AtFtsH6 is involved in the degradation of the light-harvesting complex II during high-light acclimation and senescence

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Degradation of the most abundant membrane protein on earth, the light-harvesting complex of Photosystem II (LHC II), is highly regulated under various environmental conditions, e.g., light stress, to prevent photochemical damage to the reaction center. We identified the LHC II degrading protease in *Arabidopsis thaliana* as a Zn^{2+}-dependent metalloprotease, activated by the removal of unknown extrinsic factors, similar to the proteolytic activity directed against Lhcb3 in barley. By using a reversed genetic approach, the chloroplast-targeted protease FtsH6 was identified as being responsible for the degradation. T-DNA KO *A. thaliana* mutants, lacking *ftsH6*, were unable to degrade either Lhcb3 during dark-induced senescence or Lhcb1 and Lhcb3 during high-light acclimation. The *A. thaliana ftsH6* gene has a clear orthologue in the genome of *Populus trichocarpa*. It is likely that FtsH6 is a general LHC II protease and that FtsH6-dependent LHC II proteolysis is a feature of all higher plants.

membrane protein | photosynthesis | protease

During evolution, cells have developed a complex system of molecular chaperones and proteases to control protein quality and turnover and to prevent protein damage or minimize its adverse effects on cell metabolism. Many factors trigger the degradation of proteins, including changes in environmental conditions, genetic mutations, and limitations to the availability of cofactors. Despite the multitudinous examples of proteolytic processes that take place inside the chloroplasts of higher plants and the necessity of their regulation for cell viability, our knowledge of the biochemical identity of chloroplast proteases, their substrates and physiological significance is, to date, very limited (1). For example, many questions concerning the turnover regulation of the two of the most common proteins on earth [the soluble ribulose-1,5-bisphosphate-carboxylase/oxygenase and the membrane protein LHC II, the apoprotein of the main light-harvesting complex of Photosystem II (PS II)] remain unanswered.

LHC II is located in the thylakoid membrane, where it collects energy from sunlight and transfers it, in the form of excitation energy, to the PS II reaction center. Its structure and function have been studied extensively. The excitation energy transfer within LHC II occurs on a time scale of femtoseconds (2, 3) and depends on the type, orientation, and exact position of the pigments associated with the protein moiety. Crystallographic data have revealed eight chlorophyll (chl) *a*, six chl *b*, two luteins, one neoxanthine, and one violaxanthine in the protein scaffold (4–6). The functional unit of LHC II is a trimer, representing various permutations of Lhcb1–3 apoproteins, each of ≈25 kDa. The genes coding for the three apoproteins are typically found as multiple copies in the genomes of higher plants (7). In *Arabidopsis thaliana*, for example, there are five copies of *lhcb1* and three of *lhcb2*, but only one copy of the *lhcb*3 gene (8). Lhcb1 accounts for ≈60% of the total LHC II apoprotein content, whereas Lhcb2 and Lhcb3 account for ≈30% and 10%, respectively (9). Lhcb1–3 apoproteins are similar enough to form homo- and heterotrimers in various combinations. LHC II apoproteins are highly conserved; thus, they can be expected to have distinct functional roles. Indeed, LHC II populations with different Lhcb1/Lhcb2 ratios differ significantly with respect to their PS II efficiency (10) and cation-dependent thylakoid adhesion-promoting activity (11).

Although the main function of LHC II is energy collection and transfer, it also is involved in the distribution of excitation energy between PS II and Photosystem I (PS I) (12) and in preventing damage to the photosynthetic machinery when there is too much light (13). All of these functions necessitate a very efficient and highly effective regulation of LHC II apoproteins during short- and long-term acclimation to different environments (14–16). However, despite much research, very little is known about the proteases involved in the regulation of LHC II apoproteins. It has been suggested that a light-activated serine/cysteine-type protease is involved in LHC II degradation during bean chloroplast development (17, 18) and in the degradation of LHC II trimers that have preaccumulated in mature bean thylakoid membranes (19). Another serine/cysteine-type proteolytic enzyme has been implicated in the ATP-dependent degradation of LHC II apoproteins during high-light acclimation of spinach plants (14, 20, 21). Both enzymes are claimed to be peripherally attached to the stromal side of the thylakoid membrane. Furthermore, an overexpressed peptide with the N-terminal sequence of pea Lhcb1 has been shown to be degraded *in vitro* by a stromal serine-type glutamyl endopeptidase and by a cysteine-type enzyme attached to the thylakoid membrane (22). It has also been suggested that the SppA protease might be involved in the degradative regulation of LHC II apoproteins (23). However, the LHC II protease(s) have not been conclusively identified.

Recently, we identified a metalloprotease activity responsible for Lhcb3 degradation in barley during dark-induced senescence (24). This integral thylakoid membrane protease has a pH optimum at 7.8, requires either Zn^{2+} or Mg^{2+} ions for activation, and is stimulated by ATP. Although the protease is present in control leaves, Lhcb3 degradation takes place only during senescence, perhaps after removal of as yet unidentified factors on the stromal side of the thylakoid membrane (24). A reversed genetic approach was used here to demonstrate that FtsH6 is a protease involved in LHC II degradation.

Materials and Methods

Plant Material. WT, a mutant lacking FtsH5 (var1), and T-DNA KO mutants lacking FtsH6 (SALK_012429) and FtsH11 (SALK_533047) of *A. thaliana* (var. Colombia) were grown under short-day conditions (8 h light/16 h dark with a 23°C/16°C temperature cycle) at a light intensity of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

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Abbreviations: chl, chlorophyll; HL, high light intensity.

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Mutant seeds were obtained from the Nottingham *Arabidopsis* Stock Centre. Senescence of *A. thaliana* leaves was induced by covering individual leaves with foil, while keeping the rest of the plant under normal light conditions for 2, 4, and 6 days. *A. thaliana* plants were acclimated to elevated irradiance by incubation at 600 μmol·m^{-2}·s^{-1} for 24, 48, and 72 h. Nonsenescing and nonacclimated plants were used as controls. A homozygous mutation in *ftsH5* (*var1*) gives rise to variegated plants. Homozygous mutations were confirmed by using PCR with the forward primer TCT TTC CCT CTC TCC AAC AT and the reverse primer GCT TTT TCG CAA CCT CGT AA for the *ftsH6* deletion mutant, and the forward primer TCT CTC TCT TTA TTT CTC TT and the reverse primer TCT GCC TGT GCT ATC TAA AT for the *ftsH11* deletion mutant. Amplification of the insertion was performed by using the primer GCG TGG ACC GCT TGC TGC AAC T.

***In Vitro* Lhcb1 and Lhcb3 Degradation System.** Thylakoids from fresh, senescing and, acclimated leaves were isolated as described in ref. 25, adding 0.2% BSA to the homogenization and hypotonic buffers. The thylakoids were washed for 30 min by using 0.5 M NaCl, then resuspended at a concentration of 0.6 mg of chl per ml in "optimal incubation medium" (24) and incubated in darkness at 25°C for 0–6 h. To inhibit metalloproteases, thylakoids were incubated for 6 h in the presence of 0.2 mM phosphoramidon.

Determination of Divalent Metal Cations Essential for Proteolytic Activity. Thylakoids were preincubated for 4 h in the presence of 5 mM EDTA at 0°C, then washed in an incubation medium without Mg^{2+} and Na^{2+} ions. After suspension at a concentration of 0.6 mg of chl per ml in an incubation medium containing 0.02 mM Zn^{2+}, they were incubated in darkness at 25°C for 6 h.

SDS/PAGE, Immunoblotting, and Integration Densitometry. SDS/PAGE was performed as described in ref. 26, using 14% acrylamide. For the *in vivo* experiments, samples were loaded at a concentration of 3 μg of chl per lane. In the *in vitro* experiments, the same amount of sample was loaded, corresponding to 3 μg of chl of the time point T0. After electrophoresis, the polypeptides were electrotransferred to nitrocellulose sheets and immunostained with monospecific anti-Lhcb1 or anti-Lhcb3 antibodies, as described in ref. 27. They were made visible by using enhanced chemiluminescence. For quantification of the immunostained bands, the films were scanned by using GEL-PRO ANALYZER 3.2 (Media Cybernetics, Silver Spring, MD). For each individual immunoblot, the range of linearity of the immunoresponse was checked, and only those immunoblots in which the sample dilution factor remained within the range of proportionality in all integrated areas were taken into consideration.

chl Determination. chl concentration was assayed according to the method in ref. 28.

Results

Senescence-Dependent Degradation of Lhcb3. During senescence in barley, Lhcb3 has been shown to be the main proteolytic target of all light-harvesting proteins (29). To compare the degradation of Lhcb3 during senescence in *A. thaliana* to that described for barley, several leaves of *A. thaliana* plants were covered with aluminum foil. During 6 days of dark-induced senescence of the *A. thaliana* leaves, chl disappeared gradually as indicated by yellowing of these leaves (Fig. 1*A*). chl abundance was reduced to 95%, 52%, and 47% of initial levels after 2, 4, and 6 days of senescence, respectively (Fig. 1*B*). Simultaneously, the abundance of Lhcb3 *in vivo* dropped significantly, to 77% and 46% of the initial amount after 2 and 6 days of senescence, respectively (Fig. 1*B*). Loading of the gels was performed according to chl

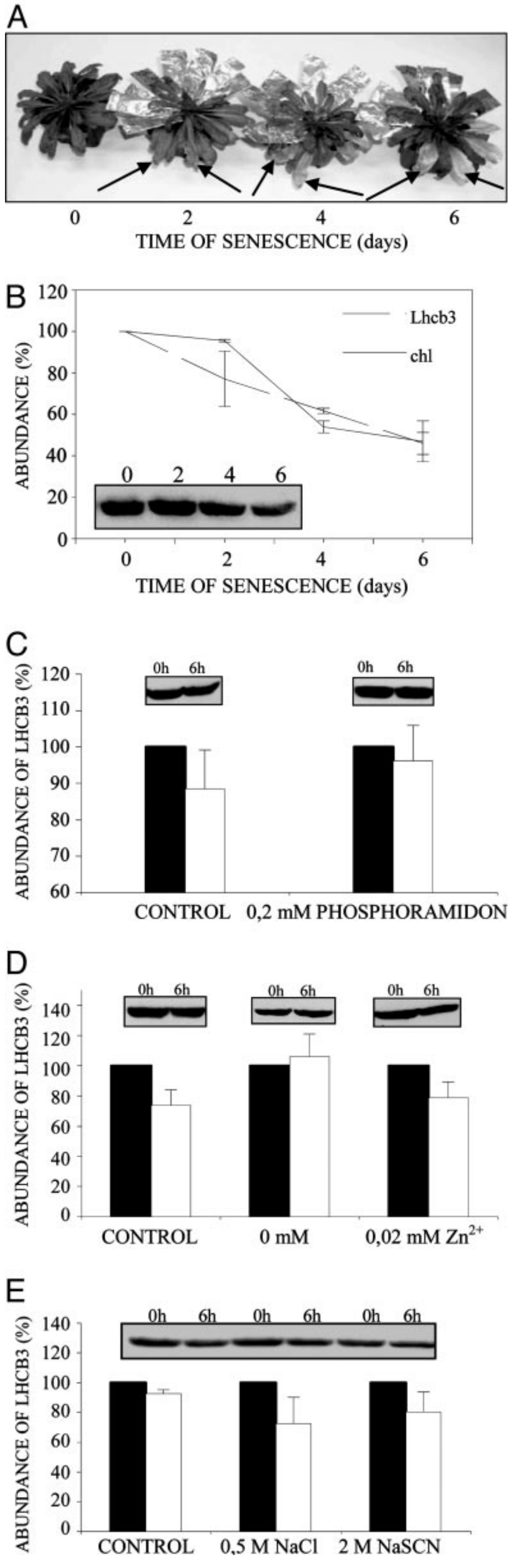

Fig. 1. *A. thaliana* senescence assay. Plants with covered leaves were grown under normal light conditions for 2, 4, and 6 days. They were then photographed after the covers were removed. (*A*) Senescing leaves are marked with arrows. (*B*) Degradation kinetics of chl and Lhcb3 during dark-induced senescence. (*C*–*E*) Effect of phosphoramidon (*C*), endogenous Zn^{2+} ions (*D*), and NaCl (*E*), a factor releasing extrinsic thylakoid membrane proteins on Lhcb3 degradation in thylakoid membranes, isolated from leaves after 2 days of senescence and incubated *in vitro* for 0–6 h. Mean values of four to six experiments are presented. The abundance of Lhcb3 at 0 h was taken as 100%.

amount in the sample; the real Lhcb3 degradation in the leaf on fresh weight basis therefore is more pronounced (only 21% left). Because gels are more precisely loaded on an equal chl basis, we will in the following express LHC II degradation on a chl basis; as a biochemical assay, this would produce the most reliable results. It has to be kept in mind that the true values of LHC II degradation, on fresh weight or per leaf basis, is larger.

To study the senescence-induced degradation of Lhcb3 further, we used the *in vitro* system for incubating thylakoids isolated from senescing barley leaves that is described in ref. 24. When thylakoids from *A. thaliana* leaves that had been senescing for 2 days were isolated and incubated *in vitro* for 6 h, a fraction of Lhcb3 was found to have been degraded (data not shown). To confirm that, in *A. thaliana* leaves, the Lhcb3-degrading protease is a zinc-dependent metalloprotease, integral to the thylakoid membrane, thylakoids were incubated *in vitro* in the presence of phosphoramidon, an inhibitor of metalloproteases. This treatment inhibited two-thirds of the Lhcb3 degradation (Fig. 1*C*), confirming that the Lhcb3 reduction in senescing *A. thaliana leaves* is due to degradation by a metalloprotease.

To identify the metal essential for the activity of the *A. thaliana* Lhcb3-degrading protease, thylakoids isolated from senescing leaves were preincubated in the presence of EDTA to remove divalent metal cations. After removal of EDTA, the thylakoids were incubated either with or without Zn^{2+} (Fig. 1*D*). In the absence of the metal, no anti-Lhcb3 proteolytic activity was observed; however, Zn^{2+} ions were able to restore Lhcb3 degradation activity to levels similar to the control. To determine the location of the Lhcb3-degrading protease in the thylakoid, membranes isolated from senescing leaves were washed with NaCl (0.5 M) to release extrinsic thylakoid membrane proteins. Washing of thylakoids caused a 4-fold acceleration of Lhcb3 degradation, indicating that the degrading protease was neither removed nor inactivated (Fig. 1*E*) and that it is integral to the thylakoid membrane. However, other factors/proteins, shielding this protease, might have been removed by the salt wash, causing enhanced activation of the protease. Taken together, these data show that the protease degrading Lhcb3 during senescence of *A. thaliana* leaves is similar to the protease found in barley leaves (24).

Degradation of Lhcb1 During Acclimation to High Light Intensity (HL). To determine whether the senescence-related metalloprotease degrading Lhcb3 was responsible for degradation of LHC II under other conditions as well, proteolysis of Lhcb1 during HL acclimation was investigated. Lhcb1 is the LHC protein that most rapidly is degraded during HL acclimation (16). *A. thaliana* leaves respond to HL conditions by a gradual loss of chl; the chl content decreased to 79%, 78%, and 66% of the initial value after 24, 48, and 72 h of HL acclimation, respectively (Fig. 2*A*). The decline in chl was accompanied by the disappearance of Lhcb1, its abundance on chl basis dropping to 76%, 62%, and 44% of the initial value after 24, 48, and 72 h, respectively (Fig. 2*A*), corresponding to 60%, 48%, and 29% on fresh-weight basis.

When thylakoids were isolated from *A. thaliana* leaves acclimated to HL for 24 h and incubated *in vitro* for 6 h, 31% of Lhcb1 was found be degraded (Fig. 2*B*). Phosphoramidon inhibited this reduction; only 5% of Lhcb1 were degraded in its presence (Fig. 2*B*). Although the absence of Zn^{2+} inhibited the reduction in Lhcb1 levels, degradation resumed after reincubation of thylakoids in the presence of Zn^{2+} (83% of initial value, 77% in the control) (Fig. 2*C*). Thus, HL acclimation-related Lhcb1 reduction is, like senescence-induced Lhcb3 degradation, caused by a Zn^{2+} metalloprotease. As demonstrated for Lhcb3, Lhcb1 degradation was accelerated after washing the thylakoids with NaCl (Fig. 2*D*), supporting the notion that the degrading protease is an integral membrane protein, which is shielded by extrinsic factors.

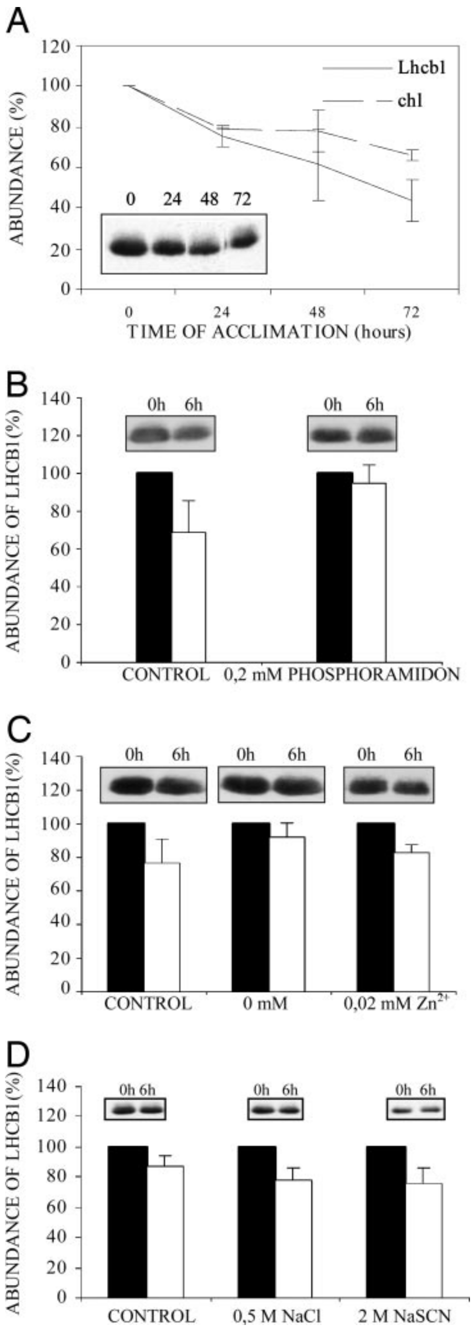

Fig. 2. Lhcb1 degradation during acclimation to elevated irradiance. *A. thaliana* plants were grown in HL (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 24, 48, and 72 h, and the following phenomena were monitored. (*A*) Degradation kinetics of chl and Lhcb1 during acclimation to HL. (*B–D*) Effect of phosphoramidon (*B*) and of endogenous Zn^{2+} ions (*C*) and NaCl (*D*), a factor releasing extrinsic thylakoid membrane proteins on Lhcb1 degradation in thylakoid membranes, isolated from leaves acclimated to HL for 24 h and incubated *in vitro* for 0–6 h. Mean values of four to six experiments are presented. The abundance of Lhcb1 at 0 h was taken as 100%.

Identification of the Protease Involved in Degradation of Lhcb1 and/or Lhcb3. Based on the biochemical properties of the Lhcb1 and Lhcb3 degrading protease, suitable candidates for this enzyme are products of *A. thaliana* genes coding for chloroplast-targeted homologs of eubacterial FtsH protease and transmembrane processing metallopeptidases. Both of these types of proteins are zinc-dependent proteases, integrally associated with the thylakoid membrane (30, 31).

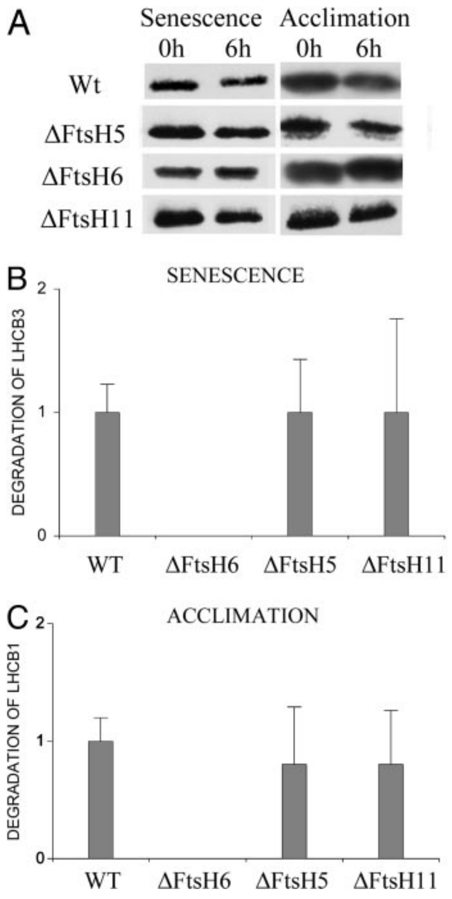

Fig. 3. Degradation of Lhcb proteins in WT and FtsH5, FtsH6, and FtsH11 mutants. (*A*) Degradation of Lhcb proteins in WT and FtsH5, FtsH6, and FtsH11 mutants. (*B*) Degradation of Lhcb3 in thylakoid membranes isolated from senescing leaves. (*C*) Degradation of Lhcb1 in thylakoid membranes isolated from leaves acclimated to HL. Values represent the means of four to six experiments. The degradation of proteins (Lhcb1 or Lhcb3) in WT was taken as 1.

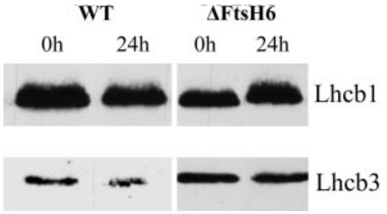

Fig. 4. *In vivo* degradation of Lhcb1 and Lhcb3 during HL acclimation in WT and FtsH6 mutant. The abundance of proteins was analyzed in thylakoids isolated from control leaves (0 h) as well as leaves acclimated to HL (600 μmol·m^{-2}·s^{-1} for 24 h) by using antibodies directed against Lhcb1 and Lhcb3, respectively.

In *Escherichia coli*, FtsH is a membrane-bound, ATP-dependent metalloprotease that belongs to the AAA-protein family (32). In the genome of *A. thaliana* there are 12 genes encoding FtsH proteases, products of 9 of which have proved to be located in the chloroplast (33). FtsH1, FtsH2, FtsH5, and FtsH8 have been identified as integral thylakoid proteins, oriented so that their ATP-binding domain and catalytic zinc-binding site face the stroma (30, 34).

Using a reversed genetic approach, we examined whether a chloroplast-targeted FtsH is involved in LHC II degradation. Because FtsH1, FtsH2, FtsH5, and FtsH8 have partially overlapping functions, we tested a loss-of function mutation of one of these proteases, FtsH5. We also identified *A. thaliana* T-DNA KO mutants for *ftsH6* and *ftsH11*; seeds from these lines were germinated and plants selfed to obtain plants homozygous for the mutation. Thus, homozygous plants lacking FtsH5 (var1, At5g42270), FtsH6 (At5g15250), and FtsH11 (At5g53170) were obtained. These mutants, as well as WT plants, were grown under standard conditions, and two LHC II protease assays were performed. ΔFtsH6 and ΔFtsH11 plants did not show any visible change in the plant phenotype [not in growth, development, or pigmentation (data not shown)]; however, lack of the *ftsH5* gene product caused, as described in ref. 35, leaf variegation. Using the LHC II protease assay, var1 (ΔFtsH5) as well as ΔFtsH11 mutants were able to degrade Lhcb3 during senescence (Fig. 3 *A* and *B*) and Lhcb1 during acclimation (Fig. 3 *A* and *C*). The minor impact on Lhcb1 degradation in the ΔFtsH5 mutant during high-light acclimation (only 80% of Lhcb1 is degraded compared with WT) is consistent with its proposed role in degrading photodamaged subunits in PS II (36). Although the role of FtsH11 is not known, our data show that it has no major function in LHC II degradation. However, in the ΔFtsH6 mutant, the level of Lhcb3 did not change during senescence (Fig. 3 *A* and *B*). Clearly, therefore, FtsH6 is involved in Lhcb3 degradation. In addition, the level of Lhcb1 did not decline in the ΔFtsH6 mutant during HL acclimation (Fig. 3 *A* and *C*). Therefore, we conclude that the FtsH6 protease is involved in both senescence-induced degradation and HL acclimation, and that it degrades both Lhcb3 and Lhcb1 during these processes.

If FtsH6 is a key protease for LHC II degradation during senescence and acclimation, the decrease in chl content during senescence and HL acclimation should, as a consequence, be retarded in the ΔFtsH6 mutant. Indeed, when chl levels were measured after 6 days of dark-induced senescence, WT plants had lost 53% of their chl, whereas ΔFtsH6 plants had only lost 33%. Similarly, 6 days of HL acclimation resulted in a 34% loss of chl in WT plants but only a 20% loss in ΔFtsH6 mutants. The loss of chl in the absence of FtsH6 is due to degradation of other chl-binding proteins (LHCI proteins and the chl *a* binding proteins of PS I and PS II).

To study the degradation of Lhcb proteins *in vivo*, WT and FtsH6 mutant plants were subjected to HL for 24 h. Immunolabeling on thylakoids isolated from these leaves shows degradation of Lhcb1 as well as Lhcb3 in WT, whereas both these proteins resist degradation in ΔFtsH6 plants (Fig. 4). The apparent accumulation of the LHCII apoproteins is due to loading of the SDS/PAGE on chl basis.

Given its important role in LHC II turnover, the FtsH6 protease should have been conserved during evolution. We identified the genes coding for FtsH proteases in the recently sequenced genome of *Populus trichocarpa* (http://genome.jgi-psf.org/Poptr1/Poptr1.home.html). By comparing the FtsH gene families of *A. thaliana* and *Populus* (12 vs. 16 genes), we were able to identify a clear FtsH6 orthologue in *Populus* (fgenesh4_pg.C_LG_XVII000398; M.G.-L., S.J., and C.F., unpublished work). The genes coding for FtsH6 in the annual plant and the tree showed a 78% similarity at the protein level. This resemblance is reflected in the overall sequence comparison (data not shown). The ATP-binding motifs, the zinc-binding motif, and the second region of homology, which are essential for proteolytic activity of the enzyme, are extremely conserved. Furthermore, the conserved lumenal domain identified in ref. 37 was found in FtsH6 of both *A. thaliana* and *Populus*. This sequence seems to be restricted to FtsH homologues from organisms performing oxygenic photosynthesis and in *A. thaliana* is present in FtsH2 (VAR2), FtsH8 (Chr1), and FtsH6 (Chr5) (37).

Because of a lack of a specific anti-FtsH6 antibody, we were not able to study the expression of this protease *in vivo* under senescence or high-light acclimation. Therefore, to obtain more information about the expression characteristics of the *A. thaliana* FtsH6 gene, we investigated the public DNA microarray data available at www.genevestigator.ethz.ch. It appears that the FtsH6 protease is mainly expressed in leaves, although its expression level is rather low. Expression is not restricted to HL conditions or senescence, although in older and senescing leaves the expression seems to be higher than in young leaves. The highest expression was found in developing rosettes. Furthermore, the expression has been shown to be up-regulated during ethylene treatment, zinc treatment, senescence, heat stress, and osmotic or salt stress, but no change has been observed during cold stress.

Discussion

We report here that FtsH6, a metalloprotease belonging to the FtsH family, is involved in the degradation of Lhcb1 during HL acclimation and Lhcb3 during dark induced senescence. It seems likely that FtsH6 is a general LHC II protease that degrades various LHC II apoproteins when they are not needed, not just during HL acclimation and senescence. Because the biochemical features of the protease in barley and *A. thaliana* are similar and the *A. thaliana ftsH6* gene has such a clear orthologue in *Populus*, we believe that FtsH6-dependent LHC II proteolysis is a feature of all higher plants.

A phylogenetic and biochemical analysis of the *A. thaliana ftsH* genes indicates the existence of four pairs of closely related genes: *ftsH1* and *ftsH5*, *ftsH2* and *ftsH8*, *ftsH7* and *ftsH9*, and the mitochondrial *ftsH3* and *ftsH10* genes (38). The FtsH6 sequence is related to the “VAR1/VAR2” group (consisting of the FtsH1, -2, -5, and -8 proteins), whose members have partially redundant functions and are involved in the biogenesis of chloroplasts and the degradation of photodamaged PsbA protein (33, 37–42). From an evolutionary perspective, it seems likely that PsbA degradation was the original function of FtsH6 and that, after ancient gene duplication, the FtsH6 gene was neofunctionalized and evolved to recognize other substrates, namely LHC II apoproteins. The set of loss-of-function mutants now available makes it possible to examine whether FtsH6 affects other photosynthesis-related proteins; this is quite likely because other chloroplast proteases seem to act on several substrates. Clp protease, for example, degrades both PsbO (43) and polypeptides of the cytochrome b_6f complex (44), whereas DegP1 protease degrades both plastocyanine and PsbO (45). Similarly, the chloroplast processing enzyme (CPE) removes transit peptides of numerous chloroplast polypeptides (46).

It should be noted that our data do not directly prove that LHC II apoproteins are the substrates of FtsH6 *in vivo*. Although this is the simplest explanation of our data, it is still possible that FtsH6 acts on another substrate, somehow activating the “real” LHC II protease. It is also possible that FtsH6 cooperates with other FtsH proteases, coexisting with them in an enzymatic heterocomplex; the occurrence of heterocomplexes comprising FtsH2 and FtsH5 has been demonstrated experimentally (33). FtsH6 may cooperate with members of other families of chloroplast proteases as well; according to current thinking, the proteolysis of photodamaged PsbA is an example of the cooperative action of proteases belonging to different families. It has been suggested that DegP2 (a member of the DegP/HtrA family of serine proteases) performs the primary cleavage event, after which the two breakdown products are removed by one or more members of the FtsH VAR1/VAR2 group (reviewed in ref. 1). A similar scenario might also describe the degradation of LHC II apoproteins.

Several researchers have reported other protease activities involved in LHC II degradation *in vivo* and *in vitro*. These proteases are localized peripherally at either the stroma thylakoid membranes (19) or at the outer surface of the grana thylakoids (14, 20, 38). Their main substrate during chl deficiency conditions (17, 18) and during acclimation to HL (14, 20, 38) was found to be the LHC II monomer. LHC II trimer (19) or LHC II N-terminal peptide (22) preaccumulated in mature chloroplasts. Some of these protease activities may cooperate with FtsH6 in the degradation of Lhcb1 and Lhcb3. In *E. coli*, FtsH was found to be able to degrade both soluble and membrane proteins, initiating proteolysis at either the N terminus or C terminus (47). The substrate must be partially unfolded, because FtsH lacks a robust unfoldase activity (48). A feature of the energy-dependent proteolytic reaction is that it occurs in a progressive manner along the polypeptide chain (49). It is, therefore, also possible that during LHC II degradation, a (serine- or cysteine-type) protease cleaves Lhcb1 or Lhcb3 before FtsH6 continues the degradation. However, the kinetics of FtsH6-catalyzed, HL-dependent degradation of LHC II seem to be quicker than those of the activity reported in ref. 20, in which was observed ATP-dependent degradation of the LHC II monomer only after a lag phase of 48–72 h. These data suggest that FtsH6-mediated proteolysis is the primary step, but it is also possible that there are multiple proteolytic activities that can act on LHC II. If, in the future, mutants lacking other proteolytic activities are isolated, the relative importance of these proteases in LHC II degradation can be addressed.

It has been found that the nonphosphorylated form of LHC II is the substrate for degradation (14), and that loss of pigments destabilizes the apoprotein (50). The N-terminal domain of LHC II was found to be essential for recognition of the protease (21). These findings are consistent with our earlier data showing that the protease degrading Lhcb3 is present in sufficient quantities even under conditions where LHC II is stable, but either the substrate or protease has to be activated in some way (24). Enhanced degradation by FtsH6 has been shown after salt-washing of the thylakoid membrane. The extrinsic proteins, shielding the stromal part of FtsH6 (or Lhcb1 and -3), might be the regulating factors. Their removal, either by dissociation or by another protease, might trigger LHC II degradation. *In vivo*, this trigger must in some way sense the “surplus” of antenna size. Both our data (24) and those of other authors (14, 20, 21) indicate that the substrate availability (or structure), rather than the presence of the protease *per se*, determines the proteolytic rate in chloroplasts.

Because of the lack of a specific anti-FtsH6 antibody, we were unable to directly investigate the expression of FtsH6 in WT plants during HL acclimation or senescence. According to published data, the expression level of FtsH6 is low, both at the mRNA and protein level (51), in plants grown under normal conditions. It has even been suggested that it is a pseudogene (38). Interestingly, ΔFtsH6 mutants have previously been analyzed and found not to be impaired in their recovery from photoinhibition *in vivo* (33). We show in this study that FtsH6 is involved in the degradation of LHC II, during both HL acclimation and senescence. The protease appears to be constitutively present in small amounts in the thylakoid, apparently not increasing in quantity during conditions when LHC II degradation occurs. It is possible that this is a general theme in chloroplast proteolysis; perhaps the turnover of many chloroplast proteins is mainly determined by the structure of the substrate, rather than the availability of the protease itself. Further studies with mutants lacking the different chloroplast proteases may verify this hypothesis.

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