



Light-inducible expression of translation factor EF-Tu during acclimation to strong light enhances the repair of photosystem II

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In photosynthetic organisms, the repair of photosystem II (PSII) is enhanced after acclimation to strong light, with the resultant mitigation of photoinhibition of PSII. We previously reported that oxidation of translation elongation factor EF-Tu, which delivers aminoacyl-tRNA to the ribosome, depresses the repair of PSII in the cyanobacterium *Synechocystis* sp. PCC 6803. In the present study, we investigated the role of EF-Tu in the repair of PSII after acclimation of *Synechocystis* to strong light. In cells that had been grown under strong light, both the repair of PSII and the synthesis of proteins de novo were enhanced under strong light, with the resultant mitigation of photoinhibition of PSII. Moreover, levels of EF-Tu were elevated, whereas levels of other components of the translation machinery, such as translation factor EF-G and ribosomal proteins L2 and S12, did not change significantly. The expression of the gene for EF-Tu was induced by light, as monitored at the transcriptional level. Elevation of the level of EF-Tu was strongly correlated with the subsequent enhancement of PSII repair in cells that had been grown under light at various intensities. Furthermore, overexpression of EF-Tu in *Synechocystis* enhanced protein synthesis and PSII repair under strong light, even after cell culture under nonacclimating conditions. These observations suggest that elevation of the level of EF-Tu might be a critical factor in enhancing the capacity for repair of PSII that develops during acclimation to strong light.

EF-Tu | photoinhibition | photosystem II | repair | strong light acclimation

Life on Earth depends almost completely on light energy from the sun. Photosynthesis uses light energy to fix carbon dioxide as carbohydrates that support the life of almost all organisms on Earth. However, excess light can damage the photosynthetic machinery and suppress photosynthetic capacity. In the photosynthetic machinery, photosystem II (PSII), a protein-pigment complex that converts light energy to chemical energy, is particularly susceptible to inactivation by strong light, a process referred to as photoinhibition of PSII (1, 2). To cope with the vulnerability of PSII, photosynthetic organisms have developed various mechanisms for protecting PSII from photoinhibition. One such protective mechanism involves acclimation; when photosynthetic organisms acclimate to strong light, PSII becomes better able to tolerate photoinhibition (3). The mitigation of photoinhibition of PSII is associated with enhanced repair of PSII in algae and plants (4, 5). However, the molecular mechanism responsible for the enhancement of repair of PSII that becomes operative during acclimation to strong light remains to be fully characterized.

PSII is repaired rapidly and efficiently by a system involving proteolytic degradation of damaged D1 protein, synthesis of the precursor to the D1 protein (pre-D1), insertion of pre-D1 into the PSII complex, processing of the carboxyl-terminal extension of pre-D1, and reactivation of PSII (6, 7). Recent studies of photoinhibition have revealed that photodamage to PSII is a light-dependent event, whereas the repair of PSII is inhibited by reactive oxygen species (ROS), which include superoxide, hydrogen

peroxide, and singlet oxygen and are abundantly produced in the photosynthetic machinery under strong light (7–10). Thus, it is likely that enhancement of the repair of PSII after acclimation to strong light might involve some form of protection of repair from inhibition by ROS.

Detailed analysis of the ROS-induced inhibition of repair of PSII in the cyanobacterium *Synechocystis* sp. PCC 6803 (hereinafter referred to as *Synechocystis*) revealed that ROS inhibit the synthesis of pre-D1 at the elongation step of translation (11, 12). Synthesis is inhibited not only of the D1 protein, but also of almost all proteins in the thylakoid membranes, suggesting that the protein synthetic machinery itself might be sensitive to ROS (11, 12). Biochemical analysis of the translational machinery of *Synechocystis* identified 2 elongation factors as the targets of ROS (13, 14): EF-Tu, which delivers aminoacyl-tRNA to the A site of the ribosome, and EF-G, which translocates peptidyl-tRNA from the A site to the P site of the ribosome. EF-Tu is inactivated via oxidation of a single cysteine residue, Cys82, with subsequent formation of both sulfenic acid and an intermolecular disulfide bond (13), while EF-G is inactivated via oxidation of Cys105 and Cys242, with subsequent formation of an intramolecular disulfide bond (15). We postulated that protection of such oxidation-sensitive elongation factors might develop during acclimation to strong light via mechanisms that support the enhancement of repair of PSII.

Significance

Photoinhibition of photosystem II (PSII) is mitigated via enhancement of the repair of PSII in photosynthetic organisms that have acclimated to strong light, but the mechanism responsible for such acclimation of PSII repair remains unknown. We report here that an elevated level of translation factor EF-Tu during the acclimation of *Synechocystis* to strong light plays a critical role in the enhancement of PSII repair via acceleration of the synthesis de novo of proteins that are required for PSII repair, such as the D1 protein. We propose a mechanism for protection of PSII from photoinhibition that becomes operative during acclimation to strong light and appears to contribute to the ability of photosynthetic organisms to tolerate strong light.

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The authors declare no competing interest.

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In the present study, we investigated the effects of strong light acclimation on the repair of PSII, protein synthesis, and levels of elongation factors in *Synechocystis*. We found that in strong light-acclimated cells, the repair of PSII and synthesis of proteins de novo were enhanced and the level of EF-Tu was elevated. There was a strong correlation between the level of EF-Tu and the extent of the ability to repair PSII. Moreover, overexpression of EF-Tu enhanced the repair of PSII via acceleration of protein synthesis de novo in nonacclimated cells. We report here that the elevated expression of EF-Tu induced during acclimation to strong light plays an important role in enhancing the subsequent repair of PSII under strong light.

Results

Repair of PSII Is Enhanced after Acclimation to Strong Light. To investigate the effect of strong light acclimation on the repair of PSII, we grew *Synechocystis* cells under light at 3 different intensities—low light (LL; 10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), moderate light (ML; 70 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), and strong light (SL; 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$)—until the optical density at 730 nm reached 1.0 ± 0.1 . We then exposed the cells to very strong light at 1,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The activity of PSII in cells grown under LL fell to 15% of the initial level within 120 min (Fig. 1A). By contrast, after 120 min, the activity of PSII in cells grown under ML remained at 40% of the initial level, and the activity in cells grown under SL remained at 65% of the initial level (Fig. 1A), suggesting that cells became more tolerant to photoinhibition of PSII as a result of acclimation to strong light. However, when cells were exposed to very strong light at 1,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the presence of lincomycin, which blocks the repair of PSII, the activity of PSII in cells grown under LL, ML, and SL fell at similar rates, indicating that enhancing the repair of PSII has no effect on the photodamage to PSII after acclimation of cells to strong light (Fig. 1B).

Protein Synthesis Is Accelerated during Acclimation to Strong Light. The repair of PSII requires the synthesis of proteins de novo, particularly of the D1 protein (4). We examined the effect of strong light acclimation on the synthesis of proteins de novo by monitoring the incorporation of ^{35}S -labeled methionine and cysteine into proteins during the exposure of cells to very strong light at 1,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. In cells grown under SL, the

rates of synthesis of many thylakoid proteins exceeded those in cells grown under LL and ML, suggesting that protein synthesis under strong light might be activated after acclimation to strong light (Fig. 2A). Moreover, the acceleration of protein synthesis was most prominent in the case of the D1 protein (Fig. 2A). The rates of synthesis of the D1 protein in cells grown under ML and SL were 2- and 3.5-fold higher, respectively, than the rate in cells grown under LL (Fig. 2B).

In addition to analysis by autoradiography, proteins on gels were stained with Coomassie brilliant blue as loading controls, and the results confirmed that basal levels of proteins were unchanged during exposure to very strong light at 1,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (SI Appendix, Fig. S1). Thus, it appeared that the enhanced repair of PSII might have been attributable to the accelerated synthesis of the D1 protein.

We also monitored the growth rate of cells under LL, ML, and SL conditions. Cells grew faster under SL than under ML and faster under ML than under LL (SI Appendix, Fig. S2). The growth rate was correlated with the rate of synthesis of the D1 protein under the 3 light conditions.

EF-Tu Level Increases during Acclimation to Strong Light. Protein synthesis under strong light is inhibited by oxidation of the elongation factors EF-Tu and EF-G in *Synechocystis* (16, 17). These observations led us to postulate that some changes in these elongation factors might occur during acclimation to strong light. We examined the effects of strong light acclimation on levels of EF-Tu and EF-G, together with other components of the translation system. After cells had been grown under LL, ML, and SL, proteins in cell extracts were separated by SDS/PAGE, and EF-Tu, EF-G, and ribosomal proteins L2, S1, and S12 were detected immunologically (Fig. 3A). The level of EF-Tu in cells grown under SL was 3 times higher than that in cells grown under LL, and the level in cells grown under ML was 2 times higher (Fig. 3B). In *Synechocystis*, there are 3 homologs of EF-G: Slr1463, Sll1098, and Sll0830. Slr1463 is phylogenetically related to chloroplast-localized EF-G; Sll1098 is a bacterial-type EF-G encoded by the *str* operon, as described in detail below (Fig. 3C); and Sll0830 is a type II form of EF-G (14). Respective levels of Slr1463, Sll1098, and Sll0830 remained almost unchanged in cells grown under LL, ML, and SL (Fig. 3B), suggesting that the total amount of EF-G might have remained unchanged during acclimation to strong light. Similarly, the level of the ribosomal protein L2, which is essential for peptidyl-transferase activity in the ribosome (18), was unchanged during strong light acclimation (Fig. 3B). The level of the ribosomal protein S1, which is required for the unfolding of structured mRNA and stabilization of the initiation complex with the small subunit of the ribosome and mRNA (19), also remained almost unchanged during acclimation to strong light (Fig. 3B). The level of the ribosomal protein S12, which is required for the interaction between the small and large subunits of the ribosome and for proper decoding by the ribosome (20, 21), was also almost unchanged during acclimation to strong light (Fig. 3B). Thus, it appears that the amount of the main body of the ribosome might have remained unchanged during acclimation to strong light.

The *sll1096* gene for S12 is a component of the *str* operon, together with genes for S7 (*sll1097*), EF-G (*sll1098*), and EF-Tu (*sll1099*) (Fig. 3C). We analyzed levels of transcripts of the *str* operon by quantitative RT-PCR. After transfer of cells from growth under light at 70 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ to very strong light at 1,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, we found that levels of *sll1098* and *sll1099* transcripts rose by approximately 3-fold in 1 h and then fell gradually (Fig. 3D). By contrast, levels of *sll1096* and *sll1097* transcripts did not change significantly during the exposure to very strong light (Fig. 3D). A previously reported analysis of transcription start sites in *Synechocystis* identified sites of light-inducible initiation of transcription in both the promoter

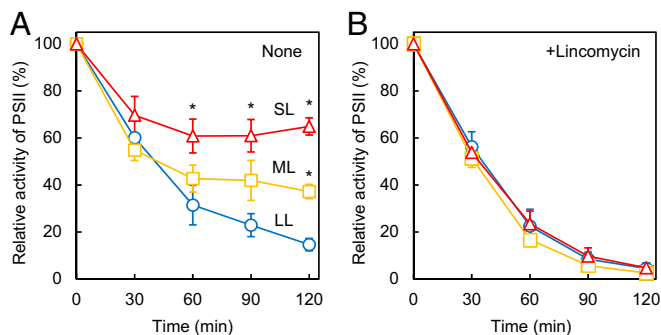


Fig. 1. Effects of light intensity during cell culture on the subsequent photoinhibition of PSII under very strong light. Cells that had been grown under light at 10 (LL), 70 (ML), and 200 (SL) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were incubated at 25 °C under very strong light at 1,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, with aeration by ambient air, in the absence (A) or presence (B) lincomycin. The activity of PSII in acclimated cells was measured in terms of the evolution of oxygen in the presence of 1 mM 1,4-benzoquinone and 1 mM $\text{K}_3\text{Fe}(\text{CN})_6$. The activities taken as 100% for cells grown under LL, ML, and SL were 846 ± 85 , 806 ± 72 , and $830 \pm 59 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl h}^{-1}$, respectively. Values are mean \pm SD (bars) of results from 3 independent experiments. Asterisks indicate statistically significant differences ($P < 0.01$, Student's *t* test).

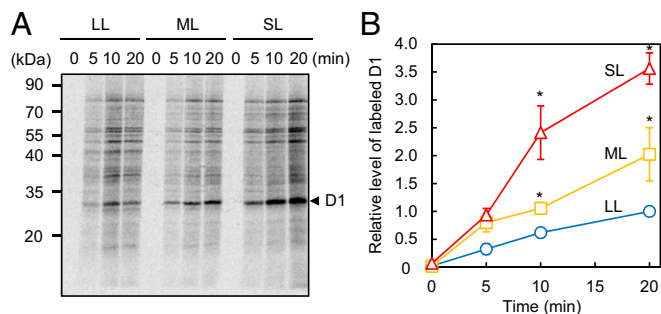


Fig. 2. Effects of light intensity during cell culture on the subsequent synthesis de novo of proteins in thylakoid membranes under very strong light. Proteins in cells that had been grown under LL, ML, and SL (Fig. 1) were pulse-labeled by incubation of cells for the indicated times under very strong light at $1,500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, with aeration by ambient air, in the presence of ^{35}S -labeled methionine and cysteine. Thylakoid membranes were isolated, and proteins ($5 \mu\text{g}$) were separated by SDS/PAGE. (A) Representative radiogram of pulse-labeled proteins from thylakoid membranes. (B) Quantitation of the relative levels of labeled D1 protein. Values are mean \pm SD (bars) of results from 3 independent experiments. The value for LL-grown cells after incubation for 20 min was taken as 1.0. Asterisks indicate statistically significant differences ($P < 0.01$, Student's t test).

and the coding region of *sl1098*, which encodes EF-G (22). Therefore, it seems plausible that the expression of genes for EF-Tu and EF-G might be controlled by strong light-inducible promoters.

EF-Tu Level Is Strongly Correlated with the Capacity for PSII Repair.

To investigate the relationship between EF-Tu level and PSII repair, we grew cells under light at various intensities and examined relative levels of EF-Tu and the tolerance of PSII to strong light, as monitored in terms of the residual activity of PSII after cells had been exposed to very strong light at $1,500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 120 min. As shown in Fig. 1, the residual activity of PSII after 120 min reflected the capacity to repair PSII, since photodamage to PSII was unaffected by the strength of the light under which cells had been grown. The correlation between the 2 parameters was 0.99, indicating that the level of EF-Tu was strongly correlated with the capacity for PSII repair (Fig. 3E).

Overexpression of EF-Tu Accelerates Protein Synthesis under Strong Light and Mitigates the Photoinhibition of PSII.

To examine the effects of elevated EF-Tu levels on protein synthesis and photoinhibition, we used a transformant of *Synechocystis* that overexpresses EF-Tu (16). In this transformant, the *sl1099* gene for EF-Tu was fused to a putative promoter region upstream of the coding region of the *sl1098* gene for EF-G. The resultant DNA fragment was introduced into a neutral site between the *slr2030* and *slr2031* genes in the *Synechocystis* genome for EF-Tu expression from both the intrinsic and neutral sites at similar levels (16, 23). We grew wild-type and transformed cells under low light at $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and compared EF-Tu expression levels. In transformed cells, EF-Tu levels were double those in wild-type cells, but levels of EF-G (Sl1098) and ribosomal proteins L2 and S12 were unaffected (Fig. 4A). We then monitored the synthesis de novo of thylakoid proteins under very strong light at $1,500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Rates of synthesis de novo of various thylakoid proteins in transformed cells were higher than those in wild-type cells (Fig. 4B). Under our conditions, the rate of synthesis de novo of the D1 protein in transformed cells was twice that in wild-type cells (Fig. 4C). These observations suggest that the overexpressed EF-Tu might accelerate protein synthesis under strong light.

Next, we examined the effects of EF-Tu overexpression on the photoinhibition of PSII. When cells were exposed to very strong

light at $1,500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, the activity of PSII in wild-type cells dropped to 20% of the initial level in 120 min, but the activity of PSII in transformed cells remained at 40% of the initial level (Fig. 4D). However, the extent of photodamage to PSII, as monitored in the presence of lincomycin, did not differ between the 2 types of cells, suggesting that EF-Tu overexpression might enhance the PSII repair.

It should be noted here that the protection of PSII against photoinhibition was apparent only under very strong light at an intensity $>1,200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (SI Appendix, Fig. S3). The photoinhibition of PSII was barely mitigated under light at lower intensities, such as $1,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, as observed in a previous study (16). Thus, it appears that EF-Tu overexpression mitigates the photoinhibition of PSII under very strong light exclusively.

Discussion

Role of EF-Tu in Protection of Protein Synthesis and PSII Repair. In the present study, we demonstrated that during acclimation to strong light, intracellular levels of EF-Tu rose in *Synechocystis* cells and both the synthesis of proteins de novo and the repair of PSII were enhanced, with the resultant mitigation of photoinhibition of PSII. Protein synthesis is sensitive to oxidative stress due to ROS that are abundantly produced in the photosynthetic machinery under strong light (11, 12). In *Synechocystis*, EF-Tu and EF-G have been identified as critical targets of ROS in the translational machinery, and these elongation factors are inactivated via oxidation of specific cysteine residues (13, 15). Expression in *Synechocystis* of mutated EF-Tu or EF-G, in which one of the target cysteine residues has been replaced by a serine residue, results in accelerated protein synthesis and enhanced repair of PSII under strong light, with the resultant mitigation of PSII photoinhibition (16, 17). However, such mutagenesis is not necessarily favorable for cells. For example, expression of the mutated EF-Tu was found to stimulate oxidative stress under strong light, as a result of the enhanced repair of PSII and subsequent activation of electron transport (16). Furthermore, replacement of all the target cysteine residues in EF-Tu or EF-G was found to be lethal (16, 17). The complete loss of such cysteine residues can be assumed to lead to disrupted regulation of protein synthesis and photosynthesis. Thus, it is likely that the oxidation-sensitive cysteine residues in EF-Tu and EF-G act as regulators that transiently depress protein synthesis under severe strong light stress. Moreover, the oxidation-sensitive cysteine residue Cys82 in EF-Tu is strongly conserved from cyanobacteria to higher plants (24).

How might cells cope with the vulnerability of elongation factors to ROS? The present study provides a clue as to a strategy for protection of elongation factors from the negative effects of oxidation. Such a strategy might involve elevation of EF-Tu levels, with enlargement of the size of a specific pool of EF-Tu, during acclimation to strong light. When oxidative stress is imposed under strong light, some of the intracellular EF-Tu is oxidized and inactivated (16). However, in strong light-acclimated cells, the reduced form of EF-Tu, or active EF-Tu, might be more abundant than seen in nonacclimated cells, even under oxidative stress, and the increased population of active EF-Tu molecules might then drive translation reactions to ensure protein synthesis and PSII repair. It seems that elevation of the EF-Tu level might be a gentler and more flexible way to protect protein synthesis from oxidative stress than modification of EF-Tu via introduction of an oxidation-insensitive cysteine residue, since the oxidation-sensitive cysteine residue in EF-Tu allows regulation of protein synthesis in a redox-dependent manner.

Overexpression of EF-Tu resulted in the accumulation of twice the normal amount of EF-Tu, with consequent enhancement of protein synthesis and PSII repair under strong light (Fig. 4). However, the protective effect of the EF-Tu overexpression on

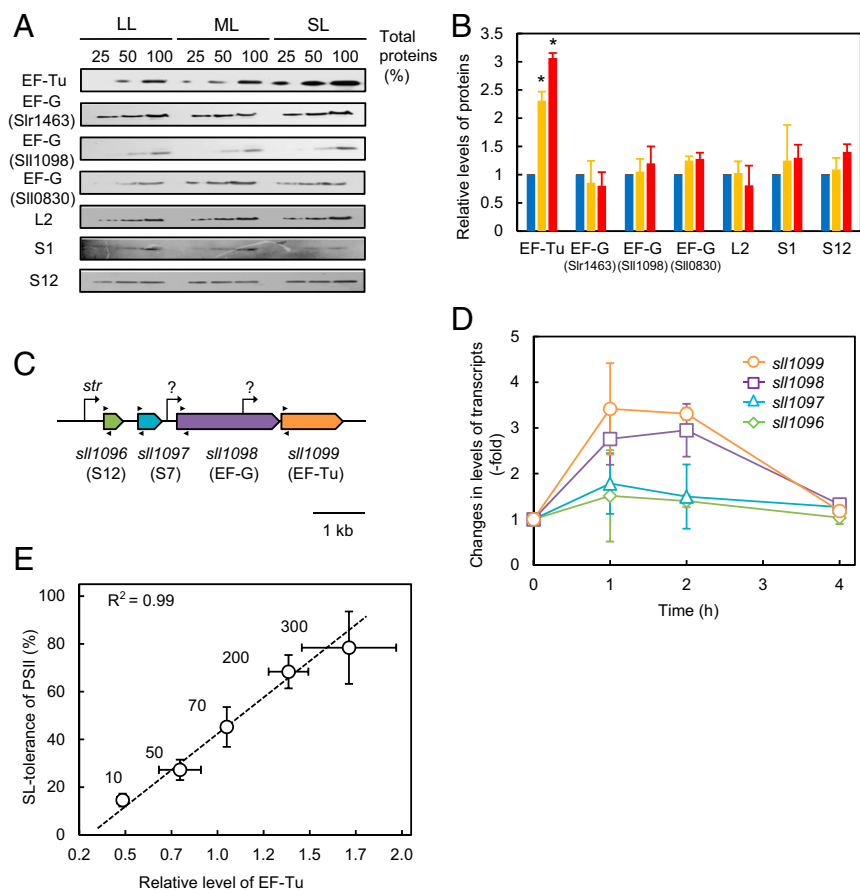


Fig. 3. Effects of light intensity during cell culture on expression levels of translation factors and ribosomal proteins. (A) Proteins in cells that had been grown under LL, ML, and SL (Fig. 1) were extracted and separated by SDS/PAGE. Proteins were detected immunologically with the corresponding specific antibodies. (B) Quantitation of relative levels of proteins in cells grown under LL (blue bars), ML (orange bars), and SL (red bars). Values for cells grown under LL were taken as 1.0. Asterisks indicate statistically significant differences ($P < 0.01$, Student's t test). (C) Schematic representation of the *str* operon and putative transcriptional start sites, including 2 putative light-inducible promoters indicated by question marks. Primers used for RT-PCR are also indicated by arrowheads. (D) Changes in levels of transcripts of genes that compose the *str* operon on a change in light intensity from 70 to 1,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Blue triangles, S12; green diamonds, S7; purple squares, EF-G; orange circles, EF-Tu. Levels of the *rnpB* transcript served as internal controls for determination of changes in levels. Values were normalized relative to respective transcripts at time 0. (E) Correlation between EF-Tu level and tolerance of PSII to strong light. In cells that had been grown under light at various intensities, EF-Tu levels were compared with the residual activities of PSII after cells had been exposed to light at 1,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 120 min. The value for cells grown under light at 10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was taken as 1.0. The numbers on the graph indicate the intensities of light under which cells had been grown ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The correlation value, R^2 , was 0.99.

PSII repair was approximately one-half that of acclimation to strong light even though levels of EF-Tu were similar (Figs. 1 and 4). These observations suggest that the protection of PSII repair that develops during acclimation to strong light requires not only an elevated EF-Tu level, but also changes in some other biomolecules. During acclimation to strong light, large amounts of carotenoids often accumulate in thylakoid and plasma membranes (25). Specific carotenoids, such as zeaxanthin and echinenone, are known to protect the repair of PSII in *Synechocystis* under strong light by scavenging singlet oxygen and quenching the triplet state of chlorophylls (8). Elevated accumulation of carotenoids during acclimation to strong light might help protect PSII repair from oxidative inhibition.

The protection of PSII from photoinhibition via EF-Tu overexpression was effective only under very strong light $>1,200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and not under less-intense light. Individual molecules of EF-Tu are approximately 8 times more abundant than ribosomes in the cell (26), but binding of EF-Tu to ribosomes is saturated when an EF-Tu:ribosome molecular ratio is 2:1 (27). Even in wild-type cells, a certain amount of active EF-Tu remains detectable under strong light at 1,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (16), and this active EF-Tu might normally be sufficient to

support protein synthesis. Alternatively, the limited effect might result from an imbalance between the elevated amount of EF-Tu and levels of other factors that are responsible for acclimation to strong light.

In transgenic wheat plants overexpressing plastidic EF-Tu, the capacity for fixation of CO_2 under heat stress was enhanced (28). This observation is in line with our results showing that elevated EF-Tu levels enhanced PSII repair. The repair of PSII is also a phenomenon susceptible to inhibition by heat stress (29, 30).

Mechanism of Light-Inducible Expression of EF-Tu. Levels of ribosomal RNA and translation factors are tightly regulated and stable. In *Escherichia coli*, a 1-to-1 stoichiometry is maintained between EF-G and the ribosome, while the number of EF-Tu molecules far exceeds that of ribosomes (26). In prokaryotes, the genes for ribosomal proteins S12, S7, EF-G, and EF-Tu form the *str* operon and are cotranscribed under a common promoter (31). However, in *Synechocystis*, we found that the EF-Tu level depended on the intensity of light under which cells were grown, with a 3-fold increase from low light (10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) to strong light (200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Fig. 3). However, under the various light conditions, levels of EF-G (Sll1098)

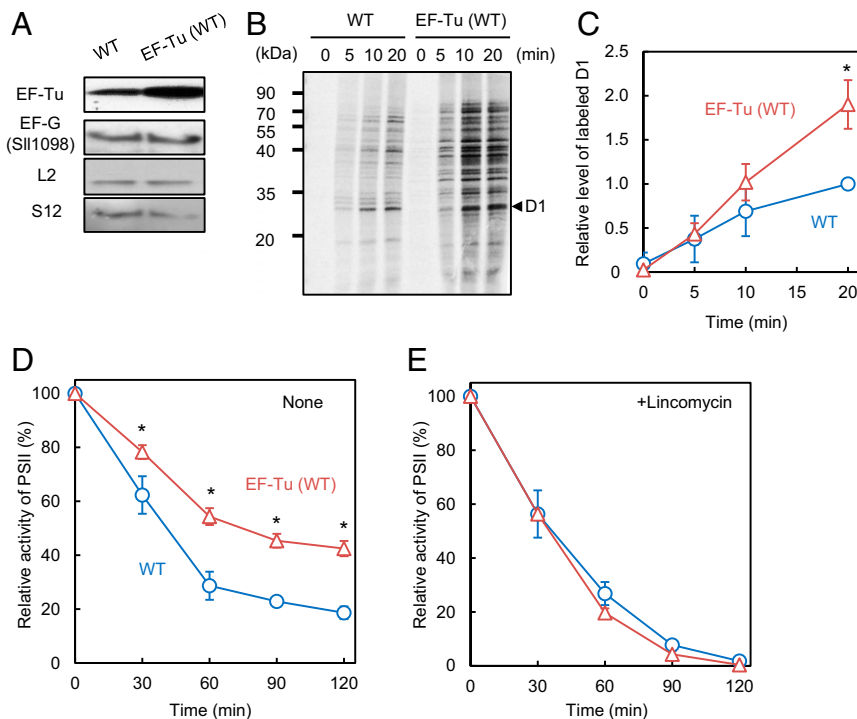


Fig. 4. Effect of overexpression of EF-Tu on the photoinhibition of PSII. (A) Proteins extracted from cells (5 μ g) from cells were separated SDS/PAGE and detected immunologically with specific antibodies against EF-Tu, EF-G2, L2, and S12. (B) A representative radiogram of pulse-labeled proteins from thylakoid membranes in cells after exposure of cells to strong light. (C) Quantitation of relative levels of labeled D1 protein in wild-type (WT; open circles) and EF-Tu-overexpressing [EF-Tu (WT); open triangles] cells. The value for wild-type cells after incubation for 20 min was taken as 1.0. (D and E) Photoinhibition of PSII under strong light. Wild-type (WT; open circles) and EF-Tu-overexpressing [EF-Tu (WT); open triangles] cells were incubated under strong light at 1,500 μ mol photons $m^{-2} s^{-1}$, with aeration by ambient air, in the absence (D) or presence (E) of lincomycin. The activities taken as 100% for wild-type and EF-Tu-overexpressing cells were 793 ± 27 and 821 ± 69 μ mol O_2 mg^{-1} Chl h^{-1} , respectively. Values are mean \pm SD (bars) of results from 3 independent experiments. Asterisks indicate statistically significant differences ($P < 0.01$, Student's t test).

remained unchanged and similar. Levels of transcripts for EF-G (S11098) and EF-Tu rose by 2.6-fold and 3.3-fold, respectively, on transfer of cells from standard light conditions (70 μ mol photons $m^{-2} s^{-1}$) to strong light (1,000 μ mol photons $m^{-2} s^{-1}$), whereas respective levels of transcripts for S12 and S7 remained unchanged (Fig. 3). Therefore, it seems likely that expression of the genes for EF-G (S11098) and EF-Tu might be induced by light, at least at the transcriptional level. Since there is a putative promoter region upstream of the coding region for EF-G (S11098), it is possible that genes for EF-G (S11098) and EF-Tu might be cotranscribed under a common light-inducible promoter. In addition, since there is another transcription start site within the coding region for EF-G (S11098) (22), the transcription of the gene for EF-Tu might also be induced by light. A transcriptomic analysis of *Synechocystis* by Mitschke et al. (22) showed that expression of the genes for EF-G (S110830 and S1r1463) is constitutive, whereas expression of the gene for EF-Tu is induced by light.

In *Bacillus stearothermophilus* and *Streptomyces ramocissimus*, expression of the gene for EF-Tu is controlled both by a promoter for the *str* operon and by its own promoter (32, 33). The gene for EF-Tu in *Synechocystis* might similarly have its own light-inducible promoter. In *E. coli*, the S7 protein suppresses the translation of transcripts for EF-G and for S7 itself (34). If similar posttranscriptional regulation is operative in the case of the *str* operon of *Synechocystis*, it might explain why synthesis of EF-Tu, but not that of EF-G, is induced at the protein level.

Proteomic analysis of a mutant of *Synechocystis* that was defective in histidine kinase 33 (Hik33), a 2-component system that responds to strong light, showed that EF-Tu level was significantly depressed in the mutant (35, 36). Thus, it seems that the

light-inducible expression of the gene for EF-Tu might be controlled by Hik33.

The light-inducible expression of EF-Tu might be specific to photosynthetic organisms. Details of the mechanism responsible for the light-induced expression of EF-Tu require clarification. The light-induced expression of EF-Tu might play important roles in the protection and control not only of photosynthesis, but also of other physiological activities, since it affects protein synthesis globally. The overall effects of this acclimation response on cellular homeostasis also require comprehensive analysis.

Materials and Methods

Strains and Culture Conditions. Cells of a glucose-tolerant strain of *Synechocystis* sp. PCC 6803 and a transformant that expresses mutated EF-Tu (16) were grown photoautotrophically at 32 $^{\circ}C$ in liquid BG-11 medium under LL at 10 μ mol photons $m^{-2} s^{-1}$, ML at 70 μ mol photons $m^{-2} s^{-1}$, or SL at 200 μ mol photons $m^{-2} s^{-1}$ with aeration by sterile air containing 1% (vol/vol) CO_2 . Cell suspensions for assays were withdrawn when the optical density at 730 nm reached 1.0 ± 0.1 .

Labeling of Proteins In Vivo. For pulse labeling of proteins, 25 mL of cell culture was supplemented with 240 kBq ^{35}S -labeled methionine and cysteine (Easy Tag EXPRE $^{35}S^{35}S$; PerkinElmer) and incubated at 25 $^{\circ}C$ under light at 1,500 μ mol photons $m^{-2} s^{-1}$ (12). Aliquots of 7 mL each were withdrawn at designated times for analysis of proteins. Labeling was terminated by the addition of nonradioactive methionine and cysteine to a final concentration of 2 mM each, followed by immediate cooling of samples on ice. Thylakoid membranes were isolated from cells, and then membrane proteins (5 μ g) were separated by SDS/PAGE on a 12.5% polyacrylamide gel containing 6 M urea (6). Labeled proteins on the gel were visualized with an imaging analyzer (FLA-7000; Fujifilm), and D1 protein levels were determined densitometrically (6).

Immunoblotting Analysis. Proteins extracted from cells were separated by SDS/PAGE on a 12.5% polyacrylamide gel and then stained with Coomassie brilliant

blue or blotted electrophoretically onto a polyvinylidene difluoride membrane (12, 37). EF-Tu, EF-G (Slr1463), EF-G (Sll1098), and EF-G (Sll0830) were detected with polyclonal antibodies that had been raised against the corresponding proteins from *Synechocystis* (14, 17). L2 and S12 were detected with polyclonal antibodies that had been raised against the corresponding proteins from *Bacillus subtilis* (provided by Dr. Takashi Inaoka, National Agriculture and Food Research Organization, Japan). S1 was detected with polyclonal antibodies that had been raised against an oligopeptide, CRQKRLAEAQGIPYE, that had been linked to keyhole limpet hemocyanin as a carrier protein at a cysteine residue (Eurofins Genomics), as described previously (37). Relative levels of proteins were determined densitometrically.

Quantification of Transcripts. Total RNA was extracted from acclimated cells by the hot-phenol method (38). One μg of total RNA was reverse-transcribed into cDNA by reverse transcriptase (Takara Bio). Specific regions of individual genes were amplified using the designed primers listed in *SI Appendix, Table S1*. *rnpB* served as the internal control gene (39). Amplified DNA was detected with SYBR Green (Toyobo), and fluorescence was monitored with the CFX Connect Real-Time PCR Detection System (Bio-Rad).

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