

Transcriptional regulation of genes encoding subunits of photosystem I during acclimation to high-light conditions in *Synechocystis* sp. PCC 6803

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**Abstract** Cyanobacteria, algae and plants reduce photosystem contents and modulate photosystem stoichiometry upon acclimation to high-light (HL) conditions to avoid the damage due to excess light energy. In order to elucidate the molecular mechanism of HL acclimation, mRNA levels of genes encoding subunits of photosystems were examined upon the change of photon flux density in the wild-type strain of *Synechocystis* sp. PCC 6803. Transcript levels and promoter activities of photosystem I (PSI) genes rapidly decreased upon the shift to HL to less than 10% of the initial level within 1 h, whereas responses of photosystem II (PSII) transcript levels were not coordinated. The prompt change of promoter activities of PSI genes, but not PSII genes, seems important for *Synechocystis* cells to regulate their photosystem contents in response to changes in photon flux density. mRNA stabilities of PSI genes also decreased during HL incubation. The down-regulation of PSI transcripts under HL did not depend on *de novo* protein synthesis, contrasting with the requirement of newly synthesized protein factor(s) for the accumulation of PSI transcripts under low light. When gene expression profiles were compared between wild-type cells and a *pmgA*-disrupted mutant that cannot modulate photosystem stoichiometry under HL, a large increase in the *psaAB* and *psaA* transcript levels was observed in the mutant under prolonged exposure to HL for 6 h. Repression of the promoter activity of the *psaA* gene by a *pmgA*-dependent mechanism was shown to be essential for the adjustment of photosystem stoichiometry under HL conditions.

**Keywords** High light acclimation - Photosystem I - Photosystem stoichiometry – *pmgA* - *Synechocystis* sp. PCC 6803 - Transcriptional regulation

**Abbreviations** HL: high light - Km<sup>r</sup> : kanamycin resistance – LL: low light - Sp<sup>r</sup> : spectinomycin resistance -

## Introduction

In response to elevated photon flux density, photosynthetic organisms change their photosynthetic apparatus to maintain a balance between energy supply (light harvesting and electron transport) and consumption (CO<sub>2</sub> fixation), and to avoid the potential photodamage. This widely-observed and physiologically important process, termed acclimation to high light (HL), has been extensively investigated from the physiological point of view. In higher plants, green algae and cyanobacteria, decreases in the contents of the photosystems and in the PSI/PSII ratio are typically observed upon the acclimation to HL (Anderson 1986; Neale and Melis 1986; Murakami and Fujita 1991; Hihara 1999; Hihara and Sonoike 2001). In the cyanobacterium *Synechocystis* sp. PCC 6803, these two responses occurred sequentially after the shift to HL conditions. Within 3 h under HL conditions, contents of chlorophyll *a* and phycocyanin on a per-cell basis drastically decreased. Namely, amounts of PSI, PSII and phycobilisome concomitantly decreased upon the shift to HL to avoid the absorption of excess light energy. After 12 h, a decrease in the PSI/PSII ratio became obvious due to the selective reduction of PSI content (Hihara et al. 1998).

To elucidate the physiological significance of the reduction in the PSI/PSII ratio upon HL acclimation, Hihara et al. (1998) characterized a mutant of *Synechocystis* sp. PCC 6803 that had a defect in *pmgA* (*sl11968*) and was unable to modulate photosystem stoichiometry under HL conditions. The selective repression of PSI content under HL conditions was lost in the mutant, leading to a failure in decrease of the PSI/PSII ratio. The *pmgA* mutants grew better than the wild type strain during a short-term exposure (e.g. 24 h) to HL (Hihara et al. 1998). Under long-term exposure to HL, however, severe inhibition of growth probably due to the generation of reactive oxygen species occurred (Hihara and Ikeuchi 1998; Sonoike et al. 2001), indicating that the decrease in the rate of electron

transport caused by the reduction in the PSI/PSII ratio is indispensable for the growth under continuous HL conditions (Hihara and Ikeuchi 1998; Sonoike et al. 2001).

Although decreases in the contents of the photosystems and the PSI/PSII ratio during HL acclimation have been physiologically characterized, molecular mechanisms controlling these processes are still poorly understood. Recently, Hihara et al. (2001) investigated the change in mRNA levels during HL acclimation of *Synechocystis* sp. PCC 6803 by using whole genome DNA microarrays. The *psa* genes encoding subunits of PSI were coordinately and remarkably down-regulated upon the shift to HL, whereas the *psb* genes coding for subunits of PSII were not. This observation indicates that transcriptional control of PSI genes may be important for HL acclimation.

In this study, mRNA levels, promoter activities and mRNA stabilities of PSI genes during HL acclimation were examined. We report here that transcriptional control of PSI genes takes an important part in acclimation responses, such as the decrease in photosystem content and the adjustment of photosystem stoichiometry. Furthermore, mRNA accumulation was compared between the wild type and the *pmgA*-disrupted mutant. The *pmgA* gene product was revealed to be important for the repression of promoter activity of the *psaA* gene encoding the reaction center subunit of PSI, and hence for the regulation of photosystem stoichiometry.

## **Materials and Methods**

### **Strains and culture conditions**

A glucose-tolerant wild-type strain of *Synechocystis* sp. PCC 6803 and the *pmgA*-disrupted mutant made by inserting the spectinomycin resistance cassette (*pmgA*::Sp<sup>r</sup>) (Hihara and Ikeuchi 1997) were grown at 32 °C in BG-11 medium (Stanier et al. 1971) with

20 mM HEPES-NaOH, pH 7.0, under continuous illumination provided by fluorescent lamps. Cells were grown in volumes of 50 ml in test tubes (3 cm in diameter) and bubbled by air. Cell density was estimated at  $A_{730}$  using a spectrophotometer (model UV-160A; Shimadzu, Kyoto, Japan). HL shift experiments were performed by transferring cells at the exponential growth phase ( $A_{730} = 0.1$  to  $0.2$ ) from LL ( $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) to HL conditions ( $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). When needed, an inhibitor of transcription, rifampicin (final  $150 \mu\text{g ml}^{-1}$ ), or an inhibitor of translation, chloramphenicol (final  $100 \mu\text{g ml}^{-1}$ ), were added to the culture.

### **Chimeric constructs of luciferase reporter genes with the promoter regions of *psaA* and *psaD***

The plasmid vector, pPT6803-1, having the promoterless *luxAB* gene, the neutral site of *Synechocystis* sp. PCC 6803 (the downstream region of *ndhB*) and the spectinomycin resistance ( $\text{Sp}^r$ ) cassette (Aoki et al. 2002) was a kind gift from Dr. S. Aoki and Prof. M. Ishiura in Nagoya University. A 415 bp fragment of the *psaA* promoter region from just downstream of the termination codon of *sll1730* to just upstream of the start codon of *psaA* was amplified by PCR with primers containing *Bsi*WI site at their 5'-termini. The PCR product was digested with *Bsi*WI and cloned into *Bsi*WI site of pPT6803-1 to obtain the reporter construct, pP*psaA-luxSp*<sup>r</sup>. A 195 bp fragment of the *psaD* promoter region from just downstream of the termination codon of *sll0698* to just upstream of the start codon of *psaD* was amplified by PCR with primers containing *Bsi*WI site at their 5'-termini. The PCR product was cloned into pPT6803-1 in the same way as in the case of the *psaAB* promoter and this reporter construct was named pP*psaD-luxSp*<sup>r</sup>. The nucleotide sequence and direction of the promoter regions in the reporter constructs were confirmed by sequencing.

The plasmid vectors having the promoterless *luxAB* gene and the kanamycin

resistance (Km<sup>r</sup>) cassette were constructed as follows. pPT6803-1 and pPpsaA-luxSp<sup>r</sup> were digested with *Bgl*III and *Mfe*I to remove the Sp<sup>r</sup> cassette. After the both restriction site of the vectors had been blunt ended with klenow enzyme, *Hinc*II fragment of the Tn5-derived Km<sup>r</sup> gene was ligated to the vectors. The direction of the Km<sup>r</sup> cassette was confirmed by digestion with *Bgl*III and *Hind*III. The resultant vectors were named pPT6803-1Km<sup>r</sup> and pPpsaA-luxKm<sup>r</sup>, respectively.

The wild type strain was transformed with both constructs of Sp<sup>r</sup> and Km<sup>r</sup>, whereas *pmgA::Sp<sup>r</sup>* was transformed with Km<sup>r</sup> constructs. The chromosome of the resultant strains contained the cassette that consisted of Sp<sup>r</sup> or Km<sup>r</sup> gene, *psaA* or *psaD* promoter and *luxAB* gene. It was confirmed that levels of luminescence derived from PpsaA-luxSp<sup>r</sup> and PpsaA-luxKm<sup>r</sup> were identical.

### DNA probes and primers

The following primers were used for amplification of PSI and PSII genes by PCR from the genomic DNA of *Synechocystis* sp. PCC 6803: *psaA* forward (5'-TTCCCCTTCCCCACGAGT-3') and reverse (5'-ACCGGCCCTACGATGAG-3'), *psaD* forward (5'-ATGACAGAACTCTCTGGA-3') and reverse (5'-TTTACCGGAGAACTTGAT-3'), *psaF* forward (5'-ATGAAACATTTGTTGGCG-3') and reverse (5'-GATTTCTGAATCCTTCAT-3'), *psbA2* forward (5'-AGTTCCAATCTGAACATC-3') and reverse (5'-GGGTAGATCAAGAATACG-3'), *psbB* forward (5'-TGTTTCGTACCGGTGCTA-3') and reverse (5'-GTCACCAACCTTAGCAAA-3'), and *psbEFLJ* forward (5'-TATTGTCACCAGCATTTCG-3') and reverse (5'-CCTAAACCAGCATAGGCTC-3'). To use PCR products directly as templates for *in vitro* transcription reaction, T7 polymerase recognition site (TAATACGACTCACTATAGGGCGA) was added to the reverse primer for

*psaA* at its 5'-terminus. Similarly, SP6 polymerase recognition site (ATTTAGGTGACACTATAGAATAC) was added to the reverse primers for *psaD*, *psaF* and *psbEFLJ*. In the cases of *psbA2* and *psbB*, PCR products were cloned into pT7Blue T-Vector (Novagen, Madison, WI) and resultant plasmids were used as templates for *in vitro* transcription reaction. *in vitro* transcription reaction was performed with a DIG RNA labeling kit (Roche, Basel, Switzerland) according to manufacturer's instructions.

### **RNA isolation and Northern-blot analysis**

Cells were collected by brief centrifugation at 4°C and stored in liquid N<sub>2</sub>. The frozen cells were thawed with 250 µl of resuspension buffer (0.3 M sucrose, 10 mM sodium acetate and 20 mM EDTA) at 65°C and immediately treated with 350 µl of lysis buffer (2% SDS (w/v) and 10 mM sodium acetate) and 100 µl of phenol at 65°C for 3 min. Cells were further treated with 500 µl of phenol at 65°C for 3 min and placed on ice. After centrifugation, the supernatant was extracted twice with phenol, once with phenol/chloroform and then once with chloroform. After precipitation with ethanol, RNA was dissolved in TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). Total RNA (2 or 3 µg) was fractionated by electrophoresis on 1.2% (w/v) agarose gel with 0.6 M formaldehyde, blotted onto a nylon membrane (Hybond N+; Amersham Biosciences, Uppsala, Sweden) and fixed by baking at 80°C for 2 h. The equal loading of RNA in all the lanes was checked by methylene blue staining. Hybridization and detection were performed according to the standard protocol for DIG hybridization (Roche). Relative transcript levels were quantified by using Scion Image software and normalized by levels of 23S rRNA.

### **Measurement of luminescence from cells harboring luciferase reporter genes**

The activity of luciferase in the liquid culture of cyanobacterial strains harboring

*Psa-luxAB* reporter genes was measured at 25°C with an ATP photometer (MONOLIGHT401; Analytical Luminescence Laboratory, San Diego, CA). 200 µl of liquid culture was transferred to a test tube (6 mm in diameter) and immediately set in the photometer. 100 µl of 0.15% (v/v) *n*-decanal was injected into the test tube with a syringe. The total luminescence from 0.5 s to 120 s after the injection of *n*-decanal was calculated.

## Results

### Regulation of accumulation of PSI transcripts upon the change of photon flux density

We first investigated mRNA levels of genes encoding the subunits of PSI and PSII during 3 h after the change of photon flux density where the initial changes in pigment contents and photosystem contents were observed. Low light (LL) grown cells of the wild-type strain of *Synechocystis* sp. PCC 6803 were shifted to HL then to LL, and the levels of mRNA were examined by Northern-blot analysis (Fig. 1). All of PSI genes examined, namely, *psaAB* and *psaA* encoding reaction center subunits, *psaD* coding for a ferredoxin docking stromal subunit and *psaF* encoding a plastocyanin docking subunit, were rapidly down-regulated upon the shift to HL and their transcripts were barely detectable after 1 h of HL exposure. After 3 h, *psaAB* and *psaA* transcripts were still undetectable, while those of *psaD* and *psaF* started to accumulate. At this point, cells were returned to LL and PSI transcripts again accumulated to the LL level. In contrast with PSI genes, PSII genes, namely, *psbA* encoding the reaction center D1 protein, *psbB* coding for core-antenna CP47 protein and *psbEFLJ* operon encoding cytochrome *b*<sub>559</sub> and other small subunits, were not coordinately regulated. These observations are consistent with the results of DNA microarray experiments (Hihara et al. 2001). It is obvious that the main component to be regulated in

response to the change of photon flux density is not the PSII genes but the PSI genes.

Next we studied transcription of PSI genes by monitoring the activities of their promoters that had been fused to the structural gene for luciferase (*luxAB*) of *Vibrio harveyi* (see Methods). Strains harboring the chimeric gene, either *PpsaA-luxSp<sup>f</sup>* or *PpsaD-luxSp<sup>f</sup>* were cultured under the same light conditions as the experiment shown in Fig. 1. The activities of *psaA* and *psaD* promoters decreased tenfold during HL exposure for 3 h and gradually restored upon the shift to LL (Fig. 2), which agrees with the changes in transcript levels shown in Fig. 1.

The level of accumulation of transcripts is determined by their stabilities as well as their transcriptional activities. To test the mRNA stabilities, we examined the kinetics of disappearance of *psaAB*, *psaA* and *psaD* transcripts after addition of an inhibitor of transcription, rifampicin, under different light conditions (Fig. 3). The half lives of the *psaAB* transcript were 10 min, 7.5 min and 5 min in LL, just after shift to HL (HL0) and after 6 h of incubation in HL (HL6), respectively. Similarly, the half lives of the *psaA* transcript were 20 min, 20 min and 8 min in LL, HL0 and HL6, respectively. In the case of the *psaD* transcript, the half lives immediately decreased from 12 min to 7 min upon the shift to HL, and further decreased to 4 min after 6 h. In addition to the repression of the promoter activity, decrease in mRNA stability may contribute to down-regulation of PSI transcripts under HL conditions to some extent.

Fig. 4A shows the effects of a translation inhibitor, chloramphenicol, on the change of PSI transcript levels upon the shift of the light regime. Chloramphenicol did not affect the repression of *psaAB*, *psaA* and *psaD* mRNA levels under HL conditions. On the other hand, it strongly inhibited the accumulation of PSI transcripts under LL conditions (Fig. 4B). The *psbA* transcript could accumulate in the presence of chloramphenicol under both HL and LL conditions (not shown), indicating that chloramphenicol did not inhibit transcription *per se*.

These results indicate that *de novo* synthesis of regulatory element(s) is required for transcriptional activation of PSI genes in LL but not for their repression in HL.

### **Regulation of accumulation of PSI transcripts under prolonged HL conditions**

In order to clarify the mechanism for regulation of photosystem stoichiometry upon the acclimation to prolonged (more than 6 h) HL conditions, we examined the difference in accumulation of PSI and PSII transcripts between wild type cells and a *pmgA*-disrupted mutant (Hihara et al. 1998) that cannot decrease the PSI/PSII ratio under HL conditions (Fig. 5). In the wild type, changes in transcript levels of PSI genes were well-coordinated, though accumulation of the *psaD* and *psaF* transcripts resumed earlier than that of the *psaA* transcript under HL conditions. The change in the transcript level of *psaAB* was almost the same as that of *psaA* (not shown). Each PSII gene showed its own response: changes in the transcript level of *psbA* and *psbB* were not so clear, while *psbEFLJ* was transiently down-regulated. In our previous study, *psbA* transcripts were transiently accumulated within 15 min after the shift to HL (Hihara et al. 2001). The induction of *psbA* genes could not be detected in the experiment shown in Fig. 5, since the earliest time point after the shift to HL was 1 h. When the levels of transcripts in the *pmgA* mutant were compared with those in the wild type, the only one remarkable difference, large increase of the *psaA* transcript level in the mutant, was observed 6 h after the shift to HL. The *psaAB* transcript also accumulated together with the *psaA* transcript in the mutant, whereas the amount of the *psaB* transcript was not affected by the presence or absence of *pmgA* (not shown).

The above observation indicates that *pmgA* specifically repressed the accumulation of *psaAB* and *psaA* transcripts after 6 h of exposure to HL. In order to clarify the mechanism of this regulation, mRNA stability and promoter activity of the *psaA* gene under prolonged HL conditions were compared between the wild type and the mutant. Fig. 6 shows the

degradation profile of the *psaAB* and *psaA* transcripts upon the addition of rifampicin to cultures incubated in HL for 6 h. Though transcripts were somewhat stabilized just after the addition of rifampicin in this experiment, no difference was observed in the profile of mRNA degradation between the two strains. Next, *PpsaA-luxKm<sup>f</sup>* chimeric gene was introduced into both the wild type and *pmgA*-mutant cells and the change in the promoter activity of the *psaA* gene was monitored during the prolonged incubation under HL conditions (Fig. 7). At first, the decrease in luciferase activity of the mutant cells was same as that of the wild-type cells. However, after 6 h of HL, the luminescence level of the mutant cells began to increase. The luciferase activities 12 h after the shift to HL, expressed as a percentage of LL level, were  $4.3 \pm 1.5\%$  and  $17.9 \pm 9.8\%$  in the wild type and the mutant, respectively. This indicates that the promoter activity of *psaA* is four times higher in the *pmgA* mutant than in the wild type under the prolonged HL conditions. The *luxAB* transcript level was also higher in the mutant harboring the *PpsaA-luxKm<sup>f</sup>* gene than in the wild-type cells (not shown).

## **Discussion**

### **Importance of transcriptional control of PSI genes for reduction of photosystem contents**

Under light-saturating conditions, cyanobacteria must decrease their antennae size and photosystem contents in order to avoid absorption of excess light energy. Within 3 h after the shift to HL, chlorophyll *a* and phycocyanin content per cell declined rapidly, which was a reflection of the decrease in phycobilisome and photosystem contents (Hihara et al. 1998). In this study, it was shown that the reduction of PSI content was due to decreased transcription of genes encoding PSI subunits but this did not occur for PSII genes. PSI transcript levels were coordinately down-regulated by about 20 fold within 1 h of HL exposure, whereas

responses of PSII transcript levels to HL were not coordinated (Figs. 1, 5). Probably, the content of PSII can be promptly decreased under HL conditions due to accelerated turnover rate of its reaction center subunits.

Promoter activities of PSI genes largely decreased under HL conditions (Fig. 2). Reduction profiles of PSI transcripts in the absence (Fig. 1) and presence (Fig. 3, circles) of the transcription inhibitor were similar, also indicating that promoter activities of PSI genes declined to almost zero level just after the shift to HL. This repression did not require newly synthesized proteins (Fig. 4A). On the other hand, when cells that had incubated under HL conditions were returned to LL, *de novo* protein synthesis was required for the increase in the promoter activities and the transcript levels of PSI genes (Figs. 1, 2, 4B). The protein factor required for up-regulation of PSI genes seemed to have a fairly short half life since the ability to accumulate PSI transcripts in LL was completely lost during 60 min of HL exposure (Fig. 4B). The prompt change of promoter activities of PSI genes seems important for *Synechocystis* cells to regulate their photosystem content in response to changes in photon flux density.

### **Importance of transcriptional control of PSI genes for adjustment of photosystem stoichiometry**

The transcript level and promoter activity of the *psaA* gene were initially the same between the wild type and the *pmgA* mutant, and the difference between two strains was only observed 6 h after the shift to HL (Figs. 5, 7). Similarly, the initial decline of pigment contents upon the shift to HL was identical between the wild type and the mutant, whereas the subsequent decrease of the PSI/PSII ratio was not observed in the mutant (Hihara et al. 1998). These observations indicate that *pmgA* is not required for the initial decrease of photosystem content but specifically involved in the modulation of photosystem

stoichiometry. In the *pmgA* mutant, the high activity of the *psaA* promoter after 6 h (Fig. 7) led to overproduction of *psaAB* and *psaA* transcripts (Fig. 5), which may explain the inability of the mutant to lower the PSI/PSII ratio upon HL acclimation. Among transcripts for PSI reaction center subunits, namely, *psaAB*, *psaA* and *psaB*, only the level of the *psaB* transcript remained unaffected in the *pmgA* mutant. Probably the *psaB* specific promoter is located downstream of the *psaA* gene and its activity is independent of a *pmgA*-dependent mechanism.

The promoter activity of the *psaA* gene must be the key for the regulation of the PSI/PSII ratio through a *pmgA*-dependent mechanism. This is in contrast with the case of regulation of photosystem stoichiometry in response to the change in light qualities. The PSI/PSII ratio increases under PSII-excited conditions and decreases under PSI-excited conditions in order to improve the quantum efficiency of photosynthesis under given light quality conditions (Melis 1991; Fujita 1997). It was reported that the level of the *psaAB* transcript was not affected by the change in light quality (Aizawa and Fujita 1997). In this case, the regulatory step is assumed to be at the translation of PsaA/B or at the insertion of chlorophyll *a* into PsaA/B polypeptide (Fujita 1997). Mechanisms for adjustment of photosystem stoichiometry must be different between acclimation to HL and that to different light qualities.

Under prolonged HL conditions, there might be another regulatory mechanism for accumulation of PSI transcripts in addition to the above mentioned *pmgA*-dependent mechanism. The transcript level of *psaD* clearly increased after 3 h of HL exposure (Figs. 1, 5), although the promoter activity was still at a low level (Fig. 2) and mRNA stability was decreased under HL conditions (Fig. 3). Similarly, the accumulation rate of the *psaA* transcript after 6 h (Fig. 5) seems faster than that expected from the promoter activity (Fig. 7) and mRNA stability (Fig. 6) both in the wild type and the mutant. These observations indicate

that a certain regulatory factor that does not locate on the promoter region affects accumulation of PSI transcripts under prolonged HL conditions. Coding regions may be one of the candidates for the regulatory element.

### **Function of PmgA deduced from its amino acid sequence**

Though the exact role of *pmgA* is still unknown, its deduced amino acid sequence implies that it may work as a kinase. N, G1, and G2 boxes required for nucleotide binding exist in proper spacing in its amino acid sequence (Hihara and Ikeuchi 1997). It is well known that both histidine kinases of the two-component system and anti-sigma factors such as RsbW and SpoIIAB from *Bacillus subtilis* have these motifs (Min et al. 1993). PmgA may belong to the family of anti-sigma factor since it lacks the conserved catalytic histidine residue of the two-component histidine kinases and its length is similar to those of anti-sigma factors. In *Bacillus subtilis*, RsbW and SpoIIAB negatively regulate the  $\sigma^B$  and  $\sigma^F$  transcription factors, respectively (Hughes and Mathee 1998). In this regulatory strategy, activity of an anti-sigma factor is controlled by direct protein-protein interaction with a specific antagonist protein, an anti-anti-sigma factor that maintains the anti-sigma factor in an inactive complex. In such a state,  $\sigma^B$ -dependent transcription of stress-response genes or  $\sigma^F$ -dependent transcription of sporulation specific genes occurs. In normal conditions, the anti-anti-sigma factor is in the phosphorylated state and cannot form a complex with the anti-sigma factor. The anti-sigma factor can directly interact with the target sigma factor to inhibit its activity. PmgA may repress the transcription of *psaAB* and *psaA* in the anti-sigma factor like manner under prolonged HL conditions. If so, there must exist protein factors that show the specific interaction with PmgA. The screening of factors that interact with PmgA is now in progress.

## **Significance of transcriptional control of PSI genes to acclimate to various environmental changes**

In this study, we showed that transcriptional control of PSI genes greatly contributes to the acclimation responses to different light intensities. It seems that transcriptional regulation of PSI genes is also important for acclimation process to other environmental changes. Recently, DNA microarray analyses on conditions of low temperature (Suzuki et al. 2001), high salinity, high osmolality (Kanesaki et al. 2002) and low CO<sub>2</sub> (Prof. T Omata in Nagoya University, personal communication) have been performed. Interestingly, down-regulation of PSI transcripts was observed under all these conditions. In cyanobacteria, transcriptional control of PSI genes must be generally required for the acclimation to various environmental conditions. To date, transcriptional control of PSII genes, especially of the *psbA* genes, has been extensively investigated (Mohamed and Jansson 1989, 1991; Bustos and Golden 1992; Tsinoiremas et al. 1994; Campbell et al. 1995; Anandan and Golden 1997; Máté et al. 1998; Tyystjärvi et al. 1998; Sippola and Aro 1999; Alfonso et al. 1999, 2000; Li and Sherman 2000; Bissati and Kirilovsky 2001; Herranen et al. 2001), since PSII was supposed to be more sensitive than PSI to almost all kinds of stresses, in particular to light stress. In contrast, there are only a few reports on the regulation of PSI genes (Smart and McIntosh 1991). However, actually, transcriptional regulation of PSI genes is important just like that of PSII genes, as shown in this study. At present, the physiological significance of the regulation of PSI has been gradually clarified (Hihara and Sonoike 2001). The signal transduction pathway from perception of environmental changes to accumulation of PSI complexes may be the main subject of the research at the next stage.

**Acknowledgements**      We thank Dr. Setsuyuki Aoki and Prof. Masahiro Ishiura (Nagoya

University) for pPT6803-1 vector, and members in Prof. Teruo Ogawa's laboratory (Nagoya University) for useful advice in measurements of luciferase activity. We also thank Prof. Junichi Ohnishi (Saitama University) for the ATP photometer and Prof. Kintake Sonoike (University of Tokyo) for critical reading of the manuscript.

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## Figure legends

**Fig. 1.** Changes in levels of transcripts coding for subunits of photosystems upon changes in photon flux density. Wild type cells of *Synechocystis* sp. PCC 6803 were grown under LL conditions (time 0) and then incubated under HL conditions for the indicated times. After incubation in HL for 180 min, cells were returned to LL conditions and incubated further for 180 min. The amounts of PSI and PSII transcripts were determined by Northern-blot analysis (3  $\mu\text{g}$  total RNA per lane).

**Fig. 2.** Changes in luminescence emitted by the bacterial luciferase under the control of the promoters of *psaA* and *psaD* genes upon the changes in photon flux density. Wild type cells harboring reporter genes (*PpsaA-luxSp<sup>r</sup>*, *PpsaD-luxSp<sup>r</sup>*) were incubated under the same light conditions as described in Fig. 1. Levels in luminescence measured with an ATP photometer were shown as a percentage of the initial level after subtraction of the background luminescence produced by the strain harboring promoter-less *luxAB* gene.

**Fig. 3.** Degradation of PSI transcripts under different light conditions in the presence of rifampicin. An inhibitor of transcription initiation, rifampicin, at a final concentration of 150  $\mu\text{g ml}^{-1}$  was added at time 0 to cultures grown under LL conditions (LL), just after transferred to HL conditions (HL0) and grown under HL conditions for 6 h (HL6). Total RNA was isolated from each aliquot of cells and subjected to Northern-blot analysis. The data represent the mean of at least three independent experiments.

**Fig. 4.** Accumulation of PSI transcripts upon changes in photon flux density in the presence

or absence of chloramphenicol. A translation inhibitor, chloramphenicol, at a final concentration of  $100 \mu\text{g ml}^{-1}$  was added to cultures 5 min before the change of light regime.

(A) LL-grown cells were incubated under HL conditions for 15 or 60 min with or without chloramphenicol. (B) Cells grown under HL conditions for 60 min were incubated under LL conditions for 15 or 60 min with or without chloramphenicol. The levels of PSI transcripts were determined by Northern-blot analysis ( $2 \mu\text{g}$  total RNA per lane).

**Fig. 5.** Changes in levels of transcripts coding for subunits of photosystems during acclimation to HL conditions. Wild type and *pmgA*-disrupted cells grown under LL conditions were transferred to HL conditions and incubated for the indicated times. The amounts of PSI and PSII transcripts determined by Northern-blot analysis were quantified and normalized by the amount of 23S rRNA. The values, in relative units to the initial (LL) levels, represent the mean and SD of at least three independent experiments.

**Fig. 6.** Degradation of *psaAB* and *psaA* transcripts of wild type and *pmgA*-disrupted cells grown under HL conditions for 6 h. An inhibitor of transcription initiation, rifampicin, at a final concentration of  $150 \mu\text{g ml}^{-1}$  was added to cultures at time 0. Total RNA was isolated from each aliquot of cells and subjected to Northern-blot analysis ( $2 \mu\text{g}$  total RNA per lane).

**Fig. 7.** Changes in luminescence emitted by the bacterial luciferase under the control of the promoter of *psaA* gene during acclimation to HL conditions. Wild type and *pmgA*-disrupted cells harboring the reporter gene (*PpsaA-luxKm<sup>f</sup>*) were grown under LL conditions (time 0) and then incubated under HL conditions for the indicated times. Levels in luminescence measured with an ATP photometer were shown as a percentage of the initial level after subtraction of the background luminescence produced by the strain harboring promoter-less

*luxAB* gene. The data represent the mean of three independent experiments.

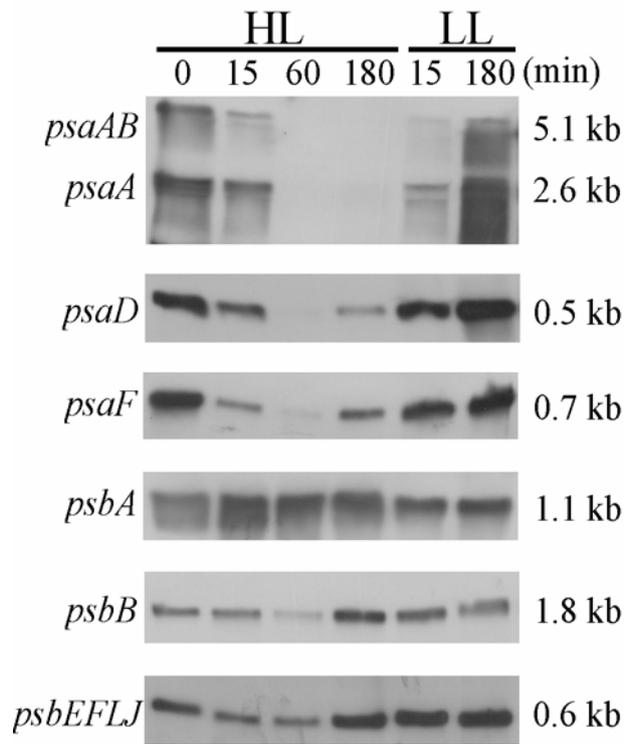


Fig. 1

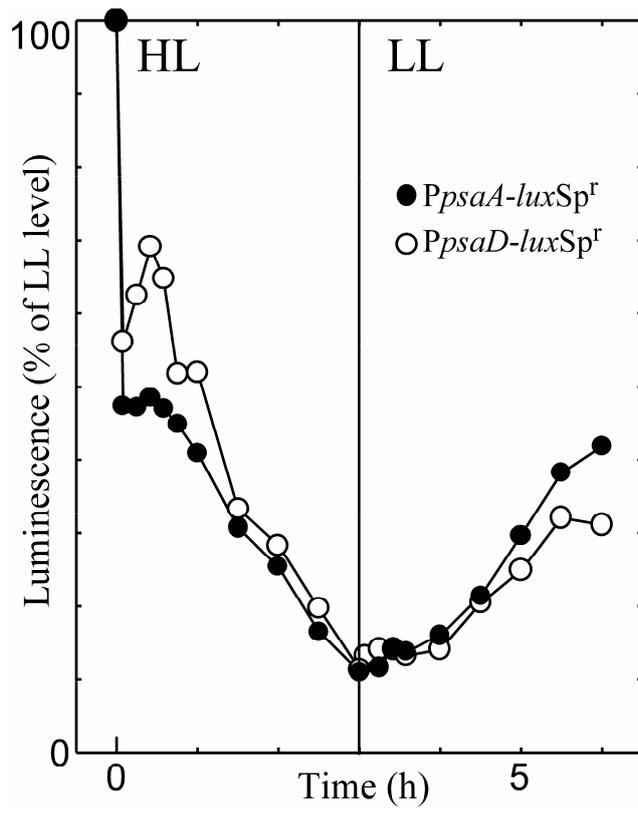


Fig. 2

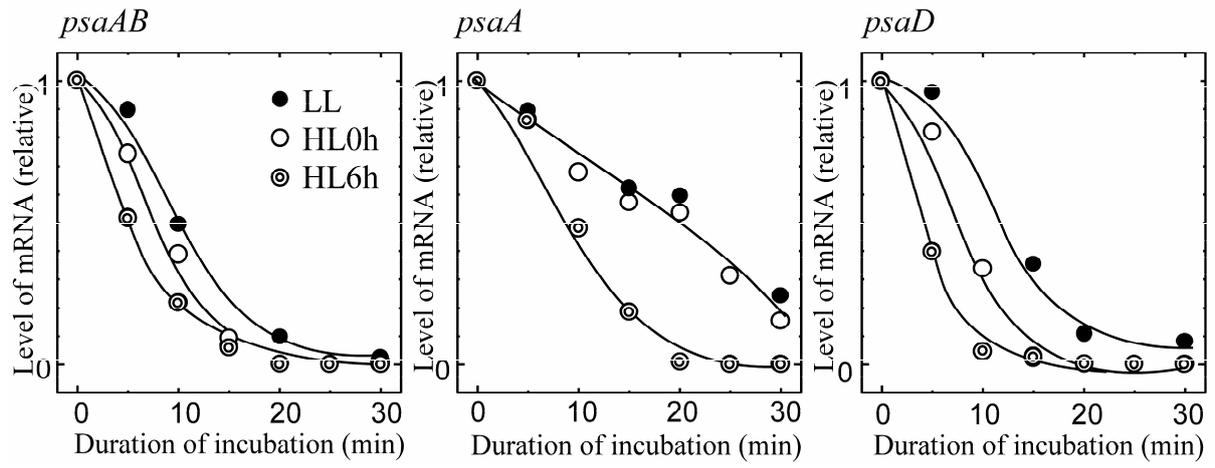


Fig. 3

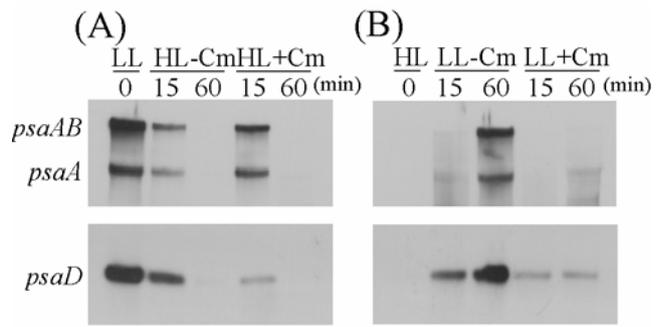


Fig. 4

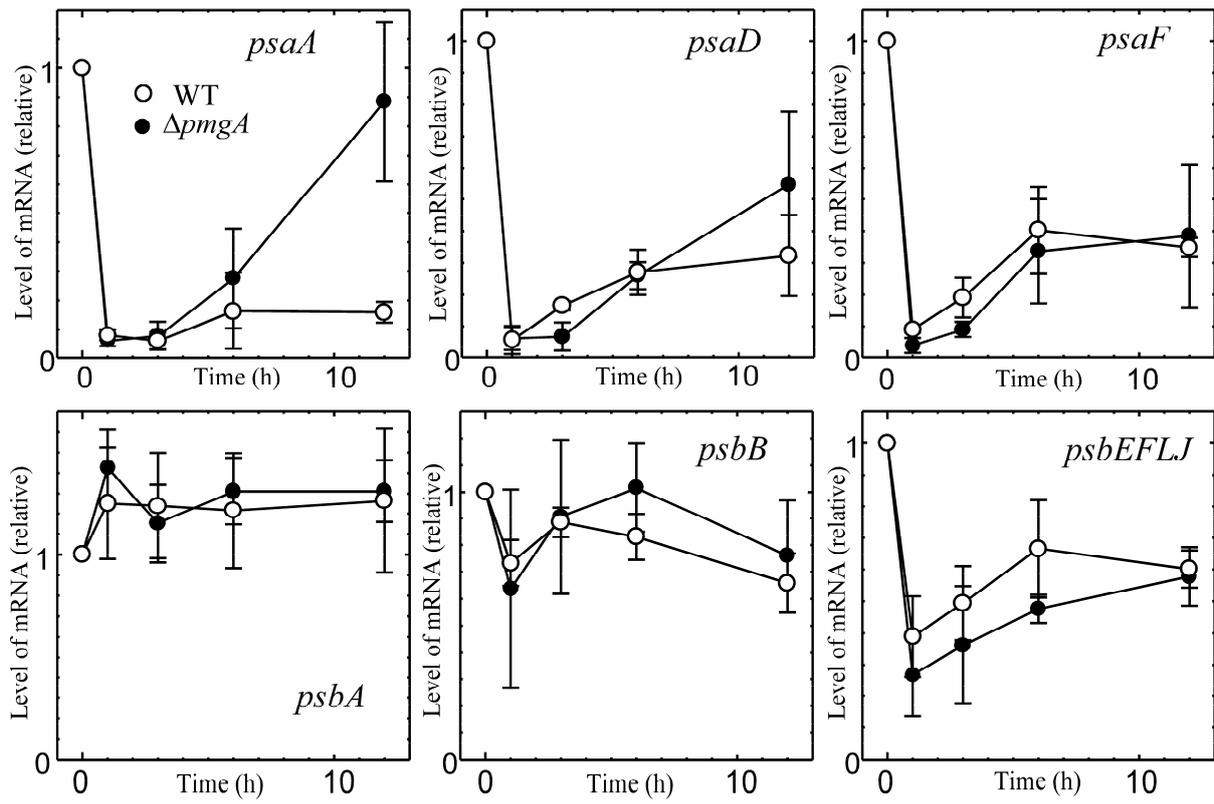


Fig. 5



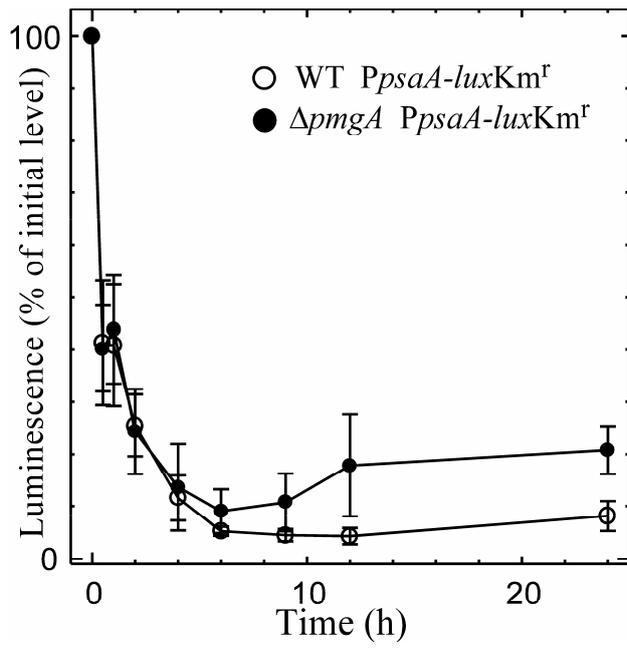


Fig. 7