Biogenesis, assembly and turnover of photosystem II units

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Assembly of photosystem II, a multiprotein complex embedded in the thylakoid membrane, requires stoichiometric production of over 20 protein subunits. Since part of the protein subunits are encoded in the chloroplast genome and part in the nucleus, a signalling network operates between the two genetic compartments in order to prevent wasteful production of proteins. Coordinated synthesis of proteins also takes place among the chloroplast-encoded subunits, thus establishing a hierarchy in the protein components that allows a stepwise building of the complex. In addition to this dependence on assembly partners, other factors such as the developmental stage of the plastid and various photosynthesis-related parameters exert a strict control on the accumulation, membrane targeting and assembly of the PSII subunits. Here, we briefly review recent results on this field obtained with three major approaches: biogenesis of photosystem II during the development of chloroplasts from etioplasts, use of photosystem II-specific mutants and photosystem II turnover during its repair cycle.

Keywords: photosystem II; assembly; chloroplast biogenesis; photosynthesis; D1 protein

1. INTRODUCTION

The smallest PSII complex capable of stable charge separation is composed of the D1/D2 heterodimer, the α - and β -subunits of *cyt b559*, and the *psbI* and *psbW* gene products. Oxygen-evolving PSII also includes at least the internal chlorophyll *a*-binding antenna proteins CP47 and CP43, and the OEC proteins PsbO, PsbP and PsbQ of 33, 23 and 17 kDa, respectively, on the lumenal side of the complex. Several LMW (less than 10 kDa) proteins, both nucleus- and chloroplast-encoded, also belong to PSII, many of them being essential for oxygen evolution, as revealed by recent studies using reverse genetics approaches (see the review in Hankamer *et al.* 2001). The formation of higher-organization PSII supercomplexes in the grana involves dimerization of the PSII monomer and the subsequent attachment of chlorophyll *a/b* binding antenna proteins.

Three-dimensional X-ray crystal-structure analysis of cyanobacterial PSII has revealed the composition and mutual orientation of 36 membrane-spanning α -helices, and the ligation of various cofactors at almost atomic resolution (Zouni *et al.* 2001). Cryoelectron crystallographic and single-particle analyses have shed further light on the structural composition of higher plant PSII (Boekema *et al.* 1995; Rhee *et al.* 1998; Hankamer *et al.* 2001), including the association of the OEC proteins and the trimeric LHCII via the minor chlorophyll *a/b*-binding proteins CP26 and CP29. Thus, great advances have been achieved during the past few years in revealing the complexity of the PSII structure, composed of more than 20 polypep-

tides. However, the role and location of several LMW polypeptides remain to be resolved.

Despite a wealth of information accumulated on the structure and function of PSII, our knowledge of the assembly of this multiprotein complex is only in its infancy. Some of the PSII polypeptides bind chlorophyll that, if present as a free pigment, can result in photo-oxidative damage through a reaction of chlorophyll triplets with oxygen. Hence, the binding of pigment cofactors has to occur in a coordinated manner, together with the insertion of the proteins into the thylakoid membrane and their assembly with other subunits of the complex. Moreover, the PSII genes reside in both the chloroplast and the nucleus, and therefore coordinated synthesis and membrane insertion of PSII subunits encoded in both compartments is a prerequisite for successful assembly of the complex. In this context, it is still a mystery why up to 16 genes, among those the genes of the most central subunits of thylakoid protein complexes, have been retained in the chloroplast genome when, during evolution, most of the genes of a chloroplast progenitor, an ancient cyanobacterium, have been transferred to the nucleus (Martin & Herrmann 1998).

We briefly review recent progress made on the assembly of PSII (see Choquet & Vallon 2000). Particular emphasis will be placed on the assembly of chloroplast-encoded gene products and the stringent coupling between the synthesis and thylakoid insertion of the membrane-spanning PSII subunits and their assembly into PSII.

2. BIOGENESIS OF PSII: THREE STRATEGIES FOR STUDYING PSII ASSEMBLY

Depending on the developmental stage of the plastid, at least three different processes can be distinguished in the building up of PSII complexes. One concerns the bio-

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genesis of thylakoid membranes and the membrane complexes during the transition from etioplasts to chloroplasts, the second concerns *de novo* biogenesis of PSII centres in light, and the third is associated with the repair of light-induced damage to PSII in mature chloroplasts. The second process is most difficult to tackle directly, and often the studies on biogenesis of PSII complexes in light have been accomplished using various PSII mutants. Although the three PSII assembly processes mentioned above clearly differ from each other, it is probable that they share common regulatory steps.

(a) *From etioplast to mature chloroplast*

Although light is required for the maturation of etioplasts to chloroplasts, many of the protein subunits of PSII are synthesized independently of light, and are already present in etioplasts. This is the case with cyt *b559* (Ohashi *et al.* 1992; Müller & Eichacker 1999), PsbH (Hird *et al.* 1991), the OEC proteins (Gamble & Mullet 1989) and D2 (Müller & Eichacker 1999). Only upon illumination, however, is the chlorophyll precursor protochlorophyllide converted into chlorophyll *a* (von Wettstein *et al.* 1995), and the synthesis of chlorophyll-binding proteins (CP43, CP47 and D1) takes place (Eichacker *et al.* 1990; Kim *et al.* 1994), thus enabling the final stable assembly of PSII.

It is now widely accepted that these development-related changes in plastid gene expression are mainly regulated at the translational and post-translational levels (reviews in Rochaix 1992; Mayfield *et al.* 1995; Zerges 2000), with transcript amounts and half-lives being similar throughout the greening process (Klein & Mullet 1986, 1987; Krupinska & Apel 1989).

The sequential synthesis of PSII proteins during greening suggests that the assembly of PSII is a stepwise process that involves the formation of intermediate PSII subcomplexes. Indeed, D2 and cyt *b559* that are found in etioplasts in 45–200 kDa complexes (Müller & Eichacker 1999), apparently function as acceptors for the assembly of D1. This has been demonstrated by radiolabelling experiments, in which the precursor form of D1 (pD1) was found to have already associated in the dark with PSII subcomplexes containing D2 and cyt *b559*. Stabilization of these assemblies upon illumination allows their accumulation and subsequent formation of higher molecular mass complexes (PSII monomers and dimers) in the chloroplast (Müller & Eichacker 1999).

(b) *Assembly in PSII protein-specific mutants*

Another very powerful approach for studies of the assembly process has been the use of mutants, either of a specific PSII subunit or of a nuclear gene that affects the expression of structural proteins of PSII. A large set of mutants lacking single proteins of specific photosynthetic complexes, including PSII, are available in cyanobacteria (Vermaas 1993; Pakrasi 1995) and *Chlamydomonas* (de Vitry & Vallon 1999), and more recently, with the development of chloroplast transformation techniques, also in higher plants.

(i) *Depletion of main PSII core subunits*

A lesion in one of the main integral subunits of PSII results, in cyanobacteria and plants, in disruption of the whole complex and in the disappearance of the other cen-

tral PSII subunits. Such is the case for cyt *b559* mutants (Pakrasi *et al.* 1990; Morais *et al.* 1998; Suorsa *et al.* 2001), D2 mutants (Erickson *et al.* 1986; Vermaas 1988; de Vitry *et al.* 1989; Kuchka *et al.* 1989; Cohen *et al.* 2001), CP47 mutants (Jensen *et al.* 1986; Vermaas 1988) and D1 mutants (de Vitry *et al.* 1989; Nilsson *et al.* 1990; Morais *et al.* 1998). In CP43 mutants, however, the other PSII core proteins are not totally missing, yet their amounts are dramatically decreased (Vermaas 1988; Rochaix *et al.* 1989). Although the consequences are similar for cyanobacteria and plants, the reasons behind the depletion of other core subunits, however, seem to differ. Inactivation of one central PSII gene in cyanobacteria has no drastic effects on the transcription of the other PSII genes, or on the translation of their respective proteins, but instead appears to accelerate the degradation of close assembly partners (Yu & Vermaas 1990, 1993). Besides this proteolytic control of gene expression, a second post-transcriptional regulatory mechanism exists in the chloroplast, namely a tight control of translation. The process by which a protein (dominant subunit) exerts translational control on the synthesis of another protein (CES subunit) of the same complex is called the CES process (see Choquet *et al.* 1998; Wollman *et al.* 1999; Choquet *et al.* 2001). The molecular mechanism underlying CES control has been elucidated for the cyt *b₆f* complex of *Chlamydomonas*, in which cyt *f* is the CES subunit and cyt *b* and subunit IV are the dominant ones (for details see Kuras & Wollman 1994; Choquet *et al.* 1998). There are also several putative examples of CES control for PSII, and future work in this field will aim to unravel the underlying molecular processes. Table 1 summarizes the putative cases of CES control of PSII proteins in *Chlamydomonas* and higher plants, studied mainly using pulse-labelling or pulse-chase approaches.

The pattern of protein interactions that emerges from the study of these PSII mutants (table 1) is in accordance with the pattern of protein accumulation in the thylakoid membrane during the transition from etioplasts to chloroplasts. The dependence of the synthesis of D2, D1, CP47 and CP43 on cyt *b559* is in line with the presence of this subunit in the thylakoid membrane already, at early phases of chloroplast development (Ohashi *et al.* 1992; Müller & Eichacker 1999). D2, in turn, is indispensable for the synthesis of D1, additionally, D2 is found in dark-grown seedlings in subcomplexes postulated to act as receptors for the assembly of other PSII subunits. Hence, it is reasonable that synthesis of D2 occurs independently of proteins such as D1, CP47 or CP43, which are assembled at subsequent stages of PSII biogenesis. Furthermore, as explained in detail in the next section, D2 has been reported to interact with D1 translation intermediates (Zhang *et al.* 1999), thus emphasizing the importance of the D2 protein for successful translation of D1.

(ii) *Depletion of low molecular weight peripheral subunits of PSII*

As opposed to deprivation of one of the major PSII core subunits that results in the disappearance of its close assembly partners, both in plants and cyanobacteria, removal of one of the LMW chloroplast-encoded PSII subunits (except for the α - and β -subunits of cyt *b559*) often has very different consequences in these two types of

Table 1. Mutants of integral PSII subunits: examples of putative CES control in *Chlamydomonas* and higher plant PSII mutants as revealed by pulse and/or pulse-chase experiments.

organism	protein deleted	synthesis of other proteins affected	reference
<i>Chlamydomonas</i>	D2	D1	Erickson <i>et al.</i> (1986) de Vitry <i>et al.</i> (1989) Cohen <i>et al.</i> (2001)
	D1	CP47	Bennoun <i>et al.</i> (1986) Jensen <i>et al.</i> (1986) de Vitry <i>et al.</i> (1989)
	CP47 cyt <i>b559</i>	D1 D2 (D1, CP47, CP43)	Jensen <i>et al.</i> (1986) Morais <i>et al.</i> (1998)
higher plants	D1	CP47	Gamble & Mullet (1989) Kim <i>et al.</i> (1994)

Table 2. Requirement of peripheral chloroplast-encoded LMW PSII subunits for assembly and function of PSII in cyanobacteria and plants.

(Abbreviations: three plus signs, 60–100% of WT; two plus signs, 30–60% of WT; one plus sign, 10–30% of WT; n.s., not studied.)

protein mutated	organism	PSII assembled?	photoautotrophic growth?	reference
PsbH	<i>Synechocystis</i>	+++	+++	Mayes <i>et al.</i> (1993)
	<i>Chlamydomonas</i>	—	—	Summer <i>et al.</i> (1997) O'Connor <i>et al.</i> (1998)
PsbI	<i>Synechocystis</i>	+++	+++	Ikeuchi <i>et al.</i> (1995)
	<i>Chlamydomonas</i>	+	+ ^a	Künstner <i>et al.</i> (1995)
PsbJ	<i>Synechocystis</i>	++	++	Lind <i>et al.</i> (1993)
	tobacco	++	—	Regel <i>et al.</i> (2001) Hager <i>et al.</i> (2002) Suorsa <i>et al.</i> (2001)
PsbK	<i>Synechocystis</i>	+++	+++	Ikeuchi <i>et al.</i> (1991)
	<i>Chlamydomonas</i>	—	—	Takahashi <i>et al.</i> (1994)
PsbL	<i>Synechocystis</i>	+	—	Anbudurai & Pakrasi (1993)
	tobacco	+ ^b	—	Suorsa <i>et al.</i> (2001)
PsbT	<i>Synechocystis</i>	n.s.	n.s.	—
	<i>Chlamydomonas</i>	+++	+++	Monod <i>et al.</i> (1994) Ohnishi & Takahashi (2001)
PsbZ	<i>Synechocystis</i>	+++	+++	S. Ulas, C. Bishop, J. Nugent and P. Mäenpää, unpublished data
	tobacco	+++	+++	Ruf <i>et al.</i> (2000) Baena-González <i>et al.</i> (2001a) Swiatek <i>et al.</i> (2001)

^a Photoautotrophic growth only in very dim light.

^b Only partial assembly.

organisms. Mutations in cyanobacteria often affect either solely the target protein, or have only a slight effect on the accumulation of other subunits of the complex (table 2). However, in chloroplasts of more developed organisms such as *Chlamydomonas* and higher plants, the engineered genetic lesions in LMW PSII proteins often have secondary effects, and in addition to the target protein, the mutants are also deprived of other PSII subunits. Among the small subunits, such an evolutionary difference holds most clearly for PsbH (Mayes *et al.* 1993; Summer *et al.* 1997), PsbI (Ikeuchi *et al.* 1995; Künstner *et al.* 1995) and PsbK (Ikeuchi *et al.* 1991; Takahashi *et al.* 1994) (table 2). The discrepancy in the requirement of these subunits for PSII assembly and/or stability among different organisms is somewhat puzzling. It is, however, conceivable that the

original role of these subunits has been mostly regulatory, with no distinct function in the assembly process itself or the stability of PSII. An example of such a subunit is the recently characterized and highly conserved PsbZ, being dispensable for PSII assembly in cyanobacteria as well as in *Chlamydomonas* and tobacco (Ruf *et al.* 2000; Baena-González *et al.* 2001a; Swiatek *et al.* 2001), but instead having an apparent regulatory function.

An additional stabilizing role might have been acquired for the PsbH, PsbI and PsbK subunits in more evolved organisms, thus making them essential for the proper assembly of PSII. A requirement for enhanced PSII stability probably relates to the intricate PSII repair cycle of plants. It is known that in plants the PSII repair process requires a shuttling of a PSII subcomplex between the

grana and stroma membranes, this being accompanied by changes in the oligomeric structure of the complex (Barbato *et al.* 1992; Baena-González *et al.* 1999). Therefore, as opposed to cyanobacteria, an increased stability of PSII might be demanded for the additional steps in the repair process. In support of this view, it was recently shown that yet another LMW chloroplast-encoded protein, PsbT (encoded by *ycf8*) is required for the efficient repair of photodamaged PSII: the absence of PsbT caused an enhanced loss of PSII activity and PSII protein subunits under strong illumination (Monod *et al.* 1994; Ohnishi & Takahashi 2001). The *psbT* gene is also present in cyanobacteria, but its function in this organism remains unknown.

(iii) *Developmental aspects*

An intriguing aspect of PSII biogenesis is its dependence on the developmental stage of the plastid. As mentioned above, several PSII proteins (cyt *b559*, D2, PsbH and OEC proteins) can already be detected in etioplasts, whereas D1, CP43 and CP47 accumulate only in light, thereby allowing the assembly of PSII to be completed. If, however, D1 or CP47 is not accumulating (e.g. in the respective mutants) after the onset of illumination, the accumulation of cyt *b559*, D2, PsbH and OEC proteins is prevented. This is apparently not a consequence of destabilization of the complex in the light due to lack of D1, but rather seems to result from a shift in the regulatory mechanisms that govern PSII gene expression upon achievement of chloroplast maturity. At the early stages of chloroplast development cyt *b559*, D2, PsbH and OEC are synthesized independently of the presence of assembly partners like D1 and CP47, while upon chloroplast maturation an obligate coupling seems to be established between synthesis of the innermost components of PSII and their assembly. Indeed, in contrast to the situation in etioplasts, the mature chloroplasts of *Chlamydomonas* D1-less mutants fail to accumulate D2 or cyt *b559* even under heterotrophic growth conditions in the dark (Morais *et al.* 1998).

Thus, both developmental cues and light are key regulatory factors during the early stages of chloroplast development. Results obtained from pulse-chase experiments with the barley *vir-115* mutant, which is deficient in D1 synthesis, also provide strong support for a link between gene expression and plastid development (Gamble & Mullet 1989). The rates of PSII protein synthesis were comparable in the WT and the *vir-115* mutant in plastids isolated from dark-grown seedlings that were illuminated for 1 hour. Nevertheless, maturation of chloroplasts upon longer illumination times (16 or 72 hours) revealed a change in regulation of gene expression, the *vir-115* mutant being unable to synthesize other intrinsic PSII core proteins to the same extent as the WT.

(c) *Maintenance control: replacement of damaged D1 during repair of PSII repair*

The water-splitting photochemistry of PSII produces various radicals and active oxygen species, which cause irreversible damage to PSII. Damaged PSII centres, however, do not usually accumulate in the thylakoid membrane due to a rapid and efficient repair mechanism. The grand design of PSII allows protection for most of its pro-

tein components, with the damage mainly targeted to a single subunit, the reaction centre D1 protein. Repair of PSII via turnover of the D1 protein is a complex process that involves reversible phosphorylation of PSII proteins and changes in the oligomeric structure of PSII (figure 1). This is combined with the shuttling of the complex between grana and stroma-exposed thylakoid domains, partial PSII disassembly and a highly specific proteolysis of the damaged D1 protein. Replacement of the damaged D1 with a new copy requires a stringent coordination between its degradation and the synthesis, insertion and assembly to PSII of the new D1 copy. Although exacerbated under higher irradiances, the turnover of D1 protein occurs at all light intensities, being by far the fastest of all the PSII subunits. Such a high turnover rate results in a preferential labelling of the D1 protein in pulse-chase experiments, and therefore provides an efficient tool for monitoring the different assembly steps of the PSII complex. It now seems clear that most of the regulation of gene expression required for the PSII repair cycle is exerted at the translational and post-translational levels. Moreover, the various assembly steps of PSII are all highly regulated, and require maintenance of proper redox conditions in the thylakoid membrane and in the stroma.

(i) *PsbA transcripts and translation initiation*

In chloroplasts, the *psbA* transcripts, encoding the D1 protein, are remarkably stable with a half-life ranging between 10 and 40 hours (Mullet & Klein 1987; Klaff & Gruissem 1991). Although some upregulation in *psbA* gene transcription and thus also in the steady-state level of *psbA* transcripts takes place at high irradiances (Kettunen *et al.* 1997; Baena-González *et al.* 2001b), the diurnal fluctuations in *psbA* transcripts are only minor as compared with cyanobacteria. Indeed, in cyanobacteria the main regulation of *psbA* gene expression resides at the level of transcription initiation (Golden *et al.* 1986; Mohamed & Jansson 1989), while in higher plants the corresponding main regulatory level resides on translation initiation of *psbA* mRNAs.

The pioneering work of Mayfield and co-workers on *Chlamydomonas* pointed for the first time to a link between chloroplast translation and photosynthesis (e.g. Somanchi & Mayfield 2001). The light-induced increase in the ATP/ADP ratio and the generation of reduced thioredoxin upon illumination were proposed to induce the binding of a nuclear-encoded regulatory protein complex to the 5'-end of the *psbA* mRNA, thereby allowing the proper association of ribosomes and the scanning of *psbA* mRNA to be initiated. Furthermore, the translation initiation regulators RB47 and RB60 were shown to be associated with thylakoid membranes (Zerges & Rochaix 1998; Trebitsh *et al.* 2001), and the signals from both PSI and PSII were shown to be required to initiate translation of the *psbA* mRNA (Trebitsh & Danon 2001).

(ii) *Targeting and insertion of D1 nascent chains to the thylakoid membrane*

In chloroplasts, the *psbA* mRNA-ribosome complexes are efficiently targeted to the thylakoid membrane in light, most probably via the binding of cpSRP54 (Nilsson *et al.* 1999), a chloroplast homologue of the SRP. In line with this observation, reverse genetics studies have revealed

Figure 1. Scheme depicting the synthesis, membrane insertion and assembly of the D1 protein during repair of damaged PSII centres (see § 2c for details).

that *Arabidopsis* cpSRP mutants fail to accumulate the D1 protein early in development (Amin *et al.* 1999). However, we have recently shown that in *Synechocystis* 6803 the targeting of the ribosome–nascent D1 chain complexes to the thylakoid membrane also requires light and is probably one important component in the regulation of D1 elongation in cyanobacterial cells (Tyystjärvi *et al.* 2001). It remains to be elucidated whether this light-induced targeting of *psbA* mRNA–ribosome complexes to the thylakoid membrane in cyanobacteria and plants is dependent on the photosynthetic redox reactions.

After targeting of the *psbA* mRNA–ribosome–nascent chain complex to the thylakoid membrane, the elongation and membrane insertion of the D1 protein are controlled by a variety of factors, including translocon association and availability of assembly partners, as well as redox control and ligation of pigments. Early pulse-labelling studies with intact isolated chloroplasts already revealed that ribosomes pause at discrete sites on *psbA* mRNA, presumably to facilitate the insertion of transmembrane domains of D1 into the thylakoid membrane, and the ligation of various cofactors to the D1 protein (Kim *et al.* 1991). More recently, *in vitro* translation in intact chloroplasts was utilized in the search of proteins possibly interacting with nascent D1 chains during the early phases of membrane insertion (Zhang *et al.* 2001). An intriguing discovery was a close interaction of ribosome–D1 nascent chain complexes with cpSecY (Zhang *et al.* 2001), a chloroplast homologue of bacterial translocation channel protein SecY. This strongly suggests that the cpSecY/E/G translo-

con in thylakoid membranes not only functions in post-translational translocation and insertion of a set of nuclear encoded thylakoid proteins (Keegstra & Cline 1999), but also guides the translocation and insertion of chloroplast-encoded membrane proteins like D1. Strong interaction of ribosomes with cpSecY indicates that the ribosomes anchor at this translocon channel, which then starts guiding the membrane insertion of the elongating D1 protein (figure 1).

(iii) Regulation of *psbA* mRNA translation elongation and co-translational assembly of D1 into PSII

Despite efficient association of the *psbA* mRNA–ribosome–nascent chain complexes with the thylakoid membrane in light, they remain under elongation arrest in mature chloroplasts if PSII complexes depleted of D1 are not available (Kettunen *et al.* 1997; Zhang *et al.* 1999). Recently, this interpretation has been further supported by studies with the *Arabidopsis* *var-2-2* mutant, which is deficient in the degradation of the damaged D1 protein (Bailey *et al.* 2002). Pulse-chase experiments revealed that the translation of the D1 protein in this mutant is severely retarded, indicating that D1 synthesis is strictly controlled by the availability of assembly partners, in this case a PSII subcomplex depleted of the D1 protein (Adir *et al.* 1990).

The *in vitro* translation in intact chloroplasts, and subsequent fractionation of the thylakoid membrane, has provided an excellent method for studies of the regulation of D1 protein elongation and concomitant assembly into PSII subcomplexes (Zhang *et al.* 1999, 2000). Applying

very short pulses of only a couple of minutes, it has been possible to chase the label into the precursor D1 via its different elongation intermediates and finally to follow the maturation of the D1 protein through C-terminal processing.

A crucial role of photosynthetic electron transfer reactions in the regulation of D1 translation elongation has been recognized for a long time (Kim *et al.* 1991, 1994; Taniguchi *et al.* 1993; Kuroda *et al.* 1996; Edhofer *et al.* 1998). Electron transport controls *psbA* mRNA translation elongation, probably by providing the system with essential reducing compounds via PSI electron flow (Kuroda *et al.* 1996; Zhang *et al.* 1999) and by maintaining a transmembrane proton gradient (Mühlbauer & Eichacker 1998; Zhang *et al.* 2000). Either PSI electron transfer or addition of a reduced thiol reactant, DTT, can restore D1 elongation under conditions where PSII electron transfer has been blocked with 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Dissipation of the proton gradient with nigericin or other uncouplers, on the other hand, completely hinders the elongation process (Mühlbauer & Eichacker 1998; Zhang *et al.* 2000). Strong dependence of D1 elongation on photosynthetic electron transfer is in accordance with several earlier experiments indicating that the synthesis of the new D1 copy and repair of PSII centres cannot proceed in darkness (e.g. Aro *et al.* 1994).

Most of the cofactors in PSII are ligated to the reaction centre proteins D1 and D2. Ligation of cofactors to the D1 protein during its turnover cycle has been suggested to occur upon pausing of ribosomes at distinct sites on the *psbA* mRNA (Kim *et al.* 1991). This has been considered to stabilize the nascent D1 chains against proteolytic degradation. Concomitantly with translation elongation, and after exiting the translocon channel, the nascent D1 chain starts interacting with its assembly partners (figure 1). A weak interaction (easily disrupted, for example, by detergents) with D2 is already established at the 17 kDa D1 intermediate stage, when D1 is composed of two transmembrane α -helices, the third one remaining in the ribosome tunnel (Zhang *et al.* 1999). This association is strengthened after four transmembrane helices of D1 have been translated and inserted into the thylakoid membrane, presumably due to the ligation of cofactors (Kim *et al.* 1991), and also to the formation of a transient disulphide bridge between the nascent D1 chain and the D2 protein (Zhang *et al.* 2000). Indeed, the elongation of the nascent D1 chain and its interaction with the D2 protein are strongly hampered if the thiol groups are chemically reduced by DTT or alkylated by N-ethylmaleimide (Zhang *et al.* 2000).

Further elongation and, finally, the termination of *psbA* mRNA translation, result in the release of the precursor D1 from ribosomes (figure 1). This is followed by fast insertion of the fifth transmembrane helix into the membrane and concomitant traverse of the C-terminus with cleavable extension into the thylakoid lumen.

(iv) *Post-translational assembly steps in the repair of PSII*

The above experiments highlight a close dependence of PSII repair on co-translational D1 protein synthesis and reassembly into PSII and on proper redox conditions in chloroplasts. The following step in PSII assembly is the

reassociation of subunits that detach from the complex during the repair process (figure 1). Partial dissociation of CP47 has been reported in some studies (van Wijk *et al.* 1996, 1997). However, one has to consider that the biochemical isolation procedures employed might give rise to artefacts, since the detergent treatments used may destabilize the PSII subcomplexes undergoing repair. The possibility of CP47 not dissociating from PSII during the repair process is supported by the fact that CP47 antibodies are able to co-immunoprecipitate D1 elongation intermediates, thus suggesting the presence of CP47 in the subcomplexes that dock the D1 nascent chains. In contrast to the uncertainty in the case of CP47, reversible dissociation and reassociation of CP43 with the PSII complex during the repair process is indisputable (Barbato *et al.* 1992; Baena-González *et al.* 1999; Zhang *et al.* 2000). Labelling in intact chloroplasts revealed that either a non-labelled and thus pre-existing, or a newly synthesized CP43 becomes associated with PSII after replacement of the damaged D1 copy (Zhang *et al.* 2000). Moreover, in contrast to antibodies against D2 and CP47, the CP43 antibodies failed to co-immunoprecipitate D1 elongation intermediates, in agreement with the absence of this protein from the complexes under repair.

Monitoring of labelled D1 protein in different PSII subcomplexes revealed the presence of either the precursor or mature D1 form in assemblies including D2 and CP47. On the contrary, the CP43 protein was stably assembled only in those PSII complexes where the D1 protein had undergone maturation by C-terminal processing (figure 1; Zhang *et al.* 2001). Whether the processing of the C-terminal extension of the pD1 protein is a prerequisite for association of CP43 with PSII, or whether the association of CP43 induces proper conformation and rapid processing of the C-terminal extension of the D1 protein, cannot be distinguished by our experimental approaches. Coordination of the processing of the D1 C-terminal extension with the assembly of CP43 might be important for 'safe' ligation of the Mn cluster and subsequent photoactivation of the OEC, which have been shown to be extremely susceptible to inactivation by light (Rova *et al.* 1998). Finally, the removal of the C-terminal extension makes possible the restoration of the water-splitting activity of PSII (Diner *et al.* 1988; Bowyer *et al.* 1992). It is noteworthy that the OEC proteins, released from PSII during its repair cycle, do not undergo rapid turnover and are thus not targeted for degradation when free in the lumen.

3. CONCLUDING REMARKS

Comparative studies between different developmental stages of the chloroplast indicate that certain PSII core subunits (D2, cyt b559) act as receptors for the synthesis and insertion of other PSII core proteins (D1, CP47, CP43), pointing to a gradual and perhaps hierarchical assembly process. Results from pulse-chase studies on PSII mutants speak in favour of such a hierarchy, as evidenced by the effect of specific 'dominant' PSII core subunits on the translation and accumulation of other subunits (table 1). This view is further supported by the finding that D1 has already interacted with D2 during the early steps of D1 biogenesis, as revealed by studies on D1

turnover during the repair of PSII centres. Such a tight coupling between the presence of a certain subunit and the synthesis of others seems to apply only for the main core subunits. More peripheral components, however, exert a general stabilizing effect on the PSII complex, the stability of PSII increasing with an increase in the number of protein subunits. This stabilizing effect is most apparent in chloroplasts of photosynthetic eukaryotes. Although in algae and higher plants most of the LMW subunits are essential for PSII assembly, there are exceptions, with subunits such as PsbZ being completely dispensable for PSII formation.

In all three cases of PSII assembly described here, the expression of PSII genes is also regulated post-transcriptionally and, depending on the organism and the protein subunit in question, either translational or post-translational processes (including proteolysis) have an important role in this control. Both cyanobacteria and plants rely on a stringent translational and post-translational regulation for the accumulation and assembly of the PSII core subunits, as exemplified by a strict dependence of D1 translation elongation on the availability of assembly partners and photosynthetic electron transfer. This obligate coupling between translation elongation and the assembly of PSII subunits, together with the strong dependence of membrane insertion and assembly of PSII subunits on photosynthetic redox reactions, are probably reasons for the maintenance of the genes of most integral PSII subunits in the plastid genome.

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Discussion

S. Styring (Department of Biochemistry, Lund University, Lund, Sweden). Is it correct that the receiving 'package' for the newly synthesized D1 protein is a complex of D2 protein, CP47 and cyt *b559*?

E.-M. Aro. This indeed seems to be the case. Pulse-chase experiments with chloroplasts isolated from mature leaves predominantly synthesize the D1 protein, and upon termination of translation the newly synthesized D1 copy is already a component of the reaction centre heteromide composed of D1, D2 and cyt *b559*. Co-immunoprecipitation experiments also seem to indicate the presence of CP47 in this reviving 'package'.

B. Andersson (Rector, Linköping University, Linköping, Sweden). To what extent do you think D1 protein integration and PSII assembly is the same during *de novo* formation of PSII or during D1 protein turnover of an existing PSII complex? Is there a difference in the coupling between D1 protein degradation and D1 integration in plants and cyanobacteria (considering that plasma mem-

branes in cyanobacteria could be involved in the assembly of photosystems)?

E.-M. Aro. Many of the partial steps during biogenesis or *de novo* formation of PSII centres are probably similar to those occurring during the rapid turnover of the D1 protein. The receiving 'package' for the D1 protein during development of chloroplasts from etioplasts has been shown to be composed of D2 and cyt *b559* (see Müller & Eichacker 1999) corresponding to the situation during D1 protein turnover. The sites of synthesis and assembly, on the other hand, may be different since during the turnover process D1 synthesis takes place on thylakoid-associated ribosomes, whereas during *de novo* synthesis of PSII complexes in cyanobacteria there are indications that this occurs on plasma membranes (Zak *et al.* 2001). During the turnover process, the degradation of D1 and the synthesis, membrane insertion and assembly of the new copy are tightly synchronized, and the process seems to be quite similar in both organisms.

T. Hundal (*Department of Biochemistry, Linköping University, Linköping, Sweden*). Do you think that the D1 fragment(s) is signalling the ribosome to synthesize new D1 protein?

E.-M. Aro. We have tried to test this hypothesis but at least so far we do not have solid data in favour of this hypothesis.

B. Diner (*Central Research and Development Department, E.I. DuPont de Nemours & Co., Wilmington, Delaware, OH, USA*). You mention that you need to process the C-terminal of the D1 protein before assembly of CP43 subunits into the subcomplex. But there is, of course, a mutant of *Scenedesmus* that cannot process the D1 protein but, nevertheless, assembles a PSII complex containing CP43.

E.-M. Aro. We can only say that the two processes, C-terminal processing of D1 and the assembly of CP43 are somehow coordinated. Considering the *Scenedesmus* mutant, it indeed is more probable that the assembly of CP43 accelerates the processing of the C-terminal extension of the D1 protein. It should be emphasized, however, that the assembly of CP43 is not a prerequisite for the processing step.

M. Wikström (*Institute of Biomedical Sciences, Department of Medical Chemistry, University of Helsinki, Helsinki, Finland*). You showed that the uncoupling of phosphorylation-inhibited translation and incorporation of the D1 protein. Is this a proton gradient effect or due to lack of ATP?

E.-M. Aro. In these experiment extra ATP was always

added and thus we believe that proton gradient itself is an important regulatory 'factor'.

P. Fromme (*Max-Volmer-Laboratorium für Biophysikalische Chemie und Biochemie, Technische Universität Berlin, Berlin, Germany*). Are the extrinsic subunits assembled only after C-terminal processing of the D1 protein?

E.-M. Aro. I do not think that the interaction with CP47 will be sufficient to maintain the Mn cluster. I would find it more reasonable that the C-terminus of the D1 protein has to be processed as a prerequisite to the assembly of the Mn cluster and the luminal extrinsic subunits.

I fully agree with you. However, the extrinsic subunits are so easily released during the fractionation steps that we do not have real data on this.

W. Junge (*Abteilung Biophysik, Universität Osnabrück, Osnabrück, Germany*). When the D1 protein is chopped up by protease, are the cofactors, chlorophylls and pheophytins kept in special devices to wait for insertion into newly synthesized D1 or floating around and possibly endangered?

E.-M. Aro. We have only limited information on what happens to the pigments and cofactors during D1 protein turnover. The presence of an inhibitor of chlorophyll or carotenoid biosynthesis (gabaculin or norflurazon, respectively) during the pulse-chase experiments, however, did not hamper the D1 synthesis and assembly processes (see Zhang *et al.* 2000). It is highly probable that the releasing pigments and cofactors are rescued by some other PSII subunits during D1 protein turnover.

Additional reference

Zak, E., Norling, B., Maitra, R., Huang, F., Andersson, B. & Pakrasi, H. B. 2001 The initial steps of biosynthesis of cyanobacterial photosystem occur in plasma membranes. *Proc. Natl Acad Sci USA* **98**, 13 443–13 448.

GLOSSARY

CES: control by epistasy of synthesis

cyt *b*: cytochrome *b*

DTT: dithiothreitol

LHC: light-harvesting complex

LMW: low molecular weight

OEC: oxygen-evolving complex

PSI: photosystem I

PSII: photosystem II

SRP: signal recognition particle

WT: wild-type