The Mutant of sl1961, Which Encodes a Putative Transcriptional Regulator, Has a Defect in Regulation of Photosystem Stoichiometry in the Cyanobacterium Synechocystis sp. PCC 6803

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In acclimation to changing light environments, photosynthetic organisms modulate the ratio of two photosynthetic reaction centers (photosystem I [PSI] and photosystem II). One mutant, which could not modulate photosystem stoichiometry upon the shift to high light, was isolated from mutants created by random transposon mutagenesis. Measurements of chlorophyll fluorescence and analysis of the reaction center subunits of PSI through western blotting in this mutant revealed that the content of PSI could not be suppressed under high-light condition. In the mutant, transposon was inserted to the sl1961 gene encoding a putative transcriptional regulator. DNA microarray analysis revealed that the expression of sl1773 was drastically induced in the sl1961 mutant upon exposure to high light for 3 h. Our results demonstrate that a transcriptional regulator, Sll1961, and its possible target proteins, including Sll1773, may be responsible for the regulation of photosystem stoichiometry in response to high light.

Energy transduction in photosynthesis depends on the coordination of two photosystems, PSII and PSI. PSII conducts a light-dependent oxidation of water and reduction of plastoquinone (for review, see Barber, 2002; Diner and Rappaport, 2002), while PSI is involved in a light-dependent electron transport from plastocyanin to NADP⁺ via ferredoxin (for review, see Fromme et al., 2001). The two light-dependent reactions are connected by cytochrome b₆f complex that catalyzes the electron transfer from plastoquinone to plastocyanin. Since PSII and PSI are functioning in tandem in photosynthetic electron flow, photosynthetic organisms must maintain the balance of two photosystems. PSII and PSI have respective light-harvesting complexes with different pigment composition, thus absorbing light of different wavelengths. The size of the light-harvesting antennae in each photosystem is also altered, depending on photon flux densities of the environments. Thus, photosynthetic organisms must modulate photosystem stoichiometry, i.e. PSI/PSII ratio, in response to environmental changes.

In cyanobacteria, PSI/PSII ratio is normally about 2 (Melis and Brown, 1980), and its ratio ranges from 1 to 4 depending on the light condition. Light that excites mainly PSII leads to the increase in PSI so as to bring about high PSI/PSII ratio of about 2 to 4, while light that excites mainly PSI induces the decrease in PSI, resulting in low PSI/PSII ratio as low as 1 (Fujita et al., 1985, 1988; Fujita and Murakami, 1987). The regulation of photosystem amounts by light quality is at the transcriptional and/or posttranscriptional level of PSI and PSII subunits (Glick et al., 1986). The changes in photosystem stoichiometry occur so as to balance the excitation energy distribution and maintain the efficient electron flow between PSII and PSI (for review, see Glazer and Melis, 1987). The regulation correlates with the redox state of plastoquinone pool and cytochrome b₆f complex (Fujita et al., 1987; Fujita, 1991; Murakami and Fujita, 1991). Light quantity, as well as light quality, induces the changes in PSI/PSII ratio in cyanobacteria (Kawamura et al., 1979; Murakami and Fujita, 1991). PSI/PSII ratio decreased upon the shift to high light through the selective suppression in the amount of PSI, in addition to the general decrease in the amount of both photosystems in cyanobacteria (for review, see Anderson, 1986; Hihara, 1999).
Many cyanobacterial mutants with different photosystem stoichiometry from that of the wild type were reported (Wilde et al., 1995, 2001; Mann et al., 2000; Shen et al., 2002; Kufryk and Vermaas, 2003; Yu et al., 2003; Wang et al., 2004). The deletion of genes encoding the subunits of PSI or PSII may lead to the decrease in relative PSI or PSII content. The disruption of genes involved in transcription, translation, assembly, or biogenesis of PSI or PSII may lead to the change in photosystem stoichiometry. One gene, sll0088, seems to encode a transcriptional regulator so that it may be involved in the regulation of photosystem stoichiometry (Yu et al., 2003; Wang et al., 2004). However, only a few cases have been reported for the regulatory mutant of photosystem stoichiometry under high-light condition (Hihara et al., 1998). One such case is the pmgA (sll1968) mutant. This mutant has normal amounts of PSI and PSII under low-light condition, but fails to change photosystem stoichiometry upon the shift to high light (Hihara et al., 1998). Thus, this mutant has a defect not in the process of synthesis, assembly, or degradation of photosystems, but in the process of regulating photosystem stoichiometry. The pmgA mutant is a kind of super mutant and grows faster than the wild type in exposure to high light for 24 h. In the prolonged high-light stress for 72 h, however, the growth of the pmgA mutant is severely suppressed, indicating that regulation of photosystem stoichiometry has physiological importance under high-light condition (Sonoike et al., 2001). The role of the regulation of photosystem stoichiometry during acclimation to high light is apparently not to maintain optimal photosynthesis but to protect the cells from oxidative damage through the suppression of photosynthetic electron transfer (Sonoike et al., 2001). The regulation of photosystem stoichiometry must be essential to survive under prolonged high-light condition. The decrease in PSI contents during acclimation to high light seems to be regulated through the repression of mRNA levels of PSI genes, judging from the data of whole-genome DNA microarray (Hihara et al., 2001). Although the mRNA levels of psaA and psaAB, which encode the subunits of PSI reaction center, are similarly down-regulated in the wild type and the pmgA mutant after 1 h of exposure to high light, the continuous suppression of the transcript levels after 6 h observed in the wild type was not seen in the pmgA mutant (Muramatsu and Hihara, 2003). It was implied that the protein that the pmgA gene encodes might belong to the family of anti-sigma factors. However, it is unknown how PmgA is involved in the transcriptional regulation of psaA and psaAB under prolonged high-light condition. Moreover, it is poorly understood how sophisticated control of PSI level can be achieved under high-light condition. In this study, we report on a putative transcriptional regulator, Sll1961, which is involved in the modulation of photosystem stoichiometry in acclimation to high light. Characterization of the disruption mutant of this gene indicates that the mutants fail to properly suppress the amount of PSI under high-light condition. Since real-time reverse transcription (RT)-PCR analysis showed that this mutation did not much affect the level of psaA and/or psaAB transcript during acclimation to high light, Sll1961 seems to function on the different signal transduction pathway from that of PmgA. The comparative study of this novel gene and the pmgA gene would lead to the breakthrough for the elucidation of the regulatory mechanism of photosystem stoichiometry.

RESULTS

Isolation of the Mutant with Altered Chlorophyll Fluorescence Kinetics

We created mutants by transposon-mediated random insertion of a chloramphenicol-resistant cassette into the chromosome of the wild-type strain. We could isolate several mutants that showed different chlorophyll fluorescence kinetics from that of the wild type. One such mutant, the 0205-79 mutant, showed fluorescence kinetics similar to the pmgA mutant that has a defect in the regulation of photosystem stoichiometry (Fig. 1). Although the difference between the wild type and these two mutants in fluorescence kinetics was very small under low-light (20 μmol m⁻² s⁻¹) condition (Fig. 1A), a large difference was observed under high-light (200 μmol m⁻² s⁻¹) condition (Fig. 1B).

Figure 1. Kinetics of fluorescence emitted from the wild type (bold line), the 0205-79 mutant (thin line), and the pmgA mutant (dot line) cells under low-light (A) or high-light (B) conditions. Cells on agar plates were dark adapted for 15 min. AL was applied for 3 s to monitor the fluorescence. Intensity of the fluorescence was normalized with the initial value at the start of AL.
Characterization of the Mutants by Pulse Amplitude Modulation Chlorophyll Fluorometer

Photosynthetic electron transport in the wild type and the mutants was characterized in detail by using pulse amplitude modulation (PAM) chlorophyll fluorometer. Changes in the intensity of chlorophyll fluorescence upon the application of multiple turnover flash or actinic light (AL) are essentially the same between low-light-acclimated cells of the wild type and the mutants (Fig. 2, A–C). When cells were grown under high-light condition, however, the sharp rise of fluorescence intensity just after exposure to AL in the wild type was not observed in the pmgA mutant and the 0205-79 mutant (Fig. 2, D–F). Upon the cessation of the AL, the fluorescence intensity rapidly decreased in high-light-acclimated cells of the wild type, while the decay of fluorescence intensity was slower and a small peak appeared in those of the pmgA mutant and the 0205-79 mutant. Slower rise of the fluorescence intensity upon application of AL as well as slower decay of that upon cessation of the AL suggested that PSI

Figure 2. Fluorescence determined by a PAM fluorometer of the wild type (A, D), the 0205-79 mutant (B, E), and the pmgA mutant (C, F) under low-light (A–C) or high-light (D–F) conditions. The fluorescence measurement was initiated by applying modulated measuring light (ML) after 5 min dark adaptation. AL was turned on (AL on) to monitor the change in the level of fluorescence. Then, AL was turned off (AL off) and far-red light was turned on (FR on) to monitor the minimal level of fluorescence (Fo) with non-photochemical quenching. During the course of fluorescence measurement, a series of multiple turnover flashes (MT flashes) was applied to obtain Fo level (in the dark) and Fm' level (under AL). Finally, dichlorophenyldimethylurea was added (+DCMU) and AL was turned on (AL on) to obtain the maximum level of fluorescence (Fm). The rise and decay of fluorescence intensity upon the application and cessation of AL were magnified in the insets.
activity is higher in the mutant cells. The transient increase of the fluorescence intensity in the dark after the cessation of the AL is usually observed when the influx of electrons to plastoquinone pool by cyclic electron flow around PSI is enhanced. The results in Figure 2 suggest that the intracellular activity of PSI in the pmgA mutant and the 0205-79 mutant is higher than that in the wild type, at least in high-light-acclimated cells.

**Determination of Photosystem Stoichiometry by Measuring Chlorophyll Fluorescence Spectra at 77 K**

We then directly determined photosystem stoichiometry by measuring chlorophyll fluorescence emission spectra at 77 K. The fluorescence peak at 695 nm is mainly emitted from PSII and that at 725 nm arises from PSI. Thus, the fluorescence intensity at 695 nm/fluorescence intensity at 725 nm ratio ($F_{695}/F_{725}$) is a good index of PSII/PSI ratio. The $F_{695}/F_{725}$ ratio was about 0.4 in the low-light-acclimated cells of the wild type, and it increased to 1.0 during first 24 h under high-light condition (Fig. 3, black circles). However, the increase in this ratio was not observed in the 0205-79 mutant (Fig. 3, white circles). The similar inability to increase PSII/PSI ratio upon high-light acclimation was reported earlier in the pmgA mutant (Hihara et al., 1998). The results suggest that the gene responsible for the mutation in the 0205-79 mutant is involved in the regulation of photosystem stoichiometry under high-light condition.

**Measurements of PSI Amounts by Western Analysis**

The relative change in photosystem stoichiometry can be caused by the change either in PSI content or in PSII content. To estimate the amount of PSI in the mutant, we performed western analysis with an antiserum raised against PsaAB, the reaction center subunit of PSI. No differences in the amounts of PsaAB were observed between the wild type and the 0205-79 mutant under low-light condition (Fig. 4). The amounts of PsaAB proteins significantly decreased in the wild type under high-light condition. This decrease was partially suppressed in the 0205-79 mutant. The failure to modulate photosystem stoichiometry in the 0205-79 mutant during acclimation to high light must be due to the insufficient suppression of PsaAB amounts under high-light condition.

**Determination of PSI Transcripts during Acclimation to High Light**

In order to examine whether the amount of PsaAB protein is regulated at transcriptional level, we performed real-time RT-PCR analysis of psaA and/or psaAB during acclimation to high light. Low-light-grown cells were shifted to high light and time course of change in psaA and/or psaAB transcripts was examined (Fig. 5). The transcript level of psaA and/or psaAB drastically decreased upon the shift to high light, and the level was partially recovered 12 h after the shift to high light. The level of psaA and/or psaAB transcripts in the 0205-79 mutant was not significantly higher than that in the wild type during acclimation to high light. The inability to keep the level of the transcript of psaA and psaAB under high-light condition in the pmgA mutant (Muramatsu and Hihara, 2003) was not observed in the 0205-79 mutant. The profile of the change in the level of the transcripts could not explain the difference of the protein level of PsaAB between the wild type and the 0205-79 mutant (Fig. 4). The gene responsible for the mutation of the 0205-79 mutant may have a role in the translational or posttranslational regulation of PsaAB.

**Growth Rate of the Wild Type and the 0205-79 Mutant**

The growth rate of the wild type and the 0205-79 mutant was not significantly different under low-light condition (data not shown). Under the light of photon flux density at 300 μmol m$^{-2}$ s$^{-1}$, the growth of the 0205-79 mutant was better than that of the wild type during the first 24 h (Fig. 6) presumably because of the higher amounts of PSI in the mutant. The wild-type cells could continue to grow in the second and third days under the light of photon flux density at 300 μmol m$^{-2}$ s$^{-1}$, but the growth of the 0205-79

![Figure 3](image-url)  
**Figure 3.** Time course of the changes in the ratio of $F_{695}/F_{725}$ (PSII/PSI) during acclimation to high light. The ratio was determined by measuring chlorophyll fluorescence emission spectra of the wild type (black circle) and the 0205-79 mutant (white circle) at 77 K. Data are the means of three samples.

![Figure 4](image-url)  
**Figure 4.** Western analysis of PsaAB in the wild type and the 0205-79 mutant grown under low- and high-light conditions for 24 h. Total proteins, corresponding to the cells of 3 mL culture of OD$_{730}$ = 0.355, were loaded on each lane.
mutant was severely suppressed, implying that the failure to decrease PSI contents might result in the photodamage under prolonged high-light condition. This phenotype is very similar to that of the *pmgA* mutant, which was also reported to have a defect in the regulation of photosystem stoichiometry in acclimation to high light (Hihara et al., 1998; Sonoike et al., 2001).

**Identification of the Gene Responsible for the Mutation in the 0205-79 Mutant**

We determined the insertion site of a chloramphenicol-resistant cassette in the 0205-79 mutant by inverse PCR. The insertion site was 704 bp downstream from the start point of the open reading frame (ORF) of *sll1961*. In the course of screening for mutants that showed different fluorescence kinetics from that of the wild type, we isolated another mutant (the 0205-91 mutant) that showed the same phenotype in chlorophyll fluorescence kinetics as the 0205-79 mutant (data not shown). We confirmed that the 0205-91 mutant could not modulate photosystem stoichiometry in acclimation to high light by determination of fluorescence emission spectra at 77 K (data not shown). In the 0205-91 mutant, the insertion site of a chloramphenicol-resistant cassette was 714 bp downstream from the start point of the ORF of *sll1961*. The result suggests that the phenotype observed in the 0205-79 mutant is due to the disruption in the *sll1961* gene. Furthermore, we created a new *sll1961* mutant by the replacement of the 476th to 1,008th nucleotide sequence of the *sll1961* gene with a spectinomycin-resistant cartridge (the *sll1961*-SpR mutant). Measurements of chlorophyll fluorescence emission spectra at 77 K confirmed that the *sll1961*-SpR mutant was unable to regulate photosystem stoichiometry in acclimation to high light (data not shown). These observations indicated that the disruption of *sll1961* is really the cause of the observed phenotype.

The *sll1961* gene contains an ORF of 1,032 bp, encoding a deduced protein of 343 amino acid residues. N-terminal region of Sll1961 contains a helix-turn-helix DNA-binding domain, suggesting that this protein may be a transcriptional regulator.

**Search for the Putative Target Genes of Sll1961**

Although Sll1961 has homology with the transcriptional regulator, the transcript level of *psaA* and/or *psaAB* was not apparently affected (Fig. 5). So, we compared genome-wide patterns of transcription between the wild type and the 0205-79 mutant (the *sll1961* mutant) by DNA microarray analysis to identify the possible target genes of Sll1961. Genes whose expression level reproducibly increased or decreased in three independent experiments were defined as the genes that were affected by the disruption of the *sll1961* gene. In low-light-acclimated cells, no significant differences of gene expression between the wild type and the *sll1961* mutant were observed (data not shown). Under high-light condition, however, some differences were observed as presented in Figure 7. The expression of one gene, *sll1773*, was reproducibly higher in the *sll1961* mutant than in the wild type after 3 h following the shift to high light (Table I). The expression of several genes such as *slr0364*, *slr2076*, and *slr2057* was lower in the *sll1961* mutant than in the wild type under high-light condition. The *slr0364*, *slr2076*, and *slr2057* genes encode a hypothetical protein, 60-kD chaperonin GroEL, and water channel protein, respectively. Northern-blot analysis (Fig. 8) showed that the expression of *sll1773* was very low either in the wild type or in the *sll1961* mutant under low-light condition. The expression of *sll1773* increased in the *sll1961* mutant during 6 h after the shift to high light, while it did not change in the wild type. We also examined the reliability of microarray data of the *slr0364*, *slr2076*, and *slr2057* genes by real-time RT-PCR. The transcript levels of *slr0364*, *slr2076*, and *slr2057* were 0.39-, 0.47-, and 0.44-fold in the 0205-79 mutant relative to the wild type after 3 h in exposure to high light.

![Figure 5](image1.png)

**Figure 5.** Transcript abundance of *psaA* in the wild type (black circle) and the 0205-79 mutant (white circle) during acclimation to high light. The abundance of *psaA* transcript relative to *rnpB* transcript was determined by real-time RT-PCR using cDNA generated from total RNA extracts from cells collected at various time points after the shift to high light.

![Figure 6](image2.png)

**Figure 6.** Growth curve of the wild type (black circle) and the 0205-79 mutant (white circle) under photon flux density at 300 μmol m$^{-2}$ s$^{-1}$. Cells grown at 20 μmol m$^{-2}$ s$^{-1}$ were shifted to 300 μmol m$^{-2}$ s$^{-1}$ at time 0 and inoculated every 24 h in order to prevent the self-shading effect.
Table 1. Genes whose expression was markedly affected in the 0205-79 mutant compared to wild type under high-light condition

<table>
<thead>
<tr>
<th>ORF</th>
<th>Gene Name</th>
<th>Product</th>
<th>0205-79/Wild Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>slr0364</td>
<td>–</td>
<td>Hypothetical protein</td>
<td>0.24 ± 0.11</td>
</tr>
<tr>
<td>slr2057</td>
<td>groEL1</td>
<td>60-kD chaperonin</td>
<td>0.25 ± 0.14</td>
</tr>
<tr>
<td>slr2057</td>
<td>app1</td>
<td>Water channel protein</td>
<td>0.31 ± 0.11</td>
</tr>
<tr>
<td>sll1773</td>
<td>pirA</td>
<td>Pirin-like protein</td>
<td>14.7 ± 6.2</td>
</tr>
</tbody>
</table>

DISCUSSION

In this study, we demonstrate that mutants of the sll1961 gene fail to regulate photosystem stoichiometry during acclimation to high light. Both the analyses of chlorophyll fluorescence quenching by PAM fluorometer and the measurements of fluorescence emission spectra at 77 K suggest that PSI content in the mutants could not be suppressed under high-light condition. Western analyses clearly revealed that the decrease in the amount of PsaAB, the reaction center subunits of PSI, under high-light condition was partially suppressed in the 0205-79 mutant (Fig. 4). All the sll1961 mutants showed normal photosystem stoichiometry under low-light condition so that they are regulatory mutants of photosystem stoichiometry. This point clearly distinguishes the sll1961 mutants from other mutants reported to have modified photosystem stoichiometry (Wilde et al., 1995, 2001; Mann et al., 2000; Shen et al., 2002; Kufryk and Vermaas, 2003). One mutant, the pmgA mutant, was reported to be a regulatory mutant of photosystem stoichiometry under high-light condition in the past (Hihara et al., 1998). The reported phenotypes of the pmgA mutant, i.e. (1) normal photosystem stoichiometry under low-light condition, (2) the suppression of the decrease of PSI content under high-light condition, and (3) the inhibition of growth under long-term high-light condition (Sonoike et al., 2001), are all quite similar to the phenotypes of the sll1961 mutants. However, transcript levels of psaA and/or psaAB were quite different between the pmgA mutant and the sll1961 mutant. Suppression of the transcript levels of psaA and/or psaAB under prolonged high-light condition was impaired in the pmgA mutant (Muramatsu and Hihara, 2003), while the transcript levels in the sll1961 mutant were similar to the wild type (Fig. 5). This suggests that PmgA is involved in the transcriptional regulation of psaA and/or psaAB, while Sll1961 is involved in translational or posttranslational regulation of PsaAB.

DNA microarray data also suggest that the expression of other PSI genes is not so much affected in the sll1961 mutant under low-light condition (data not shown) as well as under high-light condition.

DNA microarray analysis revealed that the expression of several genes under high-light condition was affected by the mutation of the sll1961 gene. The expression of sll1773 was significantly induced in the sll1961 mutant in exposure to high light for 3 h. The expression of the gene was very low in the wild type both under low- and high-light conditions (Fig. 8). The sll1773 gene encodes a pirin-like protein called PirA (Hihara et al., 2004). Pirin-like proteins are found in many different organisms ranging from Archaeabacteria to mammals (Orzaez et al., 2001). In tomato (Lycopersicon esculentum), the gene expression of pirin was induced during apoptosis (Orzaez et al., 2001), while pirin in Arabidopsis (Arabidopsis thaliana; AtPirin)-subunit of G protein, GPA1, and regulated seed germination and seedling development (Lapik and Kaufman, 2003). In the cyanobacterium Synechocystis sp. PCC 6803, the pirA (sll1773) gene is the only gene that encodes a pirin homolog, and its expression increased in the presence of NaCl, sorbitol, or ethanol at high concentration (Hihara et al., 2004).
Several explanations may be possible for the expression of pirA in the sll1961 mutant under high-light condition. Since the pirA gene is highly expressed under stressed condition in cyanobacteria (Hihara et al., 2004), its expression might be increased under severe stress condition caused by the combination of high light and disruption of the sll1961 gene. Secondly, PirA may have a role to suppress the decrease of PSI amounts under high-light condition. In the wild-type cells, Sll1961 may transcriptionally repress the expression of pirA under high-light condition and decrease the amounts of PSI. In the sll1961 mutant, Sll1961 cannot repress the expression of pirA and the decrease of PSI contents is suppressed by PirA. Since pirin is known to have a protein-protein interaction domain and regulate transcription (Dechend et al., 1999), PirA might interact with some proteins and inactivate transcription necessary for the modulation of photosystem stoichiometry. The exact role of PirA in cyanobacteria and its involvement in the modulation of photosystem stoichiometry should be examined in future.

Expression of three genes was suppressed in the sll1961 mutant compared with the wild type; slr0364, slr2076, and slr2057. The expression of slr0364 was most repressed in the sll1961 mutant compared with that in the wild type after 3 h of exposure to high light. The slr0364 gene encodes a hypothetical protein, which has a Thr-rich region. The gene seems to be a part of operon with at least the slr0366 gene whose product has also a Thr-rich region. It was reported that the expression of the slr0364 gene was induced at approximately 1 h after the shift to high light and remained continuously at high levels during acclimation to high light (Hihara et al., 2001). It is tempting to assume that Slr0364 has a role in the regulation of photosystem stoichiometry under high-light condition.

This study demonstrates that a transcriptional regulator Sll1961 is associated with modulation of photosystem stoichiometry in acclimation to high light. The results of genome-wide analysis of transcription between the wild type and the sll1961 mutant list up several candidates that may be related to the regulation of photosystem stoichiometry during acclimation to high light. The research to identify the role of these genes would be conducted in the near future to facilitate the understanding of the mechanism of regulating photosystem stoichiometry in exposure to high light.

MATERIALS AND METHODS

Strains and Growth Conditions

Synechocystis sp. PCC 6803 wild-type and mutant strains were grown in BG-11 medium (Rippka et al., 1979) with 10 mM TES. Cells in liquid culture were grown at 30°C in 50 mL glass tubes and bubbled with air under continuous illumination provided by fluorescent lamps. Photon flux density at 20 and 200 μmol m⁻² s⁻¹ was regarded as low light and high light, respectively. For creating random mutants, genes in cosmid vectors were randomly disrupted in vitro by the insertion of a transposon that has been set in two-dimensional fluorescence imaging system (FluorCam, Photon Systems Instruments), and orange AL (160 μmol m⁻² s⁻¹) from light-emitting diodes was applied for 3 s to monitor the fluorescence kinetics. The fluorescence intensity was normalized with initial value at the start point of AL.

Monitoring of Chlorophyll Fluorescence Kinetics of Dark-Adapted Cyanobacterial Cells

Cyanobacterial cells on agar plates were dark adapted for 15 min. The plates were set in two-dimensional fluorescence imaging system (FluorCam, Photon Systems Instruments), and orange AL (160 μmol m⁻² s⁻¹) from light-emitting diodes was applied for 3 s to monitor the fluorescence kinetics. The fluorescence intensity was normalized with initial value at the start point of AL.

Yield of Chlorophyll Fluorescence Determined by PAM fluorometer

Yield of chlorophyll fluorescence was determined by PAM fluorometer (PAM 100/102/103, Heinz Wals). Cells in 3 mL liquid culture were dark adapted for 5 min and then the chlorophyll fluorescence was monitored (Sonoike et al., 2001). AL at 200 μmol m⁻² s⁻¹ was used both for low-light-acclimated cells and high-light-acclimated cells.

Fluorescence Emission Spectra at 77 K

Low temperature fluorescence emission spectra at 77 K were recorded using a custom-made apparatus (Sonoike and Terashima, 1994). Cells were collected and adjusted to 5 μg chlorophyll/mL in BG-11 medium. Pigments were excited with blue light passing through a filter (Corning CS-4-46). Before measurement, cells were dark adapted for more than 10 min at room temperature to eliminate the possible effects of state transition. Chlorophyll a concentrations were determined after extraction with 100% methanol (Grimme and Boardman, 1972).

Isolation of Thylakoid Membranes

Cells of Synechocystis sp. PCC 6803 were harvested and suspended in 0.4 M Suc, 10 mM NaCl, 1 mM CaCl₂, 0.2 mM phenylmethylsulfonyl fluoride, 5 mM benazamine, and 50 mM MOPS, pH 7.0 (MOPS buffer; Sun et al., 1998). Cells were broken by a bead beater (model 1107900, Biospec Products) for three times of 30 s operation with 2 min intervals. Unbroken cells and debris were removed by low-speed centrifugation at 4,700g for 10 min. Thylakoid membranes were pelleted by centrifugation at 140,000g for 45 min. The thylakoid membranes were resuspended in the MOPS buffer.

Western Analysis

Thylakoid membranes were solubilized with 5% lithium dodecyl sulfate and 60 mM dithiobisreitol for 1 h at room temperature, and subjected to SDS-PAGE. SDS gel electrophoresis was carried out using 16% to 22% polyacrylamide gel containing 7.5 M urea (Ikeuchi and Inoue, 1988). Total proteins, corresponding to the cells of 3 mL culture of OD₇₃₀ 0.1, were loaded on each lane. Proteins were electroblotted onto poly(vinylidene difluoride) membranes (Immobilon, Millipore). The antisera against PsaAB from Thermosynechococcus elongatus BP-1 (formerly Synechococcus elongatus BP-1) was kindly provided by Prof. Isao Enami (Tokyo Science University). Reaction with antisera and immunodetection was performed according to Kashino et al. (1990).
Northern Analysis

Northern-blot analysis was performed according to Muramatsu and Hihara (2003).

cDNA Synthesis and Real-Time RT-PCR

RT-PCR was performed with an RT-PCR Core Kit (Takara) for cDNA synthesis. Real-time RT-PCR amplifications of cDNA templates were carried out using a Smart Cycler II (Cepheid) with a SYBR Premix Taq Kit (Takara). The psaA transcript accumulation was measured as the ratio of psaA RNA level to rpb8 RNA level (internal control). PCR reactions were as follows: 95°C for 10 s, followed by 40 cycles at 95°C for 1 s, and 60°C for 20 s. The psaA primers are 5'-CCCGCGAGGTGCCCAAAGTGAGC-3' and 5'-CCACAAGCAGTGATCCAGGAGA-3'. The rpb8 primers are 5'-CGCCCCAGTGCGCCGAGCGCGTGAAGA-3' and 5'-CCCGCACCTTGGCCACAACC-GGG-3'.

Identification of the Insertion Site of a Chloramphenicol-Resistant Cassette in the Mutants

Genome DNA of chloramphenicol-resistant mutant was digested with HindIII and incubated at 37°C for more than 3 h. Digested DNA fragments were self-ligated using DNA Ligation Kit (Version 2, Takara). To amplify the region flanking the inserted cassette, inverse PCR was performed using outward primers. First PCR was performed with primer AIB-A (5'-CAACAGTACTCGGTAGTGCGAC-3') and AIB-B (5'-CTGAATTACAGTGCGTCCAC-CACAAGC-3'). Each sample was subjected to 35 cycles of PCR consisting of denaturation at 93°C for 30 s and annealing at 55°C for 1.5 min and elongation at 72°C for 1.5 min. The PCR product was directly used as a template of second PCR. Second PCR was performed with primer GPS21-1 (5'-CACAGCA- TAATCGAGAACATTCA-3') and GPS21-2 (5'-GGTATTAGCAGTCGAC-GCTCAACC-3'). Second PCR was carried out under the same condition as the first one.

Sequence of the amplified flanking region was determined by the dye-terminator fluorescence detection method, using a model 310 sequence analyzer (Applied Biosystems). For sequencing, PCR amplification followed by cycle sequencing with one of the primers, GPS21-1 or GPS21-2, was performed using Thermo Sequence II dye terminator cycle sequencing Kit (Amersham Pharmacia).

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