Assembly of protein complexes in plastids

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Abstract

Photosynthetic multiprotein complexes in plants and cyanobacteria are mainly responsible for the function of the oxygenic photosynthesis. Great progress has recently been made in resolving the structures of these complexes, most of which are now known at 2 to 4Å resolution. Compared to these achievements, amazingly little is known about the biogenesis, maintenance, and stability of these macromolecular photosynthetic complexes. So far, the sequential assembly of the Photosystem II subunits is best characterized, yet the ligation of redox co-factors and other pigments still remain only poorly understood. There seems to be a general CES control of translation of the key chloroplast-encoded subunits of all thylakoid protein complexes thus ensuring a coordinated synthesis and assembly of the chloroplast- and nucleus-encoded subunits. Some light has also recently been shed on the function and abundance of the auxiliary proteins necessary for the translocation of the nucleus-encoded proteins into chloroplasts and for facilitating the assembly processes of the macromolecular photosynthetic protein complexes.

1 Introduction

Photosynthetic membrane protein complexes comprise the marvelous machinery that provides energy for all living forms on Earth. During the past few years the knowledge on the 3D structure of the photosynthetic protein complexes has advanced tremendously. Photosystem II (PSII) and Photosystem I (PSI) structures have been resolved to nearly atomic resolution, and in addition to the protein subunits, the coordinates for different ligands and co-factors are largely known (for PSII, see Zouni et al. 2001; Ferreira et al. 2004; Loll et al. 2005; for PSI see Ben-Shem et al. 2003). Similarly, the structure of the cytochrome b_6f (Cyt b_6f) complex was resolved both from the green alga *Chlamydomonas reinhardtii* (Stroebel et al. 2003) and cyanobacteria (Kurisu et al. 2003) as well as the structure of the ATP synthase (Seelert et al. 2000). It is a big challenge now to clarify the mechanisms, how these multisubunit complexes are properly assembled in the thylakoid membrane. Most of the structural subunits of these multiprotein complexes have been identified by now, yet it is not exceptional that new subunits are still recognized (e.g. Khrouchtchova et al. 2005). However, the assembly mechanisms of the subunits into the multiprotein complexes are far from being resolved, and we are only in the very beginning of understanding of the number and the functions of the

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auxiliary proteins that finally guarantee the proper synthesis, assembly, and stability of the protein subunits. Furthermore, the mechanisms of the ligation of different co-factors to the multiprotein complexes still largely remain to be elucidated.

In general, the synthesis and assembly of the main photosynthetic multiprotein complexes in plastids require concerted interactions between the nucleo-cytosolic and plastid genetic systems. These interactions occur mostly at the translational and posttranslational levels and are controlled by the nuclear-encoded regulatory factors (Rochaix 1996; Wollman et al. 1999). A common feature in the assembly of the photosynthetic multiprotein complexes in chloroplasts seems to be an assembly-dependent autoregulation of translation of the central chloroplast-encoded subunits that makes the core of all thylakoid protein complexes. This phenomenon has been defined as a CES process - Control by Epistasy of Synthesis (Wollman et al. 1999; Choquet and Vallon 2000; Wostrikoff et al. 2004; Minai et al. 2006). In the CES process, the translation of a CES protein is dependent on the presence of a specific dominant protein (or protein sub-complex) thus representing a protein-assembly-mediated autoregulation of translation. Furthermore, a strict quality control is constantly operating in chloroplasts to guarantee a proper stoichiometry of the protein subunits for efficient assembly of the complexes (Yamamoto 2001).

Besides structural subunits, the photosynthetic membrane protein complexes PSI, PSII, and the Cyt b_6f complex contain pigments and co-factors that need to be ligated to the proteins during or after the assembly of the complexes, or even concomitantly with the translation process. Assembly of the protein subunits occurs sequentially and may require the interaction with several soluble or membrane-bound chaperones, or assembly factors.

The distinct assembly steps of the multiprotein complexes in plastids are not properly known yet. PSII and the Cyt b_6f complex are by far the protein complexes whose biogenesis and assembly have been studied in greatest detail, particularly of PSII, since it is the major target for the photo-destructive processes. Experimental evidence exists on several individual assembly steps of PSII with distinct sub-complexes, the processes, which will be reviewed in the following. In addition, the assembly of PSI and Cyt b_6f are shortly reviewed, as well as the assembly of Rubisco and ferredoxin:thioredoxin reductase (FTR) as examples of the soluble plastid complexes. Focus will be put especially on the assembly of the protein subunits, since our knowledge on the mechanisms of co-factor ligation into the complexes still remains poor. Furthermore, a short survey is presented on post-translational modifications of plastid proteins that affect the turnover and assembly/disassembly of the protein subunits in the thylakoid membrane complexes.

2 Assembly of the protein complexes

2.1 Assembly of PSII

The PSII complex contains 29 different subunits, from which 15 are plastid-encoded (PsbA-PsbF, PsbH-PsbN, PsbTc, and PsbZ), the rest of them being nu-

cleus-encoded (for a review see van Wijk 2001; Rochaix 2006). The nucleus-encoded PSII proteins are synthesized on cytoplasmic ribosomes as precursor proteins, which contain an N-terminal transit peptide for plastid targeting and for determining the destination of the protein inside the chloroplast. The nucleus-encoded PSII proteins include proteins, such as PsbR, PsbW, and PsbY, three oxygen- evolving complex (OEC) proteins (PsbO, PsbP, and PsbQ), six Lhcb proteins (Lhcb1-6), and PsbS, which also belongs to the Lhcb family of proteins. The plastid-encoded PSII proteins are mainly integral membrane proteins, which are synthesized on thylakoid-bound ribosomes. Of these proteins particularly the reaction center protein D1 has been shown to be co-translationally inserted into the thylakoid membrane. Likewise, the assembly of the D1 protein to PSII during the repair process of photodamaged PSII centers was shown to occur co-translationally (Klein et al. 1988; Keegstra and Cline 1999; Zhang et al. 1999).

From the methodological point of view, the characterization of gene interruption or knockout mutants and the studies on plastid development from etioplast to chloroplast have been used to get insights into the assembly order of the protein subunits to PSII. Furthermore, isolated, intact chloroplasts have been subjected to the approaches, such as pulse and chase experiments followed by subfractionation of the various PSII subassemblies by sucrose density centrifugation (van Wijk et al. 1995; Müller and Eichacker 1999; Zhang et al. 1999). However, these latter experiments could only reveal the assembly of the major chloroplast-encoded PSII proteins D1, D2, CP43, and CP47, but failed to reveal the synthesis and assembly of the low-molecular-mass (LMM) subunits and the nucleus-encoded subunits.

A more thorough insight into the assembly steps of PSII proteins was received recently using different chromatographic methods and the two-dimensional blue native (BN)/SDS-PAGE system for separation of both the *in vitro* and *in vivo* labeled and assembled thylakoid proteins and protein complexes (e.g. Rokka et al. 2005; Nowaczyk et al. 2006). For resolving the mechanisms of assembly-dependent autoregulation of translation, an approach of chimeric gene constructs and their expression under the control of 5´UTRs of the genes of interest have been employed (Minai et al. 2006). Moreover, research on the light-induced turnover of the PSII complex has provided information that has also been applied for the research on the assembly process of a new PSII center.

2.1.1. Assembly of the PSII core monomers and dimers

The prerequisite for PSII assembly is the presence of α- and β- subunits of cytochrome b_{559} (Cyt b_{559}), which accumulate in the thylakoid membrane even in the absence of other PSII subunits (Morais et al. 1998; Müller and Eichacker 1999). Cyt b_{559} interacts with the D2 protein to form an initial complex that further serves as a receptor for the co-translational assembly of the D1 protein (Komenda et al. 2004) (Fig. 1). Indeed, evidence was recently provided indicating that the translation of D1 (a CES subunit) is strongly decreased in the absence of D2 (Minai et al. 2006). Also light is required for an efficient translation elongation and accumulation of the D1 protein, most probably due to the requirement of light for the synthesis of the pigment and other co-factor molecules that are ligated to the PSII

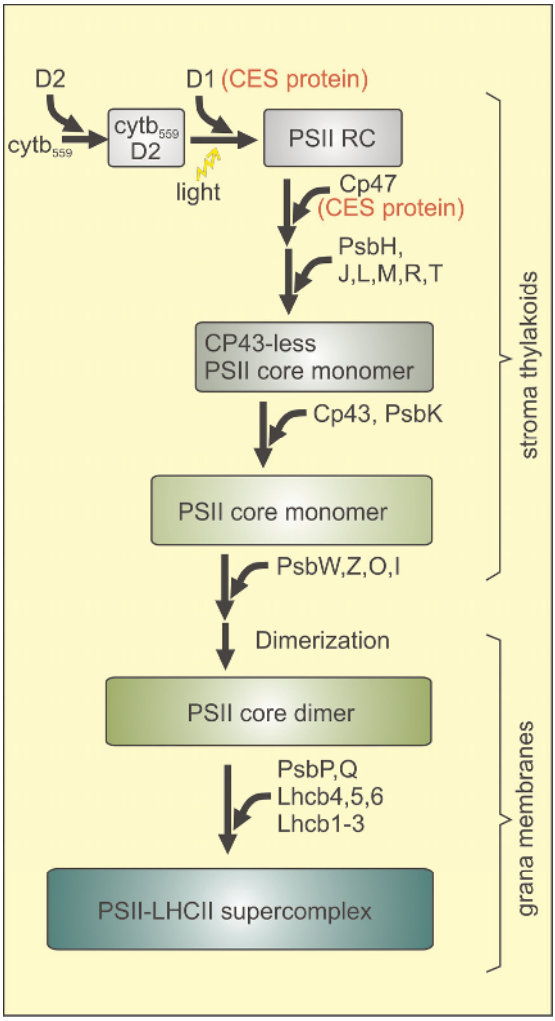

Fig. 1. Hypothetical scheme of the sequential protein assembly during biogenesis of the PSII complexes. Note that a hierarchical CES control is functioning for the translation of the D1 and CP47 proteins. So far, no data has been published on the assembly order of the PsbN and PsbY proteins, or PsbS.

complex concomitantly with the assembly process (Kim et al.1991; van Wijk and Eichacker 1996; Edhofer et al. 1998). Rapidly after the termination of translation, the D1 protein undergoes C-terminal processing (Diner et al. 1988; Bowyer et al. 1992) by the carboxyl-terminal processing protease CtpA that functions exclusively in the processing of the D1 protein in the thylakoid lumen (Anbudurai et al. 1994; Oelmüller et al. 1996).

The assembly of the D1 protein to the Cyt b_{559}/D2 subcomplex is followed by the association of the CP47 protein (Sharma et al. 1997; Müller and Eichacker 1999; Tsiotis et al. 1999; Zhang et al. 1999; Szabò et al. 2001; Rokka et al. 2005). In fact, the presence of D1 is a prerequisite for the high-level translation of the core antenna subunit CP47. The biogenesis of PSII thus involves a CES cascade where translation of D1 is dependent on the presence of D2 and the translation of CP47, in turn, is dependent on the presence of D1 (Minai et al. 2006). After the assembly of CP47, the LMM subunits PsbH, PsbL, PsbM, PsbTc, PsbR, and also PsbJ associate with the growing PSII subcomplex (Suorsa et al. 2004; Rokka et al. 2005). These LMM subunits are thought to stabilize the D1/D2/Cyt b_{559}/CP47 subassembly of PSII. The subunits PsbL, PsbM, and PsbT are located in the monomer-monomer interphase (Loll et al. 2005) and therefore are also crucial for the dimerization of PSII, whereas PsbR and PsbJ have been shown to be essential for the stable assembly of the OEC (see below). In cyanobacteria, the PsbH protein was found to be associated with CP47 and to be important for the prompt incorporation of the newly-synthesized D1 protein to the PSII complex (Komenda et al. 2005).

The subsequent assembly steps involve the association of the core antenna protein CP43 and the LMM subunit PsbK (Suorsa et al. 2004; Rokka et al. 2005) (Fig. 1). PsbK is tightly bound to CP43, and the assembly of PsbK was shown to occur only in the presence of CP43 (Sugimoto and Takahashi 2003). Furthermore, PsbK was postulated to be required for the PSII core dimerization (Zheleva et al. 1998). Due to the location of PsbK in the periphery of the dimer complex (Loll et al. 2005), this interpretation, however, needs further examination. The PsbI subunit, which was earlier reported to be a component of the PSII reaction center complex, was recently assigned to have a role in the PSII dimerization (Schwenkert et al. 2006). It is natural that the LMM subunits in the monomer/monomer interphase are crucial for dimerization. Such a role was recently experimentally proven for PsbL, which was likewise shown to be required for the stable association of CP43 (Suorsa et al. 2004).

The PsbZ protein, as well as the nuclear-encoded PsbW, are probably the last subunits that assemble to the PSII core and thereby facilitate the assembly of the minor LHCII proteins (Swiatek et al. 2001), which, in turn, are required for binding of the trimers of the light-harvesting antenna complex (LHCII) to the PSII core dimer (Shi et al. 2000; Rokka et al. 2005). Furthermore, chlorophyll (Chl) *a* synthesis enhances the accumulation and stability of monomers and, subsequently, the dimerization of the PSII core monomers (Müller and Eichacker 1999).

Light and Chl biosynthesis are essential for the synthesis and stability of the core antenna proteins CP43 and CP47 (van Wijk and Eichacker 1996). In addition to light and the availability of chlorophyll and various assembly partners, the regulation of synthesis and assembly of the PSII complex involves the presence of a variety of other factors, such as pheophytin, β-carotene, Fe, Mn, and plastoquinone. However, pigments are not always needed for the core protein accumulation; for example, in etioplasts, isolated from dark-grown barley seedlings, a PSII pre-complex has been found to exist, consisting of Cyt b_{559}, the D2 protein and the precursor form of the D1 protein (Müller and Eichacker 1999).

Only a few assembly factors important for the biogenesis of the PSII core complex have been identified so far (Table 1). A molecular chaperone HCF136 in the thylakoid lumen was first characterized with a selective role in the assembly of only the PSII reaction center complex (Meurer et al. 1998; Plucken et al. 2002) (Table 1), yet the specific molecular interactions in assisting the assembly process still remain unknown. More recently, an LPA1 (low PSII accumulation1) protein was identified in *Arabidopsis* and shown to be an integral membrane chaperone essential for the maintenance and assembly of the PSII core complex, probably through a direct interaction with the PSII reaction center protein D1 (Peng et al. 2006). Yet another membrane-localized protein HCF107, a component of a multisubunit complex, has been shown to be crucial for the assembly of PSII, affecting particularly the expression of the *psbH* gene in *Arabidopsis* (Sane et al. 2005).

HCF107, HCF136, and LPA1 probably represent only the first examples of the growing number of assembly factors, or molecular chaperones, facilitating the biogenesis of the PSII core complexes. Additional assembly factors are likely to be discovered in the near future by advanced proteomic and reverse genetics approaches. For example, some member(s) of the Alb3 family are likely to have such functions (see Section 3.2.)

2.1.2 Assembly of the proteins of the oxygen evolving complex

The OEC complex of higher plant PSII contains three extrinsic, nucleus-encoded subunits, PsbO (OEC33), PsbP (OEC23), and PsbQ (OEC16). The OEC complex is attached to the lumenal side of PSII and protects the $CaMn_4$ cluster bound to the D1 and CP43 proteins (Ferreira et al. 2004). Contrary to the membrane-embedded PSII core polypeptides that are subject to rapid proteolytic degradation when not assembled, a pool of free, assembly-competent OEC proteins has been shown to exist in the thylakoid lumen (Hashimoto et al. 1996, 1997).

One clear requirement for the assembly of OEC to the luminal side of PSII is the C-terminal processing of the precursor D1 protein. It has been demonstrated that the CP43 protein is stably assembled only if the D1 protein has undergone maturation via C-terminal processing (Zhang et al. 2000). Coordination of the C-terminal processing and the assembly of CP43 may thus be essential for stable ligation of the $CaMn_4$-cluster to the PSII core (Roose and Pakrasi 2004) and for the subsequent photoactivation of the oxygen evolving complex.

Assembly of the PsbO protein of OEC to the PSII core complex occurs in the stroma-exposed thylakoid membranes whereas the PsbP and PsbQ proteins have been found to associate with PSII in the grana thylakoids (Hashimoto et al. 1997). It was believed for a long time that the PsbO protein is the only OEC protein that directly binds to the PSII core on the lumenal side of the thylakoid membrane, and thereby provides a docking site for PsbP, which in turn binds the PsbQ protein (e.g. Miyao and Murata 1989). This model has, however, been recently challenged and evidence is accumulating supporting the concept that either all the three OEC proteins are independently bound to PSII, or only PsbO and PsbP are independently bound to PSII and one, or both, of them provide a docking site for PsbQ.

Table 1. Assembly factors, chaperones and translocator components involved in the assembly of thylakoid protein complexes.

Factor or chaperone (or nuclear locus)	function	reference
Assembly factors, chaperones		
HCF136	PSII assembly	Meurer et al. 1998; Plucken et al. 2002
HCF107	PSII assembly	Sane et al. 2005
LPA1	PSII assembly	Peng et al. 2006
TLP40	PSII assembly	Fulgosi et al. 1998
Alb3.1	PSII assembly	Ossenbühl et al. 2004
Alb3.2	PSII and PSI assembly	Gerdes et al. 2006; Göhre et al. 2006
Alb4	PSII assembly	Gerdes et al. 2006; Göhre et al. 2006
Slr1471p	PSII assembly (cyano)	Ossenbühl et al. 2006
Psb27	PSII/OEC assembly	Chen et al. 2006; Nowaczyk et al. 2006
Psb29	PSII/OEC assembly	Keren et al. 2005
Ycf3, Ycf4	PSI assembly	Boudreau et al. 1997; Naver et al. 2001
Ycf37	PSI assembly (cyano)	Wilde et al. 2001
BtpA	PSI assembly (cyano)	Bartsevich and Pakrasi 1997 Zak and Pakrasi 2000
PYG7	PSI assembly	Stöckel et al. 2006
HCF145	PSI assembly	Lezhneva and Meurer 2004
HCF101	PSI and FTR assembly	Stöckel and Oelmüller 2004
APO1	PSI and FTR assembly	Amann et al. 2004
RubA	PSI assembly (cyano)	Shen et al. 2002a, 2002b
HCF164	Cyt b_6 f assembly	Lennartz et al. 2001
HCF153	Cyt b_6 f assembly	Lennartz et al. 2006
CCDA	Cyt b_6 f assembly	Page et al. 2004
CCSA (Ycf5)	Cyt b_6 assembly	Xie and Merchant 1996; Hamel et al. 2003
CCS1-4	Cyt b_6 f assembly	Inoue et al. 1997; van Wijk 2001
CCB1-4	Cyt b_6 f assembly	Kuras et al. 1997; van Wijk 2001
HSP70	Cyt b_6 f assembly	Madueno et al. 1993
HSP70B	PSII stability and turnover; Rubisco assembly	Schroda et al. 2001; Yokthongwattana et al. 2001; Brutnell et al. 1999
BSD2	Rubisco assembly	Brutnell et al. 1999
Hsp100/ClpC1	PSI, PSII biogenesis	Sjögren et al. 2004
DnaJ	Rubisco assembly	Hartl 1996; Schlicher and Soll 1997
GrpE	Rubisco assembly	Hartl 1996; Schlicher and Soll 1997

Factor or chaperone (or nuclear locus)	function	reference
Cpn60	Rubisco and Cyt b_6 f assembly	Gatenby and Ellis 1990; Madueno et al. 1993
Cpn21	Rubisco assembly	Gatenby and Ellis 1990; Madueno et al. 1993
cpSRP54	Lhcb-protein assembly	Tu et al. 1999; Woolhead et al. 2001
cpSRP43	Lhcb-protein assembly	Tu et al. 1999; Woolhead et al. 2001
Translocator components		
Hcf106	TAT-translocation	Settles et al. 1997; Mori et al. 2001
Tha4	TAT-translocation	Mori et al. 2001
cpTatC	TAT-translocation	Mori et al. 2001
cpSecY; SecE, SecA	Plastocyanin, PsbO	Schuenemann et al. 1999

cyano = cyanobacteria

The PsbO protein attaches to the lumenal loops of the D2 and CP47 core proteins (Nield et al. 2000) and also requires the presence of CP43 for the stable assembly (Suorsa et al. 2004). For PsbP association, it was recently shown using reverse genetics approaches that the presence of the LMM protein PsbJ is an absolute requirement (Hager et al. 2002; Suorsa et al. 2004). This requirement, however, may be only indirect and result from the fact that another PSII protein, PsbR, is also missing from the PsbJ mutant thylakoids (Suorsa et al. 2006). Indeed, the PsbR protein was shown to be important for the structure and function of the OEC complex. It was demonstrated that the absence of PsbR results in a reduction of the PsbP and PsbQ proteins as well as a reduction in the light-saturated rate of oxygen evolution (Suorsa et al. 2006; Allahverdiyeva et al. 2007). These results provide evidence that PsbR is an important component in the PSII core complex, especially for the stable assembly of the PsbP protein. The third OEC protein, PsbQ, was found to be completely missing from a tobacco mutant lacking the PsbP protein (Ifuku et al. 2005) suggesting that the PsbP protein provides a docking site for the PsbQ protein (for further discussion see Suorsa and Aro, 2007). Two other PSII proteins, encoded by a single nuclear gene *psbY* (Gau et al. 1998), are also important for water oxidation (Neufeld et al. 2004) and possibly play a similar role as PsbR by stabilizing the association of the OEC proteins to the PSII core dimer.

Recent proteomic studies have revealed the existence of novel proteins in substoichiometric amounts in various purified PSII preparations (Kashino et al. 2002). Of these proteins, Psb29 was shown important for the assembly of PSII (Keren et al. 2005) but Psb27 was particularly assigned a role in the assembly of the OEC proteins to the PSII core (Roose and Pakrasi 2004). Psb27 protein seems to bind to the PSII core monomer prior to the assembly of the OEC proteins (Nowaczyk et al. 2006). Studies with the Psb27 mutant also revealed an impaired repair of the PSII centers after photoinhibition, providing evidence that the Psb27 protein possibly facilitates the assembly of OEC to the PSII core (Chen et al. 2006).

2.1.3 Assembly of the PSII-LHCII supercomplexes

The functional PSII complexes of higher plants exist as PSII-LHCII (light-harvesting chlorophyll-protein complex II) supercomplexes in the grana appressions. Of the LMM proteins of PSII, particularly the PsbZ (and PsbW) protein has been reported to be essential for the stable assembly of the PSII-LHCII supercomplexes (Swiatek et al. 2001; Rokka et al. 2005). This chloroplast-encoded protein is located in the periphery of the PSII core dimer, in a close vicinity to CP43. Overlay of the X-ray structures of spinach LHCII and the cyanobacterial PSII core onto the projection map of the cryo-EM 3D structure of the isolated PSII-LHCII supercomplexes of spinach revealed a close vicinity of PsbZ to CP26 (Lhcb5) (Loll et al. 2005). It remains to be elucidated whether the nucleus-encoded PsbW protein is located in the similar vicinity to the CP47 and CP29 (Lhcb4) proteins. Upon formation of the PSII-LHCII supercomplex, the CP29 and CP26 proteins attach the LHCII trimers, consisting of the Lhcb1 and Lhcb2 proteins, to the core dimers (Boekema et al. 1999). Furthermore, CP24 (Lhcb6) together with CP29 and CP26 most probably bind additional trimers (composed of Lhcb1-3) in the periphery of the PSII-LHCII supercomplex. The LHCII trimers are bound to the PSII dimer either strongly (S), moderately (M), or loosely (L) (Dekker and Boekema 2005). Recently, it was shown that the CP24 (Lhcb6)-deficient plants displayed a major change in the macro-organization of the PSII-LHCII supercomplexes in the grana (Kovacs et al. 2006). It was concluded that CP24 provides the linker for association of the M-trimer into the PSII complex, thereby allowing a specific macro-organization necessary for optimal function of PSII.

It is intriguing to note that the OEC proteins possibly also have specific roles in the structural integrity of the PSII-LHCII supercomplexes and their macro-organization in the grana (Dekker and Boekema 2005). Electron microscopy and single particle analysis have revealed that the PSII-LHCII supercomplexes lacking the OEC proteins differ from the native PSII supercomplexes (Boekema et al. 2000). It was concluded that the OEC proteins are needed to keep the CP29 and S-LHCII trimers at a correct distance from the PSII core in order to optimize the migration of excitation energy to the PSII core.

The Alb3 protein has been assigned an important role in the membrane insertion and assembly of the Lhcb proteins (see below more about Alb3). So far, however, no specific assembly factors have been detected to be involved in the association of the light-harvesting apparatus to the PSII core dimer, i.e., in the formation of the PSII-LHCII supercomplexes and their macro-organizations in the grana.

2.1.4 Reassembly of the PSII complexes during the photoinhibition repair cycle

The PSII complex performs a unique task in splitting water molecules to oxygen and hydrogen (protons). Such oxidizing electron transfer reactions of PSII in an atmosphere containing oxygen readily result in the formation of highly reactive radicals that are potentially harmful to the proteins and induce imbalance during

the linear electron transfer process. Situation like this may lead to photoinactivation and photodamage of PSII, when the PSII complex is unable to transfer electrons and split water molecules. A constant repair of the photodamaged PSII complexes is required for the maintenance of a sufficient level of active PSII complexes for photosynthesis. The efficiency of repair is dependent on the environmental conditions, stress factors such as high light or low temperature, impairing the efficiency of the repair process. As far as the repair process is in balance with the rate of photodamage, nonfunctional PSII complexes do not accumulate and a measurable decrease in the rate of total photosynthesis is not detected. An extensive literature has been published on the mechanisms of the PSII photoinactivation and damage to the D1 protein (for a review see Melis 1999; Prasil et al. 1992; Aro et al. 1993; Chow and Aro 2005) and therefore these subjects are not considered here in more detail.

At the protein level, most often only the D1 protein is the target for the light-induced damage, but occasionally also the D2 and PsbH proteins become damaged and require replacement during the repair cycle (Schuster et al.1988; Bergantino et al. 2003; Rokka et al. 2005). However, only the repair steps concerning the replacement of the D1 protein are considered here. It is also worth noting that the CES process seems to play no role in the recovery from photoinhibition (Minai et al. 2006).

In the beginning of the repair, the LHCII antenna dissociates from the dimer and monomerization of PSII occurs. Damaged PSII monomers then migrate from the grana to the stroma-exposed membranes, where a contact with the components required in degradation and synthesis of the D1 protein are available. OEC dissociates from PSII and a partial disassembly of the PSII core proteins takes place. The stages from photodamage to degradation of the D1 protein are regulated by phosphorylation-dephosphorylation events of the core proteins (Koivuniemi et al. 1995; Rintamäki et al. 1996) (see below). The D1 protein is degraded proteolytically, proteases from the DegP and FtsH families known to act on the process (for a review see, for example, Adam et al. 2005; Sakamoto 2006). The closest partner of the D1 protein, D2, remains most often intact in the repair process. In the synthesis of the new D1 protein, the nascent D1 protein is co-translationally inserted into the thylakoid membrane where the D2 and Cyt b_{559} act as the first assembly partners. In fact, it was demonstrated that not only the insertion into the membrane but also the assembly of the D1 protein into the PSII complex, composed of Cyt b_{559}, D2 and possibly also of CP47 and several LMM subunits, occur co-translationally during the repair process (Zhang et al. 1999, 2000; Rokka et al. 2005). Re-synthesis of the assembly partner subunits is not needed, since they are already present in the existing PSII centers under repair.

After maturation of the D1 protein, the reassembly of the internal core antenna protein CP43 occurs. CP43, residing next to the D1 protein, is always dissociated from PSII upon the repair process. Before the OEC proteins can re-associate, also most of the LMM subunits have to be assembled to the PSII complex. Finally, the properly assembled, repaired PSII monomer migrates back to the grana thylakoids, where PSII core dimerization and reactivation, with the association of the LHCII antenna proteins, take place. These last assembly steps thus accomplish the PSII

photoinhibition repair cycle providing active PSII-LHCII supercomplexes for photosynthesis.

2.2 Assembly of the PSI complex

The assembly of the PSI complex is rather poorly known due to a difficulty in isolation of various PSI subcomplexes and also to a very slow turnover rate of the PSI complexes, which results in a technical difficulty to accumulate radiolabeled amino acids into newly synthesized PSI subunits. In higher plants, the PSI core complex is composed of 14 subunits (PsaA to PsaL, PsaN, and PsaO), of which PsaA, PsaB, PsaC, PsaI, and PsaJ are plastid-encoded (Jensen et al. 2003; Ben-Shem et al. 2003). A novel subunit of PSI, the previously found phosphoprotein TMP14 (Hansson and Vener 2003) was recently identified in *Arabidopsis* (Khrouchtchova et al. 2005). This protein, designated as PSI-P, was suggested to locate in the proximity of PsaL, PsaH, and PsaO subunits, on the opposite side to the location of the LHCI antenna. Furthermore, the PSI-G subunit has been found to be bound to PsaB and to be in contact with Lhca1 (Zygadlo et al. 2006). The PSI peripheral antenna is arranged around one side of the PSI core and is composed of four different nuclear-encoded Lhca polypeptides (Lhca1-4) in higher plants. In addition, the fifth Lhca protein, which shows a different mode of regulation as compared to the other Lhca proteins, and which is present at substoichiometric amounts under standard conditions, has recently been characterized (Ganeteg et al. 2004).

A key step in the assembly of the PSI complex is the coordinate synthesis and assembly of its two chloroplast-encoded core polypeptides, PsaB and PsaA, that form, together with ca 100 Chl *a* molecules and several redox ligands, the main part of the reaction center complex (for a review see Rochaix 2006). In *Chlamydomonas*, the accumulation of PsaB was shown to be required for synthesis of the PsaA subunit that, in turn, is needed for synthesis of the PsaC subunit (Wostrikoff et al. 2004) on the stromal side of the membrane. All these three subunits, PsaA, PsaB, and PsaC, are required for stable accumulation of the PSI core complex. The rate of production of PsaB is the controlling stage in order to determine the stoichiometric expression of all subunits of the PSI core complex. There is thus a clear CES hierarchy in the sequence of polypeptide assembly during PSI biogenesis (Wostrikoff et al. 2004). Whether the other chloroplast-encoded PSI subunits PsaI and PsaJ are also CES proteins remains unknown. PsaC then coordinates the stable assembly of PsaD and PsaE, both on the stromal side of PSI (Yu et al. 1995).

Some assembly factors have been assigned a role particularly in the biogenesis of the PSI complexes (Table 1). These include the plastid-encoded Ycf3 and Ycf4 factors (Boudreau et al. 1997). Ycf3 has been found to interact directly with PsaA and PsaD, but not with the subunits of other photosynthetic complexes (Naver et al. 2001). When Ycf3 and Ycf4 were missing in the deletion mutans of *Chlamydomonas*, no stable assembly of PSI occurred, even though the PsaA, PsaB, and PsaC transcripts accumulated (Boudreau et al. 1997). In cyanobacteria, the lack of

the Ycf37 protein caused a decrease in photosynthetic activity and lowered levels of the PSI complexes, yet the mutant cells were capable of photoautorophic growth (Wilde et al. 2001). Recently, the role of a higher plant homolog for Ycf37, PYG7 was characterized in *Arabidopsis* (Stöckel et al. 2006). The plants lacking PYG7 were unable for photoautorophic growth and did not accumulate PSI complexes. However, the PSI subunits were synthesized in the mutants, indicating that the lack of the PSI complexes is due to accelerated degradation of the unassembled subunits (Stöckel et al. 2006). The lack of the HCF145 protein, on the other hand, caused dramatically decreased amounts of the PSI subunits as well, but the protein was shown to function at the mRNA level, by stabilizing the *psaA-psaB-rps14* operon (Lezhneva and Meurer 2004). In cyanobacteria, the BtpA protein has been shown to posttranscriptionally affect the accumulation of PSI (Bartsevich and Pakrasi 1997), especially under low temperature (Zak and Pakrasi 2000).

The correct assembly of the iron-sulphur clusters has been found to be essential for the accumulation of the PSI and Cyt b_6f complexes, and some proteins needed for the (general) assembly of Fe-S clusters have already been identified (Touraine et al. 2004; Yabe et al. 2004). PSI has three iron sulphur centers of type [4Fe-4S], one of which (F_x) is associated with the PsaA/B heterodimer and the two others (F_A and F_B) with PsaC. The evolutionarily conserved HCF101 protein, found to be essential for the accumulation of PSI (Stöckel and Oelmüller 2004), has been shown to function particularly in the assembly of the [4Fe-4S] clusters (Lezhneva et al. 2004). Also the APO1 protein, which is specific for vascular plants, is needed for accumulation of PSI via assembly of the [4Fe-4S] clusters (Amann et al. 2004). APO1-mediated function, however, occurs at a different stage or through a different mechanism than that os HCF101, since the phenotypes, some functional characteristics, chloroplast ultrastructure and the levels of the PSI antenna proteins differ between the *apo1* and *hcf101* mutants (Lezhneva et al. 2004; Amann et al. 2004). Nevertheless, the role of both HCF101 and APO1 in the assembly of PSI is specific for the [4Fe-4S] clusters, since both the *hcf101* and *apo1* mutants also exhibited lowered levels of the ferrodoxin-thioredoxin reductase containing [4Fe-4S] clusters (Amann et al. 2004; Lezhneva et al. 2004). Moreover, the *apo1* mutant also had reduced amounts of the NAD(P)H dehydrogenase (NDH) complexes, which likewise harbor[4Fe-4S] clusters (Amann et al. 2004). The specificity of HCF101 and APO1 for [4Fe-4S] clusters is corroborated by the fact that ferredoxin, which contains a [2Fe-2S] cluster, was present at normal levels in both the *hcf101* and *apo1* mutants (Amann et al. 2004; Lezhneva et al. 2004). In cyanobacteria, a rubredoxin protein RubA has been shown to be needed for the assembly of the F_x [4Fe-4S] cluster (Shen et al. 2002a), and the *rubA* inactivation mutant had significantly lower amounts of PSI, and was not capable of photoautotrophic growth (Shen et al. 2002b).

2.3 Assembly of the Cyt b_6f complex

The Cyt b_6f complex is a dimer, with one monomer composed of eight subunits, from which six subunits are plastid-encoded (PetA, Pet B, Pet D, PetG, PetL, and PetN) and two nuclear-encoded (PetC and PetM). The three-dimensional structure of the Cyt b_6f complex was resolved recently both from cyanobacteria (Kurisu et al. 2003) and *Chlamydomonas* (Stroebel et al. 2003). The Cyt b_6f complex is also the thylakoid protein complex, in which the CES control of the synthesis of the chloroplast-encoded proteins was first demonstrated (Choquet et al. 1998), yet the precise molecular mechanisms of the CES processes in chloroplasts remain to be elucidated.

Cytochrome f (Cyt *f*, PetA) is a CES protein because its rate of synthesis is regulated by the availability of its assembly partners, which are the chloroplast-encoded cytochrome b_6 (PetB) and the subunit IV (SU IV, PetD). In the absence of these assembly partners (or dominant subunits, Cyt b_6 and SU IV), the synthesis of Cyt *f* decreases tenfold (Kuras and Wollman 1994). The C-terminal region of Cyt *f* is important for the assembly into the complex (Mould et al. 2001). More recently, it was shown that Cyt *f* translation is autoregulated by its C-terminal domain and that this CES process for Cyt *f* expression most likely requires an interaction with the membrane-bound translational activator (Choquet et al. 2003).

One of the major Cyt b_6f subunits, the nucleus-encoded Rieske iron-sulphur protein (PetC) is synthesized in cytosol as a 26 kDa precursor and subsequently transported to the plastid. It is processed in the stroma to the mature 20 kDa protein, found to be associated with the chaperones Cpn60 and Hsp70 in the stroma and targeted to the thylakoid membrane where it is assembled into the Cyt b_6f complex (Madueno et al. 1993). For the assembly, it has been found that the presence of the Rieske [2Fe-2S] cluster, the glycine-rich region or the conserved C-terminal region is not required as a prerequisite (Kapazoglou et al. 2000). Interestingly, the Rieske protein has also been assigned a role in the assembly-mediated control of the Cyt *f* synthesis, though the effect was lower than that observed in the absence of Cyt*b* and SU IV (de Vitry et al. 2004).

The function of the small subunits PetG, PetL (ycf7), PetM, and PetN (ycf6) of the Cyt b_6f complex is not yet known properly. However, it has been demonstrated in cyanobacteria that inactivation of the *petM* gene did not affect the activity of the Cyt b_6f complex itself, but instead affected the stoichiometry of other protein complexes, suggesting that specific regulatory processes are mediated by the Cyt b_6f complex (Schneider et al. 2001). In the tobacco knockout mutant for the *petN* gene, on the contrary, the Cyt b_6f complex was totally absent, resulting in interruption in the electron transfer from PSII to PSI, these two latter complexes being, however, intact and physiologically active (Hager et al. 1999).

At least two auxiliary proteins, HCF164 (Lennartz et al. 2001) and HCF153 (Lennartz et al. 2006) have been identified that specifically regulate the accumulation of the Cyt b_6f complexes in the thylakoid membrane (Table 1). Both proteins have been found to be tightly associated with the thylakoid membrane. HCF164 is a thioredoxin-like protein and was recently shown to be able to mediate reducing equivalents across the thylakoid membrane (Motohashi and Hisabori 2006).

Among the identified target proteins for HCF164 were Cyt *f* and the Rieske protein, indicating that the interaction between HCF164, Cyt *f* and the Rieske protein might be an important prerequisite for the assembly of the Cyt b_6f complex (Motohashi and Hisabori 2006). Moreover, the CCDA protein, which is a homolog for prokaryotic thiol disulfide transporter, might be a component of the HCF164-dependent transthylakoid thioreduction pathway, and the lack of the CCDA protein caused defects in the accumulation of Cyt b_6f, and resulted in impaired photosynthesis (Page et al. 2004). The plastid-encoded CCSA protein (Xie and Merchant 1996) and the nuclear-encoded CCS1-4 proteins are needed for the *c*-heme attachment (Inoue et al. 1997; Hamel et al. 2003). In addition, the nuclear-encoded CCB1-4 proteins are specific for binding heme to Cyt b_6 (Kuras et al. 1997). For a review of the CCS and CCB proteins, see van Wijk (2001).

2.4 Assembly of soluble complexes

Increasing amount of research has recently been focused on the assembly of the thylakoid-membrane-embedded protein complexes (with the NDH complex as an exception) whereas the knowledge concerning the assembly of the chloroplast soluble complexes has not much advanced during the past few years. Here we briefly summarize the assembly processes of two stromal protein complexes, Rubisco and FTR.

2.4.1 Rubisco

In higher plants and green algae, Rubisco holoenzyme exists as a 600 kDa soluble complex of the L8S8 form. It thus consists of eight large subunits (LSU) of 55 kDa encoded by the plastome *rbcL* gene and eight small subunits (SSU) of 15-18 kDa encoded by the *rbcS* gene in the nucleus (Spreitzer 1993). Also in red algae, Rubisco is of the L8S8 form, but both subunits are plastome-encoded.

During the assembly of LSU chains, the DnaK/DnaJ/GrpE chaperone complex has been found to associate to the chains in order to maintain them in an unfolded state (Hartl 1996). Also the BSD2 protein, having homology with the DnaJ proteins, has been suggested to prevent the aggregation of the nascent LSU chains (Brutnell et al. 1999). The SSU precursors are processed during their entry into the plastid and are subsequently assembled. The Cpn60 and Cpn21 chaperonins assist in the assembly of the L8S8 holoenzyme (reviewed in Gatenby and Ellis 1990; Gutteridge and Gatenby 1995). The SSU assembly stabilizes the holoenzyme complex generating a fully active enzyme complex. In particular, the highly conserved tyrosine residues at the beta A-beta B loop of the SSU were recently identified to play a stabilizing role for the holoenzyme (Esquivel et al. 2006). SSU assembly controls LSU expression, but SSU does not have a direct effect on LSU translation. If the SSU expression is inhibited (antisense silencing in tobacco), Rubisco assembly is prevented and LSU synthesis is reduced (Rodermel et al. 1996). The assembly of Rubisco has been shown to be sensitive to oxidative stress, and it was recently proposed that during oxidative stress, the RNA recogni-

tion motif in the N-terminus of the LSU becomes exposed and binds any RNA molecule, which causes blocking of the translation and degradation of the unpaired SSU (Cohen et al. 2005, 2006). Thus, in the absence of one subunit in the complex, synthesis of another subunit decreases that has also been detected in the assembly of other photosynthetic complexes in chloroplasts (Minai et al. 2006).

2.4.2 Ferredoxin:thioredoxin reductase

The stromal FTR is a heterodimer protein of 26 kDa, consisting of the catalytical β subunit with a [4Fe-4S] cluster and a variable α subunit. The primary structure of the catalytical subunit is highly conserved between different species, whereas the variable subunit of higher plants has a N-terminal tail. The catalytical β subunit stabilizes the α subunit, since the [4Fe-4S] cluster has been shown to be important for the stability of FTR (Manieri et al. 2003). Thus, the nuclear-encoded proteins HCF101 (Lezhneva et al. 2004) and APO1 (Amann et al. 2004), essential for the assembly of the PSI [4Fe-4S] clusters, have been shown to be needed for the accumulation of FTR subunits as well.

3 Insertion of proteins to the thylakoid membrane - thylakoid translocase complexes and chaperones

3.1 Thylakoid translocases

Nucleus-encoded thylakoid proteins, first translocated to the chloroplast stroma via the envelope membrane, are generally dependent on thylakoid protein complexes, the translocases, to find their final location. They can be inserted into the thylakoid membrane or translocated to the lumen by three distinct pathways that have bacterial homologues: the SRP (signal recognition particle), the Tat (twin-arginine translocase) and the Sec (secretory) pathways. In addition, a fourth pathway exists that is considered to be 'spontaneous'. The protein composition of these translocases has been partially resolved, but very little is known about the assembly processes of the translocases themselves.

The SRP and Sec pathways translocate proteins in their unfolded state and require the activity of soluble chaperones (Mori and Cline 2001), while the Tat pathway has the rare ability to translocate proteins in their fully folded state (Clark and Theg 1997). Proteins using the SRP pathway have a single pre-sequence, which is cleaved off after the envelope translocation, while proteins using the Tat and Sec routes have bipartite pre-sequences for translocation of proteins to the thylakoid lumen. There are also differences in the energetic requirements of protein translocation between the three routes: the Sec and SRP pathways require hydrolysis of nucleoside triphosphates, ATP and GTP, respectively, even though a proton motive force may also be involved (Mant et al. 1995; Kouranov and Schnell 1996).

The chloroplast SRP is a trimer consisting of two subunits of cpSRP43 and one cpSRP54 subunit (Li et al. 1995; Tu et al. 1999). The specific substrates for SRP pathway are the Lhcb proteins, especially the Lhcb4.1 and Lhcb5 proteins have been investigated in detail (Cline 1986; Woolhead et al. 2001). The integration of an Lhcb protein into the thylakoid membrane occurs in two steps: the Lhcb protein interacts first with cpSRP to form a soluble targeting intermediate, called the transit complex, and subsequently integrates into the thylakoid membrane in the presense of GTP and FtsY (Tu et al. 1999). Furthermore, insertion of the Lhcb protein into the thylakoid membrane is known to require an additional component, Alb3 (see also below), a protein that belongs to the Oxa1-YidC family (Moore et al. 2000; Woolhead et al. 2001).

The Tat-pathway is the major route for protein export in prokaryotes, also participating in translocation of proteins to plastids (Finazzi et al. 2003). A substrate protein for the Tat-pathway contains a characteristic, conserved twin-arginine motif situated upstream of a hydrophobic stretch in the pre-sequence. The complete structure of the Tat-translocation channel is not resolved yet, but three proteins, Hcf106, Tha4, and cpTatC, have been identified as the primary components of the Tat-pathway (Settles et al. 1997; Mori et al. 2001). Such proteins as PsaN, PsbP, and PsbQ have been reported to use the Tat-pathway in their translocation (Nielsen et al. 1994; Clark and Theg 1997). The Tat-translocation has also been found to be dependent on the ΔpH across the thylakoid membrane, but this has recently been questioned by showing that the transport of the Tat-pathway substrates can take place *in vivo* in the absence of ΔpH (Finazzi et al. 2003).

The Sec-pathway translocates proteins such as plastocyanin and PsbO across the membrane to the thylakoid lumen. Components of the Sec-pathway include the membrane-bound SecY and SecE proteins, as well as the soluble stromal protein SecA (Shuenemann et al. 1999). By analogy to the bacterial Sec-pathway, it is assumed that SecA interacts with a precursor protein in the stroma and subsequently inserts itself into the membrane. SecY and SecE, in turn, form the translocation channel, maybe with some so far unidentified protein(s).

Many thylakoid proteins insert spontaneously to the membrane, without any aid of stromal components, nucleoside triphosphates, SRP, Alb3, or SecA. These include the photosynthetic reaction center proteins PsbW, PsbY, and PsaK, as well as SecE (Mant et al. 2001; Steiner et al. 2002).

The insertion mechanisms of the chloroplast-encoded proteins to the thylakoid membrane have not been thoroughly investigated. However, there is emerging evidence that the chloroplast-encoded proteins, usually synthesized on thylakoid-bound ribosomes, also use the thylakoid translocases, like SecY (Zhang et al. 2000). Alb3 interactions with the PSI and PSII reaction center proteins (Göhre et al. 2006) also propose the role of Alb3 protein in the folding and translocation of chloroplast-encoded proteins.

3.2 Chaperones

Besides the assembly factors discussed above in the context of the assembly of specific thylakoid protein complexes, several other assembly factors or molecular chaperones have been identified in chloroplasts. These chaperones include chloroplast-envelope-associated and stromal members of the Hsp70 family (for review see Jackson-Constan et al. 2001; van Wijk 2001; Schroda 2004). In addition to the general role of Hsp70 in refolding denatured proteins, some specialized functions have also been found for this chaperone. In *Chlamydomonas* it was shown that HSP70B may protect PSII under light stress and/or stabilize photodamaged PSII to allow for a coordinated repair (Schroda et al. 2001). Furthermore, in *Dunaliella salina* it was detected that a PSII repair intermediate indeed contained the HSP70B protein (Yokthongwattana et al. 2001). Moreover, folding of Rubisco by the stromal Hsp70 was shown to be assisted by the BSD2 protein, which has a high sequence similarity to the Zn-finger domain of DnaJ proteins (Brutnell et al. 1999). DnaJ (and also GrpE) proteins function as co-chaperonins in the prokaryotic Hsp70 system (Schlicher and Soll 1997). In addition, the members of the Hsp100/Clp chaperone family participate in specific functions in chloroplasts. In *Arabidopsis clpC1* mutant line lacking approximately 65% of the total Hsp100/ClpC protein, growth retardation, impaired photosynthetic capacity and reduced amounts of PSI and PSII were found, indicating that ClpC1 is essential for the normal function of the photosynthetic machinery (Sjögren et al. 2004) (For a review concerning the recent advances in the study of the Clp proteins, see Adam et al. 2006).

Also the thylakoid lumen contains a separate set of molecular chaperones, such as cpn60, cpn10, and hsc70 proteins (Schlicher and Soll 1996). Another lumenal protein TLP40 is a cyclophilin-type PPIase that is assumed to catalyze the folding of proteins newly inserted in the thylakoid membrane, or translocated into the thylakoid lumen (Fulgosi et al. 1998). This protein also functions as a phosphatase inhibitor (Vener et al. 1999). Recent characterization of the TLP40 knockout mutants has revealed that the TLP40 protein is crucial in the growth and development of *Arabidopsis* plants thus indicating its crucial importance for the biogenesis and assembly of the thylakoid protein complexes (Khrouchtchova et al. manuscript in preparation).

The Alb3 protein located in the thylakoid membrane is a member of the YidC/Oxa1/Alb3 membrane protein family, whose members are multifunctional mediators of membrane protein integration, folding and assembly into larger complexes. Their evolutionary conserved and physiologically important roles are generally linked to the assembly of the major energy-transducing membrane protein complexes (van der Laan et al. 2005). In chloroplasts, Alb3 (Alb3.1) is an important component of the thylakoid SRP pathway import complex, which is, however, not the only function of chloroplast Alb proteins in the insertion of proteins to the thylakoid membrane. Indeed, Alb3 is involved in the membrane insertion and assembly of both the nucleus- and plastid-encoded subunits of various photosynthetic membrane protein complexes (Ossenbühl et al. 2004). In *Arabidopsis*, loss of Alb3 results in an albino phenotype and a reduction in the amount of thylakoid

membranes (Sundberg et al. 1997). Although the major function of Alb3 (Alb3.1) seems to be to assist the integration and assembly of the Lhcb proteins, other members of the Alb family, Alb3.2 and Alb4, have recently been reported to also participate in the assembly of thylakoid proteins (Göhre et al. 2006; Gerdes et al. 2006). Alb3.2 was found in a large thylakoid protein complex and showed interaction with Alb3.1 and the reaction center proteins of PSI and PSII (Göhre et al. 2006). Moreover, downregulation of Alb3.1 resulted in concomitant decrease in the number of PSII and PSI reaction centers suggesting a fundamental role of Alb3.2 in the assembly of these complexes. More support for the involvement of Alb proteins in PSII biogenesis and turnover come from experiments with cyanobacterial cells where an Alb3 homolog Slr1471p was shown to directly interact with the precursor-D1 protein and facilitate the proper repair of the PSII centers (Ossenbühl et al. 2006).

4 Posttranslational modifications of chloroplast proteins

Chloroplast proteins are prone to several modifications, which occur either after nucleus-encoded proteins have been imported into chloroplasts, or upon or after protein translation in chloroplasts. The most important irreversible modifications are the N-terminal deformylation, removal of N-terminal methionine, and internal processing, whereas protein phosphorylation represents the most common reversible posttranslational modification of chloroplast proteins. Other modifications include the reversible addition and removal of functional groups by glycosylation, acylation, and nitration resulting in structural changes in proteins. Posttranslational modifications of proteins are important regulators that enhance and increase protein complexity and dynamics. They are covalent processes that change the primary structure of proteins in a sequence-specific manner. In the following, we shortly summarize the recent advances in the fields concerning N-terminal methionine excision and thylakoid protein phosphorylation in plastids. In addition, the reader is referred to the recent reviews on studies of posttranslational modifications in plants (Peck 2006; Kwon et al. 2006; Rossignol 2006; de la Fuente van Bentem et al. 2006). For imported proteins, the cleavage of the transit peptide occurs in one or two phases, depending on the final destination of the protein in chloroplast (Mori and Cline 2001) as discussed above (Section 3.1.).

4.1 N-terminal methionine excision

Although Met is the first amino acid of the newly synthesized proteins, it is usually removed from mature proteins in a process called N-terminal Met excision (NME). NME is an irreversible co-translational mechanism, completed before the nascent polypeptide chains are fully synthesized (Arfin and Bradshaw 1988). NME is best documented in plastids where the N-termini of most of the proteins encoded by the chloroplast genome have been determined (Giglione et al. 2004).

Two enzymes of sequential action are needed for NME: 1) peptidyl deformylase (PDF), which specifically removes the N-formyl group present in all nascent polypeptides synthesized in eubacteria and organelles and 2) methionine aminopeptidase (MAP), which removes the methionine specifically in all organisms (Giglione et al. 2004).

Whether the N-formyl group only, or the entire N-formylMet group, is cleaved or retained, depends mostly on the nature and bulkiness of the side chains of the second amino acid (Frottin et al. 2006). In the proteome of chloroplast-encoded proteins, however, all different possibilities exist. The excision of the N-formylMet is the most common one, this group including, among others, the reaction center proteins D1 and D2 of PSII. Additionally, a more extensive cleavage than only the N-formylMet occurs in some chloroplast proteins including RbcL, AtpI, PetA, PscC, and PsbK (Giglione et al. 2004).

In attempts to find the physiological role for NME in chloroplasts, Meinnel and colleagues (Giglione et al. 2003) tested the hypothesis whether MNE is determining the protein half-life. To this end, a specific inhibitor of PDF, actinonin, was used and found to cause a progressive loss of photosynthetic activity both in *Arabidopsis* and *Chlamydomonas* due to the destabilization of the PSII core proteins, particularly the D2 protein. Since the function of PDF is a prerequisite for MAP function, it is likely that methionine at the N-terminus of some proteins, like the D2 protein, possibly acts as a destabilizing residue. Thus, it was concluded that NME is essential for biogenesis of PSII primarily by stabilizing the D2 subunit. This conclusion is corroborated by the fact that the disruption of *PDF1B* (a gene encoding the chloroplast targeted PDF) in *Arabidopsis* led to an albino phenotype (Giglione et al. 2003). However, several proteins of various thylakoid complexes are substrates of PDF, yet the stability of only PSII and its D2 protein were primarily affected in the presence of actinonin. Therefore, the detailed mechanisms of NME in regulation of the life span of chloroplast proteins and thereby the assembly of the chloroplast protein complexes remains to be established.

4.2 Protein phosphorylation

A dynamic light- and redox-controlled protein phosphorylation system has evolved in the thylakoid membranes of chloroplasts for regulation of photosynthesis and the dynamics of the photosynthetic protein complexes (Bennett 1977, 1991; Allen 1992; Vener et al. 1998, 2007). The reversible phosphorylation concerns given amino acid residues, most commonly the tyrosine residue on the stromal side of the thylakoid membrane.

A number of PSII proteins are reversibly phosphorylated in the thylakoid membrane. Thylakoid-bound kinases are responsible for protein phosphorylation, for which several regulatory patterns have been described (Pursiheimo et al. 2003). Protein dephosphorylation, in turn, is catalyzed by the chloroplast phosphatases, being either thylakoid-bound or soluble ones (Bennett 1991). Furthermore, modulation of the thylakoid protein phosphorylation involves the thiol redox state (Rintamäki et al. 2000) and the light-induced conformational changes in the substrate

proteins (Zer et al. 1999; Jeschke et al. 2005). Thylakoid phosphoproteins include the D1, D2, CP43, and PsbH proteins of the PSII core (Bennett 1991; Vener et al. 2001; Andreuzzi et al. 2005), the Lhcb1, Lhcb2, and Lhcb4 proteins of the light-harvesting II antenna (Bennett 1991; Bergantino et al. 1995; Vener et al. 2001; Turkina et al. 2004; Tikkanen et al. 2006) as well as the PsaD protein of PSI (Hansson and Vener 2003), 9 kDa soluble phosphoprotein (TSP9) (Carlberg et al. 2003) and TMP14, the latter demonstrated recently to be a novel subunit of PSI (Khrouchtchova et al. 2005). In addition, two phosphorylation sites (Thr-2 and Ser-3) were detected recently in the Rieske Fe-S protein (PetC) of the Cyt b_6f complex in spinach, and three new threonine phosphorylation sites in the CP43 protein (Rinalducci et al. 2005).

The role of reversible phosophorylation of the above-mentioned photosynthetic proteins is not completely understood, but it has been shown to be involved in several aspects of the dynamics of photosynthetic membrane protein complexes, especially as a response to environmental cues. Light induces reversible phosphorylation of a number of PSII core proteins and of the LHCII antenna proteins Lhcb1, Lhcb2, and Lhcb 4 (Bennett 1991) via activation of the redox-dependent protein kinases, the identity of which is not yet fully elucidated.

5 Concluding remarks

Elucidation of the mechanisms, pathways, and auxiliary components involved in the synthesis, assembly, stability, and dynamics of the photosynthetic membrane protein complexes is still in its infancy. One pertinent task is to increase our understanding about the protein networks involved in auxiliary functions in guiding the assembly of the individual protein subunits to macromolecular photosynthetic complexes. Moreover, the biosynthesis and regulation of the ligation of various redox co-factors to the bioenergetic membrane protein complexes awaits extensive investigation. Table 1 summarizes our present knowledge of the assembly factors and chaperones involved in the biosynthesis of plastid protein complexes. We are now in an urgent need to get a systems biology view on the biogenesis of the photosynthetic energy providing pigment protein complexes. This will greatly facilitate, for example, the future plans to construct artificial cell factories for clean solar energy production.

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