The Deg Proteases Protect Synechocystis sp. PCC 6803 during Heat and Light Stresses but Are Not Essential for Removal of Damaged D1 Protein during the Photosystem Two Repair Cycle*

Received for publication, February 3, 2006, and in revised form, July 24, 2006 Published, JBC Papers in Press, August 15, 2006, DOI 10.1074/jbc.M601064200

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Members of the DegP/HtrA (or Deg) family of proteases are found widely in nature and play an important role in the proteolysis of misfolded and damaged proteins. As yet, their physiological role in oxygenic photosynthetic organisms is unclear, although it has been widely speculated that they participate in the degradation of the photodamaged D1 subunit in the photosystem two complex (PSII) repair cycle, which is needed to maintain PSII activity in both cyanobacteria and chloroplasts. We have examined the role of the three Deg proteases found in the cyanobacterium Synechocystis sp. PCC 6803 through analysis of double and triple insertion mutants. We have discovered that these proteases show overlap in function and are involved in a number of key physiological responses ranging from protection against light and heat stresses to phototaxis. In previous work, we concluded that the Deg proteases played either a direct or an indirect role in PSII repair in a glucose-tolerant version of Synechocystis sp. PCC 6803 (Silva, P., Choi, Y. J., Hassan, H. A., and Nixon, P. J. (2002) Philos. Trans. R. Soc. Lond. B Biol. Sci. 357, 1461–1467). In this work, we have now been able to demonstrate unambiguously, using a triple deg mutant created in the wild type strain of Synechocystis sp. PCC 6803, that the Deg proteases are not obligatory for PSII repair and D1 degradation. We therefore conclude that although the Deg proteases are needed for photoprotection of Synechocystis sp. PCC 6803, they do not play an essential role in D1 turnover and PSII repair in vivo.

An inevitable consequence of the light reactions of oxygenic photosynthesis is the formation of highly reactive molecules, such as reactive oxygen species (ROS)5 and amino acid free radicals, which can cause irreversible damage to a variety of cellular components including nucleic acids, lipids, pigments, and proteins (1, 2). The photosystem two complex (PSII), which functions as the light-driven water:plastoquinone oxidoreductase in oxygenic photosynthetic electron transport, is particularly prone to light-induced damage (3). Of the more than 25 protein subunits found in PSII, the D1 reaction center subunit appears to be the major target for photodamage (4–6). To maintain activity, a damaged PSII complex is repaired through the specific replacement of the damaged subunit (usually D1) by a newly synthesized subunit (3). Despite the importance of the PSII repair cycle for maintaining optimal photosynthetic rates in vivo, the molecular details of this repair process remain unclear.

Recently, attention has focused on the identity of the proteases that are involved in removing damaged D1 from the PSII complex. In the case of chloroplasts, in vitro experiments suggest that D1 degradation occurs in a two-step process involving the participation of two classes of protease (7). First, a member of the DegP/HtrA family of proteases (or Deg proteases), originally designated DegP2 but now renamed Deg2 (8), is thought to cleave damaged D1 between trans-membrane helices four and five on the stromal side of the membrane to generate N-terminal and C-terminal fragments of ~23 and 10 kDa, respectively. Subsequently, the 23-kDa fragment, and possibly the 10-kDa fragment, is removed from the membrane by one or more members of the FtsH protease family (9).

Selective D1 degradation also occurs in cyanobacteria such as Synechocystis sp. PCC 6803 (10). Analysis of the genome sequence of Synechocystis sp. PCC 6803 has identified three members of the Deg proteases and four members of the FtsH family of proteases (11). Mutagenesis experiments have so far demonstrated a role for one of the FtsH proteases (slr0228) in PSII repair at an early stage in D1 degradation (12). However, it remains unclear to what extent the Deg proteases are important for D1 degradation in vivo. This is a crucial question to address since recent biochemical experiments have suggested that a homologue of Deg2 in Synechocystis sp. PCC 6803 extracts could be involved in cleaving D1 during PSII repair (8, 13).

The DegP/HtrA family of proteases was initially characterized in Escherichia coli and is composed of DegP (also known as HtrA), DegQ (or HhoA), and DegS (or HhoB) (reviewed in Ref. 14). They are serine-type proteases and are found in the periplasm (DegQ), attached to the periplasmic surface of the cytoplasmic membrane (DegP), or embedded in the cytoplasmic membrane facing the periplasm (DegS). DegS is involved in

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5 The abbreviations used are: ROS, reactive oxygen species; PSII, photosystem two complex; WT, wild type; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyleneguanine. TFP, type IV pilus; TES, 2-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)-amino)ethanesulfonic acid.
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activating the $\alpha_F$-dependent transcription of stress genes and is the only member absolutely required for cell viability (14, 15). E. coli DegP has a dual function: acting as a molecular chaperone at low temperatures and acting as a protease at higher temperatures (16). The major role for DegP is thought to be the degradation of misfolded proteins (17) and denatured proteins formed, for example, during heat shock (18) and oxidative stress (19). Genetic and biochemical experiments indicate that DegP and DegQ, but not DegS, have overlapping functions (18, 20). Likewise, Deg homologues in other bacteria also show overlap in function (21, 22).

In the case of Synechocystis sp. PCC 6803, the three Deg homologues identified in the genome data base (CyanoBase, Kazusa Research Institute, Japan) are annotated HtrA (CyanoBase designation slr1204), HhoA (sll1679), and HhoB (sll1427). However, these gene products cannot be assumed to play an equivalent role to the E. coli homologues of the same name (23). Structural predictions indicate that all three members possess the serine protease domain and one of the C-terminal PDZ domains typical of this class of protease (23). The deg transcript levels increase upon light but not heat stress (24). This has reinforced the speculation that the primary role of the Deg proteases in cyanobacteria is related to photoprotection and PSII repair rather than heat stress (8).

Although the location of the Deg proteases in Synechocystis sp. PCC 6803 has not yet been established definitively, proteomics data suggest that HhoA is in the periplasm (25) and HtrA is in the outer membrane (26). At first sight, such a location would appear to exclude a role in PSII repair since functional PSII is found in the thylakoid membrane. However, PSII subcomplexes containing D1 have been found in the cytoplasmic membrane (27, 28), so it remains feasible that proteases found in the extracytoplasmic compartment might play a role in D1 degradation (29).

To address the physiological importance of the Deg proteases, we previously constructed a triple mutant in which each of the Deg proteases was insertional inactivated in the widely used glucose-tolerant strain of Synechocystis sp. PCC 6803 (30). We found that growth of the triple mutant was more sensitive than the wild type to high irradiances of visible light and that irradiance was supplemented with 5 mM glucose and the antibiotics chloramphenicol (10 $\mu$g ml$^{-1}$), erythromycin (10 $\mu$g ml$^{-1}$), and kanamycin (25 $\mu$g ml$^{-1}$).

Construction of Mutants—Interposon disruption of the Synechocystis sp. PCC 6803 deg genes encoding DegP/HtrA (slr1204), DegQ/HhoA (sll1679), and DegS/HhoB (sll1427) was performed using plasmids constructed previously (31). The three double deg mutants termed htrA$^{-}$hhoA$^{-}$ (slr1204$^{-}$sll1679$^{-}$), htrA$^{-}$hhoB$^{-}$ (slr1204$^{-}$sll1427$^{-}$), and hhoA$^{-}$hhoB$^{-}$ (sll1679$^{-}$sll1427$^{-}$) were generated in the WT strain, and all three genes were inactivated in both WT and WT-G strains to produce htrA$^{-}$hhoA$^{-}$hhoB$^{-}$ triple mutants (hereafter designated $\Delta$Deg and $\Delta$Deg-G strains, respectively). The genotypes of the mutant strains were verified by PCR using the following internal gene-specific primers: slr1204F, 5'-CTTAAGGATGGTCGTTCCTTTCC-3', and slr1204R, 5'-TCCACA-GGAATGTTCATACCCGT-3'; sll1427F, 5'-GTCTTCTAGGCCA-AAACATTCTC-3', and sll1427R, 5'-CGGTTTGAAGATGAGT-GCCAATC-3'; sll1679F, 5'-ACACGTGTAACCCGTCCGACT-3', and sll1679R, 5'-GGACGAGGGATTTAGAATTCG-3'. To generate a sigF mutant ($\Delta$SigF) of Synechocystis sp. PCC 6803 (WT background), the sigF gene (sll1564) was inactivated by insertion of an aphIII kanamycin antibiotic resistance cassette at the Splu site, 604 bp downstream of the sigF start codon. Transcription of the resistance cassette was in reverse orientation to that of sigF.

Protein Assays and Immunoblotting—Crude Synechocystis sp. PCC 6803 thylakoid membranes were prepared by glass bead breakage of cells as described in Ref. 32. Chlorophyll $a$ content was assessed by extraction into methanol and measurement of the absorbance at 666 nm (33). Protein concentrations were determined using a DC protein kit (Bio-Rad Laboratories).

Thylakoid membrane proteins were separated on 12% denaturing SDS-PAGE gels according to Ref. 32 unless otherwise stated. Gels were loaded on either chlorophyll $a$ or total protein content where indicated in the legends for Figs. 2C, 5B, 6, and 7B. Gels were then either stained with Coomassie Blue or electroblotted onto nitrocellulose (0.2-$\mu$m pore size, Bio-Rad Laboratories). Nitrocellulose membranes were incubated with specific antibodies before being probed with a horseradish peroxidase-conjugated secondary antibody (Amersham Biosciences). Proteins were visualized using a chemiluminescent kit (SuperSignal West Pico, Pierce). Primary antibodies used in this study were: (i) a C-terminal D1-specific anti-peptide antiserum (34); (ii) an anti-peptide antibody specific for E. coli FtsH, which is potentially cross-reactive with all Synechocystis sp. PCC 6803 FtsH homologues, kindly provided by Professor T. Ogura (University of Kumamoto, Japan); and (iii) a PsAD-specific antiserum donated by Professor J.-D. Rochaix (University of Geneva, Switzerland). The general oxidation state of membrane proteins was analyzed by the immunochemochemical detection of 2,4-dinitrophenylhydrazine-derivatized carbonyl groups (OxyBlot™, Chemicon International). Derivatization was performed on 15 $\mu$g of total membrane proteins for each sample.
Electron Microscopy—Synechocystis sp. PCC 6803 cells were grown in liquid BG-11 medium at 29 °C with a white light intensity of 20 μmol of photons m⁻² s⁻¹ to an OD₇₃₀ of 0.4–0.5. Undiluted cell samples were negatively stained via the droplet method (35), with 2% uranyl acetate, and imaged on Kodak SO-163 film at room temperature using a Phillips CM100 electron microscope. Micrographs were taken at 100 kV, ×15,500 magnification, and a typical defocus of 2 μm. Micrographs displaying no discernible astigmatism or drift were scanned with a Nikon LS9000 Super Coolscan densitometer with an initial step size of 6.35 μm.

Growth Assays—Synechocystis sp. PCC 6803 cells were grown in liquid or on solid BG-11 medium and subjected to: low light growth, 29 °C with 10 μmol of photons m⁻² s⁻¹ white light; high light, 29 °C with 120 μmol of photons m⁻² s⁻¹ white light; low temperature, 18 °C with 10 μmol of photons m⁻² s⁻¹ white light; high temperature, 37 °C with 10 μmol of photons m⁻² s⁻¹ white light; hydrogen peroxide, 29 °C with 10 μmol of photons m⁻² s⁻¹ white light and 10–250 μM hydrogen peroxide; cumene hydroperoxide, 29 °C with 10 μmol of photons m⁻² s⁻¹ white light and 2 mM potassium ferricyanide as artificial electron acceptors (10). For photoinhibition, Synechocystis sp. PCC 6803 cells were grown without glucose and bubbled with air at 29 °C with 10 μmol of photons m⁻² s⁻¹ white light to an OD₇₃₀ of 0.1. Plates were then exposed to a unidirectional fluorescent light; high light, 29 °C with 120 μmol of photons m⁻² s⁻¹ white light and 50–250 μM cumene hydroperoxide; high salt, 29 °C with 10 μmol of photons m⁻² s⁻¹ white light and 0.5 M NaCl. Solid medium growth assays were performed by streaking liquid grown cells diluted to an OD₇₃₀ of 0.1 onto BG-11 plates with incubation for the time indicated in the legend for Fig. 2. For the rescue of photosensitivity, cells were grown in liquid BG11 medium at 29 °C with a white light intensity of 20 μmol of photons m⁻² s⁻¹ to an OD₇₃₀ of 0.8.

Experiments—The activity of PSII was assessed by measuring the light-saturated rate of oxygen evolution from whole cells using 1 mM 2,6-dichloro-1,1-dimethylyurea (DCMU) or 10 μM atrazine and subjected to high light stress for the time indicated in the legend for Fig. 2. These concentrations of DCMU and atrazine were sufficient to block PSII activity as assessed by inhibition of growth on BG-11 agar plates and by inhibition of QA-to-QB electron transfer in cells in chlorophyll flash fluorescence experiments. For phototaxis experiments, BG-11 agar plates (1.5% agar) were spot inoculated with 5 μl of cells at an OD₇₃₀ of 0.1. Plates were then exposed to a unidirectional fluorescent white light at 15 μmol of photons m⁻² s⁻¹ and incubated at 29 °C for the time indicated in the legend for Fig. 4 (41).

Photosystem II Activity Measurements and Photoinhibition Experiments—The activity of PSII was assessed by measuring the light-saturated rate of oxygen evolution from whole cells using 1 mM 2,6-dichloro-p-benzoquinone and 2 mM potassium ferricyanide as artificial electron acceptors (10). For photoinhibition experiments, Synechocystis sp. PCC 6803 cells were grown without glucose and bubbled with air at 29 °C with 10 μmol of photons m⁻² s⁻¹ white light to an OD₇₃₀ of 0.5–0.8. Cells were harvested and resuspended in BG-11 medium to a concentration of 10 μg of chlorophyll a ml⁻¹ and subjected to a white light intensity of 100 μmol of photons m⁻² s⁻¹ for 1 h and then 1,200 μmol of photons m⁻² s⁻¹ for up to 8 h either at normal growth temperature (29 °C) or at low temperature (23 °C), with or without spectinomycin (200 μg ml⁻¹), a protein synthesis inhibitor.

Pulse-Chase Radiolabeling Experiments—Cells of WT and ΔDeg were radiolabeled using a mixture of [³⁵S]methionine and [³⁵S]cysteine (ICN) as described in Ref. 36. Pulse-chase experiments were performed as described in Ref. 32.

RESULTS

Construction of Triple and Double deg Mutants and Their Growth Characteristics in High Light—A triple mutant (ΔDeg), in which all three members of the Deg protease family were disrupted, was generated in WT Synechocystis sp. PCC 6803 by the stepwise inactivation of each gene (Fig. 1A) (see “Expri-
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A

WT Low light $\Delta$Deg

htrA hhoB

htrA hhoB

htrA hhoB

htrA hhoB

B

Low light - DCMU High light - DCMU

WT-G $\Delta$Deg-G

htrA hhoB htrA hhoB

htrA hhoB htrA hhoB

htrA hhoB htrA hhoB

htrA hhoB htrA hhoB

C

Size [kDa]

M WT $\Delta$Deg

97.4

68.0

43.0

29.0

21.0

D1

FIGURE 2. Effect of high light on the Synechocystis PCC 6803 deg mutants. A, WT, $\Delta$Deg, htrA$^{-}$hhoB, hhoA$^{-}$hhoB, and htrA$^{-}$hhoB$^{-}$ double mutants were restreaked onto BG-11 agar plates and incubated for a period of 7 days under low and high light conditions (10 and 120 $\mu$mol of photons m$^{-2}$ s$^{-1}$), respectively, see “Experimental Procedures”). Arrows indicate strains tested. B, cells of the WT-G strain and equivalent deg triple mutant ($\Delta$Deg-G) were streaked onto BG-11 agar containing 5 mM glucose with (+DCMU) or without (−DCMU) 10 $\mu$M DCMU. Plates were incubated as described in A. C, cells of WT and $\Delta$Deg were subjected to high light treatment (100 $\mu$mol of photons m$^{-2}$ s$^{-1}$, 29 °C) for 1 h (see “Experimental Procedures”). Thylakoid aliquots (7.5 $\mu$g of total protein, DC assay) prepared from cells were analyzed by immunoblotting using an OxyBlotTM kit and a D1-specific antiserum (see “Experimental Procedures”). OxyBlotTM molecular weight marker band sizes are indicated in kDa. * marks the appearance of carbonylated proteins of the range 45–50 kDa seen in $\Delta$Deg but not WT. M, OxyBlotTM protein standard.
immunocommitively following derivatization of the proteins with 2,4-dinitrophenylhydrazine. This assay is widely used to measure the degree of ROS-mediated damage to proteins (37, 38). As Fig. 2 shows, after 1 h of illumination, WT and ΔDeg exhibited similar PSII D1 protein levels. However, the ΔDeg samples showed an increased level of protein carbonylation, particularly of proteins or protein aggregates in the size range 29–60 kDa. Overall, these data suggest a role for the Deg proteases in preventing the accumulation of oxidized proteins in membranes.

Growth Characteristics of the deg Mutants at High and Low Temperatures—A classic phenotype associated with mutation of degP/htrA in E. coli is sensitivity to growth at elevated temperatures (14). To test the involvement of the Deg proteases in resistance to heat stress, growth on BG-11 agar plates was monitored at low light intensities (10 μmol of photons m⁻² s⁻¹) either at the normal growth temperature of 29 °C or at 37 °C (Fig. 3). All strains grew at 29 °C, but only ΔDeg was unable to grow at the higher temperature. All mutants were able to grow as well as WT at 18 °C and a light intensity of 10 μmol of photons m⁻² s⁻¹ (data not shown).

Effect of Oxidizing Agents and Salt Stress—Growth of the E. coli degP/htrA mutant has been shown to be sensitive to cumene hydroperoxide, an oxidizing agent that partitions into the membrane, but not to hydrogen peroxide, which can enter the cytosol (19). To investigate whether this was also true for Synechocystis sp. PCC 6803, we exposed liquid cultures of WT and ΔDeg to various concentrations of either hydrogen peroxide (over a range of 10–250 μM) or cumene hydroperoxide (from 50 to 250 μM). We found that for both compounds, the growth rates of the WT and ΔDeg strains were equally sensitive (data not shown). Under our growth conditions, hydrogen peroxide and cumene peroxide prevented cell growth in the range of 75–100 and 50–75 μM, respectively.

In Synechocystis sp. PCC 6803, salt stress induces the expression of htrA and hhoB, suggesting that both may be critical for salt stress acclimation (24, 39). To test this hypothesis we exposed cells of WT, ΔDeg, and the three double mutants to 0.5 M NaCl in liquid culture. All the mutants were able to grow as well as WT (data not shown). The doubling times were ~67 h in the presence of 0.5 M NaCl when compared with 44 h in its absence.

The Deg Proteases Are Needed for Phototaxis—It is well known that Synechocystis exhibits positive phototactic movement (40). This locomotion requires cell appendages termed type IV pili (TFP), which are also responsible for natural transformation competency (41, 42). Interestingly, we observed that the Synechocystis sp. PCC 6803 ΔDeg mutant had severely reduced motility during routine cultivation. To confirm this, we spotted cells of WT and each WT-based deg mutant onto BG-11 agar under permissive growth conditions and illuminated by a unidirectional light source. As Fig. 4A shows, the WT and all three double mutants moved toward the light to a similar extent, unlike the triple mutant, which only moved slightly. It is also worthy of note that the colony front of the hhoA ‘hhoB’ strain was smoother than WT and the other two double mutants, suggesting altered surface properties in this mutant. To further probe the reduced motility phenotype of ΔDeg, we performed electron microscopy studies to determine the presence or absence of TFP. We analyzed cells of WT, ΔDeg, and the non-motile sigF mutant (ΔSigF), which has been
shown to lack TFP (43). As expected, the WT cells (Fig. 4B) presented both thick and thin pili (41), whereas the sigF mutant (Fig. 4D) lacked the thick pili but retained shorter thin pili (43). Interestingly, the ΔDeg mutant (Fig. 4C) appeared to be hyper-piliated with thick pili when compared with both WT and the sigF mutant. We also found that the transformation efficiency of ΔDeg was severely reduced (data not shown). This phenotype is reminiscent of that observed in pilT1 and taxAY1 mutants (44, 45). The product of pilT1 is thought to be involved in pilus retraction (45), and TaxAY1 is thought to be a histidine kinase-CheY type response regulator hybrid involved in relaying light signals to the TFP apparatus (44).

**D1 Degradation and PSII Repair Do Not Require theDeg Proteases**—The major objective of this work was to assess the potential importance of the Deg proteases in PSII repair. A classical method to detect the PSII repair cycle in vivo is to monitor the rate of light-saturated oxygen evolution, which is a measure of the number of active PSII centers, as a function of time of exposure to high light irradiances, either in the presence or in the absence of an inhibitor of protein synthesis that blocks the repair cycle. For these experiments, cells were grown at 29 °C to reduce any possible negative effects on cell function caused by heat stress. In the presence of spectinomycin, both WT and ΔDeg showed similar time-dependent decays in PSII activity, which is a measure of the rate of light-induced damage to PSII. In the absence of spectinomycin, PSII activity remained high in the two strains (Fig. 5A). These data provide clear evidence to support the presence of robust PSII repair in the triple mutant. In addition, immunoblotting experiments indicated that the rate of degradation of D1 in the mutant (assessed in the samples treated with spectinomycin) was at least the same, if not greater, than that observed in the WT (Fig. 5B). In the absence of spectinomycin, D1 levels were maintained during the illumination period (Fig. 5B). Levels of immunodetectable FtsH were also similar in WT and ΔDeg (Fig. 5B).

To assess D1 turnover more directly, pulse-chase experiments were performed using [35S]methionine/cysteine. In the experiment shown in Fig. 6, radiolabeled D1 was turned over at an even greater rate in the mutant than the WT. Under these experimental conditions, there was no evidence for the accumulation of D1 aggregates, D1 cross-linked products, or D1 breakdown fragments, which are detected when PSII repair is perturbed in vivo (46).

**The Deg Proteases Are Not Required for D1 Degradation When the PSII Repair Cycle Is Impaired at Low Temperature**—To test whether the Deg proteases had a role in D1 degradation under more extreme conditions, cells were exposed to higher light irradiances at a cooler temperature. Under these conditions, PSII repair is compromised so that there is loss of PSII activity even in the absence of an inhibitor of protein synthesis (Fig. 7A). Overexposed immunoblots revealed the presence of an approximate 60-kDa D1 aggregate band (indicated by D1a), probably consisting of a D1/D2 heterodimer (47), in addition to the faster migrating D1 band at ~30 kDa (Fig. 7B). Immunoblots of spectinomycin-treated samples (Fig. 7B) indicated similar degradation rates of D1 in both strains but a slightly faster degradation rate of the D1 aggregate band in the triple mutant. Light-induced loss of D1 aggregates was also seen in samples from cells not treated with spectinomycin (Fig. 7B). The reason for the decrease in the steady-state level of D1 aggregates during illumination is unclear but might be due to an increase in the degradation rate in response to increased levels of oxidative damage to the aggregates. Overall, our data clearly
show that the Deg proteases are not essential for the removal of D1 and D1 aggregates at low temperature.

DISCUSSION

Based on a recent model for D1 degradation in chloroplasts, there has been speculation that proteolysis of photodamaged D1 protein in *Synechocystis* sp. PCC 6803 requires the direct participation of one or more members of the Deg family of proteases (8). The results contained herein show unambiguously that these proteases are not essential for the selective replacement of damaged D1 during PSII repair *in vivo*. Deg-mediated degradation of D1, if present, must therefore be a redundant process or make only a minor contribution to total D1 degradation under the experimental conditions examined here. However, it still remains feasible that Deg proteases might play a more significant physiological role in D1 degradation under conditions not tested here or in longer term acclimation processes such as those involved in reducing levels of PSII at high light (48).

In our original studies on a deg triple mutant constructed in the glucose-tolerant strain of *Synechocystis* 6803, we did observe some impairment in PSII repair (31). However, as we emphasized at the time, our results did not allow us to differentiate between a direct role for the Deg proteases in D1 degradation and an indirect role in optimizing the PSII repair cycle (31). Given the unambiguous data presented here for ΔDeg, we are now able to exclude an essential role for the Deg proteases in D1 degradation. With hindsight, it seems likely that the effects on PSII repair observed in our initial work were indirect and might have stemmed from the differences in the genetic background of the two strains examined (glucose-tolerant versus PCC 6803) or from differences in experimental growth conditions. In our original study, the cells were grown photoautotrophically at 30–33 °C, rather than at the 29 (±0.5) °C used here, which might have resulted in the cells being heat-stressed (Fig. 3) (31). Indeed, cells in the earlier study showed reduced levels of PSII detected in activity measurements (40% of WT levels) and lower levels of D1 detected immunologically (31), indicative of suboptimal growth conditions. In contrast, the ΔDeg mutant showed WT levels of PSII under the experimental growth conditions used here (Fig. 5).

We have previously shown that D1 degradation is impaired at an early stage in *ftsH* (shr0228) insertion mutants and that FtsH co-purifies with PSII (12). These observations, together with the results in this report, support a model for D1 degradation in *Synechocystis* sp. PCC 6803 in which FtsH complexes are able to remove damaged D1 without the participation of the Deg proteases. Based on the analysis of the *var2* FtsH mutant of *Arabidopsis thaliana*, we have recently proposed that this Deg-independent FtsH-mediated pathway of D1 degradation also occurs in chloroplasts (49). Although Deg2 has been assigned a
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role in D1 degradation, this conclusion was based on the results of *in vitro* assays (7). As yet, there is still no evidence, such as from the analysis of a deg2 null mutant, to support such a role in planta.

Our results clearly demonstrate that the Deg protease family is not required for cell viability in *Synechocystis* sp. PCC 6803. This contrasts with the situation in *E. coli* where DegS is required because of its role in activating the extracytoplasmic stress-response pathway through degradation of the anti-σ factor RseA (14, 15). For *Synechocystis* sp. PCC 6803, there is no obvious homologue to RseA, so this activation pathway would appear to be absent. The nature of the envelope stress-response pathway(s) in *Synechocystis* 6803 and whether they are related to the two-component pathways found in *E. coli* (50, 51) is, at the moment, unclear.

Importantly, we show that there is overlap in function between the members of the Deg proteases with regard to providing resistance to light and heat stress and enabling cells to perform positive phototaxis. The sensitivity of growth of ΔDeg to light and heat stress probably reflects an inability to remove photodamaged and thermally denatured proteins, respectively, from the cell. It is important to note that we cultivated *Synechocystis* in the light, so the overall degree of damage to protein is in principle dependent on both the temperature and the prevailing light intensity. Interestingly, the PSIi inhibitors, DCMU and atrazine, protected the *Synechocystis* Deg-G triple mutant from the effects of increased light stress. Such a phenotype suggests that the Deg proteases are required to remove proteins that have been damaged by ROS generated during linear photosynthetic electron flow. Given that two of the three members have been detected in the periplasm (HhoA) (25) and outer membrane (HtrA) (26), we suggest that ROS generated by the photosynthetic electron transport chain might damage cytoplasmic membrane proteins and also diffuse across the cytoplasmic membrane, causing damage to proteins in the extracytoplasmic compartment. This possibility is supported by the detection of carboxylated membrane proteins in the triple mutant that are absent in the WT (Fig. 2C). A role for the Deg proteases in countering the damaging effects of photosynthetic electron transport is in accordance with the observations that *deg* transcripts increase upon a dark-to-light transition (24) and that they accumulate to high levels upon the development of the photosynthetic apparatus (52).

The reduced phototaxis of the *deg* triple mutant might also be related to oxidative damage to TFP. However, our data (Fig. 4C) indicate TFP hyperpiliation of cells similar to that observed in *pillT1* and *taxAY1* mutants (44, 45). This suggests that removal of Deg proteases disturbs one or more of the following processes: (i) pilus subunit degradation; (ii) the mechanism of pilus retraction; (iii) the sensory pathway(s) involved in stimulating positive phototaxis. Taken together, our data indicate that the Deg proteases are involved in maintaining the extracytoplasmic properties of *Synechocystis* sp. PCC 6803 cells.

Surprisingly, we were unable to demonstrate the involvement of the Deg proteases in providing resistance to either salt or cold stress. DNA microarray and Northern blotting experiments have indicated that the transcripts for *htrA* accumulate rapidly following exposure to high salt and cold temperatures (24, 39). Consequently, other proteases might be able to substitute for the loss of the Deg proteases under these and possibly other stress conditions.

Acknowledgments—We are grateful to J.-D. Rochaix and T. Ogura for sending us various antisera.

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