TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. AII OSA - TOM. 238

BIOLOGICA - GEOGRAPHICA - GEOLOGICA

NOVEL PROTEINS INVOLVED IN DYNAMICS OF THE PHOTOSYNTHETIC APPARATUS

by

Sari Sirpiö

TURUN YLIOPISTO
Turku 2009

From the Laboratory of Plant Physiology and Molecular Biology
Department of Biology
FI-20014 University of Turku, Finland

Supervised by

Professor Eva-Mari Aro
Laboratory of Plant Physiology and Molecular Biology
Department of Biology
FI-20014 University of Turku, Finland

Reviewed by

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

and

Professor Kurt Fagerstedt
Department of Biological and Environmental Sciences
FI-00014 University of Helsinki, Finland

Opponent

Professor Toshiharu Shikanai
Department of Botany
Kyoto 606-8502 Kyoto University, Japan

ISBN 978-951-29-3945-9 (PRINT)
ISBN 978-951-29-3946-6 (PDF)
ISSN 0082-6979
Painosalama Oy - Turku, Finland 2009

LIST OF ORIGINAL PUBLICATIONS

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

I Suorsa, M., Sirpiö, S., Allahverdiyeva, Y., Paakkarinen, V., Mamedov, F., Styring, S. & Aro, E.-M. (2006) PsbR, a missing link in the assembly of the oxygen-evolving complex of plant photosystem II. *J. Biol. Chem.* 281(1):145-150.

II Sirpiö, S., Allahverdiyeva, Y., Suorsa, M., Paakkarinen, V., Vainonen, J., Battchikova, N. & Aro, E.-M. (2007) TLP18.3, a novel thylakoid lumen protein regulating photosystem II repair cycle. *Biochem J.* 406(3):415-425.

III Sirpiö S.*, Khrouchtchova A.*, Allahverdiyeva Y., Hansson M., Fristedt R., Vener A.V., Scheller H.V., Jensen P.E., Haldrup A. & Aro E.-M. (*equally contributing authors) (2008) AtCYP38 ensures early biogenesis, correct assembly and sustenance of photosystem II. *Plant J.* 55(4):639-651.

IV Sirpiö, S., Allahverdiyeva, Y., Holmström, M., Khrouchtchova, A., Haldrup, A., Battchikova, N. & Aro, E.-M. (2009) Novel nuclear-encoded subunits of the chloroplast NAD(P)H dehydrogenase complex. *J. Biol. Chem.* 284(2):905-912.

ABBREVIATIONS

Arabidopsis	*Arabidopsis thaliana*
ATP	adenosine triphosphate
BN	blue native
CET	cyclic electron transport around PSI
Chl	chlorophyll
Cyt	cytochrome
2-D	two-dimensional
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DMBQ	2,6-dimethyl-*p*-benzoquinone
FF-relaxation	flash-induced increase and subsequent relaxation of chlorophyll fluorescence yield
FNR	ferredoxin-$NADP^+$-oxidoreductase
FQR	ferredoxin quinone oxidoreductase
IEF	isoelectric focusing
LHC	light harvesting complex
NADPH	nicotinamide adenine dinucleotide phosphate
NDH	NAD(P)H dehydrogenase
NPQ	non-photochemical quenching of Chl fluorescence
OEC	photosystem II oxygen-evolving complex
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PMF	proton motive force
polysome	polysome-nascent chain complex
PPIase	peptidyl-prolyl isomerase
PQ	plastoquinone
PS	photosystem
SDS	sodium dodecyl sulfate
T-DNA	transferred DNA, part of the Ti (tumor inducing) plasmid of *Agrobacterium tumefaciens*

TABLE OF CONTENTS

ABSTRACT

During the past few years, a considerable number of research articles have been published relating to the structure and function of the major photosynthetic protein complexes, photosystem (PS) I, PSII, cytochrome (Cyt) b_6f, and adenosine triphosphate (ATP) synthase. Sequencing of the *Arabidopsis thaliana* (*Arabidopsis*) genome together with several high-quality proteomics studies has, however, revealed that the thylakoid membrane network of plant chloroplasts still contains a number of functionally unknown proteins. These proteins may have a role as auxiliary proteins guiding the assembly, maintenance, and turnover of the thylakoid protein complexes, or they may be as yet unknown subunits of the photosynthetic complexes. Novel subunits are most likely to be found in the NAD(P)H dehydrogenase (NDH) complex, the structure and function of which have remained obscure in the absence of detailed crystallographic data, thus making this thylakoid protein complex a particularly interesting target of investigation.

In this thesis, several novel thylakoid-associated proteins were identified by proteomics-based methods. The major goal of characterization of the stroma thylakoid-associated polysome-nascent chain complexes was to determine the proteins that guide the dynamic life cycle of PSII. In addition, a large protein complex of ≥ 1,000 kDa, residing in the stroma thylakoid, was characterized in greater depth and it was found to be a supercomplex composed of the PSI and NDH complexes.

A set of newly identified proteins from *Arabidopsis* thylakoids was subjected to detailed characterization using the reverse genetics approach and extensive biochemical and biophysical analysis. The role of the novel proteins, either as auxiliary proteins or subunits of the photosynthetic protein complexes, was revealed. Two novel thylakoid lumen proteins, TLP18.3 and AtCYP38, function as auxiliary proteins assisting specific steps of the assembly/repair of PSII. The role of the 10-kDa thylakoid lumen protein PsbR is related to the optimization of oxygen evolution of PSII by assisting the assembly of the PsbP protein. Two integral thylakoid membrane proteins, NDH45 and NDH48, are novel subunits of the chloroplast NDH complex. Finally, the thylakoid lumen immunophilin AtCYP20-2 is suggested to interact with the NDH complex, instead of PSII as was hypothesized earlier.

1. INTRODUCTION

1.1. *Arabidopsis thaliana* (*Arabidopsis*)

Arabidopsis is an annual plant that belongs to the *Brassicaceae* family of flowering plants, including cultivated species such as cabbage and radish. Even though *Arabidopsis* has no agricultural significance, it is widely used as a model organism in plant science for several reasons. *Arabidopsis* is a small plant with a short life cycle and it is capable of producing a large number of seeds – up to several thousand per individual plant. The genome of *Arabidopsis*, which is composed of five chromosomes, is one of the smallest in the plant kingdom and in the year 2000 it became the first plant genome to be fully sequenced (*Arabidopsis* Genome Initiative, 2000). The reverse genetics of *Arabidopsis* is straightforward, by using *Agrobacterium tumefaciens*-mediated gene transfer and the floral dip method (Clough and Bent, 1998). Moreover, a large number of mutant lines and genomic resources are available from public stock centers (*Arabidopsis* Biological Resource Center, European *Arabidopsis* Stock Centre etc.) (Figure 1).

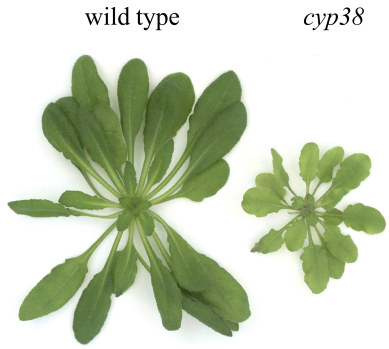

Figure 1. *Arabidopis thaliana* wild-type plant and a mutant plant deficient in immunophilin AtCYP38 (*cyp38*; GABI-Kat 744F08). The *Arabidopsis thaliana* genome contains 27235 protein-coding genes (based on TAIR8). A deficiency in only a single gene can lead to a dramatic phenotype.

1.2. Photosynthesis

From 2.5 to 3.5 billion years ago, the progenitors of the present-day cyanobacteria were evolving a novel trait, oxygenic photosynthesis. The chloroplasts of plant and algal cells are endosymbiotic remnants of free-living ancestral cyanobacteria (Barbrook et al. 1998; Curtis and Clegg, 1984; Delwiche et al. 1995; Turmel et al. 1999). The vast majority of chloroplast genes have been relocalized to the nucleus during evolution (Martin and Herrmann, 1998; Race et al. 1999) and while the genome of *Synechocystis* sp. PCC 6803, a current photosynthetic model organism, is 3.5 Mb in length and contains 3,168 potential protein-coding genes, the *Arabidopsis* chloroplast genome is only 0.3 Mb in size, with 87 potential protein-coding genes (Kaneko and Tabata 1997; Sato et al. 1999). However, the structure and function of the photosynthetic protein complexes in plants and cyanobacteria have remained rather conserved over time.

Photosynthesis comprises a series of biophysical and biochemical reactions that convert solar energy into chemical energy. Photosynthesis can be divided into two phases: the light reactions and the Calvin-Benson cycle. Light reactions take place in the thylakoid membrane network, which consists of the appressed membranes, the stroma-exposed membranes, and the soluble inner membrane space called the thylakoid lumen. In light reactions, the thylakoid membrane-embedded multi-subunit protein complexes capture the light energy and use it to make high-energy molecules. The Calvin-Benson cycle is located in the soluble compartment of the chloroplast called the stroma. In the Calvin-Benson cycle, the high-energy molecules produced in light reactions are utilized to fix inorganic carbon into chemically reduced carbohydrates. Carbohydrates such as sucrose, glucose and starch are used for energy storage and as precursors for other organic compounds, which, in turn, are used for growth and reproduction of both the photosynthetic and the heterotrophic organisms. Molecular oxygen is released as a by-product of the photosynthetic oxidation of water. Over billions of years, oxygenic photosynthesis has altered the composition of the Earth's atmosphere from anoxygenic to oxygenic, thereby enabling the development of aerobic life forms. The ozone layer in the stratosphere, which prevents the damaging ultraviolet light from reaching the Earth's surface, has been a prerequisite for life to develop outside the marine environments. The present life on Earth is entirely dependent on photosynthesis.

1.3. Thylakoid membrane protein complexes

1.3.1 The structure and function of PSII

PSII is a multi-subunit thylakoid membrane protein complex that catalyzes the light-driven electron transfer from water to plastoquinone (PQ) and, in order to fulfill this role, it is the most oxidative enzyme in the world. The PSII complex is most active in a dimeric form (Danielsson et al. 2006). The crystallographic structure of cyanobacterial (*Thermosynechococcus elongatus*) PSII has been determined at 2.9-Å resolution (Guskov et al. 2009).

PSII is located mostly in the grana membranes, and it consists of both intrinsic and extrinsic polypeptides (Andersson and Anderson 1980). PSII is composed of around 30 protein subunits (Nelson and Yocum, 2006; Shi and Schröder 2004). The minimal reaction center complex of PSII, capable of charge separation, contains five proteins: D1, D2, α and β subunits of Cyt b_{559}, and PsbI (Nanba and Satoh 1987). The D1 and D2 proteins bind the reaction center chlorophyll (Chl) P_{680} and all other redox-active cofactors needed for PSII electron transport. The PSII core complex contains the antenna proteins CP43 and CP47 with associated Chl and beta-carotene pigments, which both capture the excitation energy and pass the excitation energy collected by light harvesting complex (LHC)II to the reaction center Chl P_{680}. Electrons released from P_{680} are replaced from tyrosine Z and finally from water molecule, which is oxidized by the PSII oxygen-evolving complex

(OEC). The majority of subunits in PSII are of low molecular weight (less than 10 kDa) and are involved in PSII assembly, stabilization, dimerization, and photoprotection (Shi and Schröder 2004). An intriguing feature of many PSII and LHCII proteins (D1, D2, CP43, PsbH, Lhcb1, Lhcb2, and Lhcb4) is a reversible phosphorylation of the threonine residue at the very N-terminus of the protein (Vener 2007). Phosphorylation of protein subunits provides flexibility for PSII function under varying environmental conditions (Tikkanen et al. 2006, 2008).

1.3.2 The structure and function of the OEC complex of PSII

The OEC complex of PSII consists of the Mn_4Ca cluster and several lumenal PSII proteins and functions in water oxidation. The extrinsic proteins of OEC in higher plants include PsbO, PsbP, and PsbQ, which assist the binding of the catalytically important inorganic manganese, calcium, and chloride ions to PSII as Mn_4Ca cluster. In water oxidation Mn^{2+} ions of OEC undergo a series of oxidations called as S-states. After storing four positive charges, OEC oxidizes two water molecules and releases one oxygen molecule and four protons to the thylakoid lumen. Hence OEC participates in generation of a proton motive force (PMF), an electrochemical gradient of protons across the thylakoid membrane, which is used in order to drive ATP synthesis. In addition, PsbO and PsbP may also function to ensure the structural integrity of the PSII dimers and supercomplexes, respectively (Boekema et al. 2000).

Crystallographic analysis of the cyanobacterial PSII complex has revealed the three-dimensional structure of the Mn_4Ca cluster and identified the amino acid residues in the lumenal loops of the D1 and CP43 proteins required for the ligation of the Mn_4Ca cluster (Ferreira et al. 2004; Guskov et al. 2009; Loll et al. 2005). However, the subunit composition of OEC differs between cyanobacteria and higher plants; thus, the cyanobacterial crystallographic information as such cannot be directly applied to higher plants. In cyanobacteria OEC consists of the PsbO, PsbV (cyt c_{550}), and PsbU proteins – of which only PsbO is homologous to plant and green algae type protein (De Las Rivas et al. 2004). To date, the best model of plant PSII has been based on the published crystallographic structures of cyanobacterial PSII core and plant LHCII, PsbP, and PsbQ proteins (Nield and Barber 2006). According to this model, the PsbP and PsbQ proteins are located in different positions compared to the PsbU and PsbV proteins.

1.3.3. The structure and function of PSI, Cyt b_6f, and ATP synthase

In addition to PSII, the photosynthetic membranes contain three other abundant protein complexes: the PSI and Cyt b_6f complexes and ATP synthase. While PSI and the ATP synthase reside predominantly in the stroma-exposed membranes, the Cyt b_6f complex is distributed relatively evenly between the stroma and grana membranes (Albertsson et al. 1991; Andersson and Anderson 1980; Vallon et al. 1991).

In *Arabidopsis*, the PSI complex functions as a monomer consisting of at least 15 core subunits and 6 Lhca proteins (Jensen et al. 2007). The PsaA and PsaB subunits bind

to the reaction center Chl P_{700}. Indeed, out of the 15 core proteins, only PsaA, PsaB, and PsaC are directly involved in binding of electron transport cofactors, while others function in docking of plastocyanin and ferredoxin, in stabilization of PSI, as well as in docking of the LHCI antenna and also the LHCII subunits in state transitions (Jensen et al. 2007). The most accurate crystallographic structure of plant (*Pisum sativum*) PSI published so far is a 3.4-Å resolution structure by Amunts et al. (2007). PSI generates the most negative redox potential in nature and uses light energy for electron transport from plastocyanin to ferredoxin.

The Cyt b_6f complex shows similarity to the Cyt bc_1 complex of mitochondria, in terms of both structure and function. Cyt b_6f is composed of four major subunits – the Rieske subunit, Cyt *f*, Cyt b_6, and subunit IV – as well as four minor subunits – PetL, PetG, PetM, and PetN – and functions as a dimeric complex *in vivo* (Breyton et al. 1997). The structure of the Cyt b_6f complex has been resolved at 3.0-Å resolution (Kurisu et al. 2003, Stroebel et al. 2003). The Cyt b_6f complex functions in a cyclic process known as the Q (quinone) -cycle. In Q-cycle plastoquinol is oxidized and one of its electrons is transferred to plastocyanin, which results in release of two protons to the thylakoid lumen. The other electron of plastoquinol, in turn, is used to reduce PQ. After two electron transfer steps, plastoquinol is generated with simultaneous uptake of two protons from the stromal side of the thylakoid membrane. Via the function of the Q-cycle, two protons are translocated to the thylakoid lumen for each electron transferred. Thus, Cyt b_6f plays a key role in generation of PMF during photosynthetic electron transport (Hope 1993).

The ATP synthase of higher plant chloroplasts is F-type ATP synthase, and it bears a remarkable resemblance both in structure and function to the homologous complexes in bacteria and mitochondria (Dekker and Boekema 2005). The structure of the F-ATP synthase from bovine heart mitochondria has been solved at 2.8-Å resolution (Abrahams et al. 1994). ATP synthase is composed of three specific parts: a hydrophilic headpiece on the stromal side of thylakoids, a membrane-bound domain, and the stalk region connecting the first two domains. ATP synthase uses PMF in order to drive ATP synthesis (Dekker and Boekema 2005).

1.3.4. The structure and function of the NAD(P)H dehydrogenase (NDH) complex

In higher plant chloroplasts, the NDH complex resides in stroma-exposed thylakoid membranes and functions in cyclic electron transport around PSI (CET) and in chlororespiration (Kofer et al. 1998; Sazanov et al. 1998). The NDH complex is using stromal compounds to reduce the PQ pool and thus it plays a role in the ATP supply. Moreover, it functions in optimizing the acclimation of the photosynthetic apparatus to environmental cues (Burrows et al. 1998; Horvath et al. 2000; Quiles 2006; Rumeau et al. 2007; Wang et al. 2006). In thylakoids of mature chloroplasts the function of the NDH complex is mainly linked to regulation of the redox stage of the PQ pool, and dissipation of energy (Nixon 2000; Peltier and Cournac 2002). In etioplasts, which show

high accumulation of the NDH complex, it may also play a significant bioenergetic role (Feild et al. 1998; Nixon 2000).

The chloroplast NDH complex is homologous to mitochondrial complex I of the respiratory chain, but it resembles even more closely the cyanobacterial NDH-1 complex (Friedrich and Weiss 1997). Indeed, the cyanobacterial NDH-1L and the chloroplast NDH complex have close similarities in terms of both structure and function. The plastid genome encodes most of the NDH subunits that are also found in cyanobacteria: the NDHA-G subunits constitute the hydrophobic membrane domain and the NDHH-K subunits constitute the hydrophilic connecting domain of the complex (Battchikova and Aro 2007; Endo et al. 2008). However, NDHM-O and CRR23/NDHL, all of which are homologous to cyanobacterial proteins, are encoded by the nuclear genome (Ogawa 1992; Rumeau et al. 2005; Shimizu et al. 2008). Interestingly, none of these subunits of the NDH complex show homology to the diaphorase moiety of the mitochondrial complex 1 or to the NuoE, -F, and -G subunits of the *Escherichia coli* NDH-1 complex that carries out NAD(P)H binding and oxidation. Two-dimensional (2-D) projections of complexes isolated from *Thermosynechococcus elongatus* and analyzed by single-particle electron microscopy did, however, show a small number of NDH-1 complexes containing a long hydrophilic arm that might include the homologs of the *Esterichia coli* NuoE, -F, and –G subunits (Arteni et al. 2006). If this type of NDH complex is present in plants, it is highly likely that the NuoE-, NuoF-, and NuoG-like subunits are nuclear-encoded. Unlike plants, cyanobacteria also contain another type of NDH complex, namely NDH-1MS, which is a low CO_2-inducible complex that participates in CO_2 uptake (Zhang et al. 2004).

1.4. Photosynthetic electron transfer routes

1.4.1. The linear electron transport chain

The linear electron transport chain represents the predominant pathway of the photosynthetic light reactions. Three major thylakoid membrane protein complexes – PSII, Cyt b_6f, and PSI – cooperate in order to transport electrons from water molecules to oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$), as hypothesized by Hill and Bendall (1960) (Figure 2A). The excitation energy captured by LHCII is transferred to Chl P_{680}, the primary electron donor of PSII. The primary charge separation occurs between P_{680} and pheophytin, the primary electron acceptor. From pheophytin, the electrons are transferred to a tightly bound primary quinone acceptor, Q_A, and further to the secondary quinone acceptor, Q_B. A subsequent electron transfer step generates double-reduced Q_B^{2-}, which binds two protons from the stromal space, thus being converted to plastoquinol. Plastoquinol is released from the Q_B pocket of the D1 protein. From plastoquinol, electrons are transferred to the Cyt b_6f complex. In the Cyt b_6f complex, two protons are released into the thylakoid lumen while one electron

is transported to oxidized P_{700} of PSI through the soluble electron carrier, plastocyanin. The primary electron donor of PSI, P_{700}, is simultaneously excited by light absorbed by LHCI. From P_{700}, electrons are transferred via several electron carriers to ferredoxin and are used by ferredoxin-$NADP^+$-oxidoreductase (FNR), which reduces $NADP^+$. During the course of photosynthetic electron transport, protons are pumped across the thylakoid membrane from the stroma to the lumen, creating PMF, which is then used by ATP synthase in order to drive the synthesis of ATP.

On the donor side of PSII, P_{680}^+ is re-reduced by extracting electrons from tyrosine Z, which in turn extracts the electrons from the Mn_4Ca cluster. When four positive charges have been accumulated at the Mn_4Ca cluster as a result of four successful electron transfer steps, two water molecules are split and one oxygen molecule and four protons are released into the lumenal space.

1.4.2. Cyclic electron transport around PSI

While the photosynthetic electron transport via the linear electron transport chain has been well studied, our knowledge concerning the details of CET has remained limited even though the existence of this route has been known from the early 1960s (Arnon 1991; Shikanai 2007). Unlike linear electron transport, CET is driven solely by PSI – and the cycling of electrons around PSI leads only to the generation of PMF and to production of ATP at the expense of NADPH. Thus, plants are capable of adjusting the ratio of ATP to NADPH by CET (Shikanai 2007). This is not only important to guarantee the efficient functioning of the Calvin-Benson cycle but also to fulfill the energy requirements of various other metabolic pathways (Shikanai 2007).

The function of CET is especially important under stress conditions – including high light, heat, and drought – and also at the onset of illumination or under CO_2 limitation. Under those conditions, CET has been reported to protect the photosynthetic light reactions against oxidative stress (Bendall and Manasse 1995; Joliot and Joliot 2002; Rumeau et al. 2007). Firstly, the CET enhances protonation of the lumen, thus triggering the non-photochemical quenching of Chl fluorescence (NPQ), which, in turn protects PSII against photoinhibition (Heber and Walker 1992; Miyake et al. 2005; Müller et al. 2001). Secondly, the production of ATP at the expense of NADPH under conditions of an inefficient Calvin-Benson cycle is important for avoidance of over-reduction of the PSI acceptor side and concomitant production of reactive oxygen species and damage of PSI. In higher plants, the CET consists of two partially redundant pathways (Joet et al. 2001) known as the ferredoxin quinone oxidoreductase- (FQR-) and NDH-dependent pathways (Figure 2B).

Based on the fact that CET generates PMF, it has been suggested that a hypothetical enzyme, FQR, functions in oxidation of ferredoxin and reduction of PQ (Bendall and Manasse 1995). The FQR-dependent pathway has been shown to be sensitive to the electron transport inhibitor antimycin A, which binds to the Q_i quinone binding site of the

Cyt bc_1 complex (von Jagow and Link 1986; Tagawa et al. 1963). However, the Q_i site of Cyt b_6f is insensitive to antimycin A and antimycin A do not affect the Q-cycle of Cyt b_6f (Joet et al. 2001; Moss and Bendall 1984). Recently, the crystallographic structure of Cyt b_6f complex revealed a novel heme *x*, which is not conserved in the Cyt bc_1 complex, thus strongly suggesting that it has a role in CET (Kurisu et al. 2003; Johnson 2005; Stroebel et al. 2003). Heme *x* is capable of interacting with PQ in the central cavity of Cyt b_6f and it might thus function in the FQR-dependent pathway (Kurisu et al. 2003). One possibility is that the Cyt b_6f complex *per se* is not involved in CET but instead that ferredoxin donates electrons directly to PQ (Okegawa et al. 2005). So far, only two components of the FQR-dependent CET are known: PGR5 and PGRL1, which are both needed for efficient functioning of CET (DalCorso et al. 2008; Munekage et al. 2002; Rumeau et al. 2007). Cyt b_6f, FNR, PGRL1, and PGR5 interact with each other and it is thus possible that no distinct FQR enzyme exists, but instead that the FQR activity might result from the concerted functions of PGRL1, PGR5, and FNR (DalCorso et al. 2008; Zhang H. et al. 2001). However, PGR5 has also been suggested to have only a regulatory role during CET (Munekage et al. 2002).

In NDH-dependent CET, the electrons are transported from PSI to the PQ pool via the NDH complex. This route is known to be involved in reduction of the PQ pool in darkness, and was revealed by studies using tobacco mutants with inactivated *ndh* genes (Burrows et al. 1998; Horvath et al. 2000; Joet et al. 2001; Kofer et al. 1998; Shikanai et al. 1998). The exact electron transfer pathway of NDH-dependent CET is under intense study. So far, the electron input unit of the NDH complex has remained unknown and no consensus exists about the compound(s) feeding the electrons to the NDH complex. One possibility is that ferredoxin transfers electrons to the NDH complex via FNR, which has been shown to form a complex with NDH (Guedeney et al. 1996; Okutani et al. 2005; Quiles and Cuello 1998; Quiles et al. 2000).

Until recently, there has been debate about the importance of CET in C_3 plants such as *Arabidopsis*, especially in the absence of stress-related factors (Herbert et al. 1990). However, the finding that the *Arabidopsis* double mutant deficient in both the FQR- and NDH-dependent pathways of CET shows highly impaired growth and development has led to the conclusion that CET does indeed have a major role in C_3 plants, even under standard growth conditions (Munekage et al. 2004).

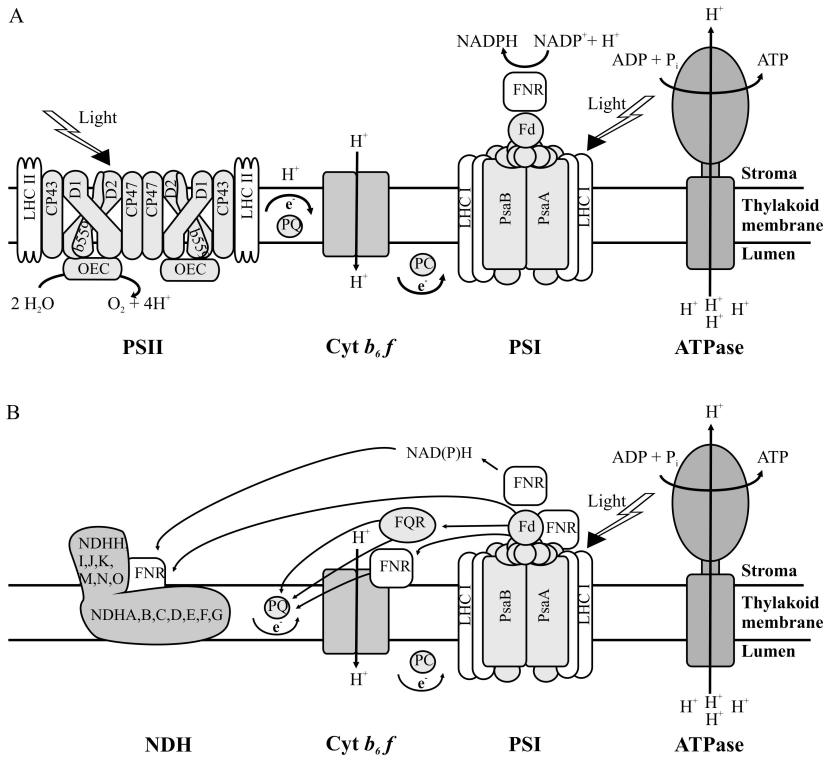

Figure 2. Simplified schemes of the photosynthetic electron transfer routes in the thylakoid membrane (modified from Endo et al. 2008). For details, see text. A. Linear electron transport. B. Hypothetical cyclic electron transport pathways around PSI. The suggestions found in the literature have been taken into account.

1.5. Photoinhibition of PSII

Whenever the light absorption of the photosynthetic machinery exceeds that used by the light reactions or that safely dissipated as heat, there is a danger of accumulation of reactive oxygen species that may damage the photosynthetic protein complexes (Barber and Andersson 1992; Demming-Adams and Adams 1992). One of the core proteins of PSII, the D1 protein, is the primary target of photodamage but the exact mechanism leading to such damage is still under debate. Several hypotheses have been proposed that emphasize the primary site of PSII photoinhibition, either on the donor side or on the acceptor side of PSII (Anderson et al. 1998; Aro et al. 1993b; Hakala et al. 2005, 2006; Ohnishi et al. 2005; Vass 1992). In principle, photoinhibition of PSII and photodamage of the D1 protein are reversible processes, and do not cause permanent damage because plants possess an efficient repair cycle for PSII.

1.5.1. Photoprotection

Plants have developed several protection mechanisms in order to avoid photodamage under high light (Demming-Adams and Adams 1992; Niyogi 1999). Photoprotection

mechanisms include the prevention of absorption of excess light, removal of excess excitation energy by dissipation of heat, and removal of reactive oxygen species formed in the photosynthetic apparatus (Demming-Adams and Adams 1992). The first category includes leaf hairing, waxy cuticle, and other morphological adaptations (Ripley et al. 1999; Robinson et al. 1993). Excess light energy can also be avoided by adjusting the leaf orientation and degree of folding, as well as by chloroplast movements (Jiang et al. 2006; Wada et al. 2003). The second category of photoprotection mechanisms includes the dissipation of light energy as heat through NPQ (Holt et al. 2004). One component of NPQ, the high-energy quenching (qE), is triggered by the protonation of lumen and is thus regulated by CET. In the presence of protonated PsbS protein, the violaxanthin de-epoxidase converts violaxanthin into zeaxanthin, which in turn deactivates excited states of Chl molecules (Demming-Adams and Adams 1996; Holt et al. 2004; Li et al. 2004; Niyogi et al. 2005). The third category of protection mechanisms includes components of the antioxidant systems, most importantly the reduced ascorbate and glutathione, which are capable of scavenging various reactive oxygen species (Asada et al. 1999).

1.5.2. The dynamic repair cycle of PSII

Despite the protection mechanisms, a constant light intensity-dependent damage of PSII occurs in the thylakoid membrane (Tyystjärvi and Aro 1996). It has been estimated that over the course of a sunny day, almost the entire population of the D1 protein is replaced (Chow et al. 2005). The repair cycle of PSII (Figure 3) occurs in multiple steps including the monomerization and partial disassembly of the dimeric PSII complex in grana stacks (Aro et al. 1993b) and migration of the PSII monomers to the stroma-exposed thylakoids (Aro et al. 2005; Baena-Gonzales et al. 1999; van Wijk et al. 1996), followed by proteolysis of the damaged D1 protein (Aro et al. 1993a; Haussuhl et al. 2001; Lindahl et al. 1996). After co-translational insertion of the newly synthesized D1 protein into the thylakoid membrane (Nilson et al. 1999; Zhang et al. 1999), the D1 protein is post-translationally processed from the C-terminus (Fujita et al. 1995), and the PSII monomers are reassembled (Zhang et al. 2000). Finally, PSII monomers migrate back to the grana thylakoids where dimerization and attachment of the LHCII antenna take place.

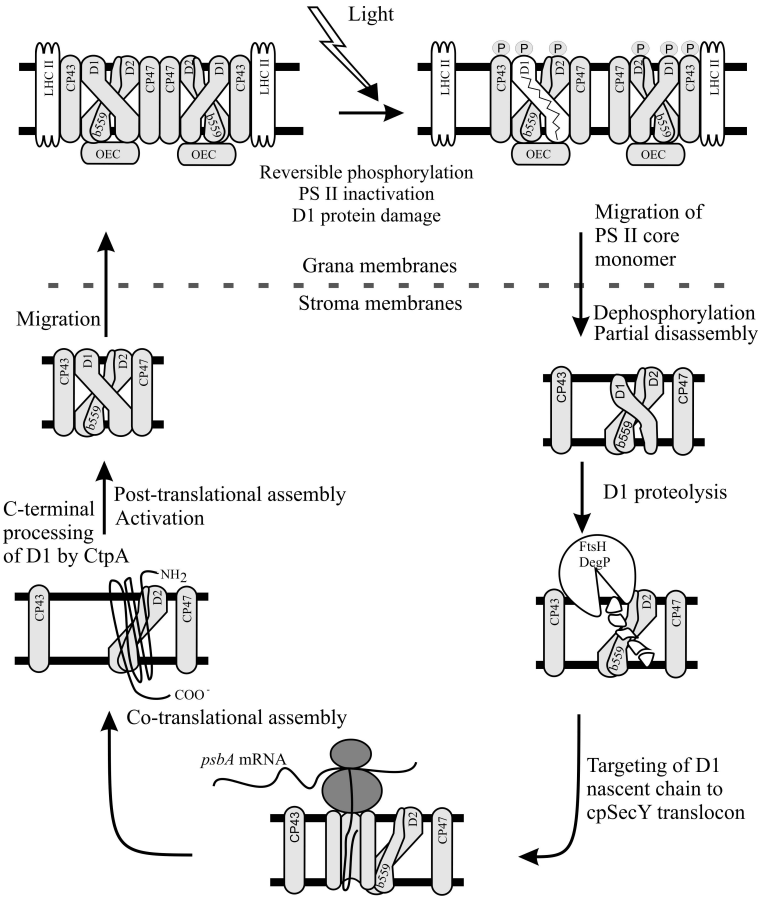

Figure 3. The dynamic repair cycle of PSII (modified from Baena-Gonzalez and Aro 2002).

1.5.3. Auxiliary proteins that assist in the repair of PSII

Dozens – possibly even hundreds – of assembly factors, chaperones, proteases, translocation proteins, kinases, and phosphatases in the chloroplast stroma, thylakoid membranes, and lumen assist the dynamic biogenesis, maintenance, and turnover of the PSII complex (Mulo et al. 2008). During the dynamic repair cycle of PSII, assistance is required in several steps, including degradation of the damaged D1 protein and *de novo* synthesis, membrane insertion, and folding of the nascent D1 protein chains, and also reassembly of the released protein subunits and different cofactors into PSII in order to ensure its proper functioning.

The identity and role of a few auxiliary proteins involved in the regulation of the repair cycle of PSII is known. The FtsH and DegP proteases are responsible for degradation of the damaged D1 protein in the stroma-exposed membranes (Adam and Ostersetzer 2001; Nixon et al. 2005). Several nuclear-encoded factors and *cis* elements control the initiation and elongation steps of translation of the new D1 protein (Marin-Navarro et al. 2007). The chloroplast signal recognition particle cpSRP54 has a role in

targeting the ribosome nascent D1 chain to the thylakoid membrane (Nilsson et al. 1999). In thylakoid membranes, the D1 protein elongation and membrane insertion through the cpSecY translocon channel occur concomitantly (Zhang L. et al. 2001). During the insertion process, LPA1 (Peng et al. 2006) and HCF136 (Meurer et al. 1998; Plücken et al. 2002) most likely guide the folding of the D1 protein, while LPA2 is responsible for reassembly of CP43 into PSII (Ma et al. 2007). PSB27, in turn, assures the correct timing of the assembly of the Mn_4Ca cluster (Chen et al. 2006).

Until recently, the thylakoid lumen was believed to be nearly deprived of proteins, apart from those directly involved in photosynthetic reactions such as the OEC proteins, plastocyanin, and violaxanthin de-epoxidase. Sequencing of the *Arabidopsis* genome, however, has revealed up to 200 proteins that are predicted to be present in the thylakoid lumen, and recent proteomic studies have identified over 80 different proteins found from this compartment, many of which still have no function assigned to them (Kieselbach and Schröder, 2003). As an example, up to 16 proteins located in the lumenal compartment have a peptidyl-prolyl isomerase (PPIase) domain (Kieselbach and Schröder, 2003; Romano et al. 2005). Members of the PPIase family are believed to facilitate the rate-limiting *cis-trans* isomerization of the peptidyl prolyl bond during protein folding. PPIases include three families classified according to ligand specificity (Göthel and Marahiel, 1999). Cyclophilins and FK506 binding proteins bind to the immunosuppressive drugs Cyclosporin A and FK506, respectively, and are collectively referred to as immunophilins. Parvulins, small single-domain proteins, constitute the third family of PPIases for which no ligand has been described.

2. AIMS OF THE STUDY

During the past decade, significant progress has been made in understanding the complex processes of the assembly, maintenance, and turnover of the photosynthetic protein complexes. Accomplishment of the genome-sequencing project of *Arabidopsis* together with the studies revealing the composition of the proteome of different chloroplast compartments have established a basis for identification and deeper characterization of a considerable number of novel proteins that have a role either as an auxiliary protein or as a subunit of the photosynthetic protein complex. To investigate the dynamics of the photosynthetic protein complexes, two approaches have been used in this thesis:

- A proteomic approach in order to find and characterize novel proteins that interact with the photosynthetic apparatus.
- Detailed characterization of newly discovered proteins involved in dynamics of the photosynthetic apparatus.

3. METHODOLOGICAL ASPECTS

3.1. Plant material and growth conditions

Arabidopsis ecotype Columbia plants were used in most of the experiments. Transferred DNA (T-DNA) insertion mutants with Columbia background were ordered either from the Gabi-Kat or SALK collections (Alonso et al. 2003; Rosso et al. 2003). Besides *Arabidopsis*, also tobacco (*Nicotiana tabacum*) cv. Petit Havanna plants were used in Paper I. Details of knock-out, silencing, and complementation constructs and also the plant growth conditions are described in the respective papers. The following *Arabidopsis* mutants were characterized in detail: SALK_114469 (*psbr*), SALK_109618 (*tlp18.3*), GABI-Kat 459D12 (*tlp18.3*), SALK_029448 (*cyp38*), GABI-Kat 744F08 (*cyp38*), antisense-AtCYP38 (*cyp38*), SALK_111363 (*ndh45*) and antisense-NDH48 (*ndh48*). In addition, the SALK_009552 and SALK_024971 lines were used to characterize the function of AtCYP20-2.

3.2. Validation of mutant lines

The mutation of the gene under study was confirmed by polymerase chain reaction (PCR) and/or by immunoblotting using protein-specific antibodies. Southern blotting according to standard procedures was performed in order to determine the number of T-DNA inserts in the insertion mutant lines (Paper IV). In Papers II and III, parallel mutant lines were used for the experiments. In addition, molecular complementation of the mutated gene was used for validation purposes (Paper III).

3.3. Isolation of chloroplasts and fractionation of the thylakoid membrane

Intact chloroplasts were isolated using Percoll step gradients (Paper II) and the thylakoid membranes were isolated as in Rintamäki et al. (1996). For measurements of photosynthetic parameters, the thylakoid membranes were isolated as described in Allahverdiyeva et al. (2007). Chloroplasts and thylakoids were fractionated using different methods in order to find novel auxiliary proteins and to investigate their location, characteristics, and functional roles in chloroplasts. Soluble stroma and thylakoid membranes were separated by centrifugation after osmotic shock of the chloroplasts (Paper IV). The stroma thylakoid-associated polysome-nascent chain complexes (polysomes) were isolated from chloroplasts in the presence of protease inhibitors using sucrose cushion centrifugation after solubilization of the thylakoid membranes with *n*-dodecyl β-D-maltoside (Paper II). Digitonin solubilization combined with differential centrifugation steps was used to subfractionate the thylakoid membrane into grana stacks and stroma-exposed membranes (Papers II and IV). Detachment of the peripheral thylakoid membrane proteins with 2M NaBr was done as described in Carlberg et al. (2003).

3.4. Separation of thylakoid proteins and protein complexes

Three electrophoretic techniques were used for separation of proteins and protein complexes. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate denatured polypeptides according to molecular mass (Laemmli, 1970). Isoelectric focusing (IEF) was used to separate proteins from thylakoid-associated polysomes (O'Farrell 1975) and blue native polyacrylamide gel electrophoresis (BN-PAGE) was used to separate the thylakoid protein complexes according to molecular mass (Kügler et al. 1997; Schägger and von Jagow 1991). Mild solubilization using *n*-dodecyl β-D-maltoside and native gradient gels (5–12.5% acrylamide) allowed the protein complexes to remain intact during the solubilization and separation steps of BN-PAGE. For 2-D gel electrophoresis, the proteins were first separated either by IEF or BN-PAGE, followed by SDS-PAGE in the second dimension.

3.5. Detection and identification of proteins

After electrophoresis, the proteins were visualized by silver staining and individual proteins were identified by immunoblotting or by mass spectrometric analysis using matrix-assisted laser-desorption-ionization time-of-flight and electrospray ionization mass spectrometry.

3.6. Synthesis, assembly, and degradation of thylakoid proteins and protein complexes

Synthesis, assembly, and degradation of thylakoid membrane proteins were studied by *in vivo* ^{35}S-methionine pulse-labelling of detached leaves (Papers II and III). Pulse-labelling and chase of radioactivity in the presence of unlabelled methionine were performed under 900 μmol photons $m^{-2}s^{-1}$ of white light. Subsequently, the thylakoid membranes were isolated and the proteins were separated either by SDS-PAGE or by 2-D BN/SDS-PAGE and detected by autoradiography.

3.7. Northern blot

The total RNA was isolated with TRIzol (Invitrogen) according to the Tri reagent total RNA isolation protocol (Sigma), and Northern blotting was performed according to a standard procedure (Papers I and IV).

3.8. Detection of hydrogen peroxide and inhibition of chloroplast translation

The accumulation of the hydrogen peroxide (Papers III and IV) was studied by the 3,3'-diaminobenzidine (DAB) method as described by Ren et al. (2002). In experiments

with inhibition of translation, 1 mM lincomycin was incorporated into leaves through the petioles overnight (Paper II).

3.9. Measurement of photosynthetic parameters

A number of different techniques were used to study the photosynthetic performance of the plants under investigation.

PSII complex: Light-saturated rates of steady-state oxygen evolution from isolated thylakoids were recorded in the presence of 1 mM DMBQ (2,6-dimethyl-*p*-benzoquinone) as an artificial electron acceptor of PSII (Paper II). PSII photochemical efficiency was recorded as a ratio of variable to maximal fluorescence, F_V/F_M (F_V is the difference between maximal, F_M, and initial, F_0, fluorescence) determined from intact leaves. Flash-induced increase and subsequent relaxation of Chl fluorescence yield (FF-relaxation) was measured either in the presence or absence of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) as described by Allahverdiyeva et al. (2004). PSII excitation pressure (1 – qP) was determined as described by Piippo et al. (2006). NPQ was determined from leaves and was calculated as $(F_M – F_M')/F_M'$ (Bilger and Björkman 1994).

PSI complex: Redox state of P_{700} was monitored from leaves by absorbance changes at 810 nm using 860 nm as a reference. The P_{700} was oxidized by far-red light and the subsequent re-reduction of P_{700}^+ in the dark was monitored (Paper II). $NADP^+$ photoreduction activity was determined from the change in absorbance at 340 nm as described by Naver et al. (1999) using a dual-beam spectrophotometer as described by Kjær and Scheller (1996).

NDH complex: Post-illumination rise in Chl fluorescence, F_0-rise, which is dependent on NDH-mediated reduction of the PQ pool in darkness, was recorded by illuminating leaves with white actinic light prior to dark transition followed by detection of the level of F_0 (Allahverdiyeva et al. 2005).

Relative amounts of PSI and PSII: Room temperature electron paramagnetic resonance spectroscopy was used to record the relative amounts of PSI and PSII. The relative amounts of PSI and PSII in the thylakoid membranes were estimated after double integration of the EPR spectra from chemically-oxidized (5 mM ferricyanide) P_{700}^+ and from the dark stable radical form $Y_D\cdot$ of the D2 protein, respectively (Danielsson et al. 2004). 77K fluorescence emission spectra of thylakoid membranes were recorded as described in Paper III.

Size of the PQ pool: In order to estimate the size of the inter-system electron pool capable of donating electrons to oxidized P_{700} in intact leaves, the changes in the oxidation curve of P_{700} on applying single-turnover and multiple-turnover pulses upon FR background were analyzed as described (Asada et al. 1992; Schreiber et al. 1988).

4. OVERVIEW OF THE RESULTS

4.1. Proteomic characterization of thylakoid-associated polysomes and stroma-exposed thylakoid membranes

In this thesis a proteomic analysis of the stroma membrane-associated polysomes was done in order to find unknown proteins that coordinate the synthesis, assembly, and turnover of the photosynthetic protein complexes (Paper II). After isolation of polysomes, proteins were separated by a combination of IEF and SDS-PAGE, and identified by mass spectrometry. In addition to subunits of the photosynthetic protein complexes and proteins involved in the translation machinery, the analysis revealed one previously characterized auxiliary protein of PSII and four functionally unknown proteins. The characterized protein was the D1 protease, Var2, which belongs to the FtsH protease family (Bailey et al. 2002). The proteins with unknown functions were TLP18.3 (AT1G54780; Paper II), NDH45 (AT1G64770; Paper IV), NDH48 (AT1G15980; Paper IV), and a PsbQ domain protein (AT1G14150), the first three of which were subjected to detailed characterization in this thesis work.

Besides the thylakoid-associated polysomal proteome, a proteome of a large thylakoid protein complex of ≥ 1,000 kDa, which was found from the stroma-exposed membranes, was analyzed by 2-D BN/SDS-PAGE (Paper IV). Five novel proteins, which at the time had unknown functions, were identified from this supercomplex by mass spectrometry. These proteins were NDH48 (AT1G15980), NDH45 (AT1G64770), AtCYP20-2 (AT5G13120), AT2G39470, and AT5G58260.

4.2. Novel supercomplex of the thylakoid membrane

The novel ≥ 1,000-kDa protein complex found from the stroma-exposed membranes turned out to be composed of the PSI and NDH complexes, based on the analysis of the subunit composition (Paper IV). Interestingly, several previously uncharacterized proteins were identified in this supercomplex. Of these, NDH45 and NDH48 were studied in detail (Paper IV). According to protein domain prediction programs (Apweiler et al. 2001; Krogh et al. 2001), the NDH48 protein contains a heptosyltransferase domain and the NDH45 protein contains a galactose mutarotase-like domain, and both proteins are predicted to be hydrophilic.

4.2.1. NDH45 and NDH48 are attached to the NDH complex

Although the computational programs predicted NDH45 and ND48 to be hydrophilic proteins, they were found exclusively in the thylakoid membrane and were strongly enriched in the stroma-exposed membranes (Paper IV). Further analysis revealed that the

majority of the NDH45 and NDH48 proteins were bound to the PSI/NDH supercomplex and only minor amounts of the proteins were present in a smaller protein complex of ~800 kDa (Paper IV). The NDH45 and NDH48 proteins were shown to be tightly bound to the PSI/NDH supercomplex, since trypsin treatment was not efficient enough to remove the proteins from the membrane (Paper IV).

4.2.2. ndh45 and ndh48 plants are deficient in the NDH complex

Arabidopsis ndh45 and *ndh48* plants did not show any visible phenotype under standard growth conditions. When the amounts of thylakoid membrane proteins were studied in *ndh45* and *ndh48* plants, the most predominant feature was a deficiency in the NDHH protein, representing the NDH complex. On the other hand, the amounts of PSI (PsaB), PSII (D1), ATP synthase (Atpβ), and the Cyt b_6f (Cyt *f*) complexes remained unaltered or slightly increased in the mutant lines compared to the wild type. In line with this, the transient rise in Chl fluorescence after the termination of actinic light (Asada et al. 1993), which is dependent on NDH-mediated reduction of the PQ pool in darkness (Mi et al. 1995), was shown to be reduced in *ndh45* and *ndh48* plants compared to the wild type.

4.2.3. NDH45 and NDH48 are subunits of the hydrophobic domain of the NDH complex

When *Arabidopsis* mutants either lacking the hydrophilic domain of the NDH complex (*ndho*) or containing strongly diminished amounts of the NDH complex (*crr2-2*) were studied, it was found that the *ndho* plants accumulated normal amounts of the NDH45 and NDH48 proteins while the *crr2-2* thylakoids contained clearly reduced amounts of the NDH45 and NDH48 proteins compared to wild type (Paper IV). Notably, when the extrinsic thylakoid membrane proteins from the wild-type, *ndho*, and *crr2-2* plants were digested with trypsin, it was found that the NDH complex partially protected NDH48 from digestion (Paper IV). As mentioned before, *ndh45* and *ndh48* thylakoids contained less of the hydrophilic NDHH protein and were thus likely to show problems in the assembly of the hydrophilic domain of the NDH complex. In summary, these results indicate that the NDH45 and NDH48 proteins are subunits of the hydrophobic domain of the NDH complex. They are located in close proximity to the interface between the hydrophobic and hydrophilic domains of the NDH complex where they function in stabilizing the assembly of the hydrophilic domain (Paper IV).

4.2.4. AtCYP20-2 co-migrates with the PSI/NDH supercomplex

AtCYP20-2 is a thylakoid lumen cyclophilin of 20 kDa, which enrich in the stroma-exposed thylakoid membrane (unpublished result). In this study, AtCYP20-2 was seen to co-migrate with the PSI/NDH supercomplex after the 2-D BN/SDS-PAGE separation (Paper IV). When the amounts of the major thylakoid membrane protein complexes were studied from the wild-type and *cyp20-2* thylakoids, no difference could be observed; nor was there any difference when the photochemical efficiency of PSII (F_V/F_M) was recorded

from wild-type and *cyp20-2* plants (unpublished results). However, minor decrease in the accumulation of the NDHH protein and activity of the NDH complex were observed in *cyp20-2* plants compared to wild type (unpublished results).

4.3. Novel auxiliary proteins of PSII

4.3.1. TLP18.3: novel lumenal protein that functions in the repair cycle of PSII

The TLP18.3 (a thylakoid lumen protein of 18.3 kDa) protein was identified from the thylakoid-associated polysome fraction. The sequence analysis of TLP18.3 revealed a C-terminal transmembrane helix. TLP18.3 protein has been reported to be located in the lumenal space of the thylakoid membrane (Peltier et al. 2002; Schubert et al. 2002), and in Paper II it was shown to be evenly distributed between the stroma-exposed membranes and grana stacks. TLP18.3 was not a tightly bound subunit of any thylakoid membrane protein complex according to analysis by 2-D BN/SDS-PAGE, although weak interactions could not be excluded (Paper II).

4.3.1.1. The phenotype of tlp18.3 plants

Arabidopsis plants lacking the TLP18.3 protein did not reveal any visible phenotype under constant growth light or high-light conditions (Paper II). Also, the Chl a/b ratio and the total amount of Chl per leaf area were similar in wild-type and mutant plants (Paper II). Even so, the growth of the *tlp18.3* plants under fluctuating light conditions led to a clearly smaller phenotype compared to the wild type (Paper II).

4.3.1.2. Photosynthetic characterization of tlp18.3 plants

Both the activity and amount of the PSI complex, measured as *in vivo* redox changes in P_{700} and by immunoblotting, respectively, were unaltered in the *tlp18.3* plants compared to wild type (Paper II). However, when the functional characteristics of PSII were studied in growth light-grown plants, minor differences were found in the oxygen evolution capacity and in the FF-relaxation between *tlp18.3* and wild-type plants. Next, the effect of the lack of the 18.3 kDa protein on the PSII complex was analyzed by immunoblotting. It was found that while the amounts of the core proteins of PSII were unaltered, the OEC proteins PsbP and PsbQ were present in slightly higher amounts in *tlp18.3* thylakoids than in the wild type (Paper II).

4.3.1.3. The repair cycle of PSII in tlp18.3 plants

Based on measurements of the PSII photochemical efficiency, the *tlp18.3* plants showed higher susceptibility of PSII to photoinhibition compared to wild type. In addition, the relative amounts of PSII monomer complexes were higher than in the wild type, indicating specific problems in the repair cycle of PSII (Paper II). Two steps of the repair cycle of PSII were clearly impaired in the *tlp18.3* plants: (i) the turnover of the damaged D1 protein, and (ii) the dimerization of the PSII complexes (Paper II). Degradation of

the damaged D1 protein takes place in stroma-exposed thylakoids, whereas the PSII dimerization occurs in the grana stacks, which is in line with the even distribution of the TLP18.3 protein between the grana and stroma thylakoids (Paper II). However, the lack of TLP18.3 protein did not lead to a severe collapse of the PSII complexes under high light, which suggests that there may be some redundance of proteins assisting these particular steps in the PSII repair cycle (Paper II).

4.3.2. AtCYP38: thylakoid lumen immunophilin crucial for PSII

AtCYP38 (a cyclophilin of 38 kDa) is a multi-domain PPIase located in the thylakoid lumen. AtCYP38 has several predicted domains, including a PPIase domain and a phosphatase-binding module (Paper III). A spinach ortholog of the AtCYP38 protein, TLP40 (a thylakoid lumen PPIase of 40 kDa), has been suggested to be a negative regulator of the thylakoid protein phosphatase (Fulgosi et al. 1998; Rokka et al. 2000; Vener et al. 1999).

4.3.2.1. The phenotype of cyp38 plants

Arabidopsis cyp38 plants were found to be hypersensitive to light and showed dramatically stunted growth compared to the wild type (Paper III). The phenotype of the plants was dependent on the intensity of growth light and photoperiod (Paper III). The *cyp38* plants were not capable of surviving under high-light conditions or short photoperiod in the absence of an additional sugar source (Paper III). Low-light conditions together with long photoperiod and sucrose supplementation were nevertheless able to rescue the mutant phenotype (Paper III).

4.3.2.2. AtCYP38 is important for the correct assembly of PSII

A finding of reduced amounts of both the PSI and PSII proteins was typical of *cyp38* plants (Paper III). Initially, it was difficult to determine whether AtCYP38 is primarily required for the assembly of PSI or PSII. However, when young seedlings instead of mature rosette leaves were investigated, it became clear that reduction in the amount of PSI is only a secondary effect (Paper III). The 50% reduction in the amount of the PSII complexes present in the *cyp38* plants relative to wild type was shown to be due to a shorter half-life of PSII in the mutant plants (Paper III). This, in turn, was due to the improper functioning of the PSII donor side, as revealed by FF-relaxation measurement in the presence of DCMU (Paper III). Based on pulse-labelling experiments, the main reason for the PSII donor-side malfunction in the *cyp38* plants was incorrect assembly of D1 and, directly or indirectly, also of OEC (Paper III). A role of AtCYP38 in the assembly of PSII is in line with the observation that the AtCYP38 protein interacts with the PSII monomer complex in BN-PAGE (Paper III).

4.3.2.3. AtCYP38 may regulate phosphorylation of PSII core and LHCII proteins

When the phosphorylation stage of the *cyp38* plants was studied, it became clear that phosphorylation of the PSII core as well as the LHCII proteins was reduced in

the mutant plants compared to wild type, even though the PQ pool was shown to be more reduced in *cyp38* than in the wild type (Paper III). In line with this, the *in vitro* dephosphorylation assay indicated that dephosphorylation of the thylakoid membrane proteins was accelerated in the *cyp38* plants compared to wild type (Paper III).

4.4. PsbR – 10 kDa protein of OEC

The gene encoding the PsbR protein is found in the nucleus of higher plants but it is missing from the genome of cyanobacteria. It has been hypothesized that PsbR, also called the 10-kDa protein of PSII, provides a site for the extrinsic OEC protein PsbP to bind to the thylakoid membrane (Ljungberg et al. 1986). The *Arabidopsis psbr* plants were shown not to have a visible phenotype under any of the light conditions studied (Paper I). However, the capacity for oxygen evolution was diminished compared to wild type, especially under low light conditions (Paper I). In addition, the *psbr* plants showed higher excitation pressure of PSII, indicating problems in the PSII electron transfer (Paper I).

When the amounts of thylakoid membrane proteins were analyzed from wild-type and the *psbr* thylakoids, it was obvious that the extrinsic OEC proteins PsbP and PsbQ were present in reduced amounts in the *psbr* plants. This difference was not derived from transcriptional regulation of the *PsbP* and *PsbQ* genes but, instead, was shown to originate from post-transcriptional regulation, most likely from defects in the assembly of these two proteins into PSII (Paper I). In addition, it was observed that the PsbJ protein is a prerequisite for the assembly of PsbR.

Moreover, the accumulation of the PSII OEC proteins was studied from wild-type plants grown either under low light conditions (50 μmol photons $m^{-2}s^{-1}$) or growth light conditions (125 μmol photons $m^{-2}s^{-1}$). Interestingly, the low light-grown plants were shown to accumulate more of the PsbP, PsbQ, and PsbR proteins than plants acclimated to the growth-light conditions (Paper I).

5. DISCUSSION

5.1. Novel proteins identified from the thylakoid membrane

Two subproteomics approaches, one dealing with the polysomes attached to the stroma membrane and the other dealing with the protein complexes of the stroma membrane, were taken in order to find novel proteins involved in the dynamics of the photosynthetic apparatus. Translation of most of the proteins encoded by the chloroplast genome takes place on ribosomes attached to the stroma-exposed thylakoid membranes (Boschetti et al. 1990). Since the D1 protein has the highest turnover rate of all the thylakoid proteins (Mattoo et al. 1984), the proteome analysis of the stroma membrane-attached polysomes was expected to result in the finding of novel auxiliary proteins, particularly those involved in the PSII repair cycle. Moreover, while the structure of the major multi-subunit thylakoid membrane protein complexes PSI, PSII, and Cyt b_6f has been resolved at 2–4-Å resolution (Amunts et al. 2007; Guskov et al. 2009; Kamiya and Shen 2003; Loll et al. 2005; Stroebel et al. 2003; Zouni et al. 2001), our knowledge of the NDH complex in stroma-exposed membranes is only in its infancy. Thus, characterization of the stroma thylakoid proteome was expected to lead to the finding of novel subunits or auxiliary proteins of the NDH complex.

Altogether, seven novel proteins were identified and the detailed characterization of four of them, TLP18.3, NDH45, NDH48, and AtCYP20-2, was accomplished in this thesis (Papers II and IV). In addition, the functional roles of two proteins, AT5G58260 and AT2G39470, which co-migrated with the PSI/NDH supercomplex (Paper IV), were recently published (Ishihara et al. 2007; Rumeau et al. 2005). AT5G58260 was identified as the NDHN subunit of the NDH complex. AT2G39470, in turn, was shown to be an auxiliary protein important for the proper functioning of NDH, and it was named PPL2. The functional role of the seventh protein, AT1G14150, still remains to be determined.

5.2. Auxiliary proteins are important in guiding the repair cycle of PSII

The light-induced photoinhibition of PSII results in irreversible damage to the D1 protein (Aro et al. 1993b; Baena-Gonzalez and Aro 2002; Barber and Andersson 1992). A substantial number of auxiliary proteins is needed in order to guarantee a precise and elaborate repair of the PSII complex (Mulo et al. 2008). The damaged D1 protein is replaced by a newly synthesized copy, and this occurs by subjecting the entire PSII complex to a repair cycle (Aro et al. 1993b; Baena-Gonzalez and Aro 2002). The damaged and phosphorylated PSII complexes migrate from the grana stacks to the stroma-exposed thylakoids, where the D1 protein is dephosphorylated and degraded (Haussuhl et al. 2001; Kapri-Pardes et al. 2007; Rintamäki et al. 1996; Zaltsman et al. 2005). The new D1 protein is synthesized and co-translationally inserted into the PSII complex through the

cpSecY translocation channel (Zhang L. et al. 2001), concomitantly with the ligation of several cofactors (Kim et al. 1991). The D1 protein is synthesized as a precursor with an extended C-terminus (Marder et al. 1984), which is later proteolytically removed (Diner et al. 1988; Fujita et al. 1995) in order to allow the binding of the Mn_4Ca cluster and the assembly of the extrinsic proteins of OEC into PSII (Bowyer et al. 1992; Roose and Pakrasi 2004). Finally, the PSII monomers migrate back to the grana thylakoids where dimerization of PSII and assembly of several small subunits take place (Hashimoto et al. 1997; Rokka et al. 2005). Auxiliary proteins that are known at present to be involved in the repair cycle include (i) kinases and phosphatases that regulate the phosphorylation of PSII and LHCII (Allen 2005; Aro and Ohad 2003; Rochaix 2007), (ii) proteases that degrade the damaged proteins, especially the D1 protein (Adam et al. 2006), (iii) regulatory elements that control the translation initiation and elongation of the D1 protein (Marin-Navarro et al. 2007), (iv) chaperones that assist in the re-assembly of the PSII subunits (Mulo et al. 2008), and (v) proteins that ensure the correct timing of PSII assembly (Chen et al. 2006).

In the work of this thesis, two novel auxiliary proteins of PSII, AtCYP38 and TLP18.3, were characterized. Both TLP18.3 and AtCYP38 are located in the lumenal space of the thylakoid membrane network (Peltier et al. 2002; Schubert et al. 2002) and they were shown to assist in the degradation of the damaged D1 protein and in the correct assembly of the newly synthesized D1 protein, respectively.

5.2.1. AtCYP38 is crucial for correct assembly of the D1 protein and for the function of OEC

The AtCYP38 protein is a multi-domain PPIase that has 82% identity with its spinach counterpart TLP40, which has been shown to possess the PPIase protein folding activity (Fulgosi et al. 1998), thus indicating that it may have a role as an auxiliary protein. However, unlike TLP40, the AtCYP38 protein is not able to perform *cis-trans* isomerization of synthetic peptides *in vitro* (Shapiguzov et al. 2006), which suggests that this protein may have a more specific function in chloroplasts.

5.2.1.1. cyp38 plants show chronic photoinhibition of PSII

The most striking feature of the *cyp38* plants is the chronic photoinhibition of PSII (Paper III; Fu et al. 2007). To gain an insight into the molecular function of AtCYP38, it was critical to pinpoint the origin of PSII malfunction in *cyp38* plants. A characteristic feature of *cyp38* thylakoids was found to be a modification of the FF-relaxation curve as compared to the wild type. Similar modification has been recorded earlier from thylakoids with an impaired donor side of PSII (Allahverdiyeva et al. 2004; Chu et al. 1995; Cser et al. 2005; Vass et al. 1999). Intriguingly, the amounts of functional PSII complexes and the extent of modifications in their donor and acceptor sides were shown to be dependent on both the amount of AtCYP38 present in the thylakoids and on the growth light intensity of plants (Paper III).

5.2.1.2. AtCYP38 is crucial for biogenesis of PSII

AtCYP38 is abundant already in the etioplast proteome (Blomqvist et al. 2006; Kanervo et al. 2008). Thus, the rapid greening process of etiolated leaves was used as a tool to determine the importance of AtCYP38 for the assembly of PSII or PSI. This experiment showed that AtCYP38 is important for the assembly of PSII specifically. Thus, it was possible to conclude that the deficiency of PSI in the full-grown *cyp38* plants results from the acclimation of PS stoichiometry to prevailing conditions (Allen and Pfannschmidt 2000).

Interestingly, the *cyp38* seedlings showed a lethal phenotype when germinated under short photoperiod in the absence of an external sugar source (Paper III). Because *cyp38* seedlings contain strongly reduced amounts of the PSII complexes (Paper III), they are likely to suffer from limited energy production even under favorable growth conditions. Earlier studies have shown that a short photoperiod enhances the risk of hydrogen peroxide production, particularly in mutants with limited energy production (Bechtold et al. 2004; Reinhold et al. 2007). In line with this, the *cyp38* seedlings accumulated hydrogen peroxide under short-day conditions (Paper III). Thus, the seedling lethality of *cyp38* under short-day conditions is most likely derived from the energy starvation together with accumulation of reactive oxygen species.

5.2.1.3. AtCYP38 primarily assists in the assembly of OEC with PSII core

Closer analysis with pulse-labelling experiments showed efficient D1 synthesis and assembly in the *cyp38* plants, even in darkness (Paper III), which is a unique feature that has never been reported before. In addition, the sequestration of newly-synthesized D1 into PSII monomers, dimers, and supercomplexes occurs in the *cyp38* plants in darkness similarly to that in wild type plants under light conditions (Paper III). On the other hand, no precursor-D1 protein could be recognized in *cyp38* thylakoids after pulse labelling, indicating exceptionally fast processing of precursor-D1 (Paper III). It is known that the assembly steps of PSII are highly coordinated. In particular, the processing of the precursor-D1 and the assembly of CP43 are closely interrelated (Zhang et al. 1999). It is conceivable that AtCYP38, upon interaction with the PSII monomer, has a crucial role in securing the proper folding of D1 and possibly also of CP43, which, in turn, provide the right orientation of amino acids to function as ligands for OEC. Such fine tuning appears to be missing in the *cyp38* plants, rendering the PSII centers highly susceptible to donor-side photoinhibition (Ohnishi et al. 2005). The function of AtCYP38 is likely to be associated with its PPIase domain. Indeed, the C-terminus and the lumenal loops of D1, as well as the crucial E-loop of CP43 – all of which are involved in the ligation of the Mn_4Ca cluster – contain several highly conserved proline residues (Anderson et al. 2002; Burnap 2004; Loll et al. 2005) as putative targets of AtCYP38.

AtCYP38 is not the only chloroplast-located PPIase proposed to function as an auxiliary protein of PSII. Stromal cyclophilin ROC4 plays a role in repair of the photodamaged

PSII (Cai et al. 2008), while lumenal immunophilin FKBP20-2 participates in the accumulation of the PSII-LHCII supercomplexes (Lima et al. 2006). Also, AtCYP20-2 has been proposed to function in the formation of PSII-LHCII supercomplexes (Romano et al. 2004), but results presented in this thesis lead to questioning of this hypothesis (see section 5.4.2).

5.2.1.4. AtCYP38 possibly has a dual role during the repair cycle of PSII

PSII proteins D1, D2, CP43, and PsbH, as well as several LHCII proteins, are reversibly phosphorylated according to environmental cues (Aro and Ohad, 2003; Rochaix 2007). The phosphorylation of the two major LHCII proteins, Lhcb1 and Lhcb2, and one minor LHCII protein, Lhcb4.2 (CP29), has been shown to regulate the distribution of excitation energy between PSII and PSI in a process called state transitions (Bellafiore et al. 2005; Tikkanen et al. 2006). The phosphorylation of the PSII core proteins has, in turn, been suggested to play a regulatory role in the PSII repair cycle by functioning as a signal for the migration of the damaged PSII from the grana to the stroma membrane (Aro et al. 1993b; Baena-Gonzalez et al. 1999; Rintamäki et al. 1996). This hypothesis was, however, challenged by a study reporting that the permanent dephosphorylation of the PSII proteins in *stn8* kinase mutants did not render PSII to enhanced photoinhibition and that the phosphorylation of PSII proteins was not crucial for D1 protein turnover and PSII repair (Bonardi et al. 2005). Since then, the debate about the issue has continued and very recently it was reported that under prolonged high-light treatment, the lack of phosphorylation of the PSII core protein does indeed hamper the migration of the damaged PSII complexes from the grana to stroma-exposed membranes by retarding the disassembly of the damaged PSII-LHCII supercomplexes (Tikkanen et al. 2008).

TLP40, the AtCYP38 ortholog in spinach, has been shown to function as a negative regulator of the intrinsic thylakoid membrane protein phosphatase involved in dephosphorylation of the PSII core proteins (Rokka et al. 2000). However, final support for this conclusion has been missing in the absence of respective mutant plants. In line with the TLP40 work, we observed that in *cyp38* thylakoids the major phosphoproteins, particularly D1, D2, and LHCII, showed a strong reduction in the *in vivo* phosphorylation level as compared to wild type (Paper III). This occurred even though the PQ pool – the redox state of which is crucial to the regulation of phosphorylation – was more reduced in *cyp38* than in the wild type. In general, such redox conditions should enhance the phosphorylation of PSII core proteins (Rintamäki et al. 1996). It is thus probable that reduction in the phosphorylation of PSII core proteins in *cyp38* is due to the fast dephosphorylation, as suggested earlier (Rokka et al. 2000). This interpretation was further supported by the results of the *in vitro* dephosphorylation assay (Paper III). The phosphorylation of LHCII proteins was also reduced, but this likely resulted from inhibition of the respective kinase (Rintamäki et al. 2000).

It is highly unlikely that the reduced phoshorylation levels of PSII proteins in *cyp38* plants would explain the severe phenotype of the mutant plants, since the *stn7/8* mutants completely lacking the phosphorylation of the PSII core and LHCII proteins did not show any distinguished phenotype under standard growth conditions (Bonardi et al. 2005). However, it has been shown earlier that dephosphorylation is a prerequisite for efficient degradation of the D1 protein (Koivuniemi et al. 1995). It is thus conceivable that the unregulated protein phosphatase in *cyp38* plants does hamper the elaborate repair cycle of PSII to some extent, especially under demanding conditions such as prolonged high-light stress (Tikkanen et al. 2008).

It is tempting to speculate that AtCYP38 functions in two different steps during the PSII repair cycle (Figure 4). When the damaged PSII complex has migrated to the stroma thylakoids, it comes into contact with the phosphatase-AtCYP38 complex, thus releasing the AtCYP38 protein into the thylakoid lumen. The release of AtCYP38 activates the thylakoid membrane phosphatase (Fulgosi et al. 1998), which, in turn, dephosphorylates the damaged PSII core complex. Subsequently, the AtCYP38 released binds to the same PSII core in order to guarantee the correct insertion and folding of the *de novo*-synthesized D1 protein.

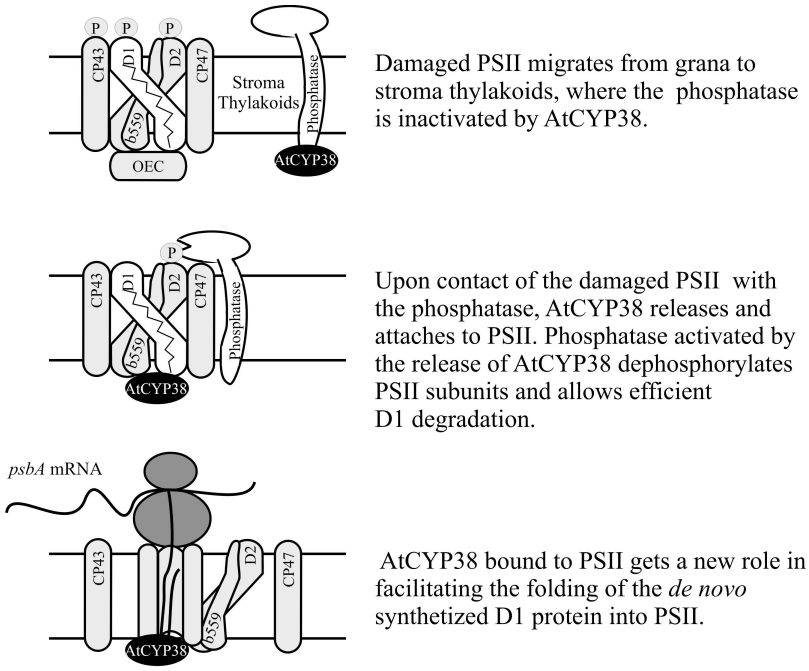

Figure 4. Hypothetical model of the dual function of AtCYP38 in PSII repair.

5.2.2. TLP18.3 fine-tunes the degradation of damaged D1 protein and the dimerization of PSII monomers

The second PSII auxiliary protein to be characterized in this thesis work was TLP18.3, a thylakoid lumen protein with a molecular mass of 18.3 kDa. Characterization of the *tlp18.3* plants revealed a higher susceptibility of PSII to photoinhibition under high light compared to wild-type plants (Paper II). The increased susceptibility to high light could have two different explanations: (i) either the PSII complexes are more susceptible to light-induced damage or (ii) the repair of the damaged PSII complexes is inefficient (Adir et al. 2003). According to the results presented here, TLP18.3 facilitates the repair of PSII centers after photodamage, and more precisely, it assists in the degradation of the damaged D1 protein and subsequent dimerization of the PSII complexes (Paper II).

Degradation of the photodamaged D1 protein in stroma membranes precedes the dimerization of the PSII complex (Rokka et al. 2005) and thus it is logical to assume that the effect of the TLP18.3 protein on the dimerization of PSII is an indirect response. However, the TLP18.3 protein was evenly distributed in the grana stacks and the stroma-exposed membranes, suggesting that it also has a function in the appressed membranes (Paper II). In addition, the *tlp18.3* plants harbored increased amounts of the OEC proteins PsbP and PsbQ (Paper II). The PsbP and PsbQ proteins are not bound to stromal PSII monomers, but are instead bound to granal PSII dimers (Hashimoto et al. 1997; Rokka et al. 2005). Moreover, earlier publications have shown that the assembly-competent OEC proteins can be stored in the thylakoid lumen (Ettinger and Theg 1991; Hashimoto et al. 1997). Taken together, these results suggest that the accumulation of the PsbP and PsbQ proteins indicates dysfunction of the dimerization of the PSII complex in *tlp18.3*.

The role of TLP18.3 as a PSII regulatory protein is in agreement with results obtained from cyanobacteria revealing the presence of the Sll1390 protein, a homolog of TLP18.3, in sub-stochiometric concentrations in an extremely pure PSII preparation extracted from *Synechocysis* sp. PCC 6803 (Kashino et al. 2002). It is thus conceivable that the function of Sll1390/TLP18.3 has been well preserved over the evolutionary time scale, which would highlight the importance of the TLP18.3 protein in the PSII repair cycle. TLP18.3 is not the only evolutionarily conserved protein regulating the dynamics of the PSII repair cycle. PSB29 (Keren et al. 2005), PSB27 (Chen et al. 2006; Mamedov et al. 2007; Nowaczyk et al. 2006; Roose and Pakrasi 2008), PPL1 (Ishihara et al. 2007; Ishikawa et al. 2005; Summerfield et al. 2005; Sveshnikov et al. 2007; Thornton et al. 2004), and ALB proteins (Ossenbuhl et al. 2006; Spence et al. 2004; Sundberg et al. 1997) also have similar roles in PSII repair both in higher plants and in cyanobacteria. Such a high number of evolutionarily conserved auxiliary proteins is interesting, since cyanobacteria lack the thylakoid heterogeneity and thus the repair cycle of PSII differs between these two organisms to some extent.

5.3. Low-molecular-weight subunit PsbR stabilizes the oxygen-evolving side of PSII

The functional roles of the PSII protein subunits that are present only in higher plants, such as the PsbR protein, have remained obscure compared to those with homologs in the cyanobacteria. This is due to the fact that the high resolution, three-dimensional structural models of PSII are based on cyanobacterial crystals – while only low-resolution structures are available from higher plants (Hankamer et al. 2001; Loll et al. 2005). In Paper I, the functional role of one of the so far poorly characterized PSII proteins, PsbR, was examined in greater detail. Early biochemical experiments had led to the conclusion that PsbR resides on the lumenal side of PSII, in close association with the OEC proteins (Roose et al. 2007). This is in line with the results presented in Paper I indicating that in the *psbr* plants, the oxygen evolving capacity is clearly diminished and the electron transfer properties of PSII are affected (Paper I, Allahverdiyeva et al. 2007). The PsbR protein has a putative transmembrane domain to anchor it to the thylakoid membrane, and a charged N-terminal domain capable of forming ion bridges with extrinsic proteins, allowing PsbR to act as a docking protein. Indeed, the PsbP and PsbQ proteins were present in reduced amounts in *psbr* thylakoids (Paper I). The exact function of PsbR appears to be ensuring the stable assembly of PsbP and, directly or indirectly, also PsbQ into the PSII core complex. These results are in accordance with an early suggestion that PsbR may be important for docking of PsbP to the PSII complex in spinach thylakoid membranes (Ljungberg et al. 1984, 1986). They are, on the other hand, contrary to the experimental data indicating that in potato *psbr* antisense plants, the PsbR protein does not have any effect on the assembly of the OEC proteins (Stockhaus et al. 1990).

Interestingly, the analysis of radioactive assembly intermediates of PSII revealed that PsbR is assembled into the CP43-less PSII monomer, while PsbO is assembled into the PSII monomer – and PsbP and PsbQ even later into the PSII dimers (Hashimoto et al. 1997; Rokka et al. 2005). It seems plausible that PsbR gets assembled into the PSII core independently of the other OEC proteins. However, a low-molecular-mass subunit of PSII, PsbJ, is required for stable integration of PsbR (Paper I).

Intriguingly, in Paper I it was found that the plants accumulate more of the PsbP, PsbQ, and PsbR proteins when grown under low-light conditions than plants grown under growth light. It was hypothesized earlier that the OEC proteins of higher plants may be important for the stacking of grana thylakoids (Dekker and Boekema 2005), which highly depends on the intensity of growth light (Aro et al. 1993a). Thus, it is possible that the increased amounts of the PsbP, PsbQ, and PsbR proteins are required for the extensive granal stacking that is characteristic of the thylakoid membrane under low-light conditions.

5.4. New insights into the structure and function of the NDH complex

The higher plant NDH complex functions in CET and chlororespiration. The NDH-dependent and FQR-dependent pathways of the CET are important in order to guarantee proper production of ATP in relation to NADPH, and for plant survival under various conditions of environmental stress (Munekage et al. 2004; Rumeau et al. 2007; Shikanai 2007). The absence of one cyclic pathway is not lethal, but the elimination of both pathways does not allow photoautotrophic growth (Munekage et al. 2004). At present, the structures of the chloroplast NDH complex and its homolog in cyanobacteria, the NDH-1L complex, are poorly characterized and only low-resolution single-particle electron microscopic images of the NDH complex are available (Arteni et al. 2006; Folea et al. 2008). Fifteen subunits of the NDH complex have been identified, both from cyanobacteria and chloroplasts (Battchikova and Aro 2007; Endo et al. 2008; Ogawa 1992; Rumeau et al. 2005; Shimizu et al. 2008). Interestingly, the subunits of the NDH complex responsible for NAD(P)H and flavin mononucleotide binding – and containing the majority of iron-sulfur clusters necessary for catalytic activity of the complex in *Escherichia coli* – have remained unknown in the NDH complexes of both cyanobacteria and chloroplasts (Battchikova and Aro 2007; Endo et al. 2008).

5.4.1. NDH complex contains several plant-specific subunits

In Paper IV, two nuclear-encoded plant-specific subunits of the NDH complex were characterized. NDH48 and NDH45 were shown to migrate together with the PSI/NDH supercomplex (Figure 5) and deficiency in either one of these proteins was shown to lead to impaired activity and accumulation of the NDH complex in thylakoid membranes (Paper IV). These data are in line with a recent publication of Takabayashi et al. (2009), where the NDH48 and NDH45 subunits were named NDF1 and NDF2, respectively. Also, a recent study with maize bundle sheath chloroplasts, rich in NDH, has shown that the NDH48 and NDH45 homologs co-migrate with the NDH complex (Majeran et al. 2008). Moreover, a co-expression analysis performed by Ishihara et al. (2007) has linked the NDH48 protein to the same regulon with the NDH auxiliary protein PPL2 and two subunits of the NDH complex, NDHN and NDHL.

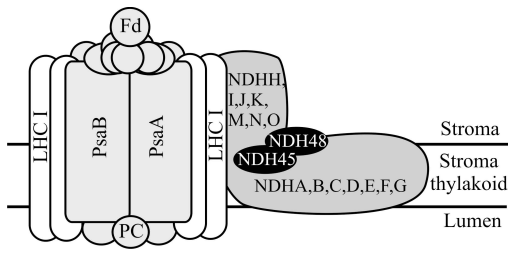

Figure 5. PSI and NDH complexes form a supercomplex in stroma thylakoids. The hypothetical locations of subunits in the hydrophilic and hydrophobic domains of the NDH complex are shown.

Neither NDH45 nor NDH48 contains domains responsible for electron transport or otherwise fulfills the prerequisite for the unknown activity unit of the NDH complex. This is also the case for the other recently identified nuclear-encoded subunits of the NDH complex: CRR3, CRR7, NDF5, and NDF6 (Ishida et al. 2009; Ishikawa et al. 2008; Munshi et al. 2005; Muraoka et al. 2006). There is, however, one exception: the recently characterized novel subunit NDF4 of the NDH complex (Takabayashi et al. 2009). NDF4 may be involved in electron transfer during NDH-dependent CET due to the fact that this protein is predicted to possess a redox-active iron-sulfur cluster. However, the role of NDF4 in the function of the NDH complex remains to be proven experimentally.

It is also possible that the NDH complex of plants and cyanobacteria uses FNR or ferredoxin as an electron donor, or completely lacks the electron input module (Battchikova and Aro 2007; Guedeney et al. 1996; Friedrich et al. 1995). The discovery of the PSI-NDH supercomplex is an interesting finding in this respect (Paper IV, Peng et al. 2008). It has been suggested earlier that a fraction of the PSI complex functions only in CET. In these considerations, the element postulated to separate the CET from linear electron transport has been either the spatial segregation of the PSI complex between the grana margins and stroma-exposed thylakoids or the formation of a CET-specific supercomplex (Albertsson 2001; Joliot and Joliot 2002, 2005). The hypothetical CET-specific supercomplex has been supposed to be composed of PSI, ferredoxin, FNR, Cyt b_6f, and plastocyanin, thus having a role in the FQR-dependent pathway (Joliot and Joliot 2002). However, the existence of such a complex has not been verified. It is tempting to speculate that in the novel PSI/NDH supercomplex, the electrons are recycled from PSI via ferredoxin (and FNR) directly to the NDH complex. The challenge of the future will be the clarification of the exact mechanisms of electron transport within the PSI/NDH supercomplex.

5.4.2. Auxiliary proteins of the NDH complex

In addition to our growing knowledge of the subunit composition of the NDH complex in higher plant chloroplasts, a multitude of nuclear-encoded auxiliary proteins that assist in the biogenesis and stability of the NDH complex have also been revealed during the past few years (Rumeau et al. 2007). The NDH complex is highly regulated at the transcriptional level. The CRR2, CRR4, CRR21, CRR22, and CRR28 proteins are members of the pentatricopeptide repeat family containing sequence-specific RNA-binding domains. CRR2 (Hashimoto et al. 2003) is involved in RNA maturation of the transcripts encoding NDHB, and both CRR4 (Kotera et al. 2005) and CRR21 (Okuda et al. 2007) assist in the maturation of *NDHD* transcripts. CRR22 and CRR28 are in turn involved in multiple editing steps of both the *NDHB* and *NDHD* transcripts (Okuda et al. 2009). In addition, HCF109 controls the stability of several plastid encoded operons, which code also subunits of the NDH complex (Meurer et al. 1996).

A nuclear-encoded factor, APO1, is required for stable assembly of several [4Fe-4S] cluster-containing complexes of chloroplasts including the NDH complex (Amann et al. 2004). CRR6 (Munshi et al. 2006) and PPL2 (Ishihara et al. 2007), located in the thylakoid membrane fraction and the thylakoid lumen, respectively, are auxiliary proteins of the NDH complex, and their functions may be linked to the assembly of the NDH complex. Two stromal proteins, CRR1 and PIFI, have also been linked to the NDH complex. CRR1 contains a NAD(P)H-binding motif and it has been suggested to be involved in biogenesis or stabilization of the NDH complex, possibly through reduction of an unknown substrate (Shimizu and Shikanai 2007). PIFI is a novel component essential for NDH-mediated non-photochemical reduction of the PQ pool in the chlororespiratory electron transport (Wang and Portis 2007). In addition, the NDH complex has been shown to be post-translationally regulated through phosphorylation of the NDHF subunit (Lascano et al. 2003).

In this study, the lumenal (Peltier et al. 2002; Schubert et al. 2002) cyclophilin AtCYP20-2 was found to co-migrate with the PSI/NDH supercomplex in 2D BN/SDS-PAGE separation of the stroma thylakoid protein complexes (Paper IV). This finding is in the line with the study of Majeran et al. (2008) in which the maize homolog of AtCYP20-2 from bundle sheath chloroplasts was found to co-migrate with the NDH complex. Surprisingly, in an earlier characterization of this protein, it was concluded that AtCYP20-2 is attached to the thylakoid membrane fractions that are enriched in PSII-LHCII supercomplexes (Romano et al. 2004). Both the PSI/NDH and the PSII-LHCII supercomplexes have a large molecular mass, and it is thus possible that Romano et al. (2004) misinterpretated their results. Based on the presence of the PPIase domain in AtCYP20-2, enrichment of AtCYP20-2 in the stroma membranes, and also co-migration of the protein with the NDH complex, it is likely that AtCYP38 is an auxiliary protein of the NDH complex.

Disruption of the *AtCYP20-2* gene did not result in any visual phenotype in the mutant plants (unpublished results). Surprisingly, only minor differences in the accumulation and activity of the NDH complex in the *cyp20-2* plants compared to wild type were recorded (unpublished results). This result is unexpected, since the AtCYP20 protein is the only lumenal cyclophilin in *Arabidopsis* that has been shown to possess PPIase activity *in vitro* (Edvardsson et al. 2003; Romano et al. 2004; Shapiguzov et al. 2006). This paradox can be explained by the fact that out of 18 immunophilins located in the chloroplast, the thylakoid lumen comprises 11 FKBP proteins and five cyclophilins, which possibly results in strong redundancy in PPIase properties (Romano et al. 2005). Indeed, the PPIase activity of another lumenal immunophilin, AtFKBP13, has been shown to be elevated in *Arabidopsis cyp20-2* knock-out mutants, suggesting that the PPIase activity of AtCYP20-2 is redundant (Edvardsson et al. 2007).

5.4.3. The NDH complex of higher plants may be involved in a carbon-concentrating mechanism

In cyanobacteria, the NDH-1MS complex is involved in CO_2 uptake (Zhang et al. 2004). The localization of NDH45 and NDH48 homologs to the NDH complex of maize bundle sheath chloroplasts led to speculation about NDH of C_4 plant thylakoids also being a part of the carbon-concentrating mechanism (Majeran et al. 2008). It is well known that carbon-concentrating mechanisms are present in all cyanobacteria, most algae, and aquatic plants as well as in C_4 plants in order to accumulate CO_2 (Raven et al. 2008). In the C_4 plants, the CO_2 binding takes place in two different compartments. In mesophyll cells phosphoenolpyruvate carboxylase binds bicarbonate originating from atmospheric CO_2 into oxaloacetate, which is further converted into malate. In bundle sheath cells malate is decarboxylated followed by the C_3 type of CO_2 fixation at elevated concentrations of CO_2. Majeran et al. (2008) suggested that the NDH complex in maize bundle sheath chloroplasts is possibly involved in hydration of CO_2 into bicarbonate, in order to reduce the diffusion rates of CO_2. According to the general view, the C_3 plants do not possess carbon-concentrating mechanisms. However, if NDH45 and NDH48 homologs in C_4 plants indeed play a role in carbon-concentrating system, the possibility of similar type of mechanism existing in C_3 plants cannot be ruled out.

6. CONCLUDING REMARKS

- Several novel proteins that function in the biogenesis, maintenance, and turnover of thylakoid protein complexes were identified from the thylakoid-associated polysome nascent chain complexes of *Arabidopsis*.
- The identity of a novel ≥ 1,000 kDa protein complex found in the stroma-exposed membranes was determined based on the subunit composition, and it was shown to be composed of the PSI and NDH complexes.
- The functional roles of two thylakoid-associated proteins, NDH45 and NDH48 that co-migrated with the PSI/NDH supercomplex were studied, and they were shown to be subunits of the hydrophobic domain of the NDH complex.
- The function of several lumenal proteins was investigated using the reverse genetics approach and in-depth functional analyses. TLP18.3 and AtCYP38 were shown to function as auxiliary proteins of PSII while the PsbR protein was found to stabilize OEC. In addition, AtCYP20-2 was suggested to have a role as an auxiliary protein of the NDH complex.
- Interestingly, the PSII OEC proteins PsbP, PsbQ, and PsbR were shown to be up-regulated under constant low-light conditions (as compared to growth-light conditions), whereas the amount of PsbO was not dependent on light intensity. This phenomenon was hypothesized to be associated with the enhanced grana stacking seen when plants are grown under low-light conditions.

7. FUTURE PERSPECTIVES

At the moment, the NDH complex can be referred to as a "mystery" complex of the thylakoid membrane. The NDH complex of higher plants has mainly been studied using the tobacco transformants with inactivations in chloroplast-encoded *ndh* genes. According to the state-of-the-art knowledge, the function of the higher plant NDH complex in the thylakoid membrane is linked to CET and chlororespiration. However, the exact mechanism of the function of the NDH complex remains unknown. Thus, one of the most interesting fields in photosynthesis research is to solve the enigma of the unknown electron input module of the NDH complex, as well as the nature of the compound(s) feeding electrons to NDH. Moreover, it is extremely tempting to speculate that the NDH complex of higher plants may have additional roles such as participation in inorganic carbon-concentration systems.

Extensive analysis of purified His-tagged NDH complexes and mutant plants showing altered Chl fluorescence has led to the finding of a number of novel subunits of the NDH complex, and also auxiliary proteins. Characterization of the subunit composition of the *Arabidopsis* PSI/NDH supercomplex is a novel approach in this field. However, even though our knowledge of the subunit composition of the higher plant NDH complex is improving, the exact roles of most of the novel NDH subunits have remained uncharacterized. Also, the assembly steps of the NDH complex are still unknown. In this respect, the detailed analysis of a novel ~800-kDa protein complex, possibly representing an assembly intermediate of the NDH complex, would be of particular interest.

Although the PSII complex has been very actively studied during the last two decades, our knowledge is still far from complete. At the moment, our knowledge concerning the repair cycle of PSII is rather good. However, it is likely that only a minor proportion of the PSII chaperones and other auxiliary proteins have been characterized so far, and many of the auxiliary proteins are still awaiting discovery. In addition, a deeper characterization of the auxiliary proteins that are already known will probably be required. Multi-domain auxiliary proteins such as AtCYP38 should be studied by targeting the mutagenesis separately to the domains of interest. Moreover, in order to study the redundant proteins like the thylakoid lumen PPIases, one should construct double, triple, and multiple mutants and subject them to detailed characterization.

ACKNOWLEDGEMENTS

This work was carried out in the Laboratory of Plant Physiology and Molecular Biology at the University of Turku. The head of the department, Professor Eevi Rintamäki is warmly thanked for her excellent guidance throughout my studies. Financial support from Academy of Finland, Biological Interactions Graduate School, and Jenny and Antti Wihuri Foundation is acknowledged.

My supervisor Professor Eva-Mari Aro is thanked for never-ending, wide-range support. You have been the best possible boss for me. I am grateful to Professors Wolfgang Schröder, and Kurt Fagerstedt for critical reading of the manuscript of this thesis, and to Hugh Kidd for revising the language.

The list of "tukihenkilö" during my Ph.D. studies is long. The major contributors of my thesis work have been Yagut, Natalia, Maija, Anastassia, Virpi, and Marjaana (in alphabetical order). Ulla-Maija, Mika, Kurt, Mirja, Maisa, Sanna, and Martti are of utmost importance to all of us, because without you no research could be done. I also thank the staff in our office room, Marja, Anna, Minna and PengPeng, and also Saija, Paula, Taina, Mirkka, Tove, Ilona, Mikko, and Julia for advises and discussions. Last but not least, all of you, both the present and former co-workers, are thanked for friendship and love. Pipola, Sweet, Pipola... I have really enjoyed my time in here.

Finally, I would like to thank my parents, sisters, and especially Jussi for being here for me.

Turku, April 2009

Sai

REFERENCES

Abrahams, J.P., Leslie, A. G., Lutter, R. and Walker, J. E. (1994) Structure at 2.8 A resolution of F1-ATPase from bovine heart mitochondria. *Nature,* **370**(6491), 621-628.

Adam, Z. and Ostersetzer, O. (2001) Degradation of unassembled and damaged thylakoid proteins. *Biochem. Soc. Trans.,* **29**(Pt 4), 427-430.

Adam, Z., Rudella, A. and van Wijk, K. J. (2006) Recent advances in the study of clp, FtsH and other proteases located in chloroplasts. *Curr. Opin. Plant Biol.,* **9**(3), 234-240.

Adir, N., Zer, H., Shochat, S. and Ohad, I. (2003) Photoinhibition - a historical perspective. *Photosynth Res.,* **76**(1-3), 343-370.

Albertsson, P.Å, Andreasson, E., Svensson, P. and Shi-Gui, Y. (1991) Localization of cytochrome f in the thylakoid membrane: Evidence for multiple domains. *Biochimica Et Biophysica Acta. Bioenergetics,* **1098**(1), 90-94.

Albertsson, P.Å (2001) A quantitative model of the domain structure of the photosynthetic membrane. *Trends Plant Sci.,* **6**(8), 349-358.

Allahverdiyeva, Y., Deak, Z., Szilard, A., Diner, B. A., Nixon, P. J. and 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. *European Journal of Biochemistry,* **271**(17), 3523-3532.

Allahverdiyeva, Y., Mamedov, F., Maenpaa, P., Vass, I. and Aro, E. M. (2005) Modulation of photosynthetic electron transport in the absence of terminal electron acceptors: Characterization of the rbcL deletion mutant of tobacco. *Biochim. Biophys. Acta,* **1709**(1), 69-83.

Allahverdiyeva, Y., Mamedov, F., Suorsa, M., Styring, S., Vass, I. and Aro, E. M. (2007) Insights into the function of PsbR protein in arabidopsis thaliana. *Biochim. Biophys. Acta,* **1767**(6), 677-685.

Allen, J.F. and Pfannschmidt, T. (2000) Balancing the two photosystems: Photosynthetic electron transfer governs transcription of reaction centre genes in chloroplasts. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.,* **355**(1402), 1351-1359.

Allen, J.F. (2005) Photosynthesis: The processing of redox signals in chloroplasts. *Curr. Biol.,* **15**(22), R929-32.

Alonso, J.M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., Stevenson, D. K., Zimmerman, J., Barajas, P., Cheuk, R., Gadrinab, C., Heller, C., Jeske, A., Koesema, E., Meyers, C. C., Parker, H., Prednis, L., Ansari, Y., Choy, N., Deen, H., Geralt, M., Hazari, N., Hom, E., Karnes, M., Mulholland, C., Ndubaku, R., Schmidt, I., Guzman, P., Aguilar-Henonin, L., Schmid, M., Weigel, D., Carter, D. E., Marchand, T., Risseeuw, E., Brogden, D., Zeko, A., Crosby, W. L., Berry, C. C. and Ecker, J. R. (2003) Genome-wide insertional mutagenesis of arabidopsis thaliana. *Science,* **301**(5633), 653-657.

Amann, K., Lezhneva, L., Wanner, G., Herrmann, R. G. and Meurer, J. (2004) ACCUMULATION OF PHOTOSYSTEM ONE1, a member of a novel gene family, is required for accumulation of [4Fe-4S] cluster-containing chloroplast complexes and antenna proteins. *Plant Cell,* **16**(11), 3084-3097.

Amunts, A., Drory, O. and Nelson, N. (2007) The structure of a plant photosystem I supercomplex at 3.4 A resolution. *Nature,* **447**(7140), 58-63.

Anderson, J.M., Park, Y. -. and Chow, W. S. (1998) Unifying model for the photoinactivation of photosystem II in vivo under steady-state photosynthesis. *Photosynt. Res.,* **56**, 1-13.

Anderson, L.B., Maderia, M., Ouellette, A. J., Putnam-Evans, C., Higgins, L., Krick, T., MacCoss, M. J., Lim, H., Yates, J. R.,3rd and Barry, B. A. (2002) Posttranslational modifications in the CP43 subunit of photosystem II. *Proc. Natl. Acad. Sci. U. S. A.,* **99**(23), 14676-14681.

Andersson, B. and Anderson, J. M. (1980) Lateral heterogeneity in the distribution of chlorophyll-protein complexes of the thylakoid membranes of spinach chloroplasts. *Biochim. Biophys. Acta,* **593**(2), 427-440.

Apweiler, R., Attwood, T. K., Bairoch, A., Bateman, A., Birney, E., Biswas, M., Bucher, P., Cerutti, L., Corpet, F., Croning, M. D., Durbin, R., Falquet, L., Fleischmann, W., Gouzy, J., Hermjakob, H., Hulo, N., Jonassen, I., Kahn, D., Kanapin, A., Karavidopoulou, Y., Lopez, R., Marx, B., Mulder, N. J., Oinn, T. M., Pagni, M., Servant, F., Sigrist, C. J. and Zdobnov, E. M. (2001) The InterPro database, an integrated documentation resource for protein families, domains and functional sites. *Nucleic Acids Res.,* **29**(1), 37-40.

Arabidopsis Genome Initiative. (2000) Analysis of the genome sequence of the flowering plant arabidopsis thaliana. *Nature,* **408**(6814), 796-815.

Arnon, D.I. (1991) Photosynthetic electron transport: Emergence of a concept, 1949–59. *Photosynthesis Res.,* **29**(3), 117-131.

Aro, E.M., McCaffery, S. and Anderson, J. M. (1993a) Photoinhibition and D1 protein degradation in peas acclimated to different growth irradiances. *Plant Physiol.,* **103**(3), 835-843.

Aro, E.M., Virgin, I. and Andersson, B. (1993b) Photoinhibition of photosystem II. inactivation,

protein damage and turnover. *Biochim. Biophys. Acta,* **1143**(2), 113-134.

Aro, E.M. and Ohad, I. (2003) Redox regulation of thylakoid protein phosphorylation. *Antioxid. Redox Signal.,* **5**(1), 55-67.

Aro, E.M., Suorsa, M., Rokka, A., Allahverdiyeva, Y., Paakkarinen, V., Saleem, A., Battchikova, N. and Rintamäki, E. (2005) Dynamics of photosystem II: A proteomic approach to thylakoid protein complexes. *J. Exp. Bot.,* **56**(411), 347-356.

Arteni, A.A., Zhang, P., Battchikova, N., Ogawa, T., Aro, E. M. and Boekema, E. J. (2006) Structural characterization of NDH-1 complexes of thermosynechococcus elongatus by single particle electron microscopy. *Biochim. Biophys. Acta,* **1757**(11), 1469-1475.

Asada, K., Heber, U. and Schreiber, U. (1992) Pool size of electrons that can be donated to P700 as determined in intact leaves: Donation to P700 from stromal components via the intersystem chain. *Plant and Cell Physiology,* **33**(7), 927-932.

Asada, K., Heber, U. and Schreiber, U. (1993) Electron flow to the intersystem chain from stromal components and cyclic electron flow in maize chloroplasts, as detected in intact leaves by monitoring redox change of P700 and chlorophyll fluorescence. *Plant and Cell Physiology,* **34**(1), 39-50.

Asada, K. (1999) The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* **50**, 601-639.

Baena-Gonzalez, E., Barbato, R. and Aro, E. (1999) Role of PSII phosphorylation in the PSII repair cycle and oligomeric structure. *Planta,* **208**, 196–204.

Baena-Gonzalez, E. and Aro, E. M. (2002) Biogenesis, assembly and turnover of photosystem II units. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.,* **357**(1426), 1451-9; discussion 1459-60.

Bailey, S., Thompson, E., Nixon, P. J., Horton, P., Mullineaux, C. W., Robinson, C. and Mann, N. H. (2002) A critical role for the Var2 FtsH homologue of arabidopsis thaliana in the photosystem II repair cycle in vivo. *J. Biol. Chem.,* **277**(3), 2006-2011.

Barber, J. and Andersson, B. (1992) Too much of a good thing: Light can be bad for photosynthesis. *Trends Biochem. Sci.,* **17**(2), 61-66.

Barbrook, A.C., Lockhart, P. J. and Howe, C. J. (1998) Phylogenetic analysis of plastid origins based on secA sequences. *Curr. Genet.,* **34**(4), 336-341.

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

Bechtold, U., Murphy, D. J. and Mullineaux, P. M. (2004) Arabidopsis peptide methionine sulfoxide reductase2 prevents cellular oxidative damage in long nights. *Plant Cell,* **16**(4), 908-919.

Bellafiore, S., Barneche, F., Peltier, G. and Rochaix, J. D. (2005) State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. *Nature,* **433**(7028), 892-895.

Bendall, D.S. and Manasse, R. S. (1995) Cyclic photophosphorylation and electron transport. *BBA-Bioenergetics,* **1229**(1), 23-38.

Bilger, W. and Björkman, O. (1994) Relationships among violaxanthin deepoxidation, thylakoid membrane conformation, and nonphotochemical chlorophyll fluorescence quenching in leaves of cotton (gossypium hirsutum L.). *Planta,* **193**(2), 238-246.

Blomqvist, L.A., Ryberg, M. and Sundqvist, C. (2006) Proteomic analysis of the etioplast inner membranes of wheat (triticum aestivum) by two-dimensional electrophoresis and mass spectrometry. *Physiol. Plantarum,* **128**(2), 368-381.

Boekema, E.J., van Breemen, J. F., van Roon, H. and Dekker, J. P. (2000) Conformational changes in photosystem II supercomplexes upon removal of extrinsic subunits. *Biochemistry,* **39**(42), 12907-12915.

Bonardi, V., Pesaresi, P., Becker, T., Schleiff, E., Wagner, R., Pfannschmidt, T., Jahns, P. and Leister, D. (2005) Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. *Nature,* **437**(7062), 1179-1182.

Boschetti, A., Breidenbach, E. and Blätter, R. (1990) Control of protein formation in chloroplasts. *Plant Sci.,* **68**, 131-149.

Bowyer, J.R., Packer, J. C., McCormack, B. A., Whitelegge, J. P., Robinson, C. and Taylor, M. A. (1992) Carboxyl-terminal processing of the D1 protein and photoactivation of water-splitting in photosystem II. partial purification and characterization of the processing enzyme from scenedesmus obliquus and pisum sativum. *J. Biol. Chem.,* **267**(8), 5424-5433.

Breyton, C., Tribet, C., Olive, J., Dubacq, J. P. and Popot, J. L. (1997) Dimer to monomer conversion of the cytochrome b6 f complex. causes and consequences. *J. Biol. Chem.,* **272**(35), 21892-21900.

Burnap, R.L. (2004) D1 protein processing and mn cluster assembly in light of the emerging photosystem II structure. *Physical Chemistry Chemical Physics,* **6**(20), 4803-4809.

Burrows, P.A., Sazanov, L. A., Svab, Z., Maliga, P. and Nixon, P. J. (1998) Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid ndh genes. *EMBO J.,* **17**(4), 868-876.

Cai, W., Ma, J., Guo, J. and Zhang, L. (2008) Function of ROC4 in the efficient repair of

photodamaged photosystem II in arabidopsis. *Photochem. Photobiol.,* **84**(6), 1343-1348.

Carlberg, I., Hansson, M., Kieselbach, T., Schröder, W. P., Andersson, B. and Vener, A. V. (2003) A novel plant protein undergoing light-induced phosphorylation and release from the photosynthetic thylakoid membranes. *Proc. Natl. Acad. Sci. U. S. A.,* **100**(2), 757-762.

Chen, H., Zhang, D., Guo, J., Wu, H., Jin, M., Lu, Q., Lu, C. and Zhang, L. (2006) A Psb27 homologue in arabidopsis thaliana is required for efficient repair of photodamaged photosystem II. *Plant Mol. Biol.,* **61**(4-5), 567-575.

Chow, W.S., Lee, H. Y., He, J., Hendrickson, L., Hong, Y. N. and Matsubara, S. (2005) Photoinactivation of photosystem II in leaves. *Photosynth Res.,* **84**(1-3), 35-41.

Chu, H.A., Nguyen, A. P. and Debus, R. J. (1995) Amino acid residues that influence the binding of manganese or calcium to photosystem II. 1. the lumenal interhelical domains of the D1 polypeptide. *Biochemistry,* **34**(17), 5839-5858.

Clough, S.J. and Bent, A. F. (1998) Floral dip: A simplified method for agrobacterium-mediated transformation of arabidopsis thaliana. *Plant J.,* **16**(6), 735-743.

Cser, K., Diner, B. A., Nixon, P. J. and Vass, I. (2005) The role of D1-Ala344 in charge stabilization and recombination in photosystem II. *Photochem. Photobiol. Sci.,* **4**(12), 1049-1054.

Curtis, S.E. and Clegg, M. T. (1984) Molecular evolution of chloroplast DNA sequences. *Mol. Biol. Evol.,* **1**(4), 291-301.

DalCorso, G., Pesaresi, P., Masiero, S., Aseeva, E., Schunemann, D., Finazzi, G., Joliot, P., Barbato, R. and Leister, D. (2008) A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in arabidopsis. *Cell,* **132**(2), 273-285.

Danielsson, R., Albertsson, P. A., Mamedov, F. and Styring, S. (2004) Quantification of photosystem I and II in different parts of the thylakoid membrane from spinach. *Biochim. Biophys. Acta,* **1608**(1), 53-61.

Danielsson, R., Suorsa, M., Paakkarinen, V., Albertsson, P. A., Styring, S., Aro, E. M. and 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**(20), 14241-14249.

Dekker, J.P. and Boekema, E. J. (2005) Supramolecular organization of thylakoid membrane proteins in green plants. *Biochim. Biophys. Acta,* **1706**(1-2), 12-39.

De Las Rivas, J., Balsera, M. and Barber, J. (2004) Evolution of oxygenic photosynthesis: Genome-wide analysis of the OEC extrinsic proteins. *Trends Plant Sci.,* **9**(1), 18-25.

Delwiche, C.F., Kuhsel, M. and Palmer, J. D. (1995) Phylogenetic analysis of tufA sequences indicates a cyanobacterial origin of all plastids. *Mol. Phylogenet. Evol.,* **4**(2), 110-128.

Demming-Adams, B. and Adams, W. (1992) Photoprotection and other responses of plants to light stress. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* **7**, 1–116.

Demming-Adams, B. and Adams W. (1996) The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends Plant Sci,* **1**, 21-26.

Diner, B.A., Ries, D. F., Cohen, B. N. and Metz, J. G. (1988) COOH-terminal processing of polypeptide D1 of the photosystem II reaction center of scenedesmus obliquus is necessary for the assembly of the oxygen-evolving complex. *J. Biol. Chem.,* **263**(18), 8972-8980.

Edvardsson, A., Eshaghi, S., Vener, A. V. and Andersson, B. (2003) The major peptidyl-prolyl isomerase activity in thylakoid lumen of plant chloroplasts belongs to a novel cyclophilin TLP20. *FEBS Lett.,* **542**(1-3), 137-141.

Edvardsson, A., Shapiguzov, A., Petersson, U. A., Schröder, W. P. and Vener, A. V. (2007) Immunophilin AtFKBP13 sustains all peptidyl-prolyl isomerase activity in the thylakoid lumen from arabidopsis thaliana deficient in AtCYP20-2. *Biochemistry,* **46**(33), 9432-9442.

Endo, T., Ishida, S., Ishikawa, N. and Sato, F. (2008) Chloroplastic NAD(P)H dehydrogenase complex and cyclic electron transport around photosystem I. *Mol. Cells,* **25**(2), 158-162.

Ettinger, W.F. and Theg, S. M. (1991) Physiologically active chloroplasts contain pools of unassembled extrinsic proteins of the photosynthetic oxygen-evolving enzyme complex in the thylakoid lumen. *J. Cell Biol.,* **115**(2), 321-328.

Feild, T.S., Nedbal, L. and Ort, D. R. (1998) Nonphotochemical reduction of the plastoquinone pool in sunflower leaves originates from chlororespiration. *Plant Physiol.,* **116**(4), 1209-1218.

Ferreira, K.N., Iverson, T. M., Maghlaoui, K., Barber, J. and Iwata, S. (2004) Architecture of the photosynthetic oxygen-evolving center. *Science,* **303**(5665), 1831-1838.

Folea, I.M., Zhang, P., Nowaczyk, M. M., Ogawa, T., Aro, E. M. and Boekema, E. J. (2008) Single particle analysis of thylakoid proteins from thermosynechococcus elongatus and synechocystis 6803: Localization of the CupA subunit of NDH-1. *FEBS Lett.,* **582**(2), 249-254.

Friedrich, T., Steinmüller, K. and Weiss, H. (1995) The proton-pumping respiratory complex I of bacteria and mitochondria and its homologue in chloroplasts. *FEBS Lett.,* **367**(2), 107-111.

Friedrich, T. and Weiss, H. (1997) Modular evolution of the respiratory NADH:Ubiquinone oxidoreductase and the origin of its modules. *J. Theor. Biol.,* **187**(4), 529-540.

Fu, A., He, Z., Cho, H. S., Lima, A., Buchanan, B. B. and Luan, S. (2007) A chloroplast cyclophilin functions in the assembly and maintenance of photosystem II in arabidopsis thaliana. *Proc. Natl. Acad. Sci. U. S. A.,* **104**(40), 15947-15952.

Fujita, S., Inagaki, N., Yamamoto, Y., Taguchi, F., Matsumoto, A. and Satoh, K. (1995) Identification of the carboxyl-terminal processing protease for the D1 precursor protein of the photosystem II reaction center of spinach. *Plant and Cell Physiology,* **36**(7), 1169-1177.

Fulgosi, H., Vener, A. V., Altschmied, L., Herrmann, R. G. and Andersson, B. (1998) A novel multi-functional chloroplast protein: Identification of a 40 kDa immunophilin-like protein located in the thylakoid lumen. *EMBO J.,* **17**(6), 1577-1587.

Guedeney, G., Corneille, S., Cuine, S. and Peltier, G. (1996) Evidence for an association of ndh B, ndh J gene products and ferredoxin-NADP-reductase as components of a chloroplastic NAD(P)H dehydrogenase complex. *FEBS Lett.,* **378**(3), 277-280.

Guskov, A., Kern, J., Gabdulkhakov, A., Broser, M., Zouni, A. and Saenger, W. (2009) Cyanobacterial photosystem II at 2.9-A resolution and the role of quinones, lipids, channels and chloride. *Nat. Struct. Mol. Biol.,* **16**(3), 334-342.

Göthel, S.F. and Marahiel, M. A. (1999) Peptidyl-prolyl cis-trans isomerases, a superfamily of ubiquitous folding catalysts. *Cell Mol. Life Sci.,* **55**(3), 423-436.

Hakala, M., Tuominen, I., Keranen, M., Tyystjärvi, T. and Tyystjärvi, E. (2005) Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of photosystem II. *Biochim. Biophys. Acta,* **1706**(1-2), 68-80.

Hakala, M., Rantamäki, S., Puputti, E. M., Tyystjärvi, T. and Tyystjärvi, E. (2006) Photoinhibition of manganese enzymes: Insights into the mechanism of photosystem II photoinhibition. *J. Exp. Bot.,* **57**(8), 1809-1816.

Hankamer, B., Morris, E., Nield, J., Gerle, C. and Barber, J. (2001) Three-dimensional structure of the photosystem II core dimer of higher plants determined by electron microscopy. *J. Struct. Biol.,* **135**(3), 262-269.

Hashimoto, A., Ettinger, W. F., Yamamoto, Y. and Theg, S. M. (1997) Assembly of newly imported oxygen-evolving complex subunits in isolated chloroplasts: Sites of assembly and mechanism of binding. *Plant Cell,* **9**(3), 441-452.

Hashimoto, M., Endo, T., Peltier, G., Tasaka, M. and Shikanai, T. (2003) A nucleus-encoded factor, CRR2, is essential for the expression of chloroplast ndhB in arabidopsis. *Plant J.,* **36**(4), 541-549.

Haussuhl, K., Andersson, B. and Adamska, I. (2001) A chloroplast DegP2 protease performs the primary cleavage of the photodamaged D1 protein in plant photosystem II. *EMBO J.,* **20**(4), 713-722.

Heber, U. and Walker, D. (1992) Concerning a dual function of coupled cyclic electron transport in leaves. *Plant Physiol.,* **100**(4), 1621-1626.

Herbert, S.K., Fork, D. C. and Malkin, S. (1990) Photoacoustic measurements in vivo of energy storage by cyclic electron flow in algae and higher plants. *Plant Physiol.,* **94**(3), 926-934.

Hill, R. and Bendall, F. (1960) Function of the two cytochrome components in chloroplasts: A working hypothesis. *Nature,* **186**(4719), 136-137.

Holt, N.E., Fleming, G. R. and Niyogi, K. K. (2004) Toward an understanding of the mechanism of nonphotochemical quenching in green plants. *Biochemistry,* **43**(26), 8281-8289.

Hope, A.B. (1993) The chloroplast cytochrome bf complex: A critical focus on function. *Biochim. Biophys. Acta,* **1143**(1), 1-22.

Horvath, E.M., Peter, S. O., Joet, T., Rumeau, D., Cournac, L., Horvath, G. V., Kavanagh, T. A., Schafer, C., Peltier, G. and Medgyesy, P. (2000) Targeted inactivation of the plastid ndhB gene in tobacco results in an enhanced sensitivity of photosynthesis to moderate stomatal closure. *Plant Physiol.,* **123**(4), 1337-1350.

Ishida, S., Takabayashi, A., Ishikawa, N., Hano, Y., Endo, T. and Sato, F. (2009) A novel nuclear-encoded protein, NDH-dependent cyclic electron flow 5, is essential for the accumulation of chloroplast NAD(P)H dehydrogenase complexes. *Plant Cell Physiol.,* **50**(2), 383-393.

Ishihara, S., Takabayashi, A., Ido, K., Endo, T., Ifuku, K. and Sato, F. (2007) Distinct functions for the two PsbP-like proteins PPL1 and PPL2 in the chloroplast thylakoid lumen of arabidopsis. *Plant Physiol.,* **145**(3), 668-679.

Ishikawa, N., Takabayashi, A., Ishida, S., Hano, Y., Endo, T. and Sato, F. (2008) NDF6: A thylakoid protein specific to terrestrial plants is essential for activity of chloroplastic NAD(P)H dehydrogenase in arabidopsis. *Plant Cell Physiol.,* **49**(7), 1066-1073.

Ishikawa, Y., Schröder, W. P. and Funk, C. (2005) Functional analysis of the PsbP-like protein (sll1418) in synechocystis sp. PCC 6803. *Photosynth Res.,* **84**(1-3), 257-262.

Jensen, P.E., Bassi, R., Boekema, E. J., Dekker, J. P., Jansson, S., Leister, D., Robinson, C. and Scheller, H. V. (2007) Structure, function and regulation of plant photosystem I. *Biochim. Biophys. Acta,* **1767**(5), 335-352.

Jiang, C.D., Gao, H. Y., Zou, Q., Jiang, G. M. and Li, L. H. (2006) Leaf orientation, photorespiration and xanthophyll cycle protect young soybean leaves against high irradiance in field. *Environ. Exp. Bot.,* **55**(1-2), 87-96.

Joet, T., Cournac, L., Horvath, E. M., Medgyesy, P. and Peltier, G. (2001) Increased sensitivity of photosynthesis to antimycin A induced by inactivation of the chloroplast ndhB gene. evidence for a participation of the NADH-dehydrogenase complex to cyclic electron flow around photosystem I. *Plant Physiol.,* **125**(4), 1919-1929.

Johnson, G.N. (2005) Cyclic electron transport in C3 plants: Fact or artefact? *J. Exp. Bot.,* **56**(411), 407-416.

Joliot, P. and Joliot, A. (2002) Cyclic electron transfer in plant leaf. *Proc. Natl. Acad. Sci. U. S. A.,* **99**(15), 10209-10214.

Joliot, P. and Joliot, A. (2005) Quantification of cyclic and linear flows in plants. *Proc. Natl. Acad. Sci. U. S. A.,* **102**(13), 4913-4918.

Kamiya, N. and Shen, J. R. (2003) Crystal structure of oxygen-evolving photosystem II from thermosynechococcus vulcanus at 3.7-A resolution. *Proc. Natl. Acad. Sci. U. S. A.,* **100**(1), 98-103.

Kaneko, T. and Tabata, S. (1997) Complete genome structure of the unicellular cyanobacterium synechocystis sp. PCC6803. *Plant Cell Physiol.,* **38**(11), 1171-1176.

Kanervo, E., Singh, M., Suorsa, M., Paakkarinen, V., Aro, E., Battchikova, N. and Aro, E. M. (2008) Expression of protein complexes and individual proteins upon transition of etioplasts to chloroplasts in pea (pisum sativum). *Plant Cell Physiol.,* **49**(3), 396-410.

Kapri-Pardes, E., Naveh, L. and Adam, Z. (2007) The thylakoid lumen protease Deg1 is involved in the repair of photosystem II from photoinhibition in arabidopsis. *Plant Cell,* **19**(3), 1039-1047.

Kashino, Y., Lauber, W. M., Carroll, J. A., Wang, Q., Whitmarsh, J., Satoh, K. and Pakrasi, H. B. (2002) Proteomic analysis of a highly active photosystem II preparation from the cyanobacterium synechocystis sp. PCC 6803 reveals the presence of novel polypeptides. *Biochemistry,* **41**(25), 8004-8012.

Keren, N., Ohkawa, H., Welsh, E. A., Liberton, M. and Pakrasi, H. B. (2005) Psb29, a conserved 22-kD protein, functions in the biogenesis of photosystem II complexes in synechocystis and arabidopsis. *Plant Cell,* **17**(10), 2768-2781.

Kieselbach, T. and Schröder, W. P. (2003) The proteome of the chloroplast lumen of higher plants. *Photosynth Res.,* **78**(3), 249-264.

Kim, J., Klein, P. G. and Mullet, J. E. (1991) Ribosomes pause at specific sites during synthesis of membrane-bound chloroplast reaction center protein D1. *J. Biol. Chem.,* **266**(23), 14931-14938.

Kjær, B. and Scheller, H. V. (1996) An isolated reaction center complex from the green sulfur bacterium chlorobium vibrioforme can photoreduce ferredoxin at high rates. *Photosynthesis Res.,* **47**(1), 33-39.

Kofer, W., Koop, H. U., Wanner, G. and Steinmüller, 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**(1-2), 166-173.

Koivuniemi, A., Aro, E. M. and Andersson, B. (1995) Degradation of the D1- and D2-proteins of photosystem II in higher plants is regulated by reversible phosphorylation. *Biochemistry,* **34**(49), 16022-16029.

Kotera, E., Tasaka, M. and Shikanai, T. (2005) A pentatricopeptide repeat protein is essential for RNA editing in chloroplasts. *Nature,* **433**(7023), 326-330.

Krogh, A., Larsson, B., von Heijne, G. and Sonnhammer, E. L. L. (2001) Predicting transmembrane protein topology with a hidden markov model: Application to complete genomes. *J. Mol. Biol.,* **305**(3), 567-580.

Kurisu, G., Zhang, H., Smith, J. L. and Cramer, W. A. (2003) Structure of the cytochrome b6f complex of oxygenic photosynthesis: Tuning the cavity. *Science,* **302**(5647), 1009-1014.

Kügler, M., Jänsch, L., Kruft, V., Schmitz, U. K. and Braun, H. -P. (1997) Analysis of the chloroplast protein complexes by blue-native polyacrylamide gel electrophoresis (BN-PAGE). *Photosynth. Res.,* **53**, 35-44.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* **227**(5259), 680-685.

Lascano, H.R., Casano, L. M., Martin, M. and Sabater, B. (2003) The activity of the chloroplastic ndh complex is regulated by phosphorylation of the NDH-F subunit. *Plant Physiol.,* **132**(1), 256-262.

Li, X.P., Gilmore, A. M., Caffarri, S., Bassi, R., Golan, T., Kramer, D. and Niyogi, K. K. (2004) Regulation of photosynthetic light harvesting involves intrathylakoid lumen pH sensing by the PsbS protein. *J. Biol. Chem.,* **279**(22), 22866-22874.

Lima, A., Lima, S., Wong, J. H., Phillips, R. S., Buchanan, B. B. and Luan, S. (2006) A redox-active FKBP-type immunophilin functions in accumulation of the photosystem II supercomplex in arabidopsis thaliana. *Proc. Natl. Acad. Sci. U. S. A.,* **103**(33), 12631-12636.

Lindahl, M., Tabak, S., Cseke, L., Pichersky, E., Andersson, B. and Adam, Z. (1996) Identification, characterization, and molecular cloning of a homologue of the bacterial FtsH protease in

chloroplasts of higher plants. *J. Biol. Chem.,* **271**(46), 29329-29334.

Ljungberg, U., Akerlund, H. E., Larsson, C. and Andersson, B. (1984) Identification of polypeptides associated with the 23 and 33 kDa proteins of photosynthetic oxygen evolution. *Biochim. Biophys. Acta,* **767**(1), 145-152.

Ljungberg, U., Akerlund, H. E. and Andersson, B. (1986) Isolation and characterization of the 10-kDa and 22-kDa polypeptides of higher plant photosystem 2. *Eur. J. Biochem.,* **158**(3), 477-482.

Loll, B., Kern, J., Saenger, W., Zouni, A. and Biesiadka, J. (2005) Towards complete cofactor arrangement in the 3.0 A resolution structure of photosystem II. *Nature,* **438**(7070), 1040-1044.

Ma, J., Peng, L., Guo, J., Lu, Q., Lu, C. and Zhang, L. (2007) LPA2 is required for efficient assembly of photosystem II in arabidopsis thaliana. *Plant Cell,* **19**(6), 1980-1993.

Majeran, W., Zybailov, B., Ytterberg, A. J., Dunsmore, J., Sun, Q. and van Wijk, K. J. (2008) Consequences of C4 differentiation for chloroplast membrane proteomes in maize mesophyll and bundle sheath cells. *Mol. Cell. Proteomics,* **7**(9), 1609-1638.

Mamedov, F., Nowaczyk, M. M., Thapper, A., Rögner, M. and Styring, S. (2007) Functional characterization of monomeric photosystem II core preparations from thermosynechococcus elongatus with or without the Psb27 protein. *Biochemistry (N. Y.),* **46**(18), 5542-5551.

Marder, J.B., Goloubinoff, P. and Edelman, M. (1984) Molecular architecture of the rapidly metabolized 32-kilodalton protein of photosystem II. indications for COOH-terminal processing of a chloroplast membrane polypeptide. *J. Biol. Chem.,* **259**(6), 3900-3908.

Marin-Navarro, J., Manuell, A. L., Wu, J. and P Mayfield, S. (2007) Chloroplast translation regulation. *Photosynth Res.,* **94**(2-3), 359-374.

Martin, W. and Herrmann, R. G. (1998) Gene transfer from organelles to the nucleus: How much, what happens, and why? *Plant Physiol.,* **118**(1), 9-17.

Mattoo, A.K., Hoffman-Falk, H., Marder, J. B. and Edelman, M. (1984) Regulation of protein metabolism: Coupling of photosynthetic electron transport to in vivo degradation of the rapidly metabolized 32-kilodalton protein of the chloroplast membranes. *Proc. Natl. Acad. Sci. U. S. A.,* **81**(5), 1380-1384.

Meurer, J., Berger, A. and Westhoff, P. (1996) A nuclear mutant of arabidopsis with impaired stability on distinct transcripts of the plastid psbB, psbD/C, ndhH, and ndhC operons. *Plant Cell,* **8**(7), 1193-1207.

Meurer, J., Plücken, H., Kowallik, K. V. and Westhoff, P. (1998) A nuclear-encoded protein of prokaryotic origin is essential for the stability of photosystem II in arabidopsis thaliana. *EMBO J.,* **17**(18), 5286-5297.

Mi, H., Endo, T., Ogawa, T. and Asada, K. (1995) Thylakoid membrane-bound, NADPH-specific pyridine nucleotide dehydrogenase complex mediates cyclic electron transport in the cyanobacterium synechocystis sp. PCC 6803. *Plant and Cell Physiology,* **36**(4), 661-668.

Miyake, C., Horiguchi, S., Makino, A., Shinzaki, Y., Yamamoto, H. and Tomizawa, K. (2005) Effects of light intensity on cyclic electron flow around PSI and its relationship to non-photochemical quenching of chl fluorescence in tobacco leaves. *Plant Cell Physiol.,* **46**(11), 1819-1830.

Moss, D.A. and Bendall, D. S. (1984) Cyclic electron transport in chloroplasts: The Q-cycle and the site of action of antimycin. *Biochim. Biophys. Acta,* **767**(3), 389-395.

Mulo, P., Sirpiö, S., Suorsa, M. and Aro, E. M. (2008) Auxiliary proteins involved in the assembly and sustenance of photosystem II. *Photosynth Res.,* **98**(1-3), 489-501.

Munekage, Y., Hojo, M., Meurer, J., Endo, T., Tasaka, M. and Shikanai, T. (2002) PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in arabidopsis. *Cell,* **110**(3), 361-371.

Munekage, Y., Hashimoto, M., Miyake, C., Tomizawa, K., Endo, T., Tasaka, M. and Shikanai, T. (2004) Cyclic electron flow around photosystem I is essential for photosynthesis. *Nature,* **429**(6991), 579-582.

Munshi, K.M., Kobayashi, Y. and Shikanai, T. (2005) Identification of a novel protein, CRR7, required for the stabilization of the chloroplast NAD (P) H dehydrogenase complex in arabidopsis. *The Plant Journal,* **44**(6), 1036-1044.

Munshi, M.K., Kobayashi, Y. and Shikanai, T. (2006) Chlororespiratory reduction 6 is a novel factor required for accumulation of the chloroplast NAD(P)H dehydrogenase complex in arabidopsis. *Plant Physiol.,* **141**(2), 737-744.

Muraoka, R., Okuda, K., Kobayashi, Y. and Shikanai, T. (2006) A eukaryotic factor required for accumulation of the chloroplast NAD(P) H dehydrogenase complex in arabidopsis. *Plant Physiol.,* **142**(4), 1683-1689.

Müller, P., Li, X. P. and Niyogi, K. K. (2001) Non-photochemical quenching. A response to excess light energy. *Plant Physiol.,* **125**(4), 1558-1566.

Nanba, O. and Satoh, K. (1987) Isolation of a photosystem II reaction center consisting of D-1 and D-2 polypeptides and cytochrome b-559. *Proc. Natl. Acad. Sci. U. S. A.,* **84**(1), 109-112.

Naver, H., Haldrup, A. and Scheller, H. V. (1999) Cosuppression of photosystem I subunit PSI-H in arabidopsis thaliana. efficient electron transfer and

stability of photosystem I is dependent upon the PSI-H subunit. *J. Biol. Chem.,* **274**(16), 10784-10789.

Nelson, N. and Yocum, C. F. (2006) Structure and function of photosystems I and II. *Annu. Rev. Plant. Biol.,* **57**, 521-565.

Nield, J. and Barber, J. (2006) Refinement of the structural model for the photosystem II supercomplex of higher plants. *Biochim. Biophys. Acta,* **1757**(5-6), 353-361.

Nilsson, R., Brunner, J., Hoffman, N. E. and van Wijk, K. J. (1999) Interactions of ribosome nascent chain complexes of the chloroplast-encoded D1 thylakoid membrane protein with cpSRP54. *EMBO J.,* **18**(3), 733-742.

Nixon, P.J. (2000) Chlororespiration. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.,* **355**(1402), 1541-1547.

Nixon, P.J., Barker, M., Boehm, M., de Vries, R. and Komenda, J. (2005) FtsH-mediated repair of the photosystem II complex in response to light stress. *J. Exp. Bot.,* **56**(411), 357-363.

Niyogi, K.K. (1999) Photoprotection revisited: Genetic and molecular approaches. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* **50**, 333-359.

Niyogi, K.K., Li, X. P., Rosenberg, V. and Jung, H. S. (2005) Is PsbS the site of non-photochemical quenching in photosynthesis? *J. Exp. Bot.,* **56**(411), 375-382.

Nowaczyk, M.M., Hebeler, R., Schlodder, E., Meyer, H. E., Warscheid, B. and Rogner, M. (2006) Psb27, a cyanobacterial lipoprotein, is involved in the repair cycle of photosystem II. *Plant Cell,* **18**(11), 3121-3131.

O'Farrell, P.H. (1975) High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.,* **250**(10), 4007-4021.

Ogawa, T. (1992) Identification and characterization of the ictA/ndhL gene product essential to inorganic carbon transport of synechocystis PCC6803. *Plant Physiol.,* **99**(4), 1604-1608.

Ohnishi, N., Allakhverdiev, S. I., Takahashi, S., Higashi, S., Watanabe, M., Nishiyama, Y. and Murata, N. (2005) Two-step mechanism of photodamage to photosystem II: Step 1 occurs at the oxygen-evolving complex and step 2 occurs at the photochemical reaction center. *Biochemistry,* **44**(23), 8494-8499.

Okuda, K., Myouga, F., Motohashi, R., Shinozaki, K. and Shikanai, T. (2007) Conserved domain structure of pentatricopeptide repeat proteins involved in chloroplast RNA editing. *Proc. Natl. Acad. Sci. U. S. A.,* **104**(19), 8178-8183.

Okuda, K., Chateigner-Boutin, A. L., Nakamura, T., Delannoy, E., Sugita, M., Myouga, F., Motohashi, R., Shinozaki, K., Small, I. and Shikanai, T. (2009) Pentatricopeptide repeat proteins with the DYW motif have distinct molecular functions in RNA editing and RNA cleavage in arabidopsis chloroplasts. *Plant Cell,* **21**(1), 146-156.

Okutani, S., Hanke, G. T., Satomi, Y., Takao, T., Kurisu, G., Suzuki, A. and Hase, T. (2005) Three maize leaf ferredoxin:NADPH oxidoreductases vary in subchloroplast location, expression, and interaction with ferredoxin. *Plant Physiol.,* **139**(3), 1451-1459.

Ossenbuhl, F., Inaba-Sulpice, M., Meurer, J., Soll, J. and Eichacker, L. A. (2006) The synechocystis sp PCC 6803 oxa1 homolog is essential for membrane integration of reaction center precursor protein pD1. *Plant Cell,* **18**(9), 2236-2246.

Peltier, J.B., Emanuelsson, O., Kalume, D. E., Ytterberg, J., Friso, G., Rudella, A., Liberles, D. A., Soderberg, L., Roepstorff, P., von Heijne, G. and van Wijk, K. J. (2002) Central functions of the lumenal and peripheral thylakoid proteome of arabidopsis determined by experimentation and genome-wide prediction. *Plant Cell,* **14**(1), 211-236.

Peltier, G. and Cournac, L. (2002) Chlororespiration. *Annu. Rev. Plant. Biol.,* **53**, 523-550.

Peng, L., Ma, J., Chi, W., Guo, J., Zhu, S., Lu, Q., Lu, C. and Zhang, L. (2006) LOW PSII ACCUMULATION1 is involved in efficient assembly of photosystem II in arabidopsis thaliana. *Plant Cell,* **18**(4), 955-969.

Peng, L., Shimizu, H. and Shikanai, T. (2008) The chloroplast NAD(P)H dehydrogenase complex interacts with photosystem I in arabidopsis. *J. Biol. Chem.,* **283**(50), 34873-34879.

Piippo, M., Allahverdiyeva, Y., Paakkarinen, V., Suoranta, U. M., Battchikova, N. and Aro, E. M. (2006) Chloroplast-mediated regulation of nuclear genes in arabidopsis thaliana in the absence of light stress. *Physiol. Genomics,* **25**(1), 142-152.

Plücken, H., Müller, B., Grohmann, D., Westhoff, P. and Eichacker, L. A. (2002) The HCF136 protein is essential for assembly of the photosystem II reaction center in arabidopsis thaliana. *FEBS Lett.,* **532**(1-2), 85-90.

Quiles, M.J. and Cuello, J. (1998) Association of ferredoxin-NADP oxidoreductase with the chloroplastic pyridine nucleotide dehydrogenase complex in barley leaves. *Plant Physiol.,* **117**(1), 235-244.

Quiles, M.J., Garcia, A. and Cuello, J. (2000) Separation by blue-native PAGE and identification of the whole NAD (P) H dehydrogenase complex from barley stroma thylakoids. *Plant Physiology and Biochemistry,* **38**(3), 225-232.

Quiles, M.J. (2006) Stimulation of chlororespiration by heat and high light intensity in oat plants. *Plant. Cell. Environ.,* **29**(8), 1463-1470.

Race, H.L., Herrmann, R. G. and Martin, W. (1999) Why have organelles retained genomes? *Trends Genet.,* **15**(9), 364-370.

Raven, J.A., Cockell, C. S. and De La Rocha, C. L. (2008) The evolution of inorganic carbon concentrating mechanisms in photosynthesis. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.,* **363**(1504), 2641-2650.

Reinhold, T., Alawady, A., Grimm, B., Beran, K. C., Jahns, P., Conrath, U., Bauer, J., Reiser, J., Melzer, M., Jeblick, W. and Neuhaus, H. E. (2007) Limitation of nocturnal import of ATP into arabidopsis chloroplasts leads to photooxidative damage. *Plant J.,* **50**(2), 293-304.

Ren, D., Yang, H. and Zhang, S. (2002) Cell death mediated by MAPK is associated with hydrogen peroxide production in arabidopsis. *J. Biol. Chem.,* **277**(1), 559-565.

Rintamäki, E., Kettunen, R. and Aro, E. M. (1996) Differential D1 dephosphorylation in functional and photodamaged photosystem II centers. dephosphorylation is a prerequisite for degradation of damaged D1. *J. Biol. Chem.,* **271**(25), 14870-14875.

Rintamäki, E., Martinsuo, P., Pursiheimo, S. and Aro, E. M. (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**(21), 11644-11649.

Ripley, B.S., Pammenter, N. W. and Smith, V. R. (1999) Function of leaf hairs revisited: The hair layer on leaves arctotheca populifolia reduces photoinhibition, but leads to higher leaf temperatures caused by lower transpiration rates. *J. Plant Physiol.,* **155**(1), 78-85.

Robinson, S.A., Lovelock, C. E. and Osmund, C. B. (1993) Wax as a mechanism for protection against photoinhibition-A study of cotyledon orbiculata. *Botanica Acta,* **106**, 307-307.

Rochaix, J.D. (2007) Role of thylakoid protein kinases in photosynthetic acclimation. *FEBS Lett.,* **581**(15), 2768-2775.

Rokka, A., Aro, E. M., Herrmann, R. G., Andersson, B. and Vener, A. V. (2000) Dephosphorylation of photosystem II reaction center proteins in plant photosynthetic membranes as an immediate response to abrupt elevation of temperature. *Plant Physiol.,* **123**(4), 1525-1536.

Rokka, A., Suorsa, M., Saleem, A., Battchikova, N. and Aro, E. M. (2005) Synthesis and assembly of thylakoid protein complexes: Multiple assembly steps of photosystem II. *Biochem. J.,* **388**(Pt 1), 159-168.

Romano, P., Edvardsson, A., Ruban, A. V., Andersson, B., Vener, A. V., Gray, J. E. and Horton, P. (2004) Arabidopsis AtCYP20-2 is a light-regulated cyclophilin-type peptidyl-prolyl cis-trans isomerase associated with the photosynthetic membranes. *Plant Physiol.,* **134**(4), 1244-1247.

Romano, P., Gray, J., Horton, P. and Luan, S. (2005) Plant immunophilins: Functional versatility beyond protein maturation. *New Phytol.,* **166**(3), 753-769.

Roose, J.L. and Pakrasi, H. B. (2004) Evidence that D1 processing is required for manganese binding and extrinsic protein assembly into photosystem II. *J. Biol. Chem.,* **279**(44), 45417-45422.

Roose, J.L., Wegener, K. M. and Pakrasi, H. B. (2007) The extrinsic proteins of photosystem II. *Photosynth Res.,* **92**(3), 369-387.

Roose, J.L. and Pakrasi, H. B. (2008) The Psb27 protein facilitates manganese cluster assembly in photosystem II. *J. Biol. Chem.,* **283**(7), 4044-4050.

Rosso, M.G., Li, Y., Strizhov, N., Reiss, B., Dekker, K. and Weisshaar, B. (2003) An arabidopsis thaliana T-DNA mutagenized population (GABI-kat) for flanking sequence tag-based reverse genetics. *Plant Mol. Biol.,* **53**(1-2), 247-259.

Rumeau, D., Becuwe-Linka, N., Beyly, A., Louwagie, M., Garin, J. and Peltier, G. (2005) New subunits NDH-M, -N, and -O, encoded by nuclear genes, are essential for plastid ndh complex functioning in higher plants. *Plant Cell,* **17**(1), 219-232.

Rumeau, D., Peltier, G. and Cournac, L. (2007) Chlororespiration and cyclic electron flow around PSI during photosynthesis and plant stress response. *Plant. Cell. Environ.,* **30**(9), 1041-1051.

Sato, S., Nakamura, Y., Kaneko, T., Asamizu, E. and Tabata, S. (1999) Complete structure of the chloroplast genome of arabidopsis thaliana. *DNA Res.,* **6**(5), 283-290.

Sazanov, L.A., Burrows, P. A. and Nixon, P. J. (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**(3), 1319-1324.

Schägger, H. and von Jagow, G. (1991) Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. *Anal. Biochem.,* **199**(2), 223-231.

Schreiber, U., Klughammer, C. and Neubauer, C. (1988) Measuring P700 absorbance changes around 830 nm with a new type of pulse modulation system. *Z Naturforsch 43c,* , 686–698.

Schubert, M., Petersson, U. A., Haas, B. J., Funk, C., Schroder, W. P. and Kieselbach, T. (2002) Proteome map of the chloroplast lumen of arabidopsis thaliana. *J. Biol. Chem.,* **277**(10), 8354-8365.

Shapiguzov, A., Edvardsson, A. and Vener, A. V. (2006) Profound redox sensitivity of peptidyl-prolyl isomerase activity in arabidopsis thylakoid lumen. *FEBS Lett.,* **580**(15), 3671-3676.

Shi, L.X. and Schroder, W. P. (2004) The low molecular mass subunits of the photosynthetic supracomplex, photosystem II. *Biochim. Biophys. Acta,* **1608**(2-3), 75-96.

Shikanai, T., Endo, T., Hashimoto, T., Yamada, Y., Asada, K. and 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**(16), 9705-9709.

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

Shimizu, H. and Shikanai, T. (2007) Dihydrodipicolinate reductase-like protein, CRR1, is essential for chloroplast NAD(P)H dehydrogenase in arabidopsis. *Plant J.,* **52**(3), 539-547.

Shimizu, H., Peng, L., Myouga, F., Motohashi, R., Shinozaki, K. and Shikanai, T. (2008) CRR23/NdhL is a subunit of the chloroplast NAD(P)H dehydrogenase complex in arabidopsis. *Plant Cell Physiol.,* **49**(5), 835-842.

Spence, E., Bailey, S., Nenninger, A., Moller, S. G. and Robinson, C. (2004) A homolog of Albino3/OxaI is essential for thylakoid biogenesis in the cyanobacterium synechocystis sp. PCC6803. *J. Biol. Chem.,* **279**(53), 55792-55800.

Stockhaus, J., Hofer, M., Renger, G., Westhoff, P., Wydrzynski, T. and Willmitzer, L. (1990) Anti-sense RNA efficiently inhibits formation of the 10 kd polypeptide of photosystem II in transgenic potato plants: Analysis of the role of the 10 kd protein. *EMBO J.,* **9**(9), 3013-3021.

Stroebel, D., Choquet, Y., Popot, J. L. and Picot, D. (2003) An atypical haem in the cytochrome b(6)f complex. *Nature,* **426**(6965), 413-418.

Summerfield, T.C., Winter, R. T. and Eaton-Rye, J. J. (2005) Investigation of a requirement for the PsbP-like protein in synechocystis sp. PCC 6803. *Photosynth Res.,* **84**(1-3), 263-268.

Sundberg, E., Slagter, J. G., Fridborg, I., Cleary, S. P., Robinson, C. and Coupland, G. (1997) ALBINO3, an arabidopsis nuclear gene essential for chloroplast differentiation, encodes a chloroplast protein that shows homology to proteins present in bacterial membranes and yeast mitochondria. *Plant Cell,* **9**(5), 717-730.

Sveshnikov, D., Funk, C. and Schröder, W. P. (2007) The PsbP-like protein (sll1418) of synechocystis sp. PCC 6803 stabilises the donor side of photosystem II. *Photosynth Res.,* **93**(1-3), 101-109.

Tagawa, K., Tsujimoto, H. Y. and Arnon, D. I. (1963) Role of chloroplast ferredoxin in the energy conversion process of photosynthesis. *Proc. Natl. Acad. Sci. U. S. A.,* **49**, 567-572.

Takabayashi, A., Ishikawa, N., Obayashi, T., Ishida, S., Obokata, J., Endo, T. and Sato, F. (2009) Three novel subunits of arabidopsis chloroplastic NAD(P)H dehydrogenase identified by bioinformatic and reverse genetic approaches. *Plant J.,* **57**(2), 207-219.

Thornton, L.E., Ohkawa, H., Roose, J. L., Kashino, Y., Keren, N. and Pakrasi, H. B. (2004) Homologs of plant PsbP and PsbQ proteins are necessary for regulation of photosystem ii activity in the cyanobacterium synechocystis 6803. *Plant Cell,* **16**(8), 2164-2175.

Tikkanen, M., Piippo, M., Suorsa, M., Sirpiö, S., Mulo, P., Vainonen, J., Vener, A. V., Allahverdiyeva, Y. and Aro, E. M. (2006) State transitions revisited-a buffering system for dynamic low light acclimation of arabidopsis. *Plant Mol. Biol.,* **62**(4-5), 779-793.

Tikkanen, M., Nurmi, M., Kangasjärvi, S. and Aro, E. M. (2008) Core protein phosphorylation facilitates the repair of photodamaged photosystem II at high light. *Biochim. Biophys. Acta,* **1777**(11), 1432-1437.

Turmel, M., Otis, C. and Lemieux, C. (1999) The complete chloroplast DNA sequence of the green alga nephroselmis olivacea: Insights into the architecture of ancestral chloroplast genomes. *Proc. Natl. Acad. Sci. U. S. A.,* **96**(18), 10248-10253.

Tyystjärvi, E. and Aro, E. M. (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**(5), 2213-2218.

Vallon, O., Bulte, L., Dainese, P., Olive, J., Bassi, R. and Wollman, F. A. (1991) Lateral redistribution of cytochrome b6/f complexes along thylakoid membranes upon state transitions. *Proc. Natl. Acad. Sci. U. S. A.,* **88**(18), 8262-8266.

van Wijk, K.J., Andersson, B. and Aro, E. M. (1996) Kinetic resolution of the incorporation of the D1 protein into photosystem II and localization of assembly intermediates in thylakoid membranes of spinach chloroplasts. *J. Biol. Chem.,* **271**(16), 9627-9636.

Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, E. and Andersson, B. (1992) Reversible and irreversible intermediates during photoinhibition of photosystem II: Stable reduced QA species promote chlorophyll triplet formation. *Proc. Natl. Acad. Sci. U. S. A.,* **89**(4), 1408-1412.

Vass, I., Kirilovsky, D. and Etienne, A. L. (1999) UV-B radiation-induced donor- and acceptor-side modifications of photosystem II in the cyanobacterium synechocystis sp. PCC 6803. *Biochemistry,* **38**(39), 12786-12794.

Vener, A.V., Rokka, A., Fulgosi, H., Andersson, B. and Herrmann, R. G. (1999) A cyclophilin-regulated PP2A-like protein phosphatase in thylakoid membranes of plant chloroplasts. *Biochemistry,* **38**(45), 14955-14965.

Vener, A.V. (2007) Environmentally modulated phosphorylation and dynamics of proteins in photosynthetic membranes. *Biochim. Biophys. Acta,* **1767**(6), 449-457.

von Jagow, G. and Link, T. A. (1986) Use of specific inhibitors on the mitochondrial bc1 complex. *Methods Enzymol.,* **126**, 253-271.

Wada, M., Kagawa, T. and Sato, Y. (2003) Chloroplast movement. *Annual Reviews in Plant Biology,* **54**(1), 455-468.

Wang, D. and Portis Jr, A. R. (2007) A novel nucleus-encoded chloroplast protein, PIFI, is involved in NAD (P) H dehydrogenase complex-mediated chlororespiratory electron transport in arabidopsis. *Plant Physiol.,* **144**(4), 1742.

Wang, P., Duan, W., Takabayashi, A., Endo, T., Shikanai, T., Ye, J. Y. and Mi, H. (2006) Chloroplastic NAD(P)H dehydrogenase in tobacco leaves functions in alleviation of oxidative damage caused by temperature stress. *Plant Physiol.,* **141**(2), 465-474.

Zaltsman, A., Ori, N. and Adam, Z. (2005) Two types of FtsH protease subunits are required for chloroplast biogenesis and photosystem II repair in arabidopsis. *Plant Cell,* **17**(10), 2782-2790.

Zhang, H., Whitelegge, J. P. and Cramer, W. A. (2001) Ferredoxin:NADP+ oxidoreductase is a subunit of the chloroplast cytochrome b6f complex. *J. Biol. Chem.,* **276**(41), 38159-38165.

Zhang, L., Paakkarinen, V., van Wijk, K. J. and Aro, E. M. (1999) Co-translational assembly of the D1 protein into photosystem II. *J. Biol. Chem.,* **274**(23), 16062-16067.

Zhang, L., Paakkarinen, V., van Wijk, K. J. and Aro, E. M. (2000) Biogenesis of the chloroplast-encoded D1 protein: Regulation of translation elongation, insertion, and assembly into photosystem II. *Plant Cell,* **12**(9), 1769-1782.

Zhang, L., Paakkarinen, V., Suorsa, M. and Aro, E. M. (2001) A SecY homologue is involved in chloroplast-encoded D1 protein biogenesis. *J. Biol. Chem.,* **276**(41), 37809-37814.

Zhang, P., Battchikova, N., Jansen, T., Appel, J., Ogawa, T. and Aro, E. M. (2004) Expression and functional roles of the two distinct NDH-1 complexes and the carbon acquisition complex NdhD3/NdhF3/CupA/Sll1735 in synechocystis sp PCC 6803. *Plant Cell,* **16**(12), 3326-3340.

Zouni, A., Witt, H. T., Kern, J., Fromme, P., Krauss, N., Saenger, W. and Orth, P. (2001) Crystal structure of photosystem II from synechococcus elongatus at 3.8 A resolution. *Nature,* **409**(6821), 739-743.