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FtsH Is Involved in the Early Stages of Repair of Photosystem II in *Synechocystis* sp PCC 6803[W]

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When plants, algae, and cyanobacteria are exposed to excessive light, especially in combination with other environmental stress conditions such as extreme temperatures, their photosynthetic performance declines. A major cause of this photoinhibition is the light-induced irreversible photodamage to the photosystem II (PSII) complex responsible for photosynthetic oxygen evolution. A repair cycle operates to selectively replace a damaged D1 subunit within PSII with a newly synthesized copy followed by the light-driven reactivation of the complex. Net loss of PSII activity occurs (photoinhibition) when the rate of damage exceeds the rate of repair. The identities of the chaperones and proteases involved in the replacement of D1 in vivo remain uncertain. Here, we show that one of the four members of the FtsH family of proteases (cyanobase designation slr0228) found in the cyanobacterium *Synechocystis* sp PCC 6803 is important for the repair of PSII and is vital for preventing chronic photoinhibition. Therefore, the *ftsH* gene family is not functionally redundant with respect to the repair of PSII in this organism. Our data also indicate that FtsH binds directly to PSII, is involved in the early steps of D1 degradation, and is not restricted to the removal of D1 fragments. These results, together with the recent analysis of *ftsH* mutants of Arabidopsis, highlight the critical role played by FtsH proteases in the removal of damaged D1 from the membrane and the maintenance of PSII activity in vivo.

INTRODUCTION

When oxygenic photosynthetic organisms are exposed to high light, their ability to convert light energy to chemical energy via photosynthesis is impaired (reviewed by Barber and Andersson, 1992; Prasil et al., 1992; Aro et al., 1993; Ohad et al., 1994). This inhibition is sometimes termed photoinhibition and is thought to be an important factor in limiting crop yields, particularly when plants are growing under conditions of environmental stress (Barber and Andersson, 1992; Long and Humphries, 1994).

A major target of photoinhibition is the photosystem II (PSII) complex of the thylakoid membrane (reviewed by Prasil et al., 1992), and in particular, the D1 reaction center subunit, which plays a role in binding many of the cofactors involved in electron transfer across the membrane (Diner and Rappaport, 2002). D1 is prone to irreversible oxidative damage by either reactive oxygen species or highly oxidizing species generated within PSII (Barber and Andersson, 1992).

To maintain PSII activity, a repair cycle operates to replace a damaged D1 polypeptide with a newly synthesized copy (Kyle et al., 1984; Ohad et al., 1984; Andersson and Aro, 2001). PSII is damaged at all light intensities, but only when the rate of damage is greater than the rate of repair is there a net loss of PSII activity (Aro et al., 1993; Keren and Ohad, 1998; Constant et al., 2000). The mechanism of D1 replacement is particularly intriguing, because only D1 of the ~25 PSII subunits is targeted for replacement. The identities of the chaperones and proteases involved in stabilizing the PSII complex during repair and removing damaged D1 in vivo remain uncertain.

The synthesis and degradation of the D1 subunit (D1 turnover) has been studied most intensively in green plants (reviewed by Aro et al., 1993). However, D1 turnover also occurs in cyanobacteria such as *Synechocystis* sp PCC 6803 (Goloubinoff et al., 1988; Komenda and Barber, 1995), but it remains unclear whether the PSII repair mechanism is conserved evolutionarily. The ease of generating mutants in Synechocystis means that it is relatively straightforward to test the role of various proteases in D1 turnover in vivo. Analysis of the complete genome sequence of Synechocystis has identified homologs of both the HtrA (or DegP) and FtsH proteases of *Escherichia coli* (reviewed by Sokolenko et al., 2002). Both types of protease are known to play roles in the degradation of misfolded membrane proteins and so are good candidates for a role in D1 degradation. HtrA is a Ser-type protease involved in the response to heat stress (reviewed by Clausen et al., 2002), whereas FtsH is a membrane-bound ATP-dependent Zn^{2+}-activated protease (Santos and De Almeida, 1975; Ogura et al., 1991; Karata et al., 1999) and a member of the AAA protein family (for ATPases associated with diverse cellular activities). Originally identified in *E. coli* on the basis of a filamentation temperature-sensitive phenotype (Santos and De Almeida, 1975), FtsH homologs have been found

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throughout nature and are implicated in the degradation of both integral and soluble proteins (Langer, 2000).

Based on experiments conducted in vitro, a two-step process has been proposed for the removal of damaged D1 from chloroplast thylakoid membranes (Spetea et al., 1999; Haußühl et al., 2001; reviewed by Adam and Clarke, 2002). First, an HtrA/DegP homolog, termed DegP2, is thought to catalyze the primary cleavage of damaged D1 in a stromally exposed region between helices 4 and 5 of the five-transmembrane D1 subunit (Haußühl et al., 2001) in a GTP-stimulated reaction (Spetea et al., 1999). Second, an FtsH homolog, termed FtsH1, and perhaps other FtsH homologs, catalyzes the degradation of the 23-kD N-terminal D1 fragment and possibly the C-terminal degradation product (Lindahl et al., 1996, 2000). In this model, therefore, FtsH is involved in the removal of D1 fragments and is not involved in the important primary cleavage event. To date, the roles of DegP2 and FtsH1 in D1 degradation have not been assessed in planta.

Here, we report the analysis of PSII repair in a specific *ftsH* mutant of Synechocystis in which one of the four FtsH homologs predicted from analysis of the genome sequence, designated slr0228 in cyanobase (http://www.kazusa.or.jp/cyano/Synechocystis/ index.html), was inactivated insertionally to create mutant slr0228::Ω (Mann et al., 2000). Previous work had indicated a role for slr0228 in the assembly of functional photosystem I (PSI) (Mann et al., 2000). Our results now also identify a role for this homolog in the early stages of PSII repair. Based on the presence of FtsH in purified PSII preparations, we propose that FtsH (slr0228) plays a direct role in D1 degradation. Based on our data and on recent results obtained with an Arabidopsis mutant (Bailey et al., 2002), the involvement of FtsH in the early steps of D1 degradation appears to be conserved in both cyanobacteria and chloroplasts. Thus, FtsH appears to play a more important role in the degradation of damaged D1 than was thought previously.

RESULTS

FtsH (slr0228) Is Required for the Resistance of Synechocystis to Moderate Light Stress

For the cyanobacterium Synechocystis, four *ftsH* homologs (designated slr0228, sll1463, slr1390, and slr1604) have been identified in the genome sequence (Kaneko and Tabata, 1997). Insertion mutants in the true wild-type background have been obtained for only slr0228 and sll1463; the other *ftsH* genes (slr1390 and slr1604) appear to be essential for cell growth (Mann et al., 2000). Studies have indicated that cells of the slr0228 insertion mutant, designated *ftsH* (slr0228::Ω), in which the coding region of slr0228 has been disrupted by the insertion of a spectinomycin resistance cassette, have reduced levels of PSI but wild-type levels of PSII (Mann et al., 2000). We found that an additional striking phenotype of the *ftsH* (slr0228::Ω) insertion mutant was the sensitivity of its growth to moderate light stress (Figure 1). At low light intensities (10 $\mu E \cdot m^{-2} \cdot s^{-1}$), the mutant grew photoautotrophically in liquid culture as well as the wild type. A relatively minor increase in the light intensity to 40 $\mu E \cdot m^{-2} \cdot s^{-1}$ led to a dramatic cessation of growth in cultures of the mutant but not the wild type and the gradual bleaching of pigment (Figure 1). By contrast, an insertion mutant of *ftsH* (sll1463) was resistant to this light stress (data not shown).

In a control experiment, a plasmid harboring a 5.1-kb HindIII fragment containing the intact slr0228 gene plus flanking regions was able to restore photoautotrophic growth to the *ftsH* (slr0228::Ω) insertion mutant under nonpermissive light conditions (100 $\mu E \cdot m^{-2} \cdot s^{-1}$ on the surface of agar plates). In this type of experiment, an intact copy of *ftsH* (slr0228) is restored to the chromosome through homologous recombination with the DNA fragment contained in the transforming plasmid. Therefore, this result suggested that the light sensitivity displayed by the *ftsH* (slr0228::Ω) mutant was caused by the disruption of *FtsH* (slr0228) rather than by a secondary mutation elsewhere in the genome.

The PSII Repair Cycle Is Impaired in the *ftsH* (slr0228) Insertion Mutant

To determine whether the loss of FtsH (slr0228) impaired the ability of the cells to repair damaged PSII, PSII activity, measured as light-saturated rates of oxygen evolution, was monitored in cells as a function of the time of exposure to high light either in the absence or the presence of lincomycin, an antibiotic that blocks the repair of PSII by inhibiting protein synthesis de novo (Figure 2A). It should be noted that for the results shown in Figures 2 and 3, the cell densities were increased to allow biochemical analyses to be performed, so there is no direct comparison with the data presented in Figure 1. In the absence of lincomycin, PSII activity in the wild type was relatively unimpaired because of an active repair cycle, whereas in the presence of lincomycin, PSII activity declined with a half-life of ~60 min. Under the latter conditions, the repair cycle was inhibited totally and the loss of PSII activity was a reflection of the rate of damage to PSII. By contrast, the *ftsH* (slr0228::Ω) mutant suffered from chronic photoinhibition even in the absence of lincomycin (Figure 2A). Under these conditions, PSII repair in the mutant was unable to match the rate of damage.

By comparing the loss of PSII activity in the presence of lincomycin, the rates of damage to PSII were found to be similar in both the wild type and the mutant. After transfer to low-light conditions, a slow recovery of PSII activity was observed in the mutant only in the absence of lincomycin (Figure 2A). Cell viability measurements excluded the possibility that the *ftsH* (slr0228::Ω) mutant was more prone to death during the period of high-light illumination (see supplemental data online). Together, these data indicated that the lack of FtsH (slr0228) caused enhanced susceptibility of PSII to photoinhibition because of an impaired PSII repair cycle rather than an increased rate of damage to PSII. When cells were exposed to lower light intensities, at which the rate of damage to PSII was reduced compared with that at higher light intensities, PSII repair in the *ftsH* (slr0228::Ω) mutant was sufficient to match the rate of damage (Figure 2B).

The Rate of D1 Degradation Is Slowed in the *ftsH* (slr0228) Mutant

Radiolabeling experiments using radioactive Met were performed to investigate the rates of D1 synthesis in the wild type and the

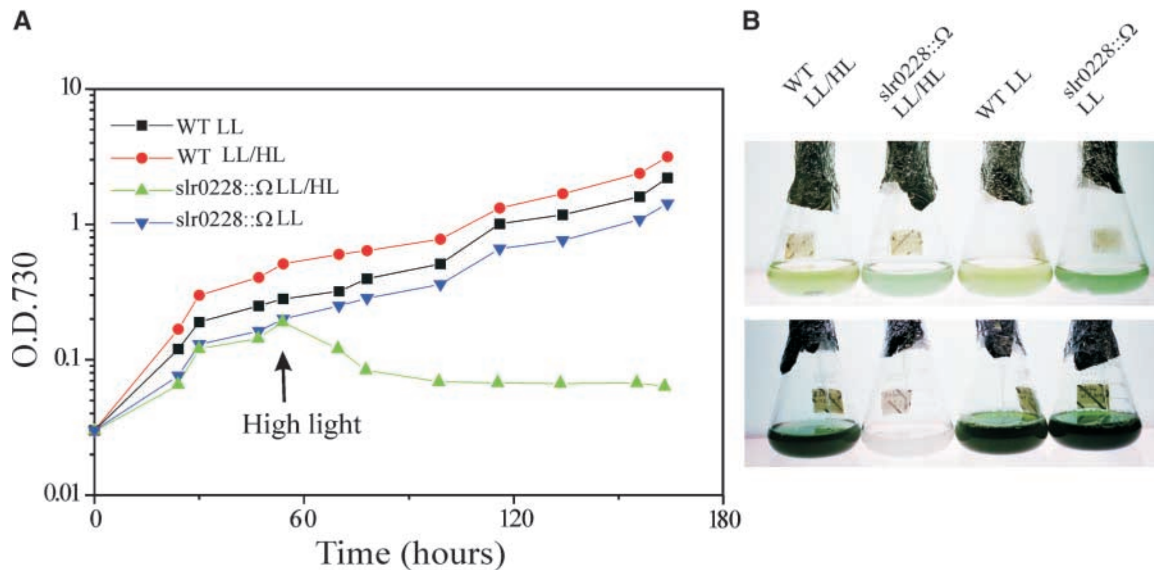

Figure 1. Photoautotrophic Growth of Synechocystis Wild Type and slr0228::Ω.

(A) All cultures were grown at 31 to 32°C in BG-11 medium with air bubbling and stirring. Initially, cultures were grown in low light (LL) (10 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 58 h. At this point (indicated by the black arrow), a wild-type (WT) culture (red line) and a mutant culture (green line) were moved to a higher light intensity (HL) (40 $\mu E \cdot m^{-2} \cdot s^{-1}$). Wild-type LL and slr0228::Ω LL continued to be grown at low light (10 $\mu E \cdot m^{-2} \cdot s^{-1}$). For each time point, duplicate measurements of OD_{730} were made and plotted as the average. The differences between the two values were less than the size of the symbols.
(B) Top photograph, cultures after 58 h; bottom photograph, the same cultures after 100 h.

mutant at low light and the subsequent rates of D1 degradation at high light (Figure 3). Lincomycin was added after the pulse period to exclude any possible effects of protein synthesis on D1 degradation (Komenda et al., 2000). The intensities of the majority of radiolabeled bands were similar in the slr0228::Ω mutant and the wild type. Most notably, the rate of D1 synthesis, as assessed by the intensity of the radiolabeled D1 band in the pulse period, was reduced in the mutant compared with that in the wild type (Figure 3B, lanes 0). In addition, the slr0228::Ω mutant showed a pronounced doublet band in the D1 region. Because the upper band of the doublet had disappeared by the first time point of the chase and there was a concomitant increase in the intensity of the lower band of the doublet, it is likely that the upper band represents a D1 precursor. Normally, C-terminal processing of precursor D1, which occurs in two steps in Synechocystis (Inagaki et al., 2001), is far too rapid to be detected on this time scale in the wild type (Figure 3B). For the wild type, the loss of radiolabeled D1 occurred with an approximate half-life of 2 h. In the slr0228::Ω mutant, however, little loss of radiolabeled D1 occurred during the high-light treatment. This experiment indicated that the D1 repair cycle in the mutant was slowed at the levels of D1 synthesis and D1 processing and importantly at the primary cleavage step in D1 degradation, which is proposed in chloroplasts to yield N-terminal 23-kD (Greenberg et al., 1987) and C-terminal 10-kD (Canovas and Barber, 1993) D1 fragments.

Immunoblot experiments also confirmed that the rate of degradation of bulk D1, assembled into PSII complexes, and not just newly synthesized D1, was slowed in the slr0228::Ω mutant (Figure 3C). In the presence of lincomycin, D1 degradation again proceeded with a half-life of ∼2 h in the wild type but was slowed dramatically in the mutant (Figure 3C). No D1 breakdown fragments were detected in either mutant or wild-type samples using a C-terminal-specific D1 antibody that had been used previously to identify D1 fragments in higher plants (Canovas and Barber (1993) (data not shown).

A pulse-chase experiment also was performed in the absence of lincomycin. As shown in Figure 3D, loss of radiolabeled D1 again was impaired in the slr0228::Ω mutant compared with that in the wild type, and there was no accumulation of D1 fragments as assessed by immunoblot analysis.

Lipid Composition in an FtsH (slr0228)–Deficient Mutant

E. coli ftsH null mutants have been found to be lethal to cell growth because of effects on the synthesis of lipopolysaccharides and phospholipids (Ogura et al., 1999). Therefore, the lipid content of an *ftsH* (slr0228) insertion mutant was investigated as a possible reason for its sensitivity to photoinhibition.

Qualitative analysis using thin layer chromatography showed that the content of neither galactolipids (composed mainly of mono- and digalactosyldiacylglycerol and sulfoquinovosyldiacylglycerol) nor phospholipids differed between the glucose-tolerant wild type (wild-type-G) and a derivative, slr0228::kan^R, in which the slr0228 gene was inactivated (data not shown). Upon further analysis of fatty acids by gas liquid chromatography, however, we found that the slr0228::kan^R mutant contained a greater proportion of saturated fatty acids than the wild type (Figure 4A), which would tend to decrease the fluidity of the

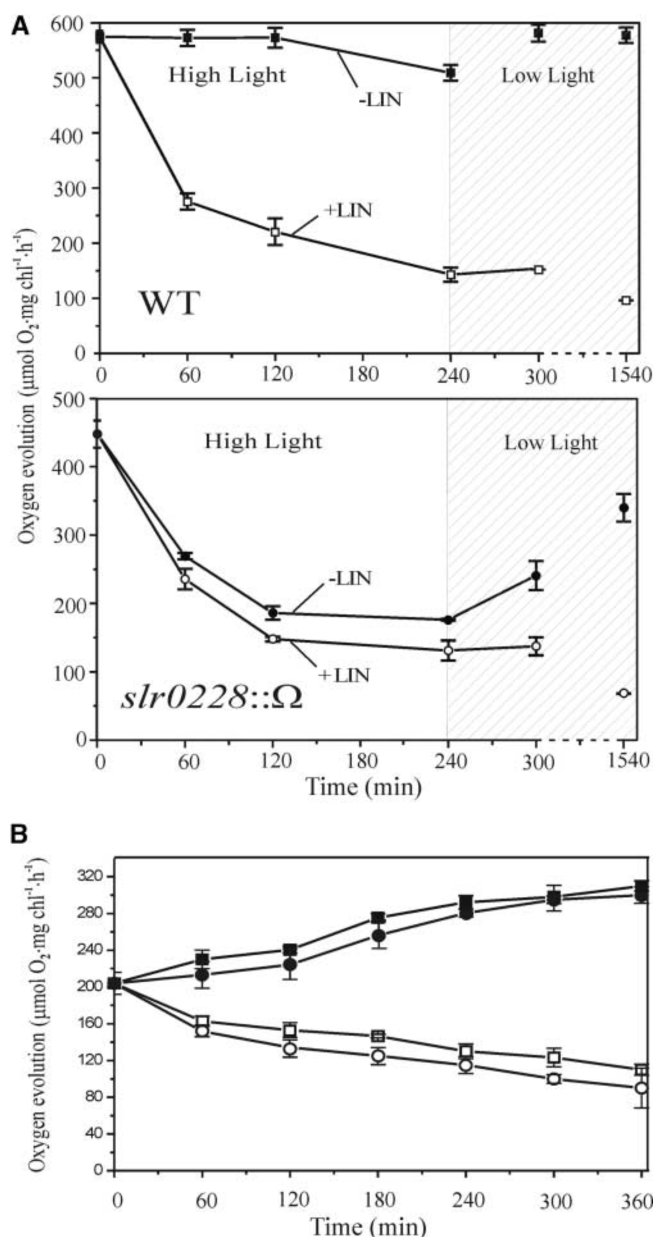

Figure 2. Effect of Light on Oxygen Evolution from Whole Cells of Synechocystis Wild Type and slr0228::Ω.

Cells at a chlorophyll concentration of 20 μg/mL were exposed to either high light (1000 $\mu E \cdot m^{-2} \cdot s^{-1}$) **(A)** or low light (100 $\mu E \cdot m^{-2} \cdot s^{-1}$) **(B)** in the absence (closed symbols) or presence (open symbols) of lincomycin (100 μg/mL). The hatched area in **(A)** represents the recovery period under light growth conditions of 10 $\mu E \cdot m^{-2} \cdot s^{-1}$. WT, wild type. In **(B)**, the wild-type data are indicated by squares and the data for the slr0228::Ω mutant are indicated by circles. Averages of two measurements of the same sample are plotted. Error bars indicate the range between the two values. Oxygen evolution was assayed in the presence of ferricyanide (1 mM) and 2,5-dimethylbenzoquinone (1 mM).

membrane. Small decreases in the level of unsaturated 18-carbon fatty acids were matched by an increase in the proportion of 16:0 molecules.

Because the composition of the thylakoid membrane has the potential to affect the dynamics of PSII repair, particularly at low temperature (Kanervo et al., 1997), it was important to determine the potential effect of the changes in fatty acids on PSII turnover in slr0228-deficient Synechocystis. Therefore, the photoinhibition phenotype of the *desA*$^-$/*desD*$^-$ strain (kindly donated by N. Murata, National Institute for Basic Biology, Okazaki, Japan) was compared with those of wild-type-G and slr0228::kan^R. This *desA*$^-$/*desD*$^-$ mutant is missing the acyl-lipid desaturases that introduce double bonds into the Δ12 and Δ6 positions of C_{18} fatty acids and so has lost all fatty acids with double bonds at those positions. It has been reported that PSII repair proceeds normally at 30°C but is impaired at 20°C (Kanervo et al., 1997). The *desA*$^-$/*desD*$^-$ cells contain no polyunsaturated fatty acids, a more severe phenotype than that of the slr0228::kan^R mutant. After a 2-h exposure to high light (1400 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 30°C, oxygen evolution had declined to almost zero in the slr0228::kan^R mutant, whereas wild-type and *desA*$^-$/*desD*$^-$ continued to evolve oxygen at similar rates. After overnight incubation at low light, the slr0228::kan^R mutant showed no detectable activity, in contrast with the wild type and the desaturase mutant (Figure 4B). This evidence suggests that the small changes in the fatty acid composition of the slr0228::kan^R mutant are not responsible for changes in the repair cycle.

Detection of FtsH in His-Tagged CP47 Preparations Isolated from Strain HT-3 of Synechocystis

The role of FtsH (slr0228) in D1 turnover in vivo may be direct, indirect, or both. To assess whether FtsH (slr0228), and possibly other *ftsH* gene products, could interact directly with PSII, experiments were performed to determine if FtsH copurified with PSII in preparations in which the PSII subunit, CP47, was His tagged (Figure 5). We hypothesized that His-tagged CP47 isolated from detergent-solubilized cyanobacterial membranes using immobilized Ni^{2+}-affinity chromatography would contain not only active PSII complexes but also complexes that were undergoing assembly or repair. We found that the Ni^{2+} resin retained immunodetectable FtsH (Figure 5A) as well as PSII complexes, as monitored by D1 immunoblot analysis (Figure 5B). The FtsH antiserum used in this work was raised to a peptide predicted from the sequence of *E. coli* FtsH and, in principle, can recognize all four FtsH homologs in Synechocystis. In parallel work, Kashino and colleagues (2002) used mass spectrometry to analyze the protein composition of the same type of His-tagged PSII preparation described here and unambiguously detected both FtsH (slr0228) and FtsH (slr1604). However, those authors could not exclude the nonspecific binding of FtsH homologs to the Ni^{2+} resin. To exclude this important possibility, we chromatographed detergent-solubilized membrane extracts from wild-type Synechocystis lacking a His tag on CP47. As expected, PSII no longer bound to the Ni^{2+} resin (Figure 5D). Importantly FtsH also was unable to bind to the resin in detectable amounts (Figure 5C). From immunoblots of concentrated fractions (lanes 5 to 8 in Figures 5A to 5D), we es-

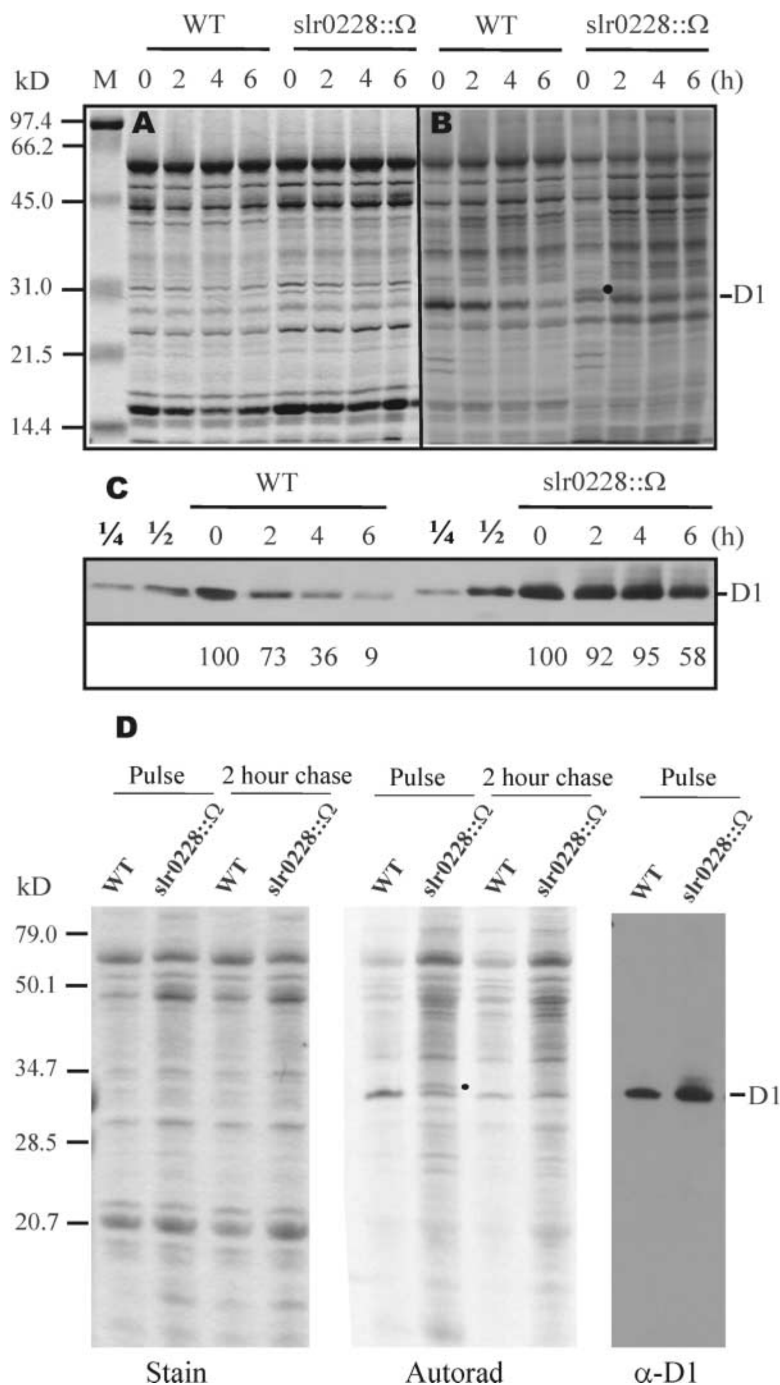

Figure 3. Synthesis and Degradation of D1 Protein in Cells Isolated from Synechocystis Wild Type and slr0228::Ω.

(A) and **(B)** Cells were labeled for 60 min at 100 μE·m^{-2}·s^{-1} in the presence of L-^{35}S-Met, lincomycin was added (100 μg/mL), and then cells were exposed for 2, 4, or 6 h at 1000 μE·m^{-2}·s^{-1}. Thylakoid membranes were isolated and analyzed by SDS-PAGE followed by Coomassie blue staining **(A)** and autoradiography **(B)**. All samples contained 1 μg of chlorophyll. The location of the radiolabeled D1 polypeptide is indicated at right (-D1). The relative migration of molecular mass standards is indicated at left. The black dot indicates the band assigned to a precursor of D1. WT, wild type.

(C) Rate of degradation of total D1 is reduced in the slr0228::Ω mutant as assessed by immunoblot analysis using D1-specific antibodies. Thylakoids were isolated under the experimental conditions described for **(A)**. Percentage D1 levels shown below the lanes were estimated by comparing the intensity of cross-reaction to a dilution series (1/2 and 1/4) of the relevant samples taken at time 0.

(D) Pulse-chase analysis of the wild type and the slr0228::Ω mutant in the absence of lincomycin with a pulse period of 60 min at 100 μE·m^{-2}·s^{-1} and a chase period of 2 h at 1000 μE·m^{-2}·s^{-1}. Thylakoid proteins were separated by SDS-PAGE and stained with Coomassie blue (Stain), and radiolabeled proteins were detected by autoradiography (Autorad). The position of D1 was confirmed by immunoblot analysis (α-D1). All samples contained 1 μg of chlorophyll. The black dot indicates the band assigned to a precursor of D1.

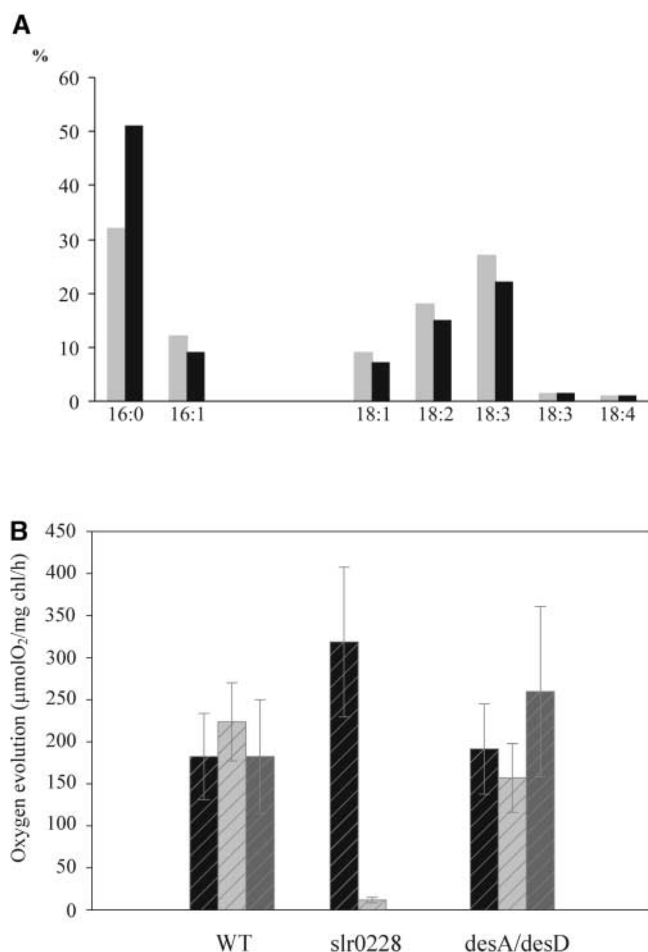

Figure 4. Lipid Composition of an *ftsH* (slr0228) Mutant and Comparison of Photoinhibition with a *desA*⁻/*desD*⁻ Mutant.

(A) Fatty acid composition of thylakoid membranes isolated from the glucose-tolerant wild-type strain (light gray bars) and the slr0228::kan^R mutant (dark gray bars). X:Y refers to the number of carbon atoms in the fatty acid (X) and the number of double bonds (Y).

(B) Oxygen evolution of the glucose-tolerant wild-type strain (WT), slr0228::kan^R (slr0228), and $desA^-/desD^-$ Synechocystis cells (desA/desD) in low light (t = 0 h, 10 μmol·m^{-2}·s^{-1}; black bars), after 2 h of light at ~1400 μmol·m^{-2}·s^{-1} (light gray bars), and after recovery in low light (19 h at 10 μmol·m^{-2}·s^{-1}; dark gray bars). Results from duplicate cultures are shown. Because of differences in the wild-type background strain and the experimental conditions, these data cannot be compared directly with the data in Figure 2. chl, chlorophyll.

timated the amount of nonspecific binding of immunodetectable FtsH to the Ni^{2+} resin to be <5% of that found in the His-tagged CP47 preparation (data not shown).

Another control experiment using a His-tagged derivative of the membrane-bound IMMUTANS protein from Arabidopsis (Prommeenate et al., 2001) confirmed that FtsH did not bind to His-tagged membrane proteins in general (Figures 5E and 5F). Other control experiments confirmed that His-tagged CP47 retained FtsH when the chromatography was conducted in the presence of high salt (500 mM NaCl) and when Co^{2+} rather than Ni^{2+} was used as the immobilized ion (data not shown).

Reduced Levels of FtsH in His-Tagged CP47 Preparations Isolated from a slr0228 Insertion Mutant

To assess whether FtsH (slr0228) was required for the binding of other FtsH homologs to PSII, we examined the levels of FtsH in PSII preparations isolated from a His-tagged CP47 strain of Synechocystis in which *ftsH* (slr0228) had been inactivated (strain HT-3/slr0228::cm^R). The results presented in Figure 6 show that the level of immunodetectable FtsH was reduced to <25% of that in the wild-type preparation. Upon careful examination of the Coomassie blue–stained gel, minor staining bands that comigrated with the immunodetectable FtsH bands were found to be reduced in intensity in the slr0228 mutant compared with the wild-type sample. Together, these results suggested that other FtsH homologs are able to bind to PSII independently of FtsH (slr0228) but at reduced levels.

FtsH Forms a Large Protein Complex

In mitochondria and *E. coli*, FtsH forms a large protein complex (Kihara et al., 1996; Steglich et al., 1999). The molecular mass of the FtsH species found in the His-tagged PSII preparation

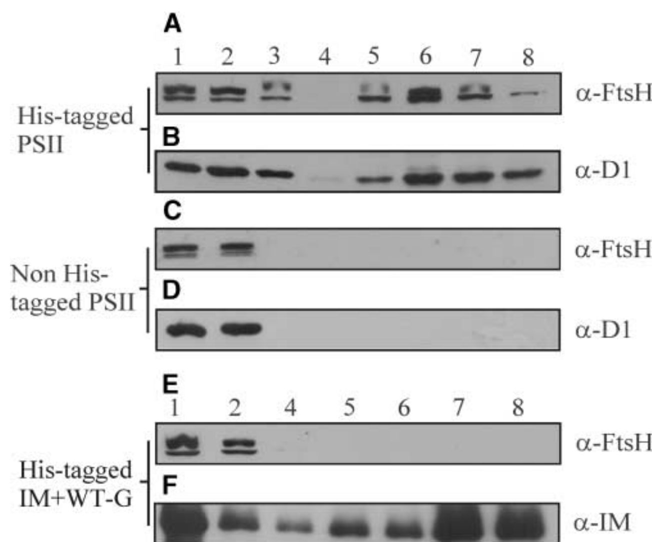

Figure 5. FtsH Homologs Bind to Isolated PSII Complexes.

Fractions obtained from immobilized Ni^{2+}-affinity chromatography of solubilized thylakoid membranes containing His-tagged PSII (**[A]** and **[B]**), non-His-tagged PSII (**[C]** and **[D]**), and non-His-tagged PSII with added His-tagged IMMUTANS (IM; **[E]** and **[F]**) were immunoblotted with antibodies (α-) specific for FtsH (**[A]**, **[C]**, and **[E]**), D1 (**[B]** and **[D]**), and IMMUTANS **(F)**. Lane 1, solubilized thylakoid membranes added to the Ni^{2+}-charged resin; lane 2, unbound material; lane 3, fifth wash; lane 4, last wash before elution; lane 5, elution with 50 mM imidazole; lane 6, elution with 100 mM imidazole; lane 7, elution with 150 mM imidazole; lane 8, elution with 300 mM imidazole. WT-G, glucose-tolerant wild type.

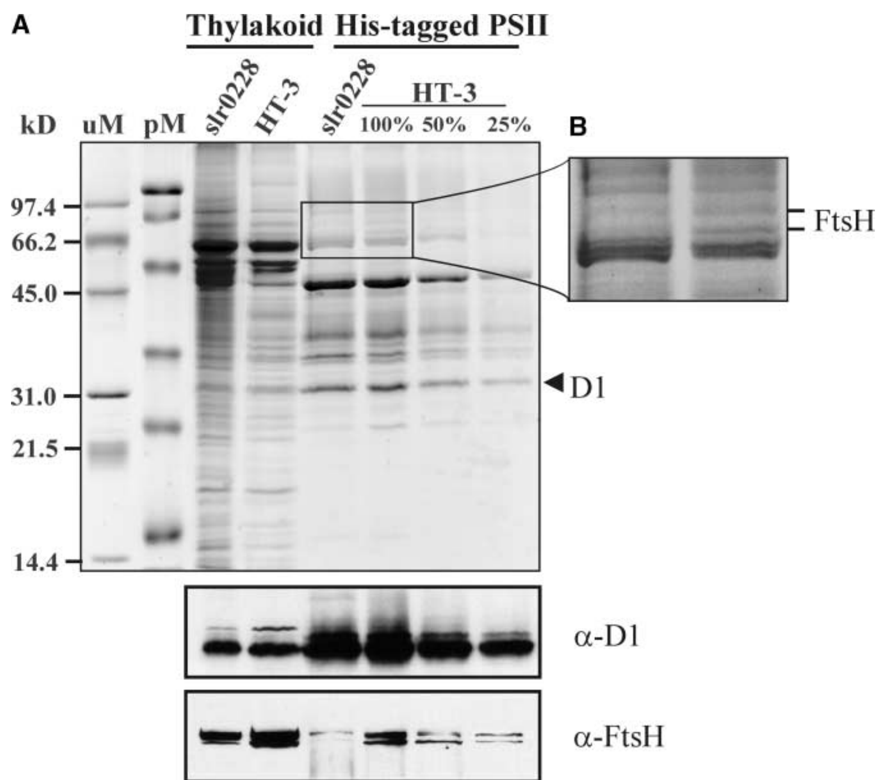

Figure 6. FtsH Homologs Are Depleted in His-Tagged PSII Isolated from a Strain Lacking FtsH (slr0228).

Analysis of FtsH proteins in thylakoid membranes and His-tagged PSII preparations isolated from Synechocystis (strain HT-3) and a derivative lacking functional FtsH (slr0228) (strain HT-3/slr0228::cm^R, designated slr0228 in **[A]**). A dilution series of the PSII preparation from HT-3 was used to quantify immunoblots.

(A) At top, a Coomassie blue–stained 12% SDS-PAGE gel containing 6 M urea; at bottom, immunoblots using antibodies (α-) specific for D1 and FtsH. Apart from the dilution series, all samples contained 1 μg of chlorophyll. The relative migration of unstained molecular mass standards (uM) is indicated at left. Lane pM contains prestained marker proteins.

(B) Magnification of the FtsH region.

isolated from strain HT-3 was assessed by blue-native PAGE in the first dimension followed by immunoblot analysis of protein separated by SDS-PAGE in the second dimension. As expected, PSII showed monomeric and dimeric complexes with predicted sizes of 250 and 500 kD, respectively (Figure 7A). Also observed were complexes assigned to monomeric and dimeric PSII complexes lacking CP43, so-called CP47 reaction centers (Rhee et al., 1998), as well as a complex probably consisting of a heterodimer of one PSII complex and one CP47 reaction center. Immunoblot experiments confirmed that under these conditions, immunodetectable FtsH, potentially consisting of one or more homologs, migrated mainly in a complex or complexes of >500 kD and did not comigrate with the majority of the PSII complexes identified on D1 and CP47 immunoblots (Figure 7B). Immunodetectable FtsH also was observed as a smear rather than as a distinct band, possibly because of heterogeneity in the sizes of the complexes containing FtsH. The lack of detectable comigration of FtsH and His-tagged CP47 on overexposed immunoblots indicated that FtsH was attached only weakly to complexes containing His-tagged CP47 and could not be isolated as a distinct complex with CP47 by blue-native PAGE.

DISCUSSION

FtsH (slr0228) Is Important for Photoprotection

The striking sensitivity of the growth of the slr0228 mutant to light emphasizes the physiological importance of this protease for cell viability after light stress. A role for slr0228 in photoprotection also is in agreement with recent DNA microarray data that indicated a strong increase in the transcript levels for slr0228 upon exposure to high light (Hihara et al., 2001). Although we have shown the involvement of FtsH (slr0228) in PSII repair in response to high light, it remains possible that FtsH (slr0228) plays a general role in the removal of unassembled or damaged membrane proteins under a variety of different stress conditions.

FtsH (slr0228) Is Required for Efficient PSII Repair in High Light

Our experiments clearly show that the PSII repair cycle is impaired in the *ftsH* (slr0228) mutant. The reduced rates of D1 synthesis, processing, and degradation observed in the slr0228::Ω

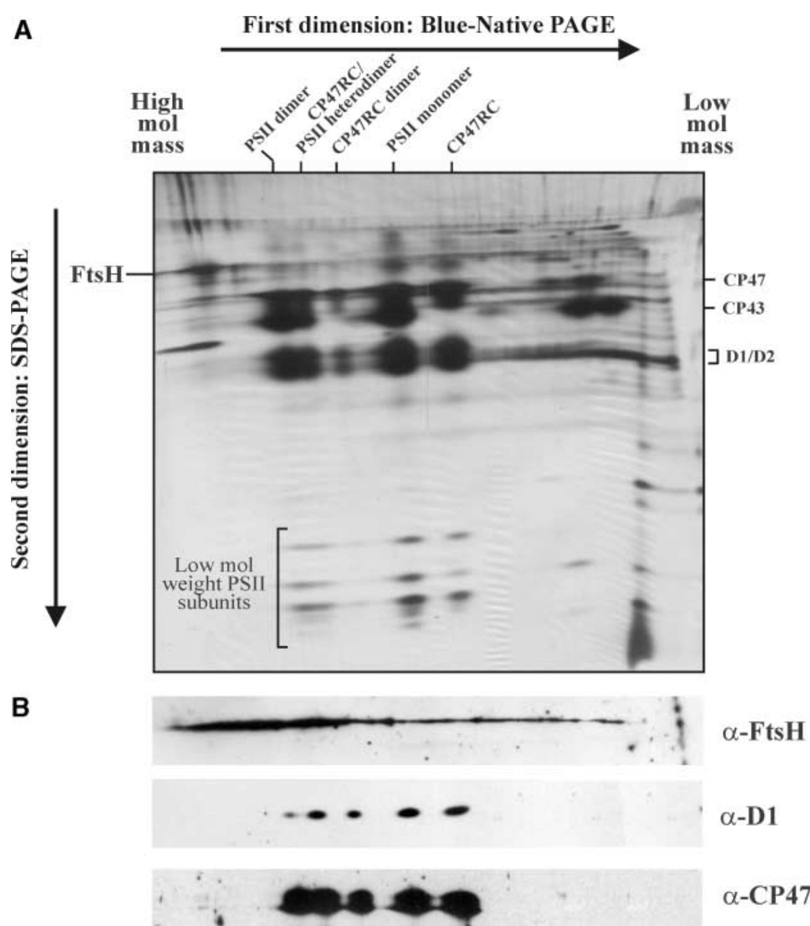

Figure 7. Analysis of His-Tagged PSII by Two-Dimensional Gel Electrophoresis.

(A) Complexes were separated in the first dimension using blue-native PAGE (5 to 16% polyacrylamide gels) followed by SDS-PAGE in the second dimension and silver staining. The positions of dimeric and monomeric PSII complexes and CP47 reaction center complexes in the first dimension are indicated by lines above the gel.

(B) Immunoblots using antibodies (α-) against FtsH, D1, and CP47.

mutant are compatible with a synchronized repair process, as suggested by Komenda and Barber (1995), in which the synthesis and incorporation of D1 into a PSII complex and the degradation of damaged D1 are mutually dependent. Therefore, in principle, impaired D1 degradation might result from impaired D1 synthesis (Komenda et al., 2000). However, the rate of D1 degradation was slowed even in the presence of lincomycin, so any effects of protein synthesis on D1 degradation can be excluded. This feature suggests a role for FtsH (slr0228) in the degradation of D1. This might be because slr0228 plays a direct role in D1 degradation or has indirect effects on the D1 repair cycle, such as on the disassembly of the damaged PSII complex before D1 degradation, the accumulation of other proteases involved in D1 degradation, the recruitment of chaperones/proteases to damaged PSII, or the presentation of damaged D1 to the degradation machinery, possibly after the migration of damaged PSII from the thylakoid to the cytoplasmic membrane, in which D1 and D2 are thought to be synthesized (Smith and Howe, 1993; Zak et al., 2001). The data shown in Figure 4 indicate that the lipid content in the mutant and the wild type is quite similar, so it is unlikely that slowing in the repair cycle is the result of a general effect of membrane fluidity on the migration of damaged PSII within the cell.

FtsH (slr0228) Is Required at an Early Stage of D1 Degradation

Notably, the degradation of D1 is impaired in the slr0228::Ω mutant at an early stage, because full-length (as assessed by SDS-PAGE) damaged D1 persists within the membrane. No D1 breakdown products were observed either immunochemically or after the radiolabeling of D1. Importantly, the lack of fragmentation of D1 in the slr0228::Ω mutant suggests that D1 cleavage and degradation are largely enzymatic under normal conditions in vivo and not reliant on the action of reactive oxygen species generated by damaged PSII (Mishra et al., 1994; Miyao et al., 1995). However, reactive oxygen species are likely to be responsible for the irreversible damage that causes the conformation

changes that in turn trigger D1 for enzymatic degradation (Sharma et al., 1997).

FtsH Homologs Bind to PSII

A direct role for FtsH (slr0228) in the repair cycle is strengthened considerably by the immunochemical detection of FtsH in a His-tagged PSII preparation (Figure 5). In parallel to our immunoblotting approach (Silva and Nixon, 2001), Kashino et al. (2001, 2002) have shown using mass spectrometry that both FtsH (slr0228) and FtsH (slr1604) are present in this type of His-tagged PSII preparation. Importantly, we show here that the presence of FtsH homologs in the His-tagged PSII preparation cannot be explained by nonspecific binding to the Ni^{2+} resin (Figure 5). Thus, FtsH is able to attach to complexes containing His-tagged CP47. Given the phenotype of the slr0228::Ω mutant, the simplest model for the function of FtsH (slr0228) is a direct role in the biogenesis and/or degradation of PSII or its subunits. The abundance of FtsH is relatively low compared with that of the other PSII subunits, as determined by Coomassie blue staining, indicative of its presence in a minor fraction of PSII (Figure 6). This might be a reflection of the presence in the preparation of PSII complexes undergoing repair. To date, attempts to identify proteases that copurify with PSII from chloroplasts have been unsuccessful, possibly because the starting material usually has been PSII from the appressed thylakoid membranes rather than from the nonappressed stromal lamellae, where PSII repair is believed to occur. In our approach, there was no initial bias in the PSII chosen for purification because our membrane samples contained all cellular membranes, including the plasma membrane.

The analysis presented here of a His-tagged PSII preparation isolated from an slr0228 insertion mutant indicates that one or more FtsH homologs are able to bind to PSII, albeit at reduced levels compared with those in wild-type preparations. The ability of other FtsH homologs to bind to PSII would explain why there is still PSII repair in the slr0228::Ω mutant at low light intensities (Figure 2B). However, at higher light intensities, in the absence of FtsH (slr0228), the degradation of damaged D1 is unable to match the increased rate of damage to PSII (Figure 2A).

VAR1 and VAR2 Are Close Relatives of FtsH (slr0228) in the Chloroplast and Also Are Important for Preventing Photoinhibition

Current predictions suggest that six to eight FtsH homologs might be targeted to the chloroplast of Arabidopsis (Adam et al., 2001; Sokolenko et al., 2002). Such a large number might reflect the targeting of different homologs to different membranes (e.g., the thylakoid and the inner envelope membrane), different substrate specificities, and different patterns of expression.

Two Arabidopsis *ftsH* mutants, *var1* (Sakamoto et al., 2002) and *var2* (Chen et al., 2000; Takechi et al., 2000), have been identified. They are both variegated, consistent with a role for FtsH in the development of chloroplasts. Based on the analysis of the slr0228 mutant described here, the effect of high light on PSII activity was examined recently in the green sectors of the *var2* mutant of Arabidopsis (lacking FtsH2). PSII was found to be more susceptible to photoinhibition than the wild type, and the degradation of D1 and D2 in lincomycin-treated detached leaves was impaired (Bailey et al., 2002). In contrast to the work presented here, selective D1 turnover has yet to be analyzed in *var2* or any plant *FtsH* mutant. Nevertheless, it is likely that *var2* is impaired in the early steps of D1 degradation (Bailey et al., 2002). Interestingly, VAR2 is the closest Arabidopsis homolog to FtsH (slr0228). More recently, the *var1* mutant of Arabidopsis (lacking FtsH5) also was shown to be important for withstanding photoinhibition, although the effects on D1 degradation have yet to be analyzed (Sakamoto et al., 2002). Besides its effects on the development of chloroplasts, the downregulation of chloroplast FtsH expression is important for the hypersensitive reaction after virus infection, probably because of the loss of photosynthetic activity in infected cells (Seo et al., 2000).

A Possible Conserved Mechanism for D1 Degradation in Cyanobacteria and Chloroplasts Involving FtsH

The analyses of the *var2* mutant (Bailey et al., 2002) and the slr0228 mutant presented here suggest a conserved role for FtsH in D1 degradation in oxygenic photosynthetic organisms. Because D1 degradation is blocked at an early stage in both mutants, we propose that FtsH is involved in the degradation of full-length damaged D1, not just breakdown fragments, as suggested previously (Lindahl et al., 2000).

Based on studies of FtsH in *E. coli* (Akiyama and Ito, 2003) and its homologs in mitochondria (Langer, 2000), suggestions can be made for how FtsH (slr0228) and its chloroplast homologs could catalyze D1 degradation. The ATPase activity of FtsH (slr0228), and possibly other homologs, might drive the pulling of damaged full-length D1, or D1 fragments, through a large FtsH pore within the membrane (Shotland et al., 1997). The data shown in Figure 7 support the idea that the FtsH homologs are able to form large protein complexes in Synechocystis. D1 proteolysis might occur from either the free N or C terminus of damaged D1 (Chiba et al., 2002) or perhaps from the ends generated by endoproteolytic cleavage (Shotland et al., 2000) of D1 in exposed regions of the protein, such as between transmembrane helices 4 and 5, close to the binding pocket of the secondary quinone. By analogy with the situation in *E. coli*, the proteolytic activity of FtsH could be stimulated by the proton-motive force across the thylakoid membrane (Akiyama, 2002) and be a highly processive reaction, so that D1 degradation products would not normally accumulate in vivo. In the case of wild-type Synechocystis, there is no indication of the light-induced accumulation of D1 fragments in vivo, and for plants, D1 fragments have been detected only under rather extreme conditions in which the normal enzymatic removal of damaged D1 might have been unable to keep pace with the production of damaged PSII (Greenberg et al., 1987; Canovas and Barber, 1993). Under such circumstances, the observed D1 fragments might be generated by reactive oxygen species or proteases unrelated to FtsH.

Analysis of D1 Degradation in Vitro and in Vivo

To understand the pathway or pathways of D1 degradation, it is important to combine data obtained in vitro with that obtained

with mutants in vivo. There are clear advantages and disadvantages to both approaches. To date, the in vitro approach has identified a role for chloroplast FtsH1 in the degradation of a D1 fragment but has failed to identify a possible role for FtsH in the degradation of full-length damaged D1. This might reflect the weak and unstable activity of FtsH used in the assay and the difficulty of measuring the small amount of full-length D1 degradation on immunoblots. At a more fundamental level, recent work with *E. coli* FtsH suggests that it is important to use a membrane system and not detergent-solubilized extracts to reconstitute the FtsH-catalyzed degradation of a membrane protein (Akiyama and Ito, 2003). Therefore, the use of membrane vesicles might be a useful approach in the future to determine whether FtsH alone can degrade full-length damaged D1 in vitro.

Are Multiple Proteases Involved in D1 Degradation in Synechocystis?

A survey of the Synechocystis genome has revealed 62 potential peptidases, many of which have close homologs in the chloroplast (Sokolenko et al., 2002). To date, the contribution of each of these to D1 degradation has not been assessed. Based on experiments in vitro, a member of the HtrA/DegP family of proteases, DegP2, has been suggested to catalyze the initial D1 cleavage event in chloroplasts (Haußühl et al., 2001). Although DegP2 is related to the HtrA/DegP family of proteases in Synechocystis, it is much larger, containing ~200 additional amino acid residues at the C terminus (Haußühl et al., 2001). In this respect, there is no obvious close homolog of the DegP2 protease in Synechocystis. Therefore, the use of DegP2 in D1 degradation in chloroplasts might have occurred after the divergence of cyanobacteria and chloroplasts. The contribution of the DegP/HtrA proteases found in Synechocystis to D1 degradation in vivo is unclear, although it is known that they are needed for growth at high light intensities (Silva et al., 2002). However, the dramatic phenotype of the slr0228 mutant described here suggests an important physiological role for this particular FtsH protease in the degradation of damaged D1. Importantly, its absence cannot be compensated for totally by other proteases within the cell, including the other members of the FtsH protease family.

METHODS

Synechocystis Strains and Growth Conditions

Table 1 describes the Synechocystis strains used in this work. The slr0228::Ω mutant and wild-type *Synechocystis* sp PCC 6803 were grown photoautotrophically in BG-11 medium (Rippka, 1972) at 28 to 30°C and at a light intensity of 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$. All strains constructed in the glucose-tolerant background were grown mixotrophically in the presence of 5 mM glucose at 28 to 30°C and at a light intensity of 20 $\mu E \cdot m^{-2} \cdot s^{-1}$. Standard methods were used to generate the Synechocystis mutants constructed in this work (Williams, 1988), and PCR was used to confirm that all copies of slr0228 had been inactivated. The *desA*$^-$/*desD*$^-$ strain (Tasaka et al., 1996) was made available by N. Murata.

Photoinhibition Experiments

The photoinhibition experiments, pulse-chase radiolabeling of cells with ^{35}S-Met, isolation of thylakoids, and oxygen evolution measurements were performed as described by Komenda and Barber (1995). Cell cultures were harvesting in early to mid exponential phase.

Isolation of His-Tagged Photosystem II

His-tagged photosystem II (PSII) complexes were isolated according to Bricker et al. (1998) with the following modifications. Thylakoid membranes were resuspended in buffer A (50 mM Mes, pH 6.0, 25% [v/v] glycerol, 20 mM $CaCl_2$, and 5 mM $MgCl_2$) and solubilized at a final concentration of 1 mg chlorophyll/mL with 1% (w/v) β-D-dodecyl maltoside (DM) at 4°C for 10 min. Unsolubilized membranes were removed by centrifugation at 150,000*g* for 30 min using a Beckman Ti70 rotor. The solubilized extract was diluted to 0.15 mg chlorophyll/mL with buffer A, and 70 μL was mixed for 1 h at 4°C with 200 μL of nitrilotriacetic acid agarose resin (Qiagen, Valencia, CA) charged with Ni^{2+}. The resin was

Table 1. Synechocystis Strains Used in This Study

Strain	Relevant Genotype	Reference
Wild-type *Synechocystis* sp PCC 6803		Pasteur Culture Collection
Synechocystis sp PCC 6803-G	Glucose-tolerant derivative of the wild type	Williams (1988)
HT-3	C-terminal His_6-tagged derivative of CP47 (PsbB); glucose-tolerant background; kan^R	Bricker et al. (1998)
slr0228::Ω	Insertion of a 2-kb Ω fragment into the AccI site of *ftsH* (slr0228) 233 bp downstream of the initiation codon; wild-type background; $spec^R$	Mann et al. (2000)
HT-3/slr0228::cm^R	Replacement of a 0.5-kb SmaI fragment within *ftsH* (slr0228) with a chloramphenicol resistance cassette; coding region disrupted 253 bp downstream of the initiation codon; HT-3 background; kan^R, cm^R	This work
slr0228::kan^R	Insertion of a kanamycin resistance cassette into *ftsH* (slr0228) at the MscI site 1144 bp downstream of the initiation codon; glucose-tolerant wild-type background; kan^R	This work
desA$^-$/*desD*$^-$	*desA*::(kan^R+ble^R)/*desD*::cm^R; glucose-tolerant wild-type background	Tasaka et al. (1996)

washed at least five times with 150 μL of buffer A containing 0.03% DM and 5 mM imidazole, and the bound proteins were eluted step-wise with 150-μL fractions of buffer A and 0.03% DM containing increasing concentrations of imidazole (50, 100, 200, and 300 mM). Fractions were analyzed on an equal-volume basis. To determine whether FtsH could bind to IMMUTANS, His-tagged IMMUTANS (14 μg) (Prommeenate et al., 2001) was incubated on ice for 10 min with a solubilized extract obtained from the glucose-tolerant strain of Synechocystis (0.15 mg chlorophyll/mL; final volume of 70 μL) and then chromatographed using the Ni^{2+}-charged resin as described above.

Gel Electrophoresis and Immunoblot Analysis

SDS-PAGE and immunodetection were performed as described by Hankamer et al. (1997). The D1-specific antiserum was raised against a C-terminal peptide and has been described by Nixon et al. (1990). Quantitation of the intensity of bands was performed using NIH Image software version 1.62. Blue-native gel electrophoresis was performed according to Schägger and von Jagow (1991) and Schägger et al. (1994) using His-tagged PSII complexes isolated as described above. Fifty-microliter samples were prepared for electrophoresis by mixing 10 μL of Coomassie Brilliant Blue G 250 stock solution (5% [w/v] in 500 mM aminocaproic acid), 20 μL of 50 mM Bis-Tris, pH 7.0, 750 mM aminocaproic acid, and 20 μL of PSII (containing 2 μg of chlorophyll) in buffer A containing 100 mM imidazole and 0.03% (v/v) DM. Electrophoresis through 5 to 16% polyacrylamide gels was performed at 100 V and 15 mA overnight at 4°C, and then the voltage was increased to 400 V for 3 to 4 h. After denaturing electrophoresis in the second dimension, gels were stained with silver as described by Blum et al. (1987) or immunoblotted.

Lipid Analysis

Membranes were prepared from Synechocystis cells essentially according to Rögner et al. (1990). Lipid and fatty acid extraction was performed as described by Kruse et al. (2000). The relative amounts of galactolipid or phospholipid were investigated using thin layer chromatography, staining with anthron or molybdenum oxide, respectively. Fatty acid composition was analyzed using gas liquid chromatography, again according to Kruse et al. (2000).

Upon request, materials integral to the findings presented in this publication will be made available in a timely manner to all investigators on similar terms for noncommercial research purposes. To obtain materials, please contact Peter J. Nixon, p.nixon@imperial.ac.uk.

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REFERENCES

Adam, Z., Adamska, I., Nakabayashi, K., Ostersetzer, O., Haussuhl, K., Manuell, A., Zheng, B., Vallon, O., Rodermel, S.R., Shinozaki, K., and Clarke, A.K. (2001). Chloroplast and mitochondrial proteases in *Arabidopsis*: A proposed nomenclature. Plant Physiol. **125,** 1912–1918.

Adam, Z., and Clarke, A.K. (2002). Cutting edge of proteolysis. Trends Plant Sci. **7,** 451–456.

Akiyama, Y. (2002). Proton-motive force stimulates the proteolytic activity of FtsH, a membrane-bound ATP-dependent protease in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **99,** 8066–8071.

Akiyama, Y., and Ito, K. (2003). Reconstitution of membrane proteolysis by FtsH. J. Biol. Chem. **278,** 18146–18153.

Andersson, B., and Aro, E.-M. (2001). Photodamage and D1 protein turnover in photosystem II. In Regulation of Photosynthesis, E.-M. Aro and B. Andersson, eds (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 377–393.

Aro, E.M., Virgin, I., and Andersson, B. (1993). Photoinhibition of photosystem II: Inactivation, protein damage and turnover. Biochim. Biophys. Acta **1143,** 113–134.

Bailey, S., Thompson, E., Nixon, P.J., Horton, P., Mullineaux, C.W., Robinson, C., and Mann, N.H. (2002). A critical role for the Var2 FtsH homologue of *Arabidopsis thaliana* in the photosystem II repair cycle in vivo. J. Biol. Chem. **277,** 2006–2011.

Barber, J., and Andersson, B. (1992). Too much of a good thing: Light can be bad for photosynthesis. Trends Biochem. Sci. **17,** 61–66.

Blum, H., Beier, H., and Gross, H.J. (1987). Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis **8,** 93–99.

Bricker, T.M., Morvant, J., Masri, N., Sutton, H.M., and Frankel, L.K. (1998). Isolation of a highly active photosystem II preparation from *Synechocystis* 6803 using a histidine-tagged mutant of CP 47. Biochim. Biophys. Acta **1409,** 50–57.

Canovas, P.M., and Barber, J. (1993). Detection of a 10 kDa breakdown product containing the C-terminus of the D1-protein in photoinhibited wheat leaves suggests an acceptor side mechanism. FEBS Lett. **324,** 341–344.

Chen, M., Choi, Y., Voytas, D.F., and Rodermel, S. (2000). Mutations in the Arabidopsis *VAR*2 locus cause leaf variegation due to the loss of a chloroplast FtsH protease. Plant J. **22,** 303–313.

Chiba, S., Akiyama, Y., and Ito, K. (2002). Membrane protein degradation by FtsH can be initiated from either end. J. Bacteriol. **184,** 4775–4782.

Clausen, T., Southan, C., and Ehrmann, M. (2002). The HtrA family of proteases: Implications for protein composition and cell fate. Mol. Cell **10,** 443–455.

Constant, S., Eisenberg-Domovitch, Y., Ohad, I., and Kirilovsky, D. (2000). Recovery of photosystem II activity in photoinhibited *Synechocystis* cells: Light-dependent translation activity is required besides light-independent synthesis of the D1 protein. Biochemistry **39,** 2032–2041.

Diner, B.A., and Rappaport, F. (2002). Structure, dynamics, and energetics of the primary photochemistry of photosystem II of oxygenic photosynthesis. Annu. Rev. Plant Biol. **53,** 551–580.

Goloubinoff, P., Brusslan, J., Golden, S., Haselkorn, R., and Edelman, M. (1988). Characterization of the photosystem II 32kDa protein in *Synechococcus* PCC 7942. Plant Mol. Biol. **11,** 441–447.

Greenberg, B.M., Gaba, V., Mattoo, A.K., and Edelman, M. (1987). Identification of a primary in vivo degradation product of the rapidly-turning-over 32 kD protein of photosystem II. EMBO J. **6,** 2865–2869.

Hankamer, B., Nield, J., Zheleva, D., Boekema, E., Jansson, S., and Barber, J. (1997). Isolation and biochemical characterisation of monomeric and dimeric photosystem II complexes from spinach and their relevance to the organisation of photosystem II *in vivo*. Eur. J. Biochem. **243,** 422–429.

Haußühl, K., Andersson, B., and Adamska, I. (2001). A chloroplast DegP2 protease performs the primary cleavage of the photodamaged D1 protein in plant photosystem II. EMBO J. **20,** 713–722.

Hihara, Y., Kamei, A., Kanehisa, M., Kaplan, A., and Ikeuchi, M.

(2001). DNA microarray analysis of cyanobacterial gene expression during acclimation to high light. Plant Cell **13,** 793–806.

Inagaki, N., Yamamoto, Y., and Satoh, K. (2001). A sequential two-step proteolytic process in the carboxyl-terminal truncation or precursor D1 protein in Synechocystis sp. PCC6803. FEBS Lett. 509, 197–201.

Kaneko, T., and Tabata, S. (1997). Complete genome structure of the unicellular cyanobacterium *Synechocystis* sp. PCC6803. Plant Cell Physiol. **38,** 1171–1176.

Kanervo, E., Tasaka, Y., Murata, N., and Aro, E.-M. (1997). Membrane lipid unsaturation modulates processing of the photosystem II reaction-center protein DS1 at low temperatures. Plant Physiol. **114,** 841–849.

Karata, K., Inagawa, T., Wilkinson, A.J., Tatsuta, T., and Ogura, T. (1999). Dissecting the role of a conserved motif (the second region of homology) in the AAA family of ATPases: Site-directed mutagenesis of the ATP-dependent protease FtsH. J. Biol. Chem. **274,** 26225–26232.

Kashino, Y., Lauber, W.M., Carroll, J.A., Wang, Q., Whitmarsh, J., Satoh, K., and Pakrasi, H.B. (2001). Characterization of purified His-tagged CP47-containing photosystem II complexes from a cyanobacterium, *Synechocystis* sp. PCC 6803. In 12th International Congress of Photosynthesis. (Melbourne, Australia: CSIRO Publishing).

Kashino, Y., Lauber, W.M., Carroll, J.A., Wang, Q., Whitmarsh, J., Satoh, K., and Pakrasi, H.B. (2002). Proteomic analysis of a highly active photosystem II preparation from the cyanobacterium *Synechocystis* sp. PCC 6803 reveals the presence of novel polypeptides. Biochemistry **41,** 8004–8012.

Keren, N., and Ohad, I. (1998). State transition and photoinhibition. In The Molecular Biology of Chloroplasts and Mitochondria in Chlamydomonas, J.-D. Rochaix, M.G. Clermont, and S. Merchant, eds (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 569–596.

Kihara, A., Akiyama, Y., and Ito, K. (1996). A protease complex in the *Escherichia coli* plasma membrane: HflKC (HflA) forms a complex with FtsH (HflB), regulating its proteolytic activity against SecY. EMBO J. **15,** 6122–6131.

Komenda, J., and Barber, J. (1995). Comparison of *psbO* and *psbH* deletion mutants of *Synechocystis* PCC 6803 indicates that degradation of D1 protein is regulated by the Q_B site and dependent on protein synthesis. Biochemistry **34,** 9625–9631.

Komenda, J., Hassan, H.A., Diner, B.A., Debus, R.J., Barber, J., and Nixon, P.J. (2000). Degradation of the photosystem II D1 and D2 proteins in different strains of the cyanobacterium *Synechocystis* PCC 6803 varying with respect to the type and level of *psbA* transcript. Plant Mol. Biol. **42,** 635–645.

Kruse, O., Hankamer, B., Konczak, C., Gerle, C., Morris, E., Radunz, A., Schmid, G.H., and Barber, J. (2000). Phosphatidylglycerol is involved in the dimerization of photosystem II. J. Biol. Chem. **275,** 6509–6514.

Kyle, D.J., Ohad, I., and Arntzen, C.J. (1984). Membrane protein damage and repair: Selective loss of a quinone-protein function in chloroplast membranes. Proc. Natl. Acad. Sci. USA **81,** 4070–4074.

Langer, T. (2000). AAA proteases: Cellular machines for degrading membrane proteins. Trends Biochem. Sci. **25,** 247–251.

Lindahl, M., Spetea, C., Hundal, T., Oppenheim, A.B., Adam, Z., and Andersson, B. (2000). The thylakoid FtsH protease plays a role in the light-induced turnover of the photosystem II D1 protein. Plant Cell **12,** 419–431.

Lindahl, M., Tabak, S., Cseke, L., Pichersky, E., Andersson, B., and Adam, Z. (1996). Identification, characterisation and molecular cloning of a homologue of the bacterial FtsH protease in chloroplasts of higher plants. J. Biol. Chem. **271,** 29329–29334.

Long, S.P., and Humphries, S. (1994). Photoinhibition of photosynthesis in nature. Annu. Rev. Plant Physiol. Plant Mol. Biol. **45,** 633–662.

Mann, N., Novac, N., Mullineaux, C., Newman, J., Bailey, S., and Robinson, C. (2000). Involvement of an FtsH homologue in the assembly of functional photosystem I in the cyanobacterium *Synechocystis* sp. PCC 6803. FEBS Lett. **479,** 72–77.

Mishra, N.P., Francke, C., van Gorkom, H.J., and Ghanotakis, D.F. (1994). Destructive role of singlet oxygen during aerobic illumination of the photosystem II core complex. Biochim. Biophys. Acta **1186,** 81–90.

Miyao, M., Ikeuchi, M., Yamamoto, N., and Ono, T. (1995). Specific degradation of the D1 protein of photosystem II by treatment with hydrogen peroxide in darkness: Implications for the mechanism of degradation of the D1 protein under illumination. Biochemistry **34,** 10019–10026.

Nixon, P.J., Metz, J.G., Rögner, M., and Diner, B.A. (1990). A *Synechocystis* PCC 6803 *psbA* deletion mutant and its transformation with a *psbA* gene from a higher plant. In Current Research in Photosynthesis, Vol. 1, M. Baltscheffsky, ed (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 471–474.

Ogura, T., et al. (1999). Balanced biosynthesis of major membrane components through regulated degradation of the committed enzyme of lipid biosynthesis by the AAA protease FtsH (HflB) in *Escherichia coli*. Mol. Microbiol. **31,** 833–844.

Ogura, T., Tomoyasu, T., Yuki, T., Morimura, S., Begg, K.J., Donachie, W.D., Mori, H., Niki, H., and Hiraga, S. (1991). Structure and function of the *ftsH* gene in *Escherichia coli*. Res. Microbiol. **142,** 279–282.

Ohad, I., Keren, N., Zer, H., Gong, H., Mor, T.S., Gal, A., Tal, S., and Domovich, Y. (1994). Light-induced degradation of the photosystem II reaction centre D1 protein in vivo: An integrative approach. In Photoinhibition of Photosynthesis, N.R. Baker and J.R. Bowyer, eds (Oxford, UK: Bios Scientific Publishers), pp. 161–177.

Ohad, I., Kyle, D.J., and Arntzen, C.J. (1984). Membrane protein damage and repair: Removal and replacement of inactivated 32-kilodalton polypeptides in chloroplast membranes. J. Cell Biol. **270,** 14919–14927.

Prasil, O., Adir, N., and Ohad, I. (1992). Dynamics of photosystem II: Mechanism of photoinhibition and recovery processes. In The Photosystems: Structure, Function and Molecular Biology, J. Barber, ed (Amsterdam: Elsevier Science Publishers), pp. 295–348.

Prommeenate, P., Lennon, A.M., and Nixon, P.J. (2001). Biochemical analysis of IMMUTANS, a potential plastoquinol oxidase of the thylakoid membrane. In 12th International Congress of Photosynthesis. (Melbourne, Australia: CSIRO Publishing).

Rhee, K.H., Morris, E.P., Barber, J., and Kühlbrandt, W. (1998). Three-dimensional structure of the plant photosystem II reaction centre at 8Å resolution. Nature **396,** 283–286.

Rippka, R. (1972). Photoheterotrophy and chemoheterotrophy among unicellular blue-green algae. Arch. Mikrobiol. **87,** 93–98.

Rögner, M., Mühlenhoff, U., Boekema, E.J., and Witt, H.T. (1990). Mono-, di- and trimeric PS I reaction center complexes isolated from the thermophilic cyanobacterium *Synechococcus* sp.: Size, shape and activity. Biochim. Biophys. Acta **1015,** 415–424.

Sakamoto, W., Tamura, T., Hanba-Tomita, Y., and Murata, M. (2002). The *VAR1* locus of *Arabidopsis* encodes a chloroplastic FtsH and is responsible for leaf variegation in the mutant alleles. Genes Cells **7,** 769–780.

Santos, D., and De Almeida, D.F. (1975). Isolation and characterization of a new temperature-sensitive cell division mutant of *Escherichia coli* K-12. J. Bacteriol. **124,** 1502–1507.

Schägger, H., Cramer, W.A., and von Jagow, G. (1994). Analysis of molecular masses and oligomeric states of protein complexes by blue native electrophoresis and isolation of membrane protein complexes by two-dimensional native electrophoresis. Anal. Biochem. **217,** 220–230.

Schägger, H., and von Jagow, G. (1991). Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. Anal. Biochem. **199,** 223–231.

Seo, S., Okamoto, M., Iwai, T., Iwano, M., Fukui, K., Isogai, A., Nakajima, N., and Ohashi, Y. (2000). Reduced levels of chloroplast FtsH protein in tobacco mosaic virus–infected tobacco leaves accelerate the hypersensitive reaction. Plant Cell **12,** 917–932.

Sharma, J., Panico, M., Shipton, C.A., Nilsson, F., Morris, H.R., and Barber, J. (1997). Primary structure characterization of the photosystem II D1 and D2 subunits. J. Biol. Chem. **272,** 33158–33166.

Shotland, Y., Koby, S., Teff, D., Mansur, N., Oren, D.A., Tatematsu, K., Tomoyasu, T., Kessel, M., Bukau, B., Ogura, T., and Oppenheim, A.B. (1997). Proteolysis of the phage λ CII regulatory protein by FtsH (HflB) of *Escherichia coli*. Mol. Microbiol. **24,** 1303–1310.

Shotland, Y., Shifrin, A., Ziv, T., Teff, D., Koby, S., Kobiler, O., and Oppenheim, A.B. (2000). Proteolysis of bacteriophage lambda CII by *Escherichia coli* FtsH (HflB). J. Bacteriol. **182,** 3111–3116.

Silva, P., Choi, Y.-J., Hassan, H.A.G., and Nixon, P.J. (2002). Involvement of the HtrA family of proteases in the protection of the cyanobacterium *Synechocystis* PCC 6803 from light stress and in the repair of photosystem II. Philos. Trans. R. Soc. Lond. B Biol. Sci. **357,** 1461–1468.

Silva, P., and Nixon, P.J. (2001). Identification of possible assembly and repair factors in photosystem two preparations of *Synechocystis* sp. PCC 6803: A new model for D1 turnover. In 12th International Congress of Photosynthesis. (Melbourne, Australia: CSIRO Publishing).

Smith, D., and Howe, C.J. (1993). The distribution of photosystem I and photosystem II polypeptides between the cytoplasmic and thylakoid membranes of cyanobacteria. FEMS Microbiol. Lett. **110,** 341–348.

Sokolenko, A., Pojidaeva, E., Zinchenko, V., Panichkin, V., Glaser, V.M., Herrmann, R.G., and Shestakov, S.V. (2002). The gene complement for proteolysis in the cyanobacterium *Synechocystis* sp. PCC 6803 and *Arabidopsis thaliana* chloroplasts. Curr. Genet. **41,** 291–310.

Spetea, C., Hundal, T., Lohmann, F., and Andersson, B. (1999). GTP bound to chloroplast thylakoid membranes is required for light-induced, multienzyme degradation of the photosystem II D1 protein. Proc. Natl. Acad. Sci. USA **96,** 6547–6552.

Steglich, G., Neupert, W., and Langer, T. (1999). Prohibitins regulate membrane protein degradation by the m-AAA protease in mitochondria. Mol. Cell. Biol. **19,** 3435–3442.

Takechi, K., Sodmergen, Murata, M., Motoyoshi, F., and Sakamoto, W. (2000). The *YELLOW VARIEGATED* (*VAR2*) locus encodes a homologue of FtsH, an ATP-dependent protease in *Arabidopsis*. Plant Cell Physiol. **41,** 1334–1346.

Tasaka, Y., Gombos, Z., Nishiyama, Y., Mohanty, P., Ohba, T., Ohki, K., and Murata, N. (1996). Targeted mutagenesis of acyl-lipid desaturases in *Synechocystis* 6803: Evidence for the important roles of polyunsaturated membrane lipids in growth, respiration and photosynthesis. EMBO J. **15,** 6416–6425.

Williams, J.G.K. (1988). Construction of a specific mutation in photosystem II photosynthetic reaction center by genetic engineering methods in *Synechocystis* 6803. Methods Enzymol. **167,** 766–778.

Zak, E., Norling, B., Maitra, R., Huang, F., Andersson, B., and Pakrasi, H.B. (2001). The initial steps of biogenesis of cyanobacterial photosystems occur in plasma membranes. Proc. Natl. Acad. Sci. USA **98,** 13443–13448.