Running head:

The factor that regulates photosystem stoichiometry

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The mutant of *sll1961*, 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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ABSTRACT

In acclimation to changing light environments, photosynthetic organisms modulate the ratio of two photosynthetic reaction centers (photosystem I and photosystem II). Two mutants, which could not modulate photosystem stoichiometry upon the shift to high light, were isolated from mutants created by random transposon mutagenesis. Measurements of chlorophyll fluorescence and analysis of the reaction center subunits of photosystem I through Western blotting in these mutants revealed that the content of photosystem I could not be suppressed under high-light condition. In these mutants, transposon was inserted to *sll1961*, which is homologous to the gene of gluconate operon transcriptional repressor (GntR) in *Bacillus anthracis* A2012. DNA microarray analysis revealed that the expression of *sll1773* was drastically induced in the *sll1961* mutant upon exposure to high light for 3 h. Our results demonstrate that a novel transcriptional regulator, Sll1961, and its possible target protein, Sll1773, may be responsible for the regulation of photosystem stoichiometry in response to high light.

INTRODUCTION

Energy transduction in photosynthesis depends on the coordination of two photosystems, photosystem II (PSII) and photosystem I (PSI). PSII conducts a light-dependent oxidation of water and reduction of plastoquinone (reviewed by Diner and Rappaport, 2002; Barber, 2002), while PSI is involved in a light-dependent electron transport from plastocyanin to $NADP^+$ via ferredoxin (for a review, see Fromme et al., 2002). The two light-dependent reactions are connected by cytochrome b_6f complex that catalyzes the electron transfer from plastoquinole 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 upon the change in light quality as well as in light quantity. For example, PSI/PSII ratio decreased upon the shift to high-light condition through the selective suppression in the amount of PSI, in addition to the general decrease of the amount of both photosystems (for reviews, 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). The deletion of genes encoding the subunits of PSI or PSII may lead to the decrease of relative PSI or PSII content. The disruption of genes involved in transcription, translation, assembly or biogenesis of PSI or PSII may all lead to the change in photosystem stoichiometry. However, only a few cases have been reported for the ‘regulatory’ mutant of photosystem stoichiometry. One such case is the *pmgA* (*sll1968*) mutant. This mutant has normal amounts of PSII and PSI under low-light condition, but fails to change the 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 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 a novel gene, *sll1961*, which is involved in the modulation of photosystem stoichiometry in acclimation to high light. Characterization of the disruption mutants of this gene indicates that the mutants fail to properly suppress the amount of PSI under high-light condition. Since real time RT-PCR analysis showed that this mutation did not much affect the level of *psaA* transcription 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 mutants 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 of such mutants, the *0205-79* mutant, showed fluorescence kinetics similar to the *pmgA* mutant, which 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 condition (Fig. 1 A), large difference was observed under high-light condition (Fig. 1 B).

Characterization by pulse amplitude modulation (PAM) chlorophyll fluorometer

Photosynthetic electron transport in the wild type and the mutant was characterized in detail by using PAM chlorophyll fluorometer. Changes in the intensity of chlorophyll fluorescence upon the application of multiple turnover flash (MT) or actinic light (AL) are essentially the same between low-light acclimated cells of the wild type and the mutants (Fig. 2A, B and C). When cells were grown under high-light condition, however, the sharp rise of fluorescence intensity just after exposure to actinic light in the wild type was not observed in the *pmgA* mutant and the *0205-79* mutant (Fig. 2D, E and F). Upon the cessation of the actinic light, 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 was observed in those of the *pmgA* mutant and the *0205-79* mutant. Slower rise of the fluorescence intensity upon application of actinic light as well as slower decay of that upon cessation of the actinic light suggested that PSI activity is higher in the mutant cells. The transient increase of the fluorescence intensity in the dark after the cessation of the actinic light 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 F_{695}/F_{725} (fluorescence intensity at 695 nm / fluorescence intensity at 725 nm) ratio 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, closed circles). However, this increase in this ratio was not observed in the *0205-79* mutant (Fig. 3, open 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 either the change in PSI content or the change 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 subunits 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 were down-regulated in the wild type under high-light condition. This down-regulation was partially repressed 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 difference of PsaAB amounts is attributed to the difference in transcriptional level, we performed real time RT-PCR analysis of *psaA* gene during acclimation to high light. Low-light grown cells were shifted to high-light condition and time course of change in *psaA* transcripts was examined (Fig. 5). The transcript level of *psaA* drastically decreased upon the shift to high light, and the level was partially recovered 12 hours after the shift to high light. The level of *psaA* transcript in the *0205-79* mutant was not higher than that in the wild type 12 h after the shift to high light. No significant differences were observed between the wild type and the *0205-79* mutant after 24 h following the shift to high light. The inability to repress the transcript of *psaA* in the *pmgA* mutant under prolonged high-light condition (Muramatsu and Hihara, 2003) was not observed in the *0205-79* mutant. The profile of the change in 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 post-translational regulation of PsaAB.

Growth rate of the wild type and the *0205-79* mutant

We examined whether the failure to regulate photosystem stoichiometry in acclimation to high light

affects the growth rate. We observed the growth of the wild type and the *0205-79* mutant during 24 h under low-light condition and during 72 h under high-light condition. The difference between the wild type and the *0205-79* mutant in the growth was not significantly observed under low-light condition (data not shown). Under high-light condition, cell cultures were adjusted to a concentration of OD_{730}=0.05 every 24 h in order to prevent the self-shading effect. The growth of the *0205-79* mutant was slightly better than that of the wild type 48 h after the shift to high light (Fig. 6) because of the inability to decrease the amounts of PSI sufficiently. The growth of the *0205-79* mutant was severely suppressed in the third day although that of the wild type was unchanged, implying that the failure to decrease PSI contents might result in the photodamage under prolonged high-light condition. This result is consistent with the report about the growth of the *pmgA* mutant which was reported to fail to modulate photosystem stoichiometry in acclimation to high light (Hihara et al., 1997; 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 by inverse PCR. The insertion site was 704 bp downstream from the start point of the open reading frame of *sll1961*. In the screening for mutants that showed different fluorescence kinetics from that of the wild type, we isolated another mutant (*0205-91* mutant) that shows the same phenotype in chlorophyll fluorescence kinetics as the *0205-79* mutant and the *pmgA* mutant (data not shown). We confirmed that the *0205-91* mutant could not modulate photosystem stoichiometry in acclimation to high light by low temperature fluorescence emission spectra analysis (data not shown). In the *0205-91* mutant, the insertion site of chloramphenicol-resistant cassette was 714 bp downstreasm from the start point of the open reading frame of the same gene, *sll1961*. The *sll1961* gene contains an open reading frame of 1032 bp, encoding a deduced protein of 343 amino acid residues. Sll1961 in *Synechocystis* sp. PCC 6803 is homologous to All1076 in *Nostoc* sp. PCC 7120, Tll2117 in *Thermosynechococcus elongatus* BP-1, Glr4252 in *Gloeobacter violaceus* PCC 7421 and SYNW0091 in *Synechococcus* sp. WH 8102 (Fig. 7). However, homologue to Sll1961 does not exist in higher plants. N-terminal region of Sll1961 contains helix-turn-helix gluconate operon transcriptional repressor (GntR) domain. This region is homologous to GntR-type transcriptional regulator in *Bacillus anthracis* and *Bacillus subtilis*.

To confirm that the failure to regulate photosystem stoichiometry was caused by the mutation of *sll1961*, we constructed a new *sll1961* mutant for spectinomycin-resistant cartridge insertion (the *sll1961*-Sp^R mutant). Measurements of chlorophyll fluorescence spectra at 77 K confirmed that the *sll1961*-Sp^R mutant was unable to regulate photosystem stoichiometry in acclimation to high light (data not shown). This observation indicated that the phenotype observed in the *0205-79* mutant was attributable to the mutation of *sll1961*.

Search for the downstream targets of Sll1961

Although Sll1961 has homology with the transcriptional regulator, GntR, it did not significantly affect the transcriptional level of *psaA*. So, we compared genome-wide patterns of transcription between the wild type and the *0205-79* mutant (the *sll1961* mutant) using DNA microarray 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 mutation of *sll1961*. In low-light acclimated cells, the significant differences of gene expression between the wild type and the *sll1961* mutant were not observed (data not shown). Under high-light condition, however, some differences were observed (Fig. 8). The expression of one gene, *sll1773*, was specifically induced in the *sll1961* mutant upon the shift to high light for 3 h (Table 1). Although the value of expression of *slr1870* was more than 7, the deviation was near 7. We excluded the *slr1870* gene from the targets of Sll1961. The expression of several genes such as *slr0364*, *slr2076* and *slr2057* was decreased in the *sll1961* mutant under the same condition.

To examine the expression of *sll1773* in the wild type and the *sll1961* mutant in detail, we performed Northern blot analysis (Fig. 9). The expression of *sll1773* was very low in low-light acclimated cells either of the wild type or the *sll1961* mutant. The expression of *sll1773* increased in the *sll1961* mutant during 6 h after the shift to high light, while it did not in the wild type.

DISCUSSION

In response to high light, photosynthetic organisms change their photosynthetic apparatus in order to avoid the photodamage. In cyanobacteria, high-light acclimation includes reduction of antenna size, activation of PSII reaction center protein turnover, enhancement of scavenging system for reactive oxygen

species, up-regulation of CO_2 fixation, state transition and regulation of photosystem stoichiometry. These phenomena have been extensively investigated from physiological point of view (Hihara, 1999). However, molecular mechanisms for high-light acclimation remain to be solved.

In this study, we demonstrate that a mutant of a novel gene, *sll1961*, fails 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 mutant could not be suppressed under high-light condition. Western analyses clearly revealed that the decrease in the amount of the reaction center subunits of PSI, PsaAB, was partially suppressed in the mutant (Fig. 4). This mutant showed normal photosystem stoichiometry under low-light condition, so that it is a regulatory mutant of photosystem stoichiometry. This point clearly distinguishes the *sll1961* mutant 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 in the past (Hihara et al., 1998). The *pmgA* mutant showed normal photosystem stoichiometry under low-light condition, and the suppression of the decrease of PSI content was observed under high-light condition. Under long-term high-light condition, the growth of the *pmgA* mutant was inhibited (Sonoike et al., 2001). These phenotypes are quite similar to that of the *sll1961* mutant. However, although mRNA levels of *psaA* and *psaAB* in the *pmgA* mutant were quite different from that in the wild type after 12 h of exposure to high light (Muramatsu and Hihara 2003), the profile of *psaA* transcript level in the wild type and the *sll1961* mutant was not able to explain the difference of PSI content (Fig. 5). This suggests that Sll1961 is involved in translational or post-translational regulation of PsaAB while PmgA is involved in transcriptional regulation of *psaA* and *psaAB*. It seems that the downstream targets of Sll1961 are not directly involved in transcription or stability of *psaA* mRNA, although the *sll1961* gene encodes a putative transcriptional regulator. DNA microarray data reveal that other PSI gene expression also showed no crucial differences between the wild type and the *sll1961* mutant under low-light condition (data not shown) as well as under high-light condition (Fig. 7).

To identify genes involved in Sll1961-mediated regulation of photosystem stoichiometry under high-light condition, DNA microarray was performed using mRNA isolated from the cells of the wild type and the *sll1961* mutant. The expression of several genes under high-light condition was affected by the

mutation of *sll1961*. The expression of *sll1773* was strikingly 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. 9). The *sll1773* gene encodes a pirin-like protein. Pirin-like proteins are found in many different organisms ranging from Archae bacteria to mammals (Orzaez et al., 2001). Pirin protein was first isolated from human by a yeast two-hybrid screening as an interactor of nuclear factor I/CCAAT box transcription factor (NFI/CTF1), which is known to stimulate adenovirus DNA replication and RNA polymerase II-driven transcription (Wendler et al., 1997). Pirin was found to interact with the ankyrin-repeat domain of the proto-oncoprotein Bcl-3 (Dechend et al., 1999). Crystal structure of human pirin by X-ray analysis indicated that pirin has metal binding residue and requires the participation of metal ion for the interaction with Bcl-3 (Zeng et al., 2003; Pang et al., 2003). In tomato, pirin was reported to be induced during apoptosis (Orzaez et al., 2001). It was reported that pirin in Arabidopsis (AtPirin) interacted with an α-subunit of G protein, GPA1, and regulated seed germination and seedling development (Lapik and Kaufman, 2003). Pirin was considered to be a multifunctional regulatory protein in plants. In the cyanobacterium *Synechocystis* sp. PCC 6803, the *sll1773* gene is the only gene that encodes a pirin homologue, and its expression increased in the addition of NaCl, sorbitol or ethanol (Hihara et al., 2004). In this study, the *sll1773* expression was induced in cells that could not modulate photosystem stoichiometry in acclimation to high light.

Several explanations may be possible for the expression of pirin gene in the *sll1961* mutant under high-light condition. First, pirin may function as a stress inducible protein. When tomato cells were subject to programmed cell death, the pirin gene expression was induced (Orzaez et al., 2001). Similarly, the *sll1773* expression might be increased when the combination of high light environment and disruption of the *sll1961* gene caused a severe stress in cyanobacteria. However, it was reported that the induction of *sll1773* expression was not related with lethal condition (Hihara et al., 2004). Secondly, pirin may have a role to suppress the decrease of PSI amounts under high-light condition. In the wild type, Sll1961 may transcriptionally represses the expression of *sll1773* under high-light condition and decrease the amounts of PSI. In the *sll1961* mutant, Sll1961 cannot repress the expression of *sll1773* and the decrease of PSI contents is suppressed by Sll1773. Since pirin is known to have a protein-protein interaction domain and regulate transcription (Dechend et al., 1997), Sll1773 might interact with some proteins and inactivate transcription

necessary for the modulation of photosystem stoichiometry.

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 Thr-rich region. The gene seems to be a part of operon with at least *slr0366* whose product has also Thr-rich region. From the data of DNA microarray, the *slr0364* gene expression 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 regulation of photosystem stoichiometry under high-light condition.

This study demonstrates that a novel 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 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 transposon, which have a chloramphenicol-resistant cassette. The wild type strains were then transformed by the cosmid vectors having transposon. The cosmid vectors having random insertion are the kind gift from Dr. H. Fukuzawa in Kyoto University. The *pmgA* (*sll1968*) mutant was constructed by insertion of the spectinomycin-resistant cassette (Hihara and Ikeuchi, 1997). To generate the *sll1961*-Sp^R mutant, the 476th to 1008th nucleoside sequence of the *sll1961* gene was replaced with the spectinomycin-resistant cassette using *Hid*III and *Sal*I

sites of the *sll1961* gene. The *sll1961*, the *sll1961*-Sp^R and the *pmgA* mutants were maintained with 20 µg/ml chloramphenicol or spectinomycin, respectively.

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, Czech Republic). Then, orange actinic light (160 µE m^{-2} s^{-1}) from LEDs was applied for 3 s to monitor the fluorescence kinetics. The fluorescence intensity was normalized with initial value at the start point of actinic light.

Yield of chlorophyll fluorescence determined by pulse amplitude modulation fluorometer

Yield of chlorophyll fluorescence was determined by pulse amplitude modulation (PAM) fluorometer (PAM 101/102/103, Heinz Waltz, Effeltrich, Germany). Cells in 3 ml liquid culture were dark-adapted for 5 min and then the modulated measuring light (ML) was turned on to obtain the minimal fluorescence level, Fo. The fluorescence level with fully reduced Q_A (Fm') was obtained by applying multiple turnover (MT) flashes (XMT-103, Heinz Waltz, Effeltrich, Germany). The stable level of fluorescence (Fs) was determined during exposure of cells to actinic light (AL) with defined photon flux density from a light source (KL 1500, Schott, Wiesbaden, Germany). The far-red light from a photodiode (FR-102, Heinz Waltz, Effeltrich, Germany) was applied just after turning off the actinic light to determine Fo', the minimum fluorescence level under non-photochemical quenching. The maximum fluorescence (Fm) was obtained by adding DCMU to the sample at a final concentration of 10 µM under actinic light (Sonoike et al., 2001).

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 4-96). 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 sucrose, 10 mM NaCl, 1 mM $CaCl_2$, 0.2 mM penylmethylsulfonyl fluoride, 5 mM benzamidine, and 50 mM MOPS pH 7.0 (Sun et al., 1998). Cells were broken by a bead-beater (Model 1107900, Biospec Products, Bartlesville, America) for three times of 30 s operation with 2 min intervals. Unbroken cells and debris were removed by low speed centrifugation at 4,700 x *g* for 10 min. Thylakoid membranes were pelleted by centrifugation at 140,000 x *g* for 45 min. The thylakoid membranes were re-suspended in MOPS buffer.

Western analysis

Thylakoid membranes were solubilized with 5% lithium dodecyl sulfate and 60 mM dithiothreitol for 1 h at room temperature, and subjected to SDS-PAGE. SDS gel electrophoresis was carried out using 16-22% polyacrylamide gel containing 7.5 M urea (Ikeuchi and Inoue, 1988). Sample containing the same number cells was loaded for each lane. Proteins were electroblotted onto PVDF membranes (Immobilon, Millipore). The antiserum against PsaAB from *Thermosynechococcus elongatus* BP-1 (formerly *Synechococcus* elongatus BP-1) was kindly provided by Dr. I. Enami (Tokyo Science University). Reaction with antiserum and immunodetection were performed according to Kashino et al. (1990).

Microarray analysis

The DNA microarray analysis was performed using CyanoCHIP ver. 1.6 (TaKaRa, Kyoto, Japan) according to Hihara et al. (2001). 5 µg of RNA was labeled using an RNA Fluorescence Labeling Core Kit (M-MLV ver. 2.0, TaKaRa, Kyoto, Japan).

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, Kyoto, Japan) for cDNA synthesis. Real-time RT-PCR amplifications of cDNA templates were carried out using a Smart Cycler II from Cepheid (Sunnyvale, CA). Amplifications were carried out with a SYBR Premix Taq Kit (TaKaRa, Kyoto, Japan). The *psaA* transcript accumulation was measured as the ratio of *psaA* RNA level to *rnpB* RNA level (internal control). PCR reactions were heated to 95˚C for 10 s, followed by 40 cycles at 95˚C for 15 s amd 60˚C for 20 s. The *psaA* primers are 5'-CCTTCGAGAAGTGGGGCAAGCCGGG-3' and 5'-CCACAGCGAGGTGCCCAAAGTGAGC-3'. The *rnpB* primers are 5'-CGCCCAGTGCGCGCGAGCGTGAGGA-3' and 5'-CCTCCGACCTTGCTTCCAACCGGGG-3'.

Identification of the insertion site

Genome DNA of chloramphenicol-resistant mutant was digested with *Hha*I and incubated at 37˚C for more than 3 h. Digested DNA fragments were self-ligated using DNA Ligation Kit (Ver. 2, TaKaRa, Kyoto, Japan). To amplify the region flanking the inserted cassette, inverse PCR was performed using outward primers. First PCR was performed with primer AIB-A (5'-CAACAGTACTGCGATGAGTGGCAG-3') and AIB-B (5'-GGTAATACTAGTGTCGACCAACCAG-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'-CACAGCATAACTGGACTGATTTCAG-3') and GPS21-2 (5'-CGTATTAGCTTACGACGCTACACC-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, Foster City, California). For sequencing, PCR amplification followed by cycle sequencing with primer GPS21-1 or GPS21-2 was performed using Thermo Sequence II dye terminator cycle sequencing Kit (Amersham Pharmacia, Uppsala, Sweden).

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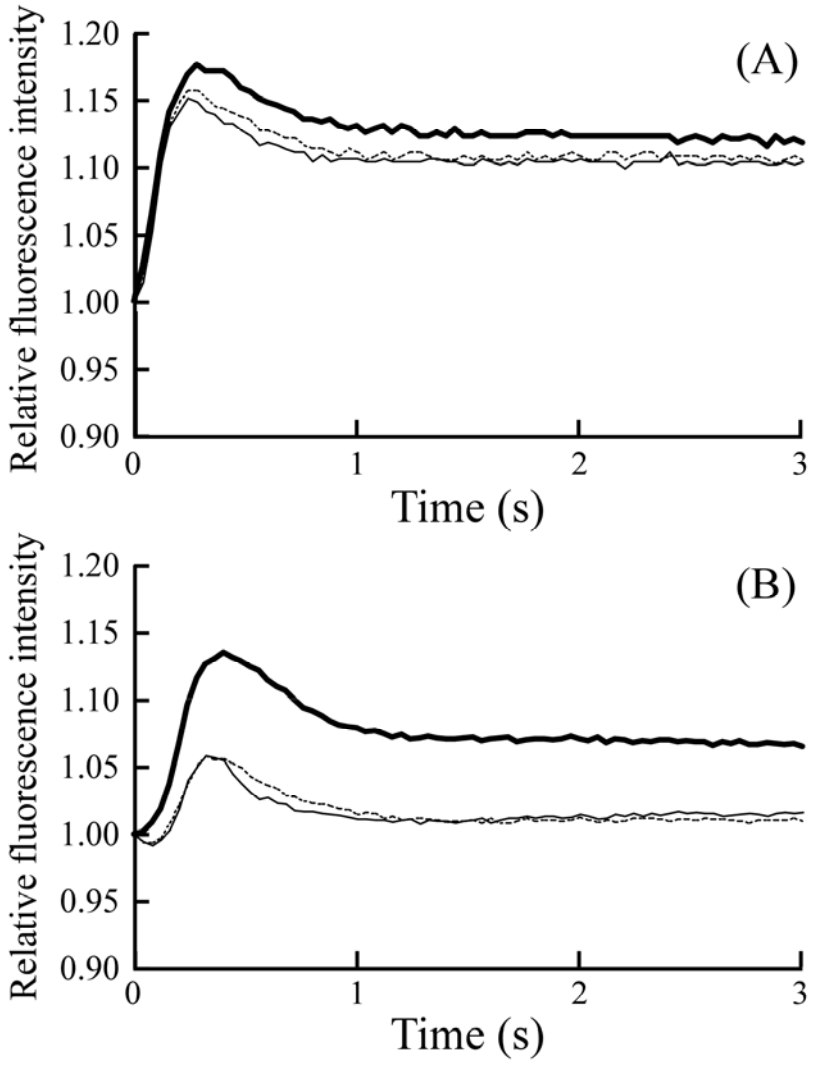
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1.10
1.05
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0.95
0.90
0
1
2
3
Time (s)
(B)
Relative fluorescence intensity
1.20
1.15
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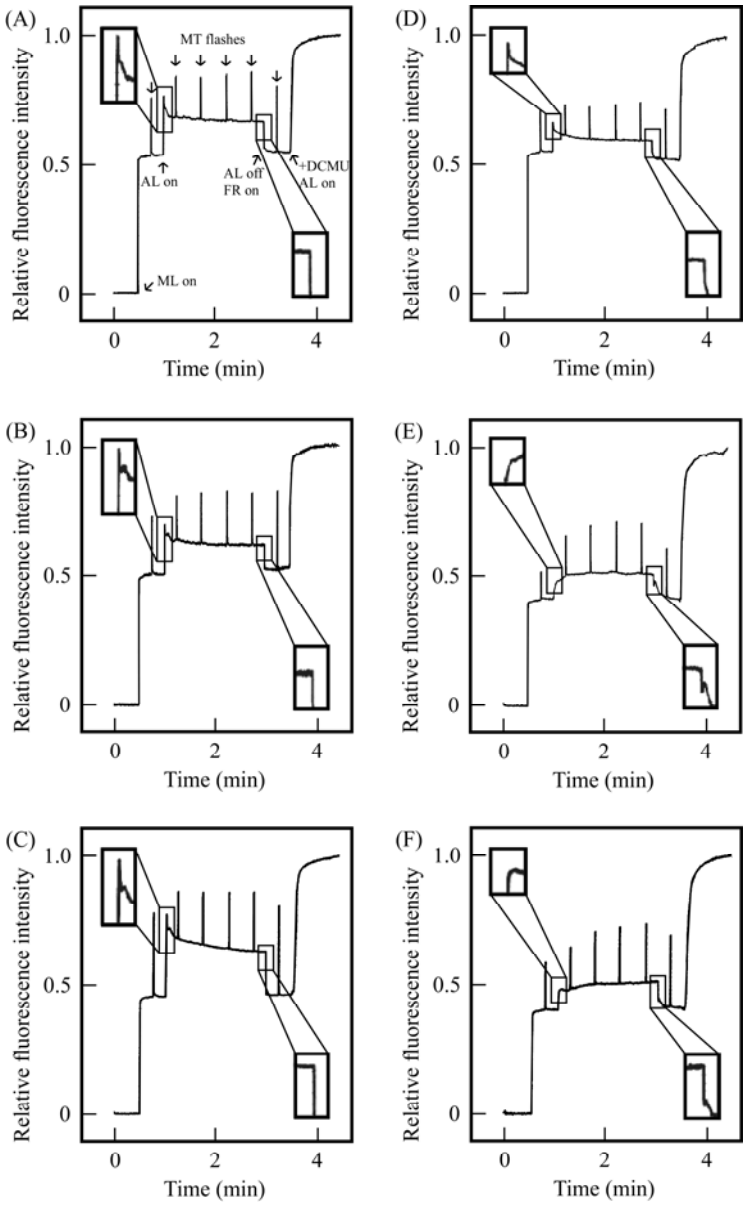
1.05
1.00
0.95
0.90
0
1
2
3
Time (s)

Fig. 1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MT flashes
AL on
AL off
FR on
+DCMU
AL on
ML on
(D)
(B)
(E)
(C)
(F)
Relative fluorescence intensity
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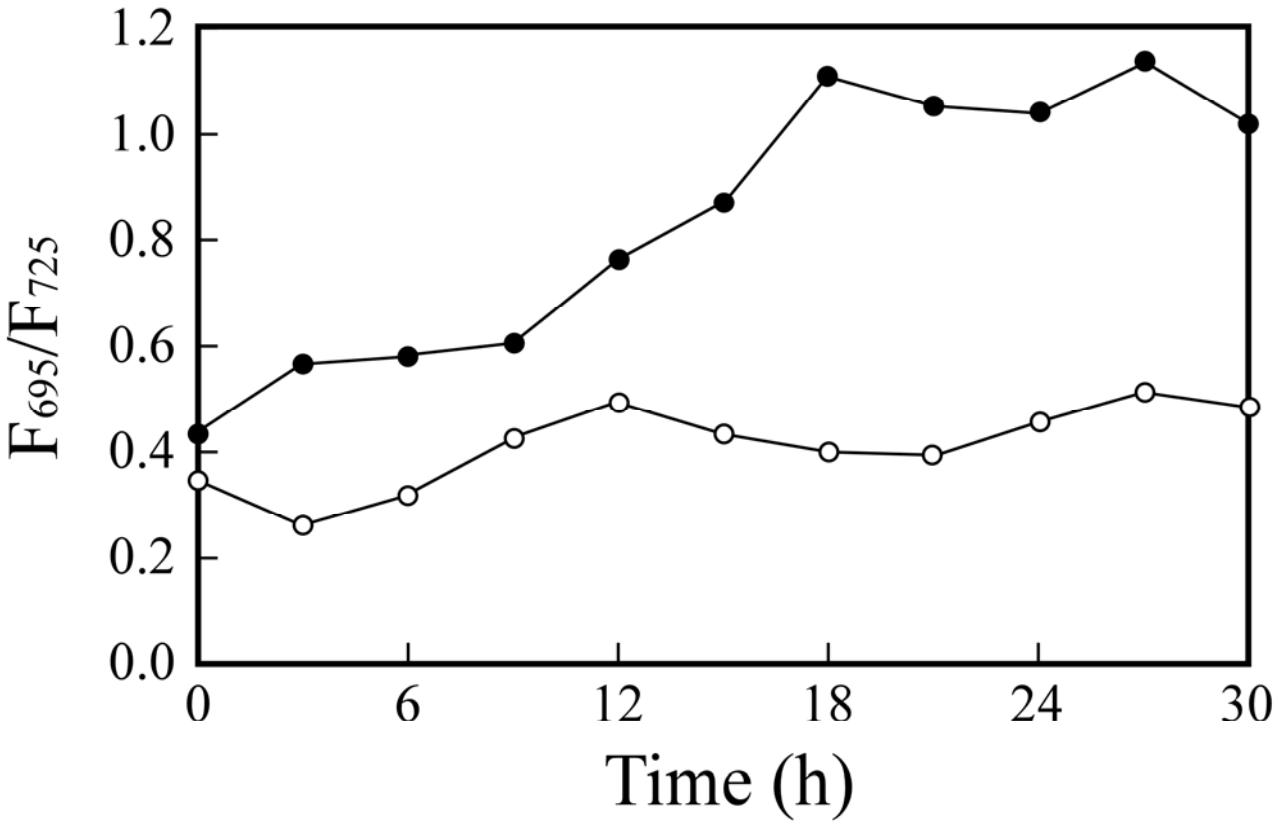
0.5
0
0
2
4
Time (min)

Fig. 2



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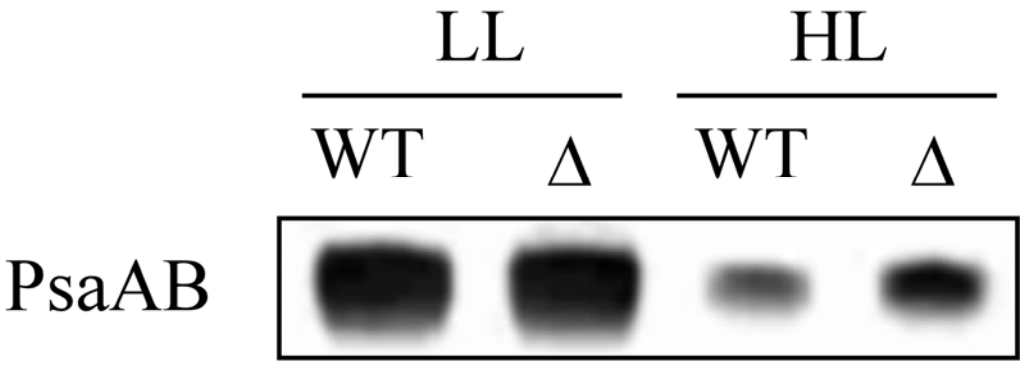

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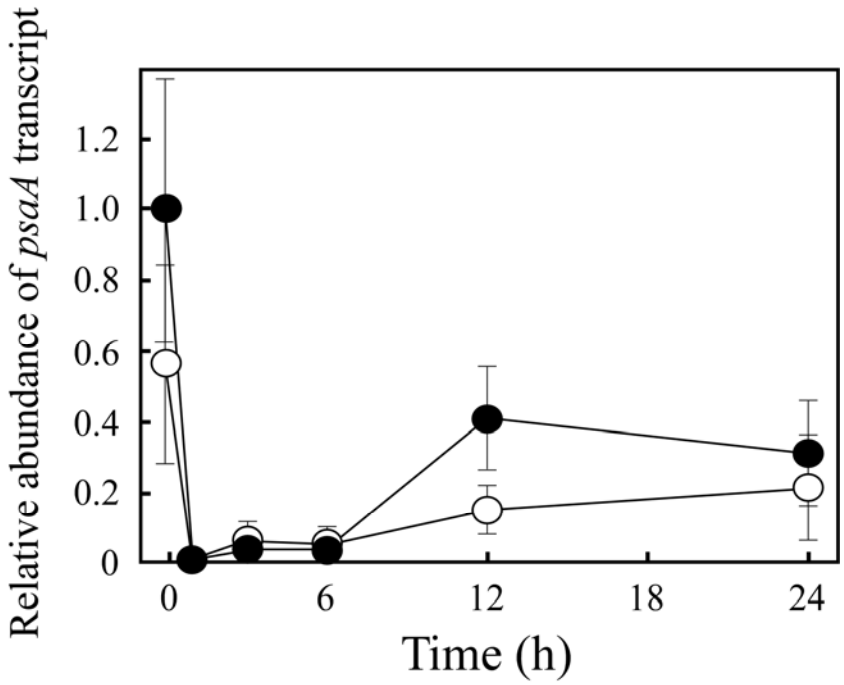
Relative abundance of psaA transcript
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0.6
0.4
0.2
0
0
6
12
18
24
Time (h)

Fig. 5

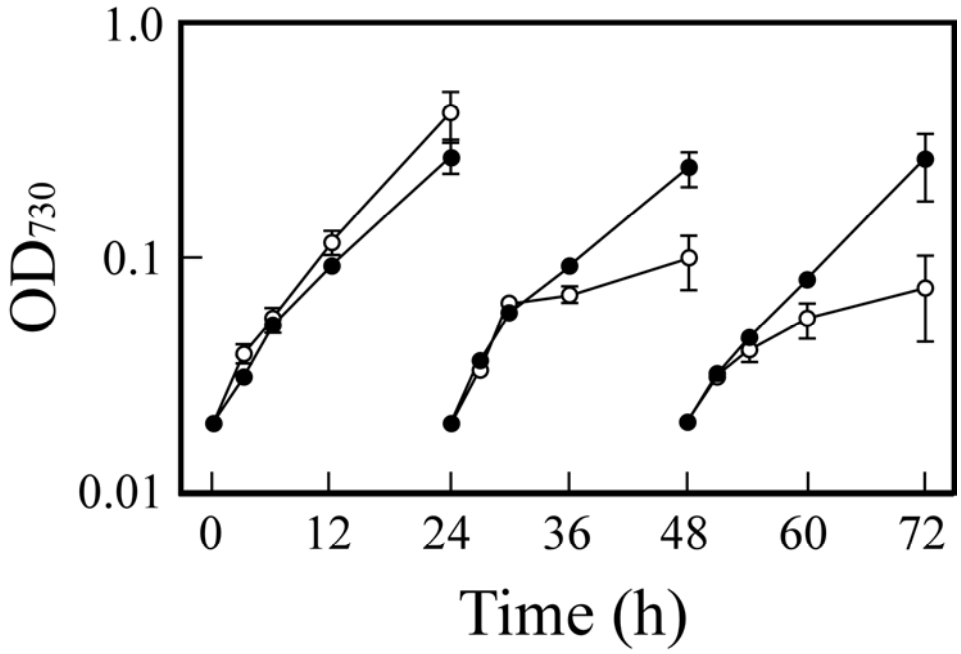

Fig. 6

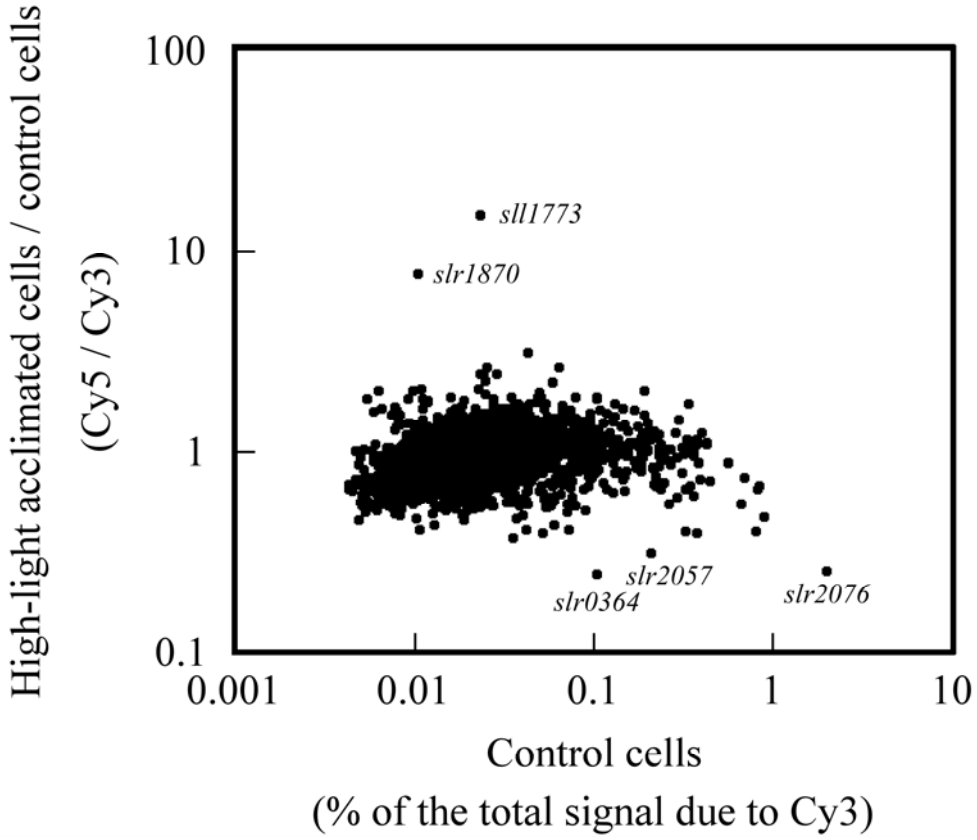
High-light acclimated cells / control cells
(Cy5 / Cy3)
100
10
1
0.1
sll1773
slr1870
slr2057
slr0364
slr2076
0.001
0.01
0.1
1
10
Control cells
(% of the total signal due to Cy3)

Fig. 7

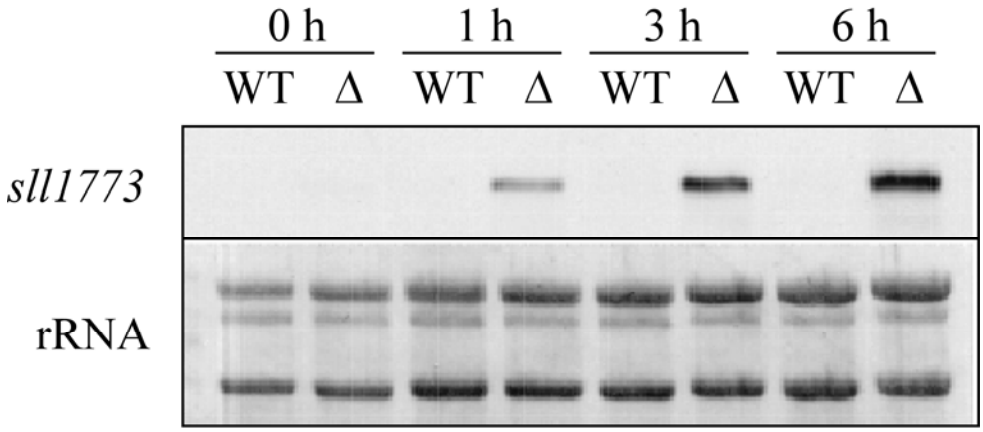

Fig. 8

FIGURE CAPTIONS AND LEGENDS

Fig. 1. Kinetics of fluorescence emitted from the wild type (black square), the *0205-79* mutant (white square) and the *pmgA* mutant (gray square) cells under low- (A) or high- (B) light conditions. Cells on agar plates were dark adapted for 15 minutes. Actinic light was applied for 3 s to monitor the fluorescence. Intensity of the fluorescence was normalized with initial value at the start of actinic light.

Fig. 2. Fluorescence determined by a pulse amplitude modulation fluorometer of the wild type (A, D), the *0205-79* mutant (B, E) and the *pmgA* mutant (C, F) under low- (A, B, C) or high- (D, E, F) light conditions. The fluorescence measurement was initiated by applying modulated measuring light after 5 minutes dark adaptation. Subsequently, a series of multi-turnover flashes (MT flashes) was applied to close PSII (reduce Q_A) temporally. Then, actinic light was turned on (AL on) to monitor the steady state level of fluorescence. Then, actinic light was turned off (AL off) and far-red light turned on (FR on) to monitor the minimal level of fluorescence with non-photochemical quenching. Finally, DCMU was added (+DCMU) and actinic light was turned on to obtain the maximum level of fluorescence (AL on). The rise and decay of fluorescence intensity upon the application and cessation of actinic light was magnified in inset.

Fig. 3. Time course of the changes in the ratio of F695/F725 (PSII/PSI) during acclimation to high light. This ratio was determined by measuring fluorescence emission spectra of the wild type (black square) and the *0205-79* mutant (white square) at 77 K. Data are the means of three samples.

Fig. 4. Western analysis of PsaAB in the wild type and the *0205-79* mutant under low- and high-light conditions. Proteins extracted from the same number of cells were loaded to each lane.

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

Fig. 6. Growth curve of the wild type (black square) and the *0205-79* mutant (white triangle) under high-light condition. 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.

Fig. 7. GntR type transcriptional regulator in cyanobacteria and *Bacillus*. The amino acid sequences of Sll1961 () in *Synechocystis* sp. PCC 6803 were compared with All1076 in *Nostoc* sp. PCC 7120 (GI:17228571), Tll2117 in *Thermosynechococcus elongatus* BP-1 (GI:22299660), Glr4252 in *Gloeobacter violaceus* PCC 7421 (GI:35214826), SYNW0091 in *Synechococcus* sp. WH 8102 (GI:33864627), gluconate operon transcriptional repressor in *Bacillus anthracis* A2012 (GI:21402132) and GntR type transcriptional regulator in *Bacillus subtilis* (GI:16077971). N-terminal region of Sll1961 contains gluconate operon transcriptional repressor domain. Black and gray boxes indicate strictly and relatively conserved amino acids, respectively. Open box shows helix-turn-helix region.

Fig. 8. DNA microarray analysis of gene expression induced by the mutation of s*ll1961* under high-light (3 h) condition. The wild type and the *sll1961* mutant were grown during 3 h after the shift to high light. RNA extracted from the wild type and the *sll1961* mutant was used for synthesis of Cy3-labeled cDNAs and Cy5-labeled cDNAs, respectively. The Cy5- and Cy3-labeled cDNAs were mixed and applied to DNA microarray.

Fig. 9. Northern analysis of the level of mRNA of *sll1773* in the wild type and the *sll1961* mutant during acclimation to high light.