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Protein Quality Control in Chloroplasts: A Current Model of D1 Protein Degradation in the Photosystem II Repair Cycle

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The chloroplast originated from endosymbiosis of photosynthetic bacteria. Thus, mechanisms essential for chloroplast biogenesis/homeostasis (protein synthesis, import from cytosol, assembly, and degradation) are predominantly governed by prokaryotic systems. Among these, the quality control system is crucial, because light energy constantly damages photosynthetic proteins and excessive light often limits plant growth by irreversibly inactivating the photosynthetic apparatuses. Here, we overview prokaryotic proteases (FtsH and Deg) which are two enzymes that play critical roles in this system. We particularly focus on Photosystem II (PSII) in thylakoid membranes, which is composed of more than 20 subunits. Among the subunits is one of the intrinsic reaction centre proteins (D1) which is considered to be the target of photodamage. Its rapid and specific turnover suggests that photodamaged D1 is degraded by these proteases and replaced with a de novo synthesized one in a system which is termed the PSII repair cycle. We discuss a current model of D1 degradation which is executed by a concerted action of particular FtsH and Deg isoforms.

Key words: chloroplast, D1 degradation, Deg protease, FtsH metalloprotease, photosynthesis.

Abbreviations: PSII, Photosystem II; LHCII, light harvesting chlorophyll complex; AAA, ATPases associated with various cellular activities.

The chloroplast is one of the distinct organelles that distinguishes plant cells from animal cells. More importantly, it is the place of photosynthesis, in which light energy is captured by pigments and transformed into chemical energy in the form of ATP and NADPH. The resultant formation of chemical energy is then used to synthesize carbohydrate by fixing carbon dioxides through the Calvin–Benson cycle (*1*). Meanwhile, chloroplasts are often subjected to receive excess light energy that ultimately causes oxidative damages in photosynthetic apparatus, implying the importance of a tightly controlled quality control system.

Since chloroplasts are thought to originate from the endosymbiosis of ancestral cyanobacterium, they have several features that are common to prokaryotes. For example, chloroplasts have their own genome and transcription/translation machinery that principally resembles bacterial ones. However, only a limited number of proteins (~80) are encoded by the chloroplast genome, produced within chloroplasts, and a vast majority of the chloroplast proteins (estimated to be more than 3000) are encoded by the nuclear genome (*2*). The genes with a nuclear origin are synthesized in cytosol and finally transported into chloroplasts post-translationally. The efficient formation of protein complexes for photosynthesis thus requires the coordinated control of protein synthesis/import, assembly and quality control. Recent studies in model plants, including *Arabidopsis thaliana*, revealed that extensively characterized prokaryotic proteases from *Escherichia coli* are also present and play important roles in chloroplasts (*3*, *4*). Here we describe our current knowledge on the quality control of chloroplast proteins through these prokaryotic proteases. In particular, we focus on Photosystem II (PSII), which is known to be highly vulnerable to photooxidative damage and requires a sophisticated quality control system (termed PSII repair cycle, Fig. 1).

PHOTOOXIDATIVE STRESS IN CHLOROPLASTS AND PSII REPAIR CYCLE

Chloroplasts are one of the plastid types and are differentiated from a 'proplastid' that is present in shoot stem cells. The colour-less proplastids contain only a few inner structures called thylakoids (sac-like membranous structure). Upon chloroplast development in leaf primordia induced by light, thylakoids are dramatically proliferated by incorporating photosynthetic antenna-, reaction centres-, electron transport-, and ATP synthase-complexes (*5*). The chloroplast is a multicopy organelle and a typical mesophyll cell in mature leaves contains over 100 chloroplasts (*6*). Within each chloroplast, thylakoids are interconnected to each other and are predominantly form

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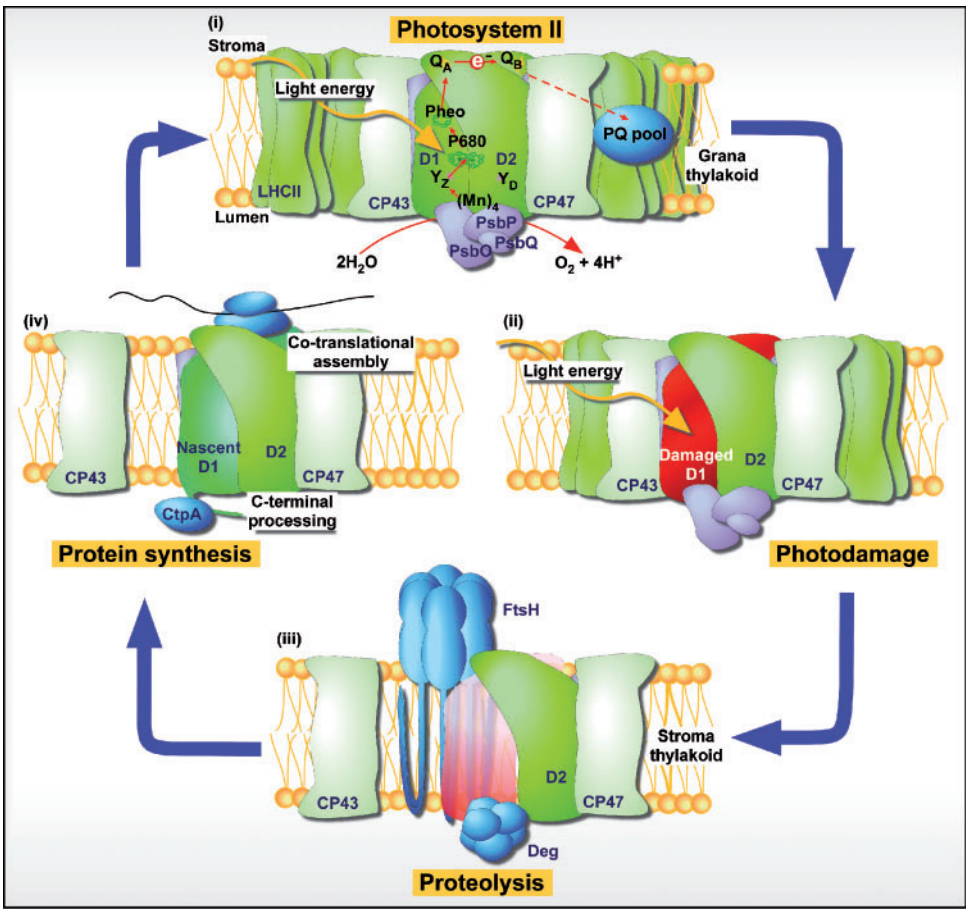

Fig. 1. **Schematic drawing of the PSII repair cycle.** (i) Functional PSII complex. The reaction center proteins (D1 and D2) bind the electron carriers involved in transferring electrons to plastoquinone. (ii) Light-induced D1 damage. Damage to PSII occurs at all light intensities. Damaged PSII migrate to stroma thylakoid and the partial disassembly of PSII occurs. (iii) D1 proteolysis. Damaged D1 protein is degraded by FtsH and Deg proteases. (iv) Synthesis of D1 nascent chain. The new D1 is co-translationally inserted into thylakoid membranes. After C-terminal processing of D1 by CtpA peptidase, repaired PSII migrates to grana thylakoid and forms a functional PSII complex. PsbO, PsbP and PsbQ, the extrinsic proteins involved in stabilization of the Mn cluster; CP43 and CP47, the inner antennae proteins; LHCII, light harvesting chlorophyll complex II; Mn_4, Mn cluster; Y_Z and Y_D, tyrosine; P680, reaction center chlorophyll; Pheo, pheophytin; Q_A and Q_B, plastquinone; PQ pool, plastquinone pool.

stacked structures which are termed grana. Thus, light acts not only as an energy source for photosynthesis, but it also serves as a positive signal for chloroplast development.

At the initial step in photosynthesis, the photonic energy of light can be harvested by pigment-containing antenna complexes that are attached to PSII (light harvesting chlorophyll complex II: LHCII). Excitation energy at the LHCII is then transferred to a chlorophyll molecule in a specific molecular environment P_{680} in the reaction centre of PSII where the primary charge separation takes place between P_{680} and a pheophytin molecule; the primary radical pair thus formed initiates the photosynthetic electron transport and oxidizes water molecules to evolve oxygen on the donor side and reduces plastoquinone on the acceptor side. Consequent formation of a proton gradient between the lumenal side (inside the thylakoid membrane) and the stromal side of chloroplasts (outside the thylakoid membrane) drives ATP synthesis through the ATPase complex (*7*–*9*).

It should be emphasized here that while light energy is the driving force for the photochemical reaction, it always damages the photosynthetic complexes at a certain frequency (*10*,*11*). Light irradiation from natural sun light always fluctuates with clouds and chloroplasts tend to receive excessive light energy that can result in photooxidation. The exposure to photooxidation leads to the irreversible damage in photosystems and consequently has an overall inhibitory effect on plant growth (*10*, *12*). In fact, chloroplasts are one of the major organelles that produce reactive oxygen species in plant cells (*13*).

Among several mechanisms known to minimize such photodamages in photosystems, protein quality control plays an important role as suggested by many experimental systems including cyanobacteria (*12*). These studies proposed a model demonstrating that a major target of photodamage is the D1 protein (*14*, *15*), one of the two proteins constituting the PSII reaction centre (*16*, *17*). This model is based on the observations that (i) D1 protein turnover occurs very rapidly and at a much faster rate

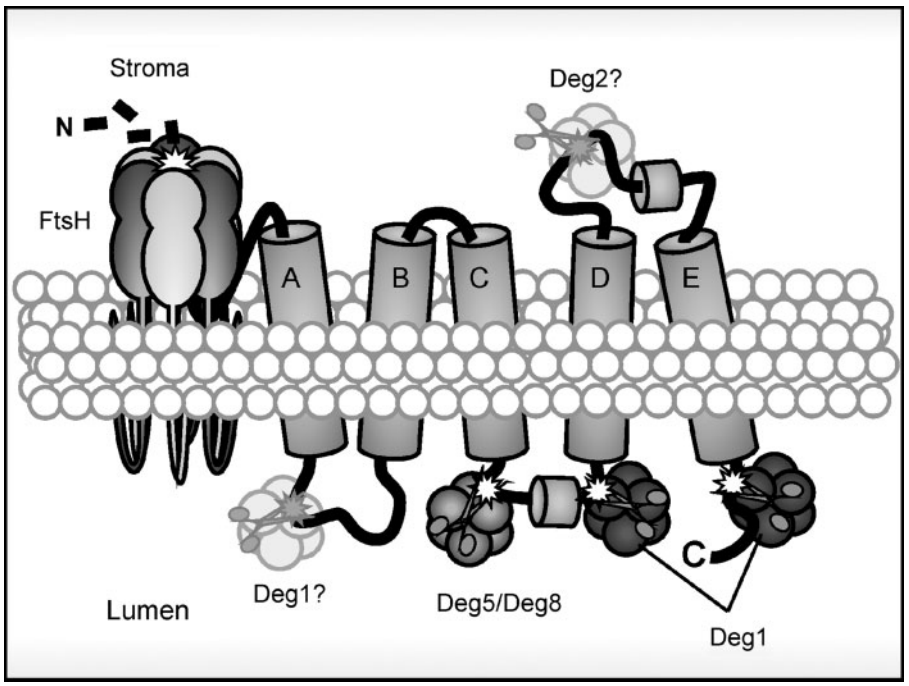

Fig. 2. **A model for proteolytic degradation of D1.** The transmembrane helices (A to E) of the D1 protein and the N and C termini are indicated. The processive proteolysis by FtsH is initiated from the N- terminal end of D1 on the stromal side. The presumed sites of cleavage by Deg proteases in the lumen are shown. The possible cleavage at AB loop by Deg1, and the previously predicted cleavage by Deg2, are indicated by grey colour. In addition to the N-terminal degradation of D1 by FtsH, the cleavages by Deg during high-light irradiation may accelerate the rate of D1 degradation because these small fragments facilitate extraction and proteolysis. FtsH-mediated proteolysis, followed by dislocation of membrane proteins as reported in *E. coli*, and/or other proteases may contribute to the degradation of these small fragments.

than another reaction centre protein (D2), and (ii) PSII is at the initial and thus rate-limiting steps of photosynthetic electron transfer (*10*, *12*, *14*, *15*). The model also predicts that rapid turnover of D1 prevents the entire PSII from being photodamaged. Since PSII consists of more than 20 subunits, functional PSII must be maintained by the so-called PSII repair cycle, where damaged D1 is specifically degraded and replaced with a newly synthesized protein molecule (Fig. 1) (*18*). The damage in PSII is proportional to light intensity (*10*, *11*). Excess light energy beyond the capacity of PSII repair results in photoinhibition and as a consequence, irreversibly damaged D1 proteins accumulate (*10*, *12*). One way to assess photoinhibition in chloroplasts is to measure PSII activity under prolonged exposure to high light. A mutant deficient in the PSII repair cycle exhibits decreased PSII activity under such conditions.

PROTEASES INVOLVED IN D1 DEGRADATION

As mentioned before, D1 turnover is so prominent in chloroplasts that much attention has been paid to identify proteases that are involved in this process. Meanwhile, genomic information gained from studies in model plants has provided us with possible prokaryotic proteases that are localized in chloroplasts. Such identified candidate proteases include ATP-dependent Clp, FtsH, Lon and non-dependent Deg proteases (*3*, *4*). Recent studies in *Arabidopsis* have demonstrated that FtsH and Deg play predominant roles in D1 degradation (Fig. 2).

Degradation by FtsH—FtsH is a thylakoid-membrane bound metalloprotease that belongs to an AAA subfamily of the AAA+ protein super family (AAA denotes ATPases associated with various cellular activities) (*19*, *20*). It contains both ATPase and zinc-binding catalytic protease domains that are encoded within a single polypeptide (*21*, *22*). Structural studies of bacterial FtsHs demonstrate that FtsH forms a hexameric ring structure (*22*). It should be noted that FtsH is essentially encoded by a single gene in *E. coli* and constitutes a homohexamer (*22*, *23*). On the other hand, multiple FtsH isomers are present in photosynthetic bacteria and chloroplasts and constitute a hetero-complex (*24–27*). Genomic database analyses have confirmed that *Arabidopsis* encodes 12 FtsH genes (*28*). Subsequent transient assays with GFP fusion proteins demonstrated that nine FtsHs (FtsH1, 2, 5, 6, 7, 8, 9, 11 and 12) are localized in thylakoid membranes (Table 1) (*28–30*). Predominantly, FtsH isomers (FtsH1, 2, 5 and 8) protease domains topologically face the stromal side of thylakoids (*24*, *31*, *32*). Similar to the *E. coli* FtsH, plant FtsHs are suggested to form a hexameric structure (*24–27*).

Among the chloroplast isomers, FtsH1, 2, 5 and 8 have been shown to represent major isomers constituting the FtsH hetero-complex in thylakoid membranes (*24–27*). So far, these four FtsHs are the only ones detectable by

Table 1. **FtsH and Deg protease isomers in Arabidopsis chloroplasts.**

Name	Location identified[a]	Protease type	Mutant phenotype	Reference
FtsH1	Thylakoid (s)	AAA metalloprotease (type A)	No phenotype	(*28*)
FtsH2	Thylakoid (s)	AAA metalloprotease (type B)	Yellow variegated	(*30–32*)
FtsH5	Thylakoid (s)	AAA metalloprotease (type A)	Yellow variegated	(*28*, *29*)
FtsH6	–	AAA metalloprotease	No phenotype	(*28*, *52*)
FtsH7	–	AAA metalloprotease	–	
FtsH8	Thylakoid (s)	AAA metalloprotease (type B)	No phenotype	(*28*)
FtsH9	–	AAA metalloprotease	–	
FtsH11	–	AAA metalloprotease	Thermosensitive; Dual targeting to mitochondria and chloroplasts; *i*-AAA protease	(*53*, *54*)
FtsH12	–	AAA metalloprotease	–	
Deg1	Thylakoid (l)	Serine protease	No knockouts reported (Lethal?)	(*41*, *45*)
Deg2	Thylakoid (s)	Serine protease	No phenotype	(*42*, *44*)
Deg5	Thylakoid (l)	Serine protease	High-light sensitive	(*43*)
Deg8	Thylakoid (l)	Serine protease	High-light sensitive	(*43*)

[a]l and s indicate lumenal and stromal side of the thylakoid membranes, respectively.

proteome analysis (*33*, *34*) and the precise role of the remaining FtsHs is currently unclear. The FtsH hetero-complex is comprised by two types of isomers: (Type A as represented by FtsH1 and FtsH5, and Type B as represented by FtsH2 and FtsH8) (*24–27*). Importance of the two-types of isomers has been drawn by genetic analysis as follows. A deficiency of FtsH2 or FtsH5 causes leaf variegation (i.e. appearance of leaves with green and white sectors like house plants) and a lack of both isomers enhances variegation (i.e. increase of white sectors) (*29*). In contrast, the lack of both Type A (FtsH1/FtsH5) or both Type B (FtsH2/FtsH8) results in a seedling lethal phenotype which is far more severe than variegation (*26*). It has also been revealed that isomers within the same type are functionally interchangeable, but isomers between the different types are not. Together, it has been demonstrated that at least one isomer from each type appears to be necessary for a stable accumulation of the FtsH complex. Interestingly, the presence of the two-type isomers is well conserved in most land plants and alga (*24*, *35*), suggesting that the hetero-complex is an adaptive form for chloroplasts. One advantage for the hetero-complex seems to be the heterogeneity of the protease domains. Our preliminary results suggest that the abolishment of protease activity in FtsH2 has little effect on plant viability and protease activity. These data suggest that all six protease domains are not necessary in the FtsH complex if provided from either one of the isomers. Stoichiometry and tissue-specific composition of each isomer in the hetero-complex is currently unclear. Most of the isomers appear to be expressed without apparent tissue specificity.

Involvement of FtsH in D1 degradation was initially implicated by indirect evidence that the mutant lacking FtsH2 or FtsH5 not only shows leaf variegation but also exhibits high-light sensitive PSII activities (typical phenotype resulting from photoinhibition) (*29*, *36*). Several *in vitro* experiments also implicated that photodamaged D1 is degraded by FtsH (*37*, *38*). In contrast, *in vivo* evidence is necessary to fulfil the role of FtsH in the PSII repair. Although Bailey *et al*. (*36*) reported attenuation of light dependent D1 and D2 degradation in a FtsH2 deficient mutant, their results were limited due to the use of variegated leaves. We recently showed that non-variegated mutants lacking FtsH2 or FtsH5 apparently impair light-dependent D1 degradation *in vivo* when D1 synthesis is inhibited by lincomycin (D1 is synthesized in chloroplasts). While these data consistently indicate the predominant role of FtsH in PSII repair, residual D1 degradation observed in FtsH2/FtsH5 deficient mutants is also apparent. Here we raise two possibilities that are not mutually excluded: the contribution of other FtsH isomers and/or the presence of proteases other than FtsHs.

Degradation by Deg—Deg is a serine protease and unlike FtsH, it is not ATP-dependent. In addition to the protease domain, Deg is characterized by the presence of PDZ domains that are necessary for protein–protein interactions and recognizing substrate proteins. Previous studies in *E. coli* and human have demonstrated that Deg forms a homo-trimer, and the two trimers associate with each other to form a hexamer (*39*). Database search of Deg homologues demonstrated that Deg proteases comprise a protein family of 16 Deg proteins in *Arabidopsis* (*40*). Immunoblot analyses indicated that four Degs (Deg1, 2, 5 and 8) are present in chloroplasts and are loosely attached to thylakoid membranes (Table 1) (*41–43*).

A characteristic feature of thylakoids is the presence of Degs in both sides of the membrane: Deg1, 5 and 8 are located on the lumenal side and Deg2 is localized on the stromal side (*41–43*). In an earlier study, the stromal Deg2 was suggested to participate in D1 degradation in the PSII repair (*42*). It was reasonable to consider that Deg2 is involved in the initial endo-proteolytic cleavage and subsequently D1 is processively degraded by FtsH. However, this possibility was currently excluded based on the observation that a Deg2-deficient mutant has no effect on PSII photoinhibition (*44*). Thus, Deg2 seems to have little effect, if any, on the PSII repair cycle. Instead, the lumenal Deg1, 5 and 8 proteins are currently believed to participate in the degradation of D1 protein. The possibility of heterocomplex formation among these isomers is currently unclear, but a

pull-down assay using His-DEG5 and His-DEG8 fusion proteins confirmed the interaction between DEG5 and DEG8 (*43*). Knock-out mutants of Deg1 are not available, whereas knock-down by RNAi had a profound effect on chloroplast development. In this Deg1 knock-down line, PSII activity is light sensitive, and interestingly, several degradation intermediates that are derived from the C-terminal part of D1 were decreased in abundance (*45*). Thus, Deg1 appears to play a role in PSII repair but it is fundamentally important for chloroplast development. Supporting this essential function of Deg1 is the observation that the lack of Deg1 results in the concomitant decrease of FtsH and Deg2 protein (*45*). In addition to Deg1, 5 and 8 also appear to play a role in the repair of PSII. The *deg5*/*deg8* double mutant was shown to be viable and is characterized by impairment of D1 degradation when it is exposed to strong light. Under this condition, the double mutant accumulated a very limited amount of a degradation intermediate derived from the N-terminal part of D1 (*43*).

A PROPOSED MODEL OF D1 DEGRADATION IN THE PSII REPAIR CYCLE

Centrally located in PSII, D1 is embedded in thylakoid membranes with five transmembrane domains (Fig. 2) with its N-terminal end exposed to the stromal side (*16*, *17*). Maturation of the D1 protein requires a processing step at the C-terminal end by a novel luminal localized CtpA peptidase (*46*, *47*). Thus, the highly hydrophobic nature of the D1 protein demands coordinated degradation conducted by FtsH and Deg (and possibly other proteases) at both sides of the thylakoid membrane. How do these proteases act on D1 degradation? It should be noted that FtsH is a peptidase that recognizes an end of peptides and is responsible for processive hydrolysis of peptide bonds (*20*). This processive degradation depends on unfoldase activity provided by the ATPase domain. Consequently, these circumstances indicate that FtsH can degrade D1 by itself. In contrast, Deg is an endo-peptidase that cleaves intermembrane peptide regions exposed to the lumenal side (*39*). Thus, complete digestion of D1 is unlikely to occur by Deg alone, and may require additional proteases. Based on these observations, it is strongly suggested that the heteromeric FtsH complex is predominantly involved in D1 degradation in the PSII repair cycle. Deg proteases assist effective D1 degradation by increasing the number of D1 endoproteolytic intermediates that are accessible to FtsH (Fig. 2). This assumption correlates well with the fact that the expression of most Deg isomers is light-dependent, whereas FtsH2/5 genes are constitutively expressed (*33*).

The supplemental role of Deg in the PSII repair is also supported by studies in cyanobacterium *Synechocystis* sp. PCC6803. This photosynthetic bacterium has four genes encoding an FtsH homologue (*27*). One of the FtsH homologues (slr0228) showing highest similarity to *Arabidopsis* FtsH2 plays an important role in PSII repair and in the degradation of photodamaged D1 (*48*, *49*). Whether FtsH is present as a hetero-complex remains unclear at this time. The slr0228 protein appears to physically interact with D1 (*48*). Interestingly, D1 degradation was dramatically attenuated when the N-terminal 20 amino acids were removed from D1 (*50*). These data exemplified that the predominant role of FtsH in PSII repair is shared between *Synechocystis* and *Arabidopsis*. In strong contrast to this observation, knockout of all Deg activities in *Synechocystis* does not interfere with D1 degradation and has little or no effect on PSII repair (*51*). One interpretation of these observations is that a primary pathway of D1 degradation involves FtsH alone through the recognition of photodamaged D1 at its N-terminal end. The evolution of chloroplasts gained an additional and supplementary mechanism by incorporating Deg proteases, because an efficient PSII repair had been indispensable for adapting to strong light conditions. Fragmentation of D1 at the lumenal side may accelerate the processivity of FtsH proteolysis and secure the effective removal of hydrophobic D1 protein (Fig. 2). Likewise, there is a possibility that Deg2 acts on processing D1 on the stromal side, although the lack of Deg2 by itself does not significantly affect PSII photoinhibition (*44*).

To fully understand the quality control of PSII, several questions remain to be answered by future research. First, the recognition of photodamaged D1 proteins by FtsH and Deg needs to be elucidated. Is there any other factor that is involved in this step? So far, none of the proteins interacting with FtsHs have been detected in chloroplasts. Second, the disassembly/reassembly of PSII in the repair cycle needs to be understood and coupled with the degradation mechanism. These processes are mutually associated, since it was shown that partially disassembled PSII complexes substantially accumulate in the mutant lacking FtsH2. Finally, the precise reason for specifically degrading photodamaged D1 in chloroplasts remains unclear and should be evaluated. We assume that the accumulation of PSII intermediates as notably detected in the FtsH2 mutant is highly toxic and consequently results in the formation of reactive oxygen species (based on our unpublished data). It is therefore possible that the rapid protein turnover of D1 is crucial for removing such intermediates.

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CONFLICT OF INTEREST

None declared.

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