyake C, Tomizawa K, Endo T, Tasaka M, Shikanai T (2004) Cyclic electron flow around photosystem I is essential for photosynthesis. Nature **429:** 579-582

Munekage Y, Hojo M, Meurer J, Endo T, Tasaka M, Shikanai T (2002) PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in Arabidopsis. Cell **110:** 361-371

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

Murakami R, Ifuku K, Takabayashi A, Shikanai T, Endo T, Sato F (2002) Characterization of an *Arabidopsis thaliana* mutant with impaired psbO, one of two genes encoding extrinsic 33-kDa proteins in photosystem II. FEBS Lett **523:** 138-142

Murray JW, Barber J (2006) Identification of a calcium-binding site in the PsbO protein of photosystem II. Biochemistry **45:** 4128-4130

Nalin CM, McCarty RE (1984) Role of a disulfide bond in the gamma subunit in activation of the ATPase of chloroplast coupling factor 1. J Biol Chem **259:** 7275-7280

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

Nelson TJ, Quattrone A, Kim J, Pacini A, Cesati V, Alkon DL (2003) Calcium-regulated GTPase activity in the calcium-binding protein calexcitin. Comp Biochem Physiol B Biochem Mol Biol **135:** 627-638

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

Nickelsen J, Rengstl B (2013) Photosystem II assembly: from cyanobacteria to plants. Annu Rev Plant Biol **64:** 609-635

Nishiyama Y, Allakhverdiev SI, Murata N (2011) Protein synthesis is the primary target of reactive oxygen species in the photoinhibition of photosystem II. Physiol Plant **142:** 35-46

Niyogi KK, Li XP, Rosenberg V, Jung HS (2005) Is PsbS the site of non-photochemical quenching in photosynthesis? J Exp Bot **56:** 375-382

Nomura H, Komori T, Kobori M, Nakahira Y, Shiina T (2008) Evidence for chloroplast control of external Ca^{2+}-induced cytosolic Ca^{2+} transients and stomatal closure. Plant J **53:** 988-998

Nomura H, Komori T, Uemura S, Kanda Y, Shimotani K, Nakai K, Furuichi T, Takebayashi K, Sugimoto T, Sano S, Suwastika IN, Fukusaki E, Yoshioka H, Nakahira Y, Shiina T (2012) Chloroplast-mediated activation of plant immune signalling in Arabidopsis. Nat Commun **3:** 926

Obayashi T, Okamura Y, Ito S, Tadaka S, Aoki Y, Shirota M, Kinoshita K (2014) ATTED-II in 2014: evaluation of gene coexpression in agriculturally important plants. Plant Cell Physiol **55:** e6

Okuno D, Iino R, Noji H (2011) Rotation and structure of FoF1-ATP synthase. J Biochem **149:** 655-664

Peng L, Fukao Y, Fujiwara M, Takami T, Shikanai T (2009) Efficient operation of NAD(P)H dehydrogenase requires supercomplex formation with photosystem I via minor LHCI in Arabidopsis. Plant Cell **21:** 3623-3640

Peng L, Ma J, Chi W, Guo J, Zhu S, Lu Q, Lu C, Zhang L (2006) LOW PSII ACCUMULATION1 is involved in efficient assembly of photosystem II in *Arabidopsis thaliana*. Plant Cell **18:** 955-969

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

Peng L, Shimizu H, Shikanai T (2008) The chloroplast NAD(P)H dehydrogenase complex interacts with photosystem I in Arabidopsis. J Biol Chem **283:** 34873-34879

Pesaresi P, Hertle A, Pribil M, Kleine T, Wagner R, Strissel H, Ihnatowicz A, Bonardi V, Scharfenberg M, Schneider A, Pfannschmidt T, Leister D (2009) Arabidopsis STN7 kinase provides a link between short- and long-term photosynthetic acclimation. Plant Cell **21:** 2402-2423

Peter GF, Thornber JP (1991) Biochemical composition and organization of higher plant photosystem II light-harvesting pigment-proteins. J Biol Chem **266:** 16745-16754

Petroutsos D, Busch A, Janssen I, Trompelt K, Bergner SV, Weinl S, Holtkamp M, Karst U, Kudla J, Hippler M (2011) The chloroplast calcium sensor CAS is required for photoacclimation in *Chlamydomonas reinhardtii*. Plant Cell **23:** 2950-2963

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous-equations for assaying chlorophyll-A and chlorophyll-B extracted with 4 different solvents – verification of the concentration of chlorophyll standards by atomic-absorption spectroscopy. Biochim. Biophys. Acta **975:** 384-394

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, Ibrahim IM, Allen JF (2013) Evolutionary rewiring: a modified prokaryotic gene-regulatory pathway in chloroplasts. Philos Trans R Soc Lond B Biol Sci **368:** 20120260

Puthiyaveetil S, Ibrahim IM, Allen JF (2012) Oxidation-reduction signalling components in regulatory pathways of state transitions and photosystem stoichiometry adjustment in chloroplasts. Plant Cell Environ **35:** 347-359

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

Rintamäki 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

Roberts IN, Lam XT, Miranda H, Kieselbach T, Funk C (2012) Degradation of PsbO by the Deg protease HhoA Is thioredoxin dependent. PLoS One **7:** e45713

Rocha AG:,U.C. (2013) Identification of CP12 as a Novel Calcium-Binding Protein in Chloroplasts. Plants **2:** 530-540

Rocha AG, Vothknecht UC (2012) The role of calcium in chloroplasts--an intriguing and unresolved puzzle. Protoplasma **249:** 957-966

Rochaix JD, Lemeille S, Shapiguzov A, Samol I, Fucile G, Willig A, Goldschmidt-Clermont M (2012) Protein kinases and phosphatases involved in the acclimation of the photosynthetic apparatus to a changing light environment. Philos Trans R Soc Lond B Biol Sci **367:** 3466-3474

Roh MH, Shingles R, Cleveland MJ, McCarty RE (1998) Direct measurement of calcium transport across chloroplast inner-envelope vesicles. Plant Physiol **118:** 1447-1454

Rokka A, Suorsa M, Saleem A, Battchikova N, Aro EM (2005) Synthesis and assembly of thylakoid protein complexes: multiple assembly steps of photosystem II. Biochem J **388:** 159-168

Rott M, Martins NF, Thiele W, Lein W, Bock R, Kramer DM, Schottler MA (2011) ATP synthase repression in tobacco restricts photosynthetic electron transport, CO2 assimilation, and plant growth by overacidification of the thylakoid lumen. Plant Cell **23:** 304-321

Rumberg B, Siggel U (1969) pH changes in the inner phase of the thylakoids during photosynthesis. Naturwissenschaften **56:** 130-132

Rumeau D, Peltier G, Cournac L (2007) Chlororespiration and cyclic electron flow around PSI during photosynthesis and plant stress response. Plant Cell Environ **30:** 1041-1051

Sai J, Johnson CH (2002) Dark-stimulated calcium ion fluxes in the chloroplast stroma and cytosol. Plant Cell **14:** 1279-1291

Sakamoto W, Tamura T, Hanba-Tomita Y, Murata M, Sodmergen (2002) The VAR1 locus of Arabidopsis encodes a chloroplastic FtsH and is responsible for leaf variegation in the mutant alleles. Genes Cells **7:** 769-780

Sazanov LA, Burrows PA, Nixon PJ (1998) The plastid ndh genes code for an NADH-specific dehydrogenase: isolation of a complex I analogue from pea thylakoid membranes. Proc Natl Acad Sci U S A **95:** 1319-1324

Schweitzer C, Schmidt R (2003) Physical mechanisms of generation and deactivation of singlet oxygen. Chem Rev **103:** 1685-1757

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

Shikanai T (2007) Cyclic electron transport around photosystem I: genetic approaches. Annu Rev Plant Biol **58:** 199-217

Shikanai T, Endo T, Hashimoto T, Yamada Y, Asada K, Yokota A (1998) Directed disruption of the tobacco ndhB gene impairs cyclic electron flow around photosystem I. Proc Natl Acad Sci U S A **95:** 9705-9709

Shimizu M, Kato H, Ogawa T, Kurachi A, Nakagawa Y, Kobayashi H (2010) Sigma factor phosphorylation in the photosynthetic control of photosystem stoichiometry. Proc Natl Acad Sci U S A **107:** 10760-10764

Shutova T, Nikitina J, Deikus G, Andersson B, Klimov V, Samuelsson G (2005) Structural dynamics of the manganese-stabilizing protein-effect of pH, calcium, and manganese. Biochemistry **44:** 15182-15192

Sonoike K (2011) Photoinhibition of photosystem I. Physiol Plant **142:** 56-64

Sonoike K, Terashima I, Iwaki M, Itoh S (1995) Destruction of photosystem I iron-sulfur centers in leaves of *Cucumis sativus L*. by weak illumination at chilling temperatures. FEBS Lett **362:** 235-238

Spetea C, Hundal T, Lohmann F, Andersson B (1999) GTP bound to chloroplast thylakoid membranes is required for light-induced, multienzyme degradation of the photosystem II D1 protein. Proc Natl Acad Sci U S A **96:** 6547-6552

Stael S, Rocha AG, Wimberger T, Anrather D, Vothknecht UC, Teige M (2011) Cross-talk between calcium signalling and protein phosphorylation at the thylakoid. J Exp Bot **63:** 1725-1733

Sun C, Johnson JM, Cai D, Sherameti I, Oelmuller R, Lou B (2010) *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. J Plant Physiol **167:** 1009-1017

Sun X, Peng L, Guo J, Chi W, Ma J, Lu C, Zhang L (2007) Formation of DEG5 and DEG8 complexes and their involvement in the degradation of photodamaged photosystem II reaction center D1 protein in Arabidopsis. Plant Cell **19:** 1347-1361

Suorsa M, Aro EM (2007) Expression, assembly and auxiliary functions of photosystem II oxygen-evolving proteins in higher plants. Photosynth Res **93:** 89-100

Suorsa M, Regel RE, Paakkarinen V, Battchikova N, Herrmann RG, Aro EM (2004) Protein assembly of photosystem II and accumulation of subcomplexes in the absence of low molecular mass subunits PsbL and PsbJ. Eur J Biochem **271:** 96-107

Suorsa M, Sirpiö S, Allahverdiyeva Y, Paakkarinen V, Mamedov F, Styring S, Aro EM (2006) PsbR, a missing link in the assembly of the oxygen-evolving complex of plant photosystem II. J Biol Chem **281:** 145-150

Surek B, Kreimer G, Melkonian M, Latzko E (1987) Spinach ferredoxin is a calcium-binding protein. Planta **171:** 565-568

Szechynska-Hebda M, Karpinski S (2013) Light intensity-dependent retrograde signalling in higher plants. J Plant Physiol **170:** 1501-1516

Takabayashi A, Kishine M, Asada K, Endo T, Sato F (2005) Differential use of two cyclic electron flows around photosystem I for driving CO2-concentration mechanism in C4 photosynthesis. Proc Natl Acad Sci U S A **102:** 16898-16903

Tang RH, Han S, Zheng H, Cook CW, Choi CS, Woerner TE, Jackson RB, Pei ZM (2007) Coupling diurnal cytosolic Ca^{2+} oscillations to the CAS-IP3 pathway in Arabidopsis. Science **315:** 1423-1426

Terashima M, Petroutsos D, Hudig M, Tolstygina I, Trompelt K, Gabelein P, Fufezan C, Kudla J, Weinl S, Finazzi G, Hippler M (2012) Calcium-dependent regulation of cyclic photosynthetic electron transfer by a CAS, ANR1, and PGRL1 complex. Proc Natl Acad Sci U S A **109:** 17717-17722

Thordal-Christensen,H:Zhang,Z:Wei,Y:Collinge,D.B (1997) Subcellular localization of H_2O_2 in plants. H_2O_2 accumulation in papillae and hypersensitive response furing the barley powdery mildew interaction. **11:** 1187-1194

Tikhonov AN (2013) pH-dependent regulation of electron transport and ATP synthesis in chloroplasts. Photosynth Res **116:** 511-534

Tikhonov AN (2012) Energetic and regulatory role of proton potential in chloroplasts. Biochemistry (Mosc) **77:** 956-974

Tikkanen M, Grieco M, Kangasjärvi S, Aro EM (2010) Thylakoid protein phosphorylation in higher plant chloroplasts optimizes electron transfer under fluctuating light. Plant Physiol **152:** 723-735

Tikkanen M, Grieco M, Nurmi M, Rantala M, Suorsa M, Aro EM (2012) Regulation of the photosynthetic apparatus under fluctuating growth light. Philos Trans R Soc Lond B Biol Sci **367:** 3486-3493

Tikkanen M, Mekala NR, Aro EM (2014) Photosystem II photoinhibition-repair cycle protects Photosystem I from irreversible damage. Biochim Biophys Acta **1837:** 210-215

Tikkanen M, Nurmi M, Kangasjärvi 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, Suorsa M, Gollan PJ, Aro EM (2012) Post-genomic insight into thylakoid membrane lateral heterogeneity and redox balance. FEBS Lett **586:** 2911-2916

Tjus SE, Scheller HV, Andersson B, Moller BL (2001) Active oxygen produced during selective excitation of photosystem I is damaging not only to photosystem I, but also to photosystem II. Plant Physiol **125:** 2007-2015

Trissl HW, Wilhelm C (1993) Why do thylakoid membranes from higher plants form grana stacks? Trends Biochem Sci **18:** 415-419

Trost P, Fermani S, Marri L, Zaffagnini M, Falini G, Scagliarini S, Pupillo P, Sparla F (2006) Thioredoxin-dependent regulation of photosynthetic glyceraldehyde-3-phosphate dehydrogenase: autonomous vs. CP12-dependent mechanisms. Photosynth Res **89:** 263-275

Tsiotis G, Psylinakis M, Woplensinger B, Lustig A, Engel A, Ghanotakis D (1999) Investigation of the structure of spinach photosystem II reaction center complex. Eur J Biochem **259:** 320-324

Tyystjärvi E (2013) Photoinhibition of Photosystem II. Int Rev Cell Mol Biol **300:** 243-303

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

Tyystjärvi E, Hakala M, Sarvikas P (2005) Mathematical modelling of the light response curve of photoinhibition of photosystem II. Photosynth Res **84:** 21-27

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 Walraven HS, Scholts MJ, Zakharov SD, Kraayenhof R, Dilley RA (2002) pH-dependent Ca^{2+} binding to the F0 c-subunit affects proton translocation of the ATP synthase from Synechocystis 6803. J Bioenerg Biomembr **34:** 455-464

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

Vothknecht UC, Westhoff P (2001) Biogenesis and origin of thylakoid membranes. Biochim Biophys Acta **1541:** 91-101

Waadt R, Kudla J (2008) In Planta Visualization of Protein Interactions Using Bimolecular Fluorescence Complementation (BiFC). CSH Protoc **2008:** pdb.prot4995

Wang P, Duan W, Takabayashi A, Endo T, Shikanai T, Ye JY, Mi H (2006) Chloroplastic NAD(P)H dehydrogenase in tobacco leaves functions in alleviation of oxidative damage caused by temperature stress. Plant Physiol **141:** 465-474

Wang WH, Chen J, Liu TW, Chen J, Han AD, Simon M, Dong XJ, He JX, Zheng HL (2014) Regulation of the calcium-sensing receptor in both stomatal movement and photosynthetic electron transport is crucial for water use efficiency and drought tolerance in Arabidopsis. J Exp Bot **65:** 223-234

Wang WH, Yi XQ, Han AD, Liu TW, Chen J, Wu FH, Dong XJ, He JX, Pei ZM, Zheng HL (2011) Calcium-sensing receptor regulates stomatal closure through hydrogen peroxide and nitric oxide in response to extracellular calcium in Arabidopsis. J Exp Bot **63:** 177-190

Wei L, Guo J, Ouyang M, Sun X, Ma J, Chi W, Lu C, Zhang L (2010) LPA19, a Psb27 homolog in Arabidopsis thaliana, facilitates D1 protein precursor processing during PSII biogenesis. J Biol Chem **285:** 21391-21398

Weinl S, Held K, Schlucking K, Steinhorst L, Kuhlgert S, Hippler M, Kudla J (2008) A plastid protein crucial for Ca^{2+}-regulated stomatal responses. New Phytol **179:** 675-686

Wientjes E, Drop B, Kouril R, Boekema EJ, Croce R (2013) During state 1 to state 2 transition in *Arabidopsis thaliana*, the photosystem II supercomplex gets phosphorylated but does not disassemble. J Biol Chem **288:** 32821-32826

Yamamoto H, Kato H, Shinzaki Y, Horiguchi S, Shikanai T, Hase T, Endo T, Nishioka M, Makino A, Tomizawa K, Miyake C (2006) Ferredoxin limits cyclic electron flow around PSI (CEF-PSI) in higher plants--stimulation of CEF-PSI enhances non-photochemical quenching of Chl fluorescence in transplastomic tobacco. Plant Cell Physiol **47:** 1355-1371

Yamamoto H, Peng L, Fukao Y, Shikanai T (2011) An Src homology 3 domain-like fold protein forms a ferredoxin binding site for the chloroplast NADH dehydrogenase-like complex in Arabidopsis. Plant Cell **23:** 1480-1493

Yi X, Hargett SR, Frankel LK, Bricker TM (2009) The PsbP protein, but not the PsbQ protein, is required for normal thylakoid architecture in *Arabidopsis thaliana*. FEBS Lett **583:** 2142-2147

Yu F, Park S, Rodermel SR (2004) The Arabidopsis FtsH metalloprotease gene family: interchangeability of subunits in chloroplast oligomeric complexes. Plant J **37:** 864-876

Zakharov SD, Ewy RG, Dilley RA (1993) Subunit III of the chloroplast ATP-synthase can form a $Ca^{(2+)}$-binding site on the lumenal side of the thylakoid membrane. FEBS Lett **336:** 95-99

Zhang L, Paakkarinen V, van Wijk KJ, Aro EM (1999) Co-translational assembly of the D1 protein into photosystem II. J Biol Chem **274:** 16062-16067

Zhang L, Paakkarinen V, van Wijk KJ, Aro EM (1999) Co-translational assembly of the D1 protein into photosystem II. J Biol Chem **274:** 16062-16067

Zhang N, Portis AR,Jr (1999) Mechanism of light regulation of Rubisco: a specific role for the larger Rubisco activase isoform involving reductive activation by thioredoxin-f. Proc Natl Acad Sci U S A **96:** 9438-9443