A Critical Role for the Var2 FtsH Homologue of Arabidopsis thaliana in the Photosystem II Repair Cycle in Vivo*

Received for publication, June 25, 2001, and in revised form, November 1, 2001

Published, JBC Papers in Press, November 20, 2001, DOI 10.1074/jbc.M105878200

Shaun Bailey‡, Elinor Thompson§, Peter J. Nixon‖, Peter Horton‡, Conrad W. Mullineaux‡, Colin Robinson‡, and Nicholas H. Mann‡**

From the ‡Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, the §Department of Biology, University College London, Darwin Building, Gower Street, London WC1E 6BT, the ‖Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, and the ‡Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2TN United Kingdom

Using a var2-2 mutant of Arabidopsis thaliana, which lacks a homologue of the zinc-metalloprotease, FtsH, we demonstrate that this protease is required for the efficient turnover of the D1 polypeptide of photosystem II and protection against photoinhibition in vivo. We show that var2-2 leaves are much more susceptible to light-induced photosystem II photoinhibition than wild-type leaves. Furthermore, the rate of photosystem II photoinhibition in untreated var2-2 leaves is equivalent to that of var2-2 and wild-type leaves, which have been treated with lincomycin, an inhibitor of the photosystem II repair cycle at the level of D1 synthesis. This is in contrast to untreated wild-type leaves, which show a much slower rate of photosystem II photoinhibition due to an efficient photosystem II repair cycle. The recovery of var2-2 leaves from photosystem II photoinhibition is also impaired relative to wild-type. Using Western blot analysis in the presence of lincomycin we show that the D1 polypeptide remains stable in leaves of the var2-2 mutant under photoinhibitory conditions that lead to D1 degradation in wild-type leaves and that the abundance of DegP2 is not affected by the var2-2 mutation. We conclude, therefore, that the Var2 FtsH homologue is required for the cleavage of the D1 polypeptide in vivo. In addition, we identify a conserved luminal domain in Var2 that is unique to FtsH homologues from oxygenic phototrophs.

The Photosystem II (PSII)† complex is a large protein-pigment assembly that catalyzes the light-dependent oxidation of water to molecular oxygen in chloroplasts and cyanobacteria. At the core of PSII lies the D1/D2 heterodimer, which binds the pigments and co-factors necessary for primary photochemistry (1). The D1 polypeptide is also important because of its high rate of turnover (2). This high turnover rate is related to the vulnerability of PSII to light, with D1 being the main target for photoinactivation and subsequent damage. An efficient repair cycle for D1 is therefore of paramount importance in oxygenic phototrophs. When the rate of photoinactivation and damage of D1 exceeds the capacity for repair, photoinhibition occurs, resulting in a decrease in the maximum efficiency of PSII photochemistry.

A key feature of the D1 repair cycle is the degradation of the damaged polypeptide. It is generally accepted that damaged D1 is initially cleaved at a site on the stromal loop between transmembrane helices D and E yielding a 23-kDa N-terminal fragment (3) and a 10-kDa C-terminal fragment (4). This cleavage step is believed to be initiated by structural changes within the D1 polypeptide (5), although the precise nature of the cleavage event remains unclear. One proposal is that the action of active oxygen species acts to cleave the D1 polypeptide during strong illumination (6). However, the temperature dependence of the process (7) and its sensitivity to protease inhibitors (8) indicates the involvement of enzymatic proteolysis by an unidentified protease. Following cleavage, the breakdown fragments of D1 are rapidly degraded and the PSII complex reassembles with the co-translational integration of a newly synthesized polypeptide.

Several proteases have been identified in photosynthetic organisms (reviewed in Ref. 9), and a number of studies have addressed the possibility that one or more may be involved in D1 turnover/assembly. One such protein is the stromal DegP2 protease, which has been shown to cleave D1 in in vitro assays (10). Another protease implicated in D1 turnover, following in vitro analysis, is the thylakoid FtsH homologue, FtsH1 (11). In Arabidopsis thaliana FtsH1 has been shown to degrade the 23-kDa breakdown product of D1 in isolated thylakoid membranes and purified PSII core complexes. Both studies are, however, limited in that the analysis was carried out in vitro, and the true in vivo role of both DegP2 and FtsH1 remains unclear.

The FtsH protease belongs to the AAA (ATPases associated with a variety of cellular activities) protein superfamily whose members are widely distributed among prokaryotes and eukaryotes. They are involved in a number of diverse cellular functions, including organelle biosynthesis, transcriptional regulation, membrane fusion, and proteolysis (reviewed in Ref. 12). All AAA family members are characterized by the presence of one or two highly conserved ATPase domains containing Walker A and B ATPase motifs. FtsH is further characterized by the presence of a zinc-metalloprotease motif (reviewed in Ref. 13).

The ftsH gene was first identified in Escherichia coli (14), where it encodes a 71-kDa polypeptide involved in various functions, including protein degradation (15, 16). FtsH-related homologues have also been implicated in protein degradation in eukaryotic organelles, for example in yeast mitochondria.
Recently, we have demonstrated a role for FtsH in photosynthesis following the disruption of a gene encoding an FtsH homologue in the cyanobacterium *Synechocystis PCC 6803*. The mutant strain, designated slr0228::Ω, was shown to be impaired in the maintenance of photosystem I (PSI), with levels decreased by 60% relative to wild-type (19). Furthermore, the slr0228::Ω mutant shows enhanced PSI photoinhibition due to a decrease in the rate of D1 degradation. The Slr0228 FtsH homologue of *Synechocystis* is closely related to Var2, a second homologue of FtsH that has recently been identified in *Arabidopsis* (20, 21). Mutations in the var2 locus of *Arabidopsis* give rise to extensive leaf variegation due to impaired thylakoid membrane biogenesis. Green sectors in the var2 mutants, however, contain morphologically normal chloroplasts despite lacking the Var2 homologue. This phenomenon gave us the opportunity to assess the involvement of Var2 in D1 turnover using *in vivo* assays of PSI photoinhibition and D1 degradation in whole leaves.

In the present study we identify a unique conserved lumenal domain in Var2 that is not shared by *Arabidopsis* FtsH1 but is common to FtsH homologues from a diverse range of oxygenic phototrophs, including Slr0228 from *Synechocystis*. In addition we find that, in the absence of Var2, PSI photoinhibition is more extensive due to a critical role for the FtsH homologue in the primary cleavage of the D1 polypeptide. This report therefore identifies for the first time a chloroplast protease that is involved in *in vivo* protection of plants from photoinhibition.

**MATERIALS AND METHODS**

**Plant Material**—Wild-type seeds of *A. thaliana* (L.) Heynh. cv. Columbia and the var2-2 mutant were grown under a long photoperiod (16 h light, 8 h dark). A growth irradiance of 100 μmol m⁻² s⁻¹ was provided by fluorescent tubes in a Fi-totron growth chamber, model 600G3/THTL (Fisons, Loughborough, UK). Temperature was maintained at 20 °C.

**Light and Lincomycin Treatment of Leaves**—Detached leaves were floated adaxial side up on water with the temperature regulated at 20 °C. Irradiance of either 300 or 1800 μmol m⁻² s⁻¹ was provided through fiber optics fed via a Schott lamp (Schott Glass Ltd., Stafford, UK) and filtered for heat using a Calflex C filter.

Chloroplast-encoded protein synthesis was blocked using lincomycin. Detached leaves were incubated with their petioles submerged in 1 ml solutions of lincomycin at an irradiance of 20 μmol m⁻² s⁻¹ for 3 h prior to photoinhibitory light treatment. The temperature was maintained at 20 °C during incubation.

**Fluorescence Measurements**—Room temperature chlorophyll fluorescence was measured using a PAM 101 fluorometer (Heinz Waltz, Effeltrich, Germany). All measurements were made at 20 °C with saturating CO₂. Photoinhibition was assayed by calculating the ratio of maximum to variable fluorescence (Fv/Fm) as a measure of the maximal photochemical efficiency of PSII. In all experiments Fv/Fm was determined initially following dark adaptation overnight at ~5 μmol m⁻² s⁻¹. Following photoinhibitory light treatment, either in the presence or absence of lincomycin, leaf disks were dark-adapted for 15 min prior to Fv/Fm measurement to allow for the relaxation of rapidly reversible fluorescence quenching components.

The fast-relaxing, energy-dependent component of non-photochemical quenching, qE (22), and the photochemical quenching parameter qP (23) were calculated following 1 h of actinic illumination at either 300 or 1800 μmol m⁻² s⁻¹ using the following equations:

\[ qE = \frac{(Fm/Fm_\alpha) - (Fm/Fm')}{(Fm/Fm')} \quad \text{(Eq. 1)} \]

\[ qP = \frac{(Fm' - F0) - (Fm' - Fm)}{Fm' - F0} \quad \text{(Eq. 2)} \]

where Fm is the maximum fluorescence yield, Fm’ is the maximum fluorescence following illumination for 1 h, Fm’’ is the maximum fluorescence yield after 10-min dark relaxation subsequent to 1-h illumination, F0 is the steady-state fluorescence yield in the light following 1 h illumination, and Fm’’ is the maximum yield in the dark following 1-h illumination.

Fluorescence spectra at 77 K were recorded using a LS50 luminescence spectrometer with a liquid-nitrogen-cooled, low temperature housing (PerkinElmer Life Sciences, Gaithersburg, MD). Excitation was at 445 nm (5-nm bandwidth), for chlorophyll a absorption. Spectra were measured over 600–750 nm (5-nm bandwidth) to reveal fluorescence emitted from PSI (at approximately 682 nm) and PSII (at approximately 732 nm). Approximately 20 mg of leaf tissue was ground to a powder in liquid nitrogen, then mixed to a homogenous frozen suspension with 5 ml of cold grinding buffer (0.33 M sorbitol, 5 mM MgCl₂, 5 mM EDTA, 10 mM HEPES, pH 7.6) to ensure that there was no fluorescence re-absorption. The diluted plant tissue was then stored in liquid nitrogen in 4-mm silicon tubes until used for recording spectra. Spectra were normalized to PSI fluorescence to allow comparison of mutant and wild-type plants.

**Other Methods**—Chlorophyll was extracted from leaf disks by grinding in 90% (v/v) acetone or from thylakoids by diluting in 80% acetone. Following removal of leaf debris by centrifugation (1500 × g, 5 min), chlorophyll content was determined according to Porra et al. (24). For the preparation of thylakoids, leaves were homogenized in semi-frozen grinding media (0.33 M sorbitol, 5 mM MgCl₂, 5 mM EDTA, 100 mM HEPES, pH 7.6). The homogenized solution was filtered through four layers of muslin followed by two layers of muslin and one layer of cotton wool. The homogenate was centrifuged at 4000 × g for 10 min. The pellet was resuspended in a small volume of wash buffer (0.33 M sorbitol, 1 mM MgCl₂, 1 mM EDTA, 50 mM HEPES, pH 7.6) before being centrifuged at 4000 × g for 10 min. The pellet was then osmotically shocked by resuspension in 5 mM MgCl₂ for at least 30 s before the addition of an equal volume of 0.66 M sorbitol. Thylakoids were either used fresh or immediately frozen in liquid nitrogen.

**SDS-PAGE** was carried out essentially according to Laemmli (25), including 6 M urea in both the stacking and resolving gels. Solubilized thylakoids (1 μg of Chl equivalent or 30 μg of protein) were separated on a 15% (w/v) acrylamide gel and blotted onto Hybond C nitrocellulose membrane (Amersham Biosciences, Inc., UK). D1 and D2 antisera were raised against synthetic peptides and were specific for the C terminus of PsbS antisera were raised against purified protein from spinach (26). Deg2 antisera were raised against His-tagged Deg2 from *A. thaliana* overexpressed in *Escherichia coli* (10). Bands were quantitated using TotalLab (Nonlinear Dynamics Ltd.) software.

**RESULTS**

*In Vivo Assays of Light-induced PSII Photoinhibition*—The chlorophyll a fluorescence parameter Fv/Fm measures the maximum efficiency of PSII photochemistry. It correlates with both the number of functional PSII reaction centers (28) and the quantum yield of light-induced O₂ evolution (29) and has, therefore, been extensively used as an *in vivo* measure of PSII photoinhibition. To test whether the var2-2 mutant is affected in terms of PSII photoinhibition we compared Fv/Fm *in vivo*, in detached leaves of wild-type *Arabidopsis* and the FtsH mutant var2-2. Fv/Fm values were measured during photoinhibitory irradiance (1800 μmol m⁻² s⁻¹) in the presence or absence of lincomycin, which inhibits D1 synthesis and hence blocks the repair of damaged PSII (Fig. 1). In the absence of lincomycin, WT leaves showed an initial decrease in Fv/Fm to about 50% of the overnight dark-adapted values. After about 2 h there was no further decrease in Fv/Fm. In the presence of lincomycin the decrease in Fv/Fm in WT leaves was more rapid and continued until Fv/Fm values approached zero. Because D1 synthesis is inhibited in the presence of lincomycin, the rate of photoinhibition, as measured by the decrease in Fv/Fm, reflects the rate of PSII photoactivation. When Fv/Fm was monitored in the var2-2 mutant in the presence of lincomycin, during the photoinhibitory light treatment, the decrease was similar to that of wild-type leaves in the presence of lincomycin. This suggests that both wild-type and var2-2 leaves have the same rate of PSII photoactivation. In addition, the decrease in Fv/Fm values in var2-2 leaves during photoinhibition in the absence of lincomycin also proceeded with the same rapid kinetics as lincomycin-treated WT and var2-2 leaves, strongly suggesting that the D1 repair cycle is impaired in var2-2.

---

2 P. Silva and P. J. Nixon, personal communication.
To further characterize the susceptibility of var2-2 leaves to PSII photoinhibition and to assess the capacity for recovery from photoinhibition, Fv/Fm values were monitored in wild-type and var2-2 leaves following treatment with both moderate (300 μmol m^{-2}s^{-1}) and high (1800 μmol m^{-2}s^{-1}) irradiance, and during the subsequent dark recovery period. As shown in Fig. 2, following 1 h of illumination at 300 μmol m^{-2}s^{-1} the wild-type leaves maintained the same high values of Fv/Fm recorded after overnight dark adaptation (time 0), whereas var2-2 leaves showed a decrease in Fv/Fm from 0.695 to below 0.5. One hour of illumination at 1800 μmol m^{-2}s^{-1} resulted in a decrease in Fv/Fm in wild-type leaves from 0.8 to just below 0.6, indicating photoinhibition under these high light conditions as expected. However, var2-2 levels decreased from 0.73 to 0.32 during the same period of irradiance clearly demonstrating enhanced photoinhibition in var2-2 leaves relative to wild-type. In addition, the recovery from photoinhibition was much slower in var2-2 leaves when compared with wild-type. Fv/Fm values, measured in wild-type leaves dark-adapted overnight (unattached during dark adaptation), have high values of above 0.8 (time 0), var2-2 leave, on the other hand, failed to reach the high values of Fv/Fm expected for leaves dark-adapted for this period of time. Furthermore, following 1-h irradiance at 1800 μmol m^{-2}s^{-1} WT leaves showed clear increases in Fv/Fm during the first 6 h of the subsequent dark recovery period. After 20 h the wild-type leaves had almost reached the same high values of Fv/Fm recorded after overnight dark adaptation. In contrast var2-2 leaves showed no sign of recovery in the first 6 h following illumination at either 300 or 1800 μmol m^{-2}s^{-1}, and although there was some recovery after 20 h this failed to restore the Fv/Fm values to those measured following overnight dark adaptation.

**Photosynthetic Characteristics**—We have compared a number of photosynthetic characteristics that may potentially contribute to PSII photoinhibition in var2-2 and wild-type leaves. The ratio of chlorophyll a to chlorophyll b (Chl a/b) has been shown to correlate well with both the size of the PSII light-harvesting antenna and the level of thylakoid membrane stacking (30). Furthermore, the susceptibility of PSII to photoinhibition has been correlated with Chl a/b (31). Table I shows values of Chl a/b for var2-2 and WT leaves of Arabidopsis. Both values are equivalent and are consistent with a large PSII antenna following growth at low irradiance.

**In Vivo Analysis of D1 Degradation**—The more commonly used approach to study D1 turnover is pulse labeling with [35S]methionine. We initially attempted to carry out such studies but found that var2-2 mutant leaves labeled very much more slowly than the wild-type and that a greatly extended (>4×) labeling period was needed to obtain D1 signals comparable to the wild-type. This in itself is consistent with impaired D1 turnover. Mutant leaves are sickly, and following incubation periods sufficient to label D1, the leaf material has degenerated to the point where a chase is no longer technically possible. Therefore, we elected to adopt a Western blotting approach. The ability to degrade the D1 polypeptide in vivo following light-induced damage was assayed in wild-type and var2-2 leaves using Western blot analysis in the absence and presence of lincomycin. Because D1 synthesis is inhibited following lincomycin treatment the degradation of existing D1 results in a decrease in polypeptide content relative to untreated leaves. As shown in Fig. 4A the D1 polypeptide was decreased by 32% relative to untreated leaves in wild-type leaves following 3-h treatment with lincomycin at low irradiance (20 μmol m^{-2}s^{-1}). After 2-h subsequent exposure to photoinhibitory irradiance, the remaining D1 polypeptide was reduced by 66% in wild-type leaves. In contrast, the D1 polypeptide showed no decrease in the var2-2 mutant following...
The D1 polypeptide throughout, suggesting that D1 degradation is matched by synthesis, thereby demonstrating the efficacy of the lincomycin treatment.

Western blot analysis using antibody specific to the other PSII core polypeptide, D2, was also carried out following the same treatment of leaves as described for D1. Again there were losses in the D2 polypeptide in wild-type leaves following lincomycin treatment at low light and more dramatically following exposure to photoinhibitory irradiance. These losses are not, however, as marked as those observed for the D1 polypeptide. As with D1 the D2 polypeptide remained stable following exposure of var2-2 leaves to photoinhibitory irradiance despite an initial loss following lincomycin treatment at low light.

To demonstrate that the turnover of the core PSII polypeptides represents specific degradation and not just destabilization of the photosynthetic apparatus, the content of the minor PSII polypeptide, PsbS, was assayed following lincomycin and photoinhibitory light treatment. As shown in Fig. 4A the PsbS polypeptide content remains unchanged throughout in both wild-type and var2-2 leaves. However, it is interesting to note that the PsbS levels in untreated var2-2 leaves are lower than those of wild-type, and this observation may account for the lower values of qE for the mutant as has been shown for a psbS mutant of Arabidopsis (34). The thylakoid protease DegP2 has already been implicated in D1 turnover (10). To establish whether the effect of the var2-2 mutation was indirectly affecting D1 turnover via a reduction of DegP2 a Western blot was carried out on thylakoid proteins from the wild-type and var2-2 mutant with anti-DegP2 antibodies. No reduction in the abundance of DegP2 was observed in the var2-2 mutant (Fig. 4C).

Sequence Alignments of a Conserved FtsH Lumenal Domain—To investigate the relationship between FtsH homologues from photosynthetic and non-photosynthetic organisms the amino acid sequences from Arabidopsis FtsH1 and Var2 were aligned with FtsH sequences from Synechocystis PCC 6803 and the E. coli FtsH using the MACAW program, which employs the segment pair overlap method to detect small regions of similarity between sequences. This alignment revealed that Var2 and the Slr0228 FtsH homologue from Synechocystis contain a conserved 81-amino acid sequence feature that is not present in FtsH1, E. coli FtsH, or any other Synechocystis FtsH homologue. Further analysis of Slr0228 using the TMHMM (version 2.0) program to predict transmembrane helices revealed that this 81-amino acid feature lay between two very strongly predicted transmembrane helices running from residues 15–37 and 115–137. Given the predicted orientation of these helices and assuming that Slr0228 is located in the thylakoid membrane, the conserved 81-amino acid feature would constitute a lumenal domain. Var2 is already known to be localized to the thylakoid membrane (20). When the sequence of the putative conserved 81-amino acid lumenal domain from Slr0228 was used to do a protein-protein BLAST search of the NCBI non-redundant data base, a number of sequences were returned, all of which shared extensive similarity (Fig. 5). Interestingly, all of the sequences were exclusively FtsH homologues from oxygenic phototrophs. We propose that this 81-amino acid conserved lumenal (CL) domain represents a key identifier of a sub-family of FtsH homologues whose members are restricted to oxygenic photosynthetic organisms. Two further
members of this sub-family are encoded on chromosomes 1 and 5 of *A. thaliana* indicating the existence of a multigene family.

**DISCUSSION**

As part of a PSII repair cycle, damaged D1 polypeptides may be rapidly degraded and replaced by newly synthesized polypeptides (reviewed in Ref. 35). This repair cycle is of great importance to oxygenic phototrophs because when the rate of photoinactivation and damage of D1 exceeds the capacity for repair, photoinhibition occurs, resulting in a decrease in the maximum efficiency of PSII photochemistry. This may ultimately affect the viability of the whole organism. Despite intensive research, the mechanism of D1 degradation has remained largely uncharacterized. Analysis *in vitro* indicates that a stromal DegP type protease is capable of performing the initial cleavage of D1 (10); however, the role of DegP2 in D1 turnover *in vivo* remains unclear. In contrast, the *in vivo* analysis of photoinhibition and D1 degradation presented here suggests that a thylakoid FtsH homologue is required for this initial cleavage step. Using the Arabidopsis *var2*-2 mutant, which has a missense mutation at the end of the second transmembrane domain and fails to accumulate the Var2 FtsH homologue in the membrane, we have shown that PSII is more susceptible to photoinhibition in the absence of this protease (*Fig. 1*). We suggest therefore that an impaired PSII repair cycle forms the basis of the enhanced sensitivity of PSII to photoinhibition in the *var2*-2 mutant. Dark relaxation of *Fv/Fm* following light treatment is slower in *var2*-2 leaves than wild-type (*Fig. 2*). In addition, the kinetics of formation of photoinhibition in untreated *var2*-2 leaves are identical to wild-type leaves, which have been treated with lincomycin and therefore are unable to carry out D1 repair (*Fig. 1*). Taken together, these results strongly indicate that the D1 repair cycle is diminished in the absence of Var2. Western blot analysis of the D1 polypeptide following lincomycin and high-light treatment provides direct evidence for decreased turnover of D1 in the *var2*-2 mutant (*Fig. 4*). The stability of the 32-kDa polypeptide in *var2*-2 suggests that the Var2 FtsH homologue may be involved in the initial cleavage step of photo-damaged D1 polypeptides. Furthermore, the PSII core polypeptide, D2, which is also known to undergo damage and repair under photo-inhibitory irradiance (38), also remains stable in the *var2*-2 mutant following treatment with lincomycin and high-light (*Fig. 2*). This is in contrast to wild-type leaves, which show a marked decrease in D2 polypeptide content. The exact nature of the involvement of FtsH in the repair cycle of PSII is unclear, but it seems likely that the protease is directly involved in D1 degradation following lincomycin treatment. Other possibilities for the involvement of Var2 in D1 turnover exist, including a possible role in phosphorylation in wild-type leaves.

A number of factors may account for this enhanced susceptibility of PSII to photoinhibition. These include greater PSII antenna size and thylakoid membrane stacking (31), decreased capacity for energy-dependent quenching of absorbed photons (36) and enhanced PSII excitation pressure (37), all of which can be measured as Chl a/b ratio, qE and qP, respectively. However, the Chl a/b ratios of both wild-type and *var2*-2 leaves are essentially the same (*Table 1*), and, although *var2*-2 leaves do show decreased levels of qE formation and lower values of qP following light treatment (*Table 1*), the rate of PSII photoinactivation in the presence of lincomycin is the same as wild-type (*Fig. 1*). We suggest therefore that an impaired PSII repair cycle forms the basis of the enhanced sensitivity of PSII to photoinhibition in the *var2*-2 mutant. Dark relaxation of *Fv/Fm* following light treatment is slower in *var2*-2 leaves than wild-type (*Fig. 2*). In addition, the kinetics of formation of photoinhibition in untreated *var2*-2 leaves are identical to wild-type leaves, which have been treated with lincomycin and therefore are unable to carry out D1 repair (*Fig. 1*). Taken together, these results strongly indicate that the D1 repair cycle is diminished in the absence of Var2. Western blot analysis of the D1 polypeptide following lincomycin and high-light treatment provides direct evidence for decreased turnover of D1 in the *var2*-2 mutant (*Fig. 4*). The stability of the 32-kDa polypeptide in *var2*-2 suggests that the Var2 FtsH homologue may be involved in the initial cleavage step of photo-damaged D1 polypeptides. Furthermore, the PSII core polypeptide, D2, which is also known to undergo damage and repair under photo-inhibitory irradiance (38), also remains stable in the *var2*-2 mutant following treatment with lincomycin and high-light (*Fig. 2*). This is in contrast to wild-type leaves, which show a marked decrease in D2 polypeptide content. The exact nature of the involvement of FtsH in the repair cycle of PSII is unclear, but it seems likely that the protease is directly involved in D1 turnover *in vivo*. The possibility of an indirect effect via DegP2 has been excluded (*Fig. 4C*). Other possibilities for the involvement of Var2 in D1 turnover exist, including a possible role in determining the phosphorylation state of D1, because it has been proposed that phosphorylated D1 remains stable (39). Var2 may also have a direct role in D2 turnover, although it is possible that the degradation of D2 requires prior degradation of D1 and that another thylakoid protease may be involved in D2 turnover. Such a suggestion relating to the connectivity of regulation between D1 and D2 has previously been made (40). Indeed, the possibility that a number of proteases, particularly...
FtsH proteases, may also perform D1 degradation in vivo cannot be ruled out. Photoactivation and damage of the D1 polypeptide are known to proceed by at least two separate mechanisms, namely donor and acceptor side photoinhibition (reviewed in Ref. 41), which give rise to distinct breakdown products. Data base analysis suggests that A. thaliana contains at least five homologues of FtsH. An involvement of these proteases during D1 degradation via multiple pathways is likely. Such an overlap in function of the various FtsH homologues and possibly other thylakoid proteases, could account for our observation that both the D1 and D2 polypeptides undergo some degradation in the presence of lincomycin at low light (Fig. 4A).

Our data showing that FtsH homologues are required for the initial cleavage of the D1 polypeptide are strengthened by the finding that the var2-2 phenotype with respect to D1 turnover is also mirrored in the slr0228::Ω mutant of Synechocystis, which lacks one of four FtsH homologues encoded in the genome.2 The sequence analysis presented here also reveals that Var2 and the Slr0228 FtsH homologue share another feature in common; the conserved lumenal domain (Fig. 5). This domain is exclusive to FtsH-like proteins from oxygenic phototrophs.

We propose that this domain identifies a sub-family of FtsH homologues restricted to and essential for oxygenic photosynthetic organisms. The discovery of this domain may represent a significant step forward in our understanding of the specificity and regulation of FtsH homologues.

In conclusion we have demonstrated that the Var2 FtsH homologue of Arabidopsis is required for the protection of PSII from photoinhibition in vivo via a role in the efficient turnover of the PSII core polypeptides, D1 and D2.

Acknowledgments—We are grateful to Drs. I. Adamska and C. Funk for the kind gifts of the DegP2 and PsbS antisera, respectively.

REFERENCES
1. Nanba, O., and Satoh K. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 109–112
2. Mattow, A. K., Hoffman-Falk, H., Marder, J. B., and Edelman, M. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 1380–1384
3. Greenberg, B. M., Gaba, V., Mattoo, A. K., and Edelman, M. (1987) EMBO J. 6, 2865–2869
4. Canovas, P. M., and Barber, J. (1993) FEBS Lett. 324, 341–344
5. Jansen, A. K., Depka, B., Trebst, A., and Edelman, M. (1993) J. Biol. Chem. 268, 21246–21252
6. Miyao, M. (1994) Biochemistry 33, 9722–9730
7. Aro, E. M., Hundal, T., Carlberg, I., and Andersson, B. (1990) Biochim. Biophys. Acta 1019, 269–275
8. De Las Rivas, J., Shipton, C. A., Pontious, M., and Barber, J. (1993) Biochemistry 32, 6944–6950
9. Adam Z. (2000) Biochimie (Paris) 82, 647–654
10. Haufljui, K., Andersson, B., and Adamska, I. (2001) EMBO J. 20, 713–722
11. Lindahl, M., Speetca, C., Hundal, T., Oppenheim, A. B., Adam, Z., and Andersson, B. (2000) Plant Cell 12, 419–432
12. Patel, S., and Laitterich, M. (1998) Trends Cell Biol. 8, 65–67
13. Schumann, W. (1999) FEMS Microbiol. Rev. 23, 1–11
14. Santos, D., and Almeida, D. F. P. (1975) J. Bacteriol. 124, 1502–1507
15. Herman, C., Ogura, T., Tomoyasu, T., Hiraga, S., Akiyama, Y., Ito, K., Thomas, R., D’ari, R., and Bouloc, P. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10861–10865
16. Tomoyasu, T., Gamar, J., Boku, B., Kanemori, M., Mori, H., Rutman, A. J., Oppenheim, A. B., Yura, T., Yananaka, K., Niki, H., Hiraga, S., and Ogura, T. (1995) EMBO J. 14, 2551–2566
17. Weber, E. B., Hanekamp, T., and Thurness, P. E. (1996) Mol. Biol. Cell 7, 307–317
18. Oster etzer, O., and Adam, Z. (1997) Plant Cell 9, 857–865
19. Manoh, N. H., Novar, N., Mullineaux, C. W., Newman, J., Bailey, S., Robinson, C. (2000) FEBS Lett. 479, 72–77
20. Chen, M., Choi, Y.-D., Votias, D. F., and Rodermel, S. (2000) Plant J. 22, 303–313
21. Takechi, K., Sodmergen, Murata, M., Motome, F., and Sakamoto, W. (2000) Plant Cell Physiol. 41, 1334–1346
22. Thiele, A., Winter, K., and Krause, G. H. (1997) J. Plant Physiol. 151, 286–292
23. Van Kooten, O., and Snell, J. F. H. (1990) Photosynth. Res. 25, 147–150
24. Porra, R. J., Thompson, W. A., and Kreidemann, P. E. (1989) Biochim. Biophys. Acta 972, 163–170
25. Laemml, U. K. (1970) Nature 227, 680–685
26. Dalla Chiessa, M., Friso, G., Deak, Z., Vass, L., Barber, J., and Nixon, P. J. (1997) Eur. J. Biochem. 248, 731–740
27. Ljungberg, U., Arkelund, H.-E., and Andersson, B. (1986) Eur. J. Biochem. 158, 477–482
28. Oquist, G., Chow, W. S., and Anderson, J. M. (1992) Plant Physiol. 98, 450–460
29. Demming, B., and Bjorkman, O. (1987) Planta 171, 171–184
30. Leong, T. Y., and Anderson, J. M. (1984) Photosynth. Res. 3, 105–115
31. Aro, E. M., McCaffrey, S., and Anderson, J. M. (1986) Plant Physiol. 83, 853–843
32. Horton, P., and Black, M. T. (1981) Biochim. Biophys. Acta 635, 53–62
33. Horton, P., Ruban, A. V., and Walters, R. G. (1998) Annu. Rev. Plant Physiol. 49, 655–684
34. Li, X. P., Bjorkman, O., Shih, C., Grossman, A. R., Rosenquist, M., Jansson, S., and Aro, E. M. (2000) Nature 403, 391–395
35. Aro, E. M., Oquist, G., and Anderson, B. (1993) Biochim. Biophys. Acta 1143, 113–134
36. Deenig-Adams, B., and Adams, W. W. (1992) Annu. Rev. Plant Physiol. 43, 599–626
37. Humer, N. P. A., Oquist, G., and Sarban, F. (1998) Trends Plant Sci. 3, 224–230
38. Schuster, G., Timberg, R., and Ohad, I. (1988) Eur. J. Biochem. 177, 403–410
39. Rintamaki, E., Kettunen, R., and Aro, E. M. (1996) J. Biol. Chem. 271, 6470–6475
40. Jansen, M. A. K., Greenberg, B. M., Edelman, M., Mattoo, A. K., and Gaba, V. (1996) Photochem. Photobiol. 63, 814–817
41. Long, S. P., and Humphries, S. (1994) Annu. Rev. Plant Physiol. 45, 633–662
A Critical Role for the Var2 FtsH Homologue of Arabidopsis thaliana in the Photosystem II Repair Cycle in Vivo
Shaun Bailey, Elinor Thompson, Peter J. Nixon, Peter Horton, Conrad W. Mullineaux, Colin Robinson and Nicholas H. Mann

J. Biol. Chem. 2002, 277:2006-2011.
doi: 10.1074/jbc.M105878200 originally published online November 20, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M105878200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 41 references, 11 of which can be accessed free at
http://www.jbc.org/content/277/3/2006.full.html#ref-list-1