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Review

The roles of chloroplast proteases in the biogenesis and maintenance of photosystem II☆

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ABSTRACT

Photosystem II (PSII) catalyzes one of the key reactions of photosynthesis, the light-driven conversion of water into oxygen. Although the structure and function of PSII have been well documented, our understanding of the biogenesis and maintenance of PSII protein complexes is still limited. A considerable number of auxiliary and regulatory proteins have been identified to be involved in the regulation of this process. The carboxy-terminal processing protease CtpA, the serine-type protease DegP and the ATP-dependent thylakoid-bound metalloprotease FtsH are critical for the biogenesis and maintenance of PSII. Here, we summarize and discuss the structural and functional aspects of these chloroplast proteases in these processes. This article is part of a Special Issue entitled: SI: Photosystem II.

1. Introduction

The light-driven photosynthetic reactions in plants and algae take place within four multi-subunit protein complexes in the chloroplast thylakoid membranes, including photosystem II (PSII), the cytochrome b_6f complex, photosystem I (PSI) and the ATP synthase complex [1,2]. PSII catalyzes the light-induced transfer of electrons from water to plastoquinone, leading to oxygen evolution. The PSII complex is composed of more than 20 subunits and associated cofactors, with a reaction center consisting of the D1 and D2 proteins, α and β subunits of cytochrome b559, and the PsbI protein. The D1 and D2 proteins form a heterodimer that binds all the factors for primary charge separation [3]. The PSII core complex also contains CP47, CP43, an oxygen-evolving complex, and at least 13 low-molecular-mass proteins [1,2]. The CP43 and CP47 proteins, two inner chlorophyll a-binding proteins, are closely associated with the PSII reaction center, providing a conduit for excitation energy transfer from the exterior antennae to the reaction center [4]. CP43 is also important for coordinating the CaMn4 cluster involved in water oxidation [5]. In the thylakoid membranes, the active form of the PSII core is present as a dimer that is surrounded by LHCII trimers [1,2,5]. The formation of the final functional PSII complex depends on the tight coordination of synthesis and assembly of all the PSII subunits.

The recently determined PSII structures from plants, cyanobacteria and algae have provided us with substantial knowledge regarding many aspects of the molecular events of PSII function. However, our knowledge of the biogenesis and maintenance of the PSII protein complexes *in vivo* is still limited. It is known that the biogenesis of PSII protein complexes is regulated at the transcriptional, translational and post-translational levels by a considerable number of auxiliary and regulatory proteins [6,7]. In addition, this process also requires the coordination of actions between the nucleus and the chloroplasts because some of the protein subunits of the PSII complex are encoded by the nuclear genome, synthesized in the cytosol and subsequently targeted to the chloroplast via an N-terminal transit peptide [8]. The nuclear-encoded proteins are directed to the stroma and the thylakoid lumen, where they form functional protein complexes by combining with chloroplast-encoded subunits [9–11]. Additionally, the PSII complex is very flexible and responds dynamically to developmental status and environmental stresses [12–14]. For instance, the PSII complex is subject to unavoidable photodamage under all irradiances, which would lead to an inhibition of PSII activity when the irradiance is so high that the rate of damage exceeds the rate of repair [15–17]. To minimize such photodamage to the photosystems, the chloroplasts possess a PSII repair mechanism for the maintenance of PSII function [13,17]. During this PSII repair cycle, many auxiliary and regulatory proteins are required for sequential repair steps including the degradation of photodamaged PSII proteins, *de novo* protein synthesis,

Abbreviations: PSII, photosystem II; CTP, carboxy-terminal processing protease; PSI, photosystem I; LHCII, light harvesting complex II; SPP, stromal processing peptidase

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membrane insertion, folding of the nascent proteins and the reassembly of the released proteins and various cofactors into the reconstituted PSII [13,18,19]. Genetic approaches combined with proteomic analysis have allowed us to identify a few auxiliary and regulatory proteins involved in the biogenesis and maintenance of the PSII complex including assembly factors, chaperones, proteases, translocation proteins, kinases and phosphatases [18,19].

Proteases regulate a wide range of functions throughout the life cycle of the cell. They are involved in the control of the cell cycle, gene expression, differentiation, protein targeting, protein sorting, protein folding, protein quality control and programmed cell death [20]. Because chloroplasts are believed to have originated as endosymbiotic ancestral cyanobacteria, it is not surprising that all chloroplast proteases identified to date are homologs of bacterial proteases [21–23]. Chloroplast proteases are distributed among the chloroplast envelopes, the chloroplast stroma, the thylakoid membranes and the thylakoid lumen [21–23]. Chloroplast proteases identified to date include Clp, FtsH, Lon, DegP, CTP and SPP; the existence of several other chloroplast proteases and peptidases has been inferred from sequence information. These proteases are expected to play important roles in numerous aspects of the biogenesis and maintenance of chloroplasts, ranging from the removal and degradation of signal sequences [24–26] and the maintenance of homeostasis in chloroplasts [27–29] to the degradation of partially assembled complexes or damaged proteins [21,22]. Here, we focus on the proteases involved in regulating the biogenesis and maintenance of the PSII protein complex. More detailed descriptions of the types and functions of chloroplast proteases can be found in the reviews [23,30,31].

2. D1 processing

The D1 protein is encoded by the chloroplast *psbA* gene and translated by thylakoid membrane-bound ribosomes. The D1 protein is synthesized as a precursor form with an extended sequence of 9–16 amino acid residues at its carboxyl terminus [32–34] and cotranslationally or post-translationally inserted into the PSII complex [35,36]. However, only after proteolytic cleavage of the C-terminal extension to produce the mature form of the D1 protein and the subsequent assembly of the catalytic manganese cluster can the binding of extrinsic proteins on the luminal side of the PSII complex proceed [34,37,38]. Processing of the precursor D1 is thus a prerequisite for the formation of a PSII complex capable of splitting water [34,36,37]. In chloroplasts, the processing of precursor D1 is performed by a specific luminal endoprotease, CtpA.

Carboxyl-terminal processing proteases (Ctps) are a relatively newly described group of serine proteases that are responsible for the C-terminal processing of proteins. Although they are found in a broad range of organisms-bacteria, archaea, algae, plants and animals, their physiological functions are poorly understood, compared with the amino-terminal processing of bacterial proteins. CtpA was initially identified in cyanobacteria by the genetic approach [39,40]. The CtpA protein of *Synechocystis* shows significant similarity to the Prc protein, a carboxy-terminal processing protease in *E. coli* [39]. The structure of CtpA in *Scenedesmus obliquus* has been determined to 1.8-Å resolution using the multiwavelength anomalous dispersion method [41]; it is monomeric and composed of three folding domains. The middle domain is topologically homologous to known PDZ motifs and is proposed to be the site at which the substrate C-terminus binds [41]. The CtpA protein has been purified from the chloroplasts of pea, spinach and barley [37,42,43].

A *ctpA* mutant strain of *Synechocystis* had no PSII-mediated water oxidation activity but had normal cytochrome b6f and photosystem I activities [39]. The PSII complexes in the mutant cells had functional PSII reaction centers, but they were unable to accept electrons from water [40]. The D1 protein in the mutant cells was 2 kDa larger than that in wild-type cells due to the presence of a C-terminal extension, which indicated the involvement of CtpA in the D1 precursor processing *in vivo* [40]. Using site-specific mutations at 14 conserved residues of CtpA in the *Synechocystis*, it was shown that five residues (Ser313, Lys338, Asp253, Arg255, and Glu316) are critical for the *in vivo* activity of CtpA [44]. The C-terminal extension of D1 is excised with the sequential two-step processing in *Synechocystis* sp. PCC 6803 [45,46]. However, it is removed by a single proteolytic step in higher plants. A processing intermediate of D1 (termed iD1) was detected in *Synechocystis* sp. PCC 6803, which might originate from cleavage of the D1 precursor after residue Ala352 while this cut is not essential for the final cleavage step after residue Ala344 [46]. The CtpA protease plays a crucial role in forming iD1 as indicated by the analysis of various CtpA and CtpB null mutants [46]. Besides CtpA and CtpB, there might be additional proteases required for D1 maturation because low levels of C-terminal processing still occur *in vivo* in the absence of CtpA and CtpB [45]. An obvious candidate for this proteolytic activity is the Tsp-like carboxyl-terminal protease CtpC [46].

Three putative CtpA homologs have been identified in *Arabidopsis*. The chloroplast protein levels and the formation of thylakoid membrane complexes were similar to those of wild-type plant in an *Arabidopsis* mutant of one gene (*At3g57680*). Accumulation of the D1 precursor was not detected in this mutant [47]. This observation suggests that there may be functional redundancy between the three CTP homologs in *Arabidopsis*, although the roles of two other *Arabidopsis* CTPs in D1 processing remain to be determined.

Recombinant CtpA exhibits a relatively weak affinity for its substrates *in vitro*; thus, it has been suggested that additional components are required to facilitate the processing of the D1 precursor by CtpA [48]. The PratA protein has been implicated in facilitating D1 processing in *Synechocystis* [49,50]. However, no direct interaction between Prat and CtpA has yet been detected, although a direct interaction between PratA and the D1 precursor has been demonstrated [50]. Interestingly, the Psb27 protein was found to be more abundant in the *Synechocystis ctpA* mutant, suggesting a possible role of Psb27 as an assembly factor during PSII biogenesis [40]. Recently, Wei et al. showed that LPA19, a Psb27 homolog, is involved in the processing of the D1 precursor protein in *Arabidopsis*. Chloroplast protein labeling assays have shown that the C-terminal processing of the D1 protein was impaired in an *lpa19* mutant. The LPA19 protein has been shown to interact specifically with the soluble C terminus present in the D1 precursor as well as the mature D1 through yeast two-hybrid analyses [51]. However, the relationship between LPA19 and CtpA remains to be determined.

3. D1 degradation

The reaction center D1 protein of PSII is generally considered to be the main target of photodamage by visible light [13]. The D1 protein has five transmembrane α-helices (designated A to E) connected by stromal and lumenal loops (Fig. 1). Several biochemical studies have suggested that photodamaged D1 protein is cleaved at distinct sites within soluble loops followed by secondary proteolysis of the primary cleavage products [52,53].

3.1. Involvements of the deg proteases

Deg proteases are periplasmic ATP-independent serine proteases that are widely distributed throughout bacteria, Archaea and Eukarya. DegP, one of the Deg proteases, is named after the null mutant phenotype (for *degradation of periplasmic proteins*) in *E. coli* [54]. The DegP protease comprises an N-terminal proteolytic domain featuring a catalytic site of the trypsin type with His–Asp–Ser as the catalytic triad and a C-terminal domain with two PDZ domains [55]. The PDZ domains regulate proteolytic activity and are necessary for the formation of functional oligomeric and protein–protein interactions [56,57]. The crystal structure of *E. coli* DegP had been resolved,

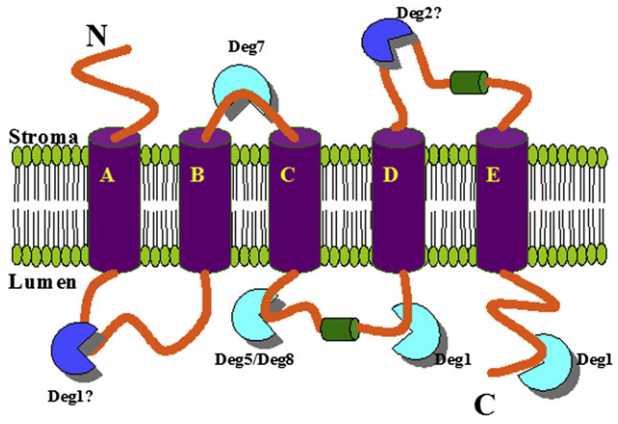

Fig. 1. Schematic representation of the D1 protein and its possible degradation mediated by Deg proteases in *Arabidopsis* chloroplasts. Five transmembrane helices of the D1 proteins, connected by stromal and luminal loops, are marked A to E. The positions of the N and C termini are indicated. The presumed sites of cleavage by Deg proteases in the luminal and stromal loops are shown. The possible cleavage in the AB loop by Deg1 [77] and the previously predicted cleavage in the DE loop by Deg2 [67] are indicated in gray.

revealing that DegP oligomerizes into a hexamer formed by the staggered association of two trimeric rings, with its proteolytic sites enclosed within a central chamber [58]. Recently, the structures of large 12-mer ($DegP_{12}$) and 24-mer ($DegP_{24}$) oligomeric cages have also been elucidated by X-ray crystallography [59] and cryo-electron microscopy [60]. These results indicate that hexameric DegP represents the resting, proteolytically inactive state of the protein, in which the conformation of the active site is completely distorted. It quickly transforms into the proteolytically active multimers 12-mer-Degp and 24-mer-DegP upon the binding of misfolded proteins [59–61]. In *E. coli*, DegP has been demonstrated to be responsible for the degradation of misfolded or otherwise aberrant periplasmic and membrane proteins and is essential for the viability of *E. coli* at a high temperature [54]. DegP has both chaperone activity, predominant at low temperature, and proteolytic activity, present at high temperature [62,63]. However, the switch between proteolytic and chaperon activity of DegP was not dependent on the temperature under some conditions. In fact, DegP protein may act as a chaperone at high temperatures and as a protease at low temperatures as well in *E. coli* [64,65]. This raises a possibility that other factors rather than the temperature regulate the chaperone/protease transition of DegP protein [65]. Similar phenomenon was also observed in plant DegP protease [66].

In *Arabidopsis*, 16 DegP-encoding genes have been identified (denoted *deg1* to *deg16*). Each of the chloroplastic Deg proteases consists of a protease domain at its C-terminus and a PDZ domain at its N-terminus except for Deg5, which has no obvious PDZ domain. Besides the protease and PDZ domains, the long sequences which are not similar to any well known conserved domains are present between the N and C-terminal regions of chloroplast Deg proteins from *Arabidopsis* [67]. Of the 16 Deg proteases, Deg1, Deg2, Deg5, Deg8 and Deg7 have been found to be localized in the chloroplasts by a GFP-fusion assay and proteomic analysis [67–73]. Deg1, Deg5 and Deg8 are peripherally attached to the luminal side of the thylakoid membranes [69–71,73], whereas Deg2 and Deg7 are located on the stromal side of thylakoid membranes [67,72]. Another Deg protease of *Arabidopsis*, Deg 15 is targeted to peroxisomes [74]. Like the Deg proteases in *E. coli*, *Arabidopsis* chloroplast Deg proteins also form protein complexes. Size-exclusion chromatography suggested that recombinant *Arabidopsis* Deg1 was present as a mixture of monomers and hexamers [75]. Blue native gel and pull-down analyses have shown that *Arabidopsis* Deg5 and Deg8 form protein heterocomplexes in the lumen and that the ratio of Deg5 to Deg8 is ~1:1 [73].

An early *in vitro* study suggested that the Deg2 protein cleaved photodamaged D1 protein in the stromal-exposed DE loop to produce N-terminal 23-kDa and C-terminal 10-kDa fragments; the 23-kDa fragment was subsequently degraded [67]. However, an *Arabidopsis* mutant lacking Deg2 had normal D1 protein turnover under high light conditions [76]. This finding suggested that the participation of Deg2 in D1 degradation is not essential *in vivo*. However, analysis of an *Arabidopsis* mutant with a nonfunctional *deg7*, the other stromal-side Deg protease, showed that this mutant was more sensitive to high light stress and that the turnover rate of D1 was reduced compared with the wild-type plant [72]. This study indicated that Deg7 participates in the process of D1 repair. *In vivo* experiments with recombinant Deg7 protein have suggested that Deg7 can indeed catalyze the cleavage of photodamaged D1 protein at the stromal loop connecting the B and C transmembrane helices [72]. The *deg2deg7* double mutant and the *deg7* single mutant showed similar degrees of sensitivity to high light in terms of growth and PSII activity [72]. This result also confirmed that Deg2 seems to have little effect on PSII repair *in vivo*.

The luminal-side Deg proteins, Deg1, 5, and 8, are currently shown to participate in the degradation of the D1 protein [73,77]. Transgenic *Arabidopsis* plants with reduced levels of Deg1 were more sensitive to photoinhibition and accumulated more of the D1 protein, probably in an inactive form. These plants accumulated less of the 16-kDa and C-terminal degradation products of D1 [77]. One of these products, a 5.2-kDa fragment, can also be generated *in vitro* upon the addition of recombinant Deg1 to inverted thylakoid membranes [77]. These results indicate that Deg1 is indeed involved in the degradation of the D1 protein during the process of PSII repair from photoinhibition and that the cleavage sites are located in the CD-loop immediately downstream of transmembrane helix E [77]. Studies with the *deg5*, *deg8* and *deg5deg8* mutants have also demonstrated that Deg5 and Deg8 participate in the degradation of photodamaged D1 protein and are important for efficient turnover of the D1 protein [73]. The *deg5deg8* double mutant showed increased photosensitivity and reduced rates of D1 degradation compared with single mutants of *deg5* and *deg8*. A 16-kDa N-terminal degradation fragment of the D1 protein was detected in wild-type plants but not in the *deg5deg8* mutant following *in vivo* photoinhibition [73]. DegG5 and Deg8 appeared to form a protease complex and to have a synergistic function in the primary cleavage of the CD loop of the PSII reaction center protein D1, producing an N-terminal 16-kDa fragment and a C-terminal 18-kDa fragment [73]. Taking these data into account, a model of our understanding of the degradation of the D1 protein mediated by chloroplastic Degs is illustrated in Fig. 1.

The proteolytic activities of chloroplast Deg proteases have been well documented [67,72,73,77]. However, whether chloroplast Deg proteases also have chaperone activities remains unknown. Recently, experiments by Sun et al. (2010) using recombinant Deg1 protein have demonstrated that chloroplast Deg1 also has chaperone activities, like the *E. coli* ortholog DegP; however, the chaperone activity of Deg1 appears to be associated with its protease domain rather than its PDZ domain [66]. Transgenic plants with reduced levels of Deg1 accumulated normal levels of different subunits of the major photosynthetic protein complexes but reduced levels of PSII dimers and supercomplexes [66]. *In vivo* pulse-chase protein labeling experiments have shown that the assembly of newly synthesized proteins into PSII dimers and supercomplexes was impaired in the Deg1-deficient transgenic lines, although the rate of chloroplast protein synthesis was unaffected [66]. It appears that Deg1 is involved in the regulation of the PSII assembly process. However, this function of Deg1 is related to its chaperone activity rather than its protease activity. In fact, the synthesis rate and steady-state levels of photosynthetic proteins were not altered in these transgenic plants

[66]. Protein overlay assays have provided direct evidence that Deg1 interacts with the PSII reaction center protein D2 [66]. It is likely that Deg1 assists in the assembly of the PSII complex, probably by serving as a specific chaperone for the PSII reaction center D2 protein.

3.2. Involvement of FtsHs

FtsHs are membrane-bound ATP-dependent metalloproteases that belong to a larger family of AAA + proteins. There is only a single FtsH protein in most bacteria, and it is necessary for bacterial growth [78]. The FtsH protein contains one or two transmembrane domains located in its N terminus, an ATPase domain, and a proteolytic domain located in its C terminus [79]. The C-terminal proteolytic domain contains the His-Glu-X-X-His motif characteristic of zinc-dependent metalloproteases [83]. In *E. coli*, FtsH forms a hexameric ring structure like those of other AAA + proteins, and its catalytic sites are located at the interior of the hexamer, as indicated by the analysis of the FtsH crystal structure [80–83].

Chloroplastic FtsH was first identified immunologically from spinach thylakoids and was found to be an integral thylakoid membrane protein [84]. The *Arabidopsis* nuclear genome contains 12 *FTSH* genes. Of the 12 *FTSH* gene products, 9 (FtsH1, FtsH2, FtsH5, FtsH6, FtsH7, FtsH8, FtsH9, FtsH11 and FtsH12) have been found to be targeted to chloroplasts by transient-expression assay with GFP fusion proteins [85], whereas FtsH11 was suggested to be targeted to both chloroplasts and mitochondria [86]. FtsH1, FtsH2, FtsH5 and FtsH8 are the four major isomers of chloroplastic FtsH complexes [87]. Proteomic analysis of isolated thylakoid membranes has shown that these proteins are localized to thylakoid membranes, with their catalytic C-terminal regions facing the stromal side of the membrane [84,85]. Of these FtsH proteins, FtsH2 is the most abundant in chloroplasts, followed by FtsH5 and then by much lower levels of FtsH8 and FtsH1 [88]. The FtsH1 and FtsH8 mutants do not display obvious phenotypes, whereas deficiencies of FtsH2 and FtsH5 result in severe and weak leaf variegation phenotypes [89–91]. It seems that the levels of these four FtsH proteins are correlated with the severity of the phenotypes.

The compositions of chloroplast FtsH complex have been analyzed with the biochemical and genetic approaches. In spinach thylakoid membranes FtsH exists in monomeric, dimeric, and hexameric forms, whereas it is hexameric in PSII [92]. In *Arabidopsis*, FtsH may form heteromeric hexamers, which are made of two different types of monomers. Both the FtsH2 and FtsH5 proteins comigrate on native gels in sucrose gradients and in size-exclusion chromatography [85,87]. Additionally, monospecific antibodies to each of these proteins coimmunoprecipitate the other, suggesting that FtsH2 and FtsH5 are present in a heteromeric complex [85,87]. Comparison of FtsH protein levels in *ftsh2* and *ftsh5* mutants has demonstrated that deficiencies in either FtsH2 or FtsH5 cause a concomitant decrease in levels of the other [85]. In contrast, the leaf variegation of the *ftsh2* mutant was rescued by the overexpression of FtsH8 but not FtsH5, whereas the leaf variegation of an *ftsh5* mutant was rescued by the overexpression of FtsH1 [87,93]. Furthermore, crosses between *ftsh2* and *ftsh8* mutants and between *ftsh5* and *ftsh1* mutants resulted in severe phenotypes including albinism, heterotrophy, disruption of flowering, and severely reduced male fertility [94], although the *ftsh2ftsh5* double mutant remained viable but with severe leaf variegation [90]. These results suggest that a functional FtsH heterocomplex is composed of isomers of type A (FtsH1 and FtsH5) and type B (FtsH2 and FtsH8), although the stoichiometry of each isomer in the heterocomplex remains unclear. The major isoforms representing each type are FtsH2 and FtsH5 while FtsH1 and FtsH8 appear to function as minor isomers in the heterocomplex.

The involvement of FtsHs in D1 degradation has been confirmed by evidence that mutants lacking FtsH2 or FtsH5 are much more susceptible to photodamage [95,96]. A study by Bailey et al. showed that leaves of *ftsh2* mutants were much more susceptible to light-induced PS II photoinhibition than those of wild-type plants. The recovery of *ftsh2* mutants from PS II photoinhibition was also impaired [95]. The D1 protein remained stable in the leaves of the *ftsh2* mutant under photoinhibitory conditions that led to D1 degradation in wild-type leaves [95]. Kato et al. performed *in vivo* D1 degradation assays with nonvariegated leaves of other mutants, *var2 fug1* and *var1 fug1*, in which the leaf variegations in *ftsh2* and *ftsh5* were suppressed, and demonstrated that D1 degradation by FtsH2 and FtsH5 was impaired under conditions of different light intensities [96]. The *ftsh2* mutant accumulated less PSII supercomplex and more PSII intermediate lacking CP43 (termed RC47) than wild-type plants. This evidence suggested that the D1 degradation was impaired at the step of RC47 formation during PSII repair [96]. These results are similar to those obtained for the FtsH (slr0228) of *Synechocystis* species PCC6803 [97,98]. As the exposed N-terminal tail of the D1 subunit is required for rapid D1 degradation in *Synechocystis*, the FtsH-mediated degradation of damaged D1 may proceed from its N-terminal end [99]. Damage to D1 may result in a conformational change in the PSII dimer and a partial disassembly of the PSII monomer, which would allow the entrance of FtsH to RC47 and the degradation of D1 from its N-terminus [96].

3.3. Cooperation of deg and FtsH proteins

Based on early work utilizing *in vitro* studies, it has been suggested that the degradation of photodamaged D1 in chloroplasts is a two-step process involving the initial cleavage of a stromally exposed loop connecting transmembrane helices D and E of D1 by the Deg2 protease followed by the removal of the N-terminal fragment by the FtsH protease [53]. The recombinant FtsH1 protein can only degrade the 23-kDa D1 fragment present in isolated PSII core complexes but not the full-length D1 protein *in vitro* [100]. This evidence suggests that perhaps FtsH protease complexes are mainly responsible for the progressive degradation of substrates from either the free N- or C-terminus followed by the action of Deg or other proteases [53].

Analysis of a *Synechocystis* sp. PCC6803 triple mutant lacking all three Deg proteases has revealed that the rapid and selective turnover of D1 still occurred [101]. This evidence, together with the significant function of the FtsH protease slr0228 (a homolog of FtsH2 in *Arabidopsis*) in PSII repair, led to the proposal that rapid and selective D1 turnover might be mediated by FtsH alone and might not require prior cleavage by a Deg protease [96,102]. In this model, it was suggested that damaged D1 is degraded in a highly processive reaction starting from its N-terminus, but the damaged D1 must initially exist in a destabilized state because FtsH is a weak unfoldase [102]. Oxidative damage to amino acid residues as well as damage to and the loss of cofactors such as pigments and metal ions and partial disassembly of the complex to form the RC47 complex could lead to the destabilization of D1 protein [102].

In *Arabidopsis*, the *ftsh2* mutant exhibited greater sensitivity to high-light treatment than the *deg7* mutant [72]. Taking into account the significant function of FtsH2 in PSII repair in *Synechocystis* [94,95], it appears that FtsH proteases, especially FtsH2, play a crucial role in D1 degradation during PSII repair, rather than acting only in the removal of D1 breakdown products, as proposed in the earlier two-step model. Neither Deg5 nor Deg8 is essential for D1 turnover in *Arabidopsis* under normal growth conditions because the turnover rates of D1 were not affected in *deg5* and *deg8* single mutants or in the *deg5deg8* double mutant [103]. Deg1, Deg5, Deg7 and Deg8 are involved in cleaving the intermembrane peptide regions of D1 exposed to the stromal or the luminal side of the chloroplast membrane. The efficiency of D1 degradation may thus be enhanced by the increased number of D1 degradation intermediates that are accessible by FtsH, facilitating and accelerating the FtsH-mediated degradation of D1.

4. Degradation of other PSII proteins

Although the D1 protein is the primary target of photodamage in the PSII complex, the PSII proteins D2, CP47, CP43, LHCII, the oxygen-evolving complexes and the small PSII subunit PsbH have also been shown to undergo photodamage and replacement in PSII [13,104,105].

Our recent work has shown that Deg7 is involved in the primary cleavage of other photodamaged proteins, including D2, CP47, and CP43 [72]. A 29-kD fragment of D2 is detected after treatment of thylakoid membranes with recombinant Deg7, which indicates that the cleavage site of photodamaged D2 is located at its N terminus [72]. The detection of 19-kD fragments of CP47 and CP43 suggests that the cleavage occurs at the stromal loop connecting transmembrane domains D and E of CP47 and CP43 [72]. The *deg7* mutant is more sensitive to high light stress than the wild-type plants, which suggests that the proteolytically activity of Deg7 is important for maintaining PSII function *in vivo* [72].

The PsbO protein, a 33 kDa extrinsic subunit of photosystem II, is located at the lumenal side of the thylakoid membranes. Chassin et al. demonstrated that recombinant Deg1 is able to degrade full-length expressed PsbO *in vitro* [75]. Li et al. used the transgenic plants with reduced Deg1 to investigate whether the PsbO protein is also the substrate of Deg1 *in vivo*. The transgenic plants accumulated degradation products of the PsbO protein while the levels of full-length PsbO were not affected [106]. Furthermore, the PsbO degradation products could be efficiently degraded by the recombinant Deg1 [106]. These results suggest that Deg1 is involved in the degradation of the PsbO degradation fragments, but not in the initial cleavage event itself.

LHC II is located in the thylakoid membranes and functions in harvesting energy from sunlight and transferring it, in the form of excitation energy, to the PS II reaction center [107]. The degradation of LHCII is highly regulated under various environmental conditions. Under normal growth conditions, the degradation of LHCII is very slow and almost undetectable [108,109]. However, the degradation of LHCII is accelerated upon switching from low to high light or from light to dark and also upon dark-induced senescence [108,110–113]. Under these conditions, different types of proteases may be involved in LHCII degradation. Serine- or cysteine-type proteases of spinach can reversibly bind to stroma thylakoids and degrade nonphosphorylated LHCII monomers, but not trimers, during high-light acclimation [110–113]. A serine-type protease of bean etioplasts has been found to degrade LHC II apoprotein monomers during its assembly with pigments [107]. This protease is attached to the stroma thylakoids and nonappressed thylakoid membranes and activated by Mg^{2+} and light [113]. Using a reverse-genetics approach, Zelisko et al. determined that the chloroplast-targeted protease FtsH6 is responsible for the degradation of LHCII [114]. Lhcb3 was unable to be degraded during dark-induced senescence in the *Arabidopsis ftsh6* mutant. Lhcb1 or Lhcb3 was also unable to be degraded during high-light acclimation in this mutant [114]. EGY1, an ATP-independent metalloprotease in the thylakoid membranes, may also participate in the degradation of LHCII. In the *egy1* mutant, the grana stacks were reduced, and the levels of LHCI and LHCII were significantly decreased [115].

5. Conclusions and perspectives

In this review, we focus on the current knowledge of the chloroplast proteases so far reported to be involved in regulation of PSII functions (Table 1). Deg and FtsH proteases have been assigned functions in the biogenesis and maintenance of the PSII complex, especially in the repair of PSII centers. An interesting question is how Deg, FtsH and possibly other proteases cooperate in the degradation of photodamaged D1 protein. *Arabidopsis* mutants with reduced Deg1 levels also contain less FtsH protease than the wild type, while *ftsh* mutants contain less Deg1. These findings suggest that there is a physical interaction between Deg1 and FtsH in D1 degradation during PSII repair [77]. However, the Deg1 protein is a lumen-exposed protease, whereas FtsH is a stroma-exposed protease. How this cooperation is achieved is an unresolved issue. The roles of specific FtsH and Deg proteases in this process remain to be characterized. In cyanobacteria, D1 degradation is not affected by the absence of the Deg proteases [101]. The mechanism of cooperation between the Deg and FtsH proteases in cyanobacteria thus seems distinct from that in higher plants.

Table 1
Summary of the characterized *Arabidopsis* proteases.

Protein name	Accession	Protease type	Location[a]	References
Processing proteases				
CtpA1	AT3G57680	Serine	L	[47]
Deg proteases				
Deg1	AT3G27925	Serine	L	[66,69,77,106]
Deg2	AT2G47940	Serine	S	[67,76]
Deg5	AT5G39830	Serine	L	[73,103]
Deg7	AT3G03380	Serine	S	[72]
Deg8	AT5G39830	Serine	L	[73,103]
Deg15	AT1g28320	Serine	Per	[74]
FtsH proteases				
FtsH1	AT1G50250	Metallo	T	[85,93,94]
FtsH2	AT2G30950	Metallo	T	[85,89,91,93–96]
FtsH5	AT5G42270	Metallo	T	[85,90,93,94]
FtsH6	AT5G15250	Metallo	T	[85,114]
FtsH7	AT3G47060	Metallo	E	[21,85]
FtsH8	AT1G06430	Metallo	T	[85,93,94]
FtsH9	AT5G58870	Metallo	T	[85]
FtsH11	AT5G53170	Metallo	E M	[85,86]
Other				
EGY1	AT5G35220	Metallo	T	[115]

[a] Abbreviation: L — lumen; S — stroma; T — thylakoid membranes; E — envelop membranes; M — mitochondria; Per — peroxisome.

With the recent advances in chloroplast proteomics analysis and the sequencing of the *Arabidopsis* genome, most (if not all) potential chloroplast proteases have been identified. Although a few chloroplast proteases have been relatively well studied, the functional significance of many of the chloroplast proteases is not yet understood. One current challenge is to identify the substrates of chloroplast proteases and reveal their substrate recognition mechanisms. The recent emerging advances in proteomics and mass spectrometry analysis may help in the further identification of putative substrates [116].

Elucidating the complexity and diversity of chloroplast protease function is another challenge. In contrast to the bacterial proteases that are single-copy, most of the proteases in photosynthetic organisms are present in multiple copies. These proteases usually form large complexes like those in bacteria; however, the compositions and actions of these protease complexes in chloroplasts are distinct from those of bacteria. Thus, the actions and functions of chloroplast proteases have become more complex and diverse during the evolution of photosynthetic organisms. For instance, our work has shown that, unlike DEGP in *E. coli*, *Arabidopsis* Deg1 does not show an apparent temperature-dependent switch between protease and chaperone activities. The contribution of the PDZ domain to the chaperone activity of *Arabidopsis* Deg1 is also different from that of *E. coli* DEGP [66].

The behaviors of chloroplast FtsH complex are also distinct to those of *E. coli*. Unlike the bacterial homohexamers, the chloroplast FtsH in *Arabidopsis* exists as heterohexamers comprising of two types of isomers [85,87,93]. The presence of the two-type isomers is well conserved in the chloroplast of most land plants and alga [117]. Thus it raises an interesting question why the chloroplast has such a heterohexamer complex instead of a homohexamer complex. One

reason may be its advantage on the heterogeneity of the protease domains compared to that of homohexamer complex [117]. Actually, the FtsH homohexamer complex without the protease activity provided by Type B isomers still functions normally in the chloroplast [118]. These protease domains in heterohexamers may be in excess and act redundantly to the function of heterohexamer complex.

The chloroplast contains multiple protease systems, and it is likely that there are a number of proteins that can be degraded by more than one protease system. Conversely, one type of protease can also degrade several types of substrates. Thus, there appears to be a chloroplast protease network with significant functions in chloroplast development and maintenance. Further work in this field will allow for better understanding of the complete chloroplast protease network.

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