The mechanism of down-regulation of photosystem I content
under high-light conditions in the cyanobacterium Synechocystis
sp. PCC 6803.

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Abbreviations: HL, high light; LL, low light; PS, photosystem; LA, levulinic acid; ALA, 5-aminolevulinic acid; WT, wild type.
Summary

Down-regulation of photosystem I (PSI) content is an essential process for cyanobacteria to grow under high-light (HL) conditions. In a pmgA (sll1968) mutant of *Synechocystis* sp. PCC 6803, all the levels of PSI content, chlorophyll and transcripts of the *psaAB* genes encoding reaction center subunits of PSI could not be maintained low during HL incubation, although causal relationship among these phenotypes remains unknown. In this study, we modulated the activity of *psaAB* transcription or that of chlorophyll synthesis to estimate their contribution to the regulation of PSI content under HL conditions. Analysis of the *psaAB*-OX strain, in which the *psaAB* genes were overexpressed under HL conditions, revealed that the amount of *psaAB* transcript could not affect PSI content by itself. Suppression of chlorophyll synthesis by an inhibitor, levulinic acid, in pmgA mutant revealed that chlorophyll availability could be a determinant of PSI content under HL. It was also suggested that chlorophyll content under HL conditions is mainly regulated at the level of 5-aminolevulinic acid synthesis. We conclude that, upon the shift to HL conditions, activities of *psaAB* transcription and of 5-aminolevulinic acid synthesis are strictly down-regulated by regulatory mechanism(s) independent of PmgA during the first 6 h, and then a PmgA-mediated regulatory mechanism becomes active after 6 h onward of HL incubation to maintain these activities at low level.
Introduction

While light is essential for growth of photosynthetic organisms, excess light energy leads to the production of reactive oxygen species and to eventual inactivation of photosynthesis. To avoid such damages, photosynthetic organisms must acclimate to high-light (HL) conditions by altering their photosynthetic apparatus. For example, they decrease the amount of antenna pigments (Anderson, 1986; Melis, 1991; Anderson et al., 1995; Walters, 2005), carry out state transition (Fujimori et al., 2005a), increase the capacity of CO₂ fixation (Bjorkman, 1981; Anderson, 1986) and activate the scavenging system for reactive oxygen species (Grace & Logan, 1996; Niyogi, 1999). Regulation of the amount of photosystems is another way to acclimate to HL (Anderson, 1986; Neal & Melis, 1986; Murakami & Fujita, 1991; Hihara & Sonoike, 2001). In cyanobacteria, the decrease of photosystem I (PSI) content is more prominent than that of photosystem II (PSII), leading to the decrease of photosystem stoichiometry (PSI/PSII ratio) under HL conditions (Murakami & Fujita, 1991; Hihara & Sonoike, 2001). Several components involved in the regulation of photosystem stoichiometry were reported so far (Hihara et al., 1998; Fujimori et al., 2005b; Ozaki et al., 2007). The physiological significance of this regulation has been shown by the characterization of a pmgA (sll1968) mutant of the cyanobacterium Synechocystis sp. PCC 6803. This mutant has defect in keeping PSI content at low level under HL conditions, while its PSII content is regulated
normally as in wild-type (WT) cells (Hihara et al., 1998). The large amount of PSI complex in the pmgA mutant causes higher electron transport activity, leading to an enhanced rate of photosynthesis. Although a higher rate of photosynthesis in the pmgA mutant contributes to a growth advantage over the WT during a short-term exposure (ca. 24 h) to HL, disadvantage appears under prolonged HL conditions. The growth of the pmgA mutant is severely inhibited after 48 h of HL exposure, probably because of the generation of reactive oxygen species at the PSI reducing side (Sonoike et al., 2001). Apparently, decrease in the PSI content should be indispensable for growth under HL conditions. However, the mechanism by which the amount of PSI complex is modulated under HL has remained unknown.

In the course of characterization of the pmgA mutant, we noticed two mutant phenotypes that may lead to the elucidation of the regulatory mechanism of PSI content. First, we realized that the pmgA mutant has defect in the transcriptional regulation of psaAB genes, encoding the reaction center subunits of photosystem I, under HL conditions. In both WT cells and the pmgA mutant, the transcript levels of psaAB genes rapidly decreased upon the shift to HL conditions. After 6 h of HL exposure, psaAB transcripts were maintained at low level in WT cells, whereas they began to accumulate enormously in the pmgA mutant (Muramatsu & Hihara, 2003). As for the transcription of other PSI genes, the pmgA mutant did not show such an obvious defect. Apparently, the
regulation of the *psaAB* transcription is the key factor for the HL acclimation, even though the transcription of other PSI genes were also down-regulated cooperatively. Secondly, we found that the *pmgA* mutant has higher amount of chlorophyll on a per cell basis than WT cells after 6 h of HL exposure (Hihara *et al.*, 1998). Although this may be a consequence of the increased PSI content, there is a possibility that enhanced chlorophyll synthesis is the cause of the increased PSI content in the mutant, not the result of it. These observations imply that the regulation of *psaAB* transcription or of chlorophyll synthesis might have a crucial role for the down-regulation of PSI content under HL conditions. In this study, we estimated the contribution of *psaAB* transcription and chlorophyll synthesis to the regulation of PSI content by modulating these activities during HL acclimation. Moreover, the role of PmgA in the regulation of these activities is discussed in terms of two phase-mechanism of the repression of PSI content under HL conditions.

**Methods**

**Strains and culture conditions**

A glucose-tolerant WT strain of *Synechocystis* sp. PCC 6803 was grown at 31°C in BG-11 liquid medium (Stanier *et al.*, 1971) with 20 mM HEPES-NaOH, pH 7.0. Cells were grown in test tubes (3 cm in diameter) and bubbled with air. Unless otherwise stated, cultures were grown under continuous
illumination at 20 μmol photons m$^{-2}$ s$^{-1}$ provided by fluorescent lamps. The
$pmgA$ (sll1968) disrupted mutant, which was made by insertion of the
spectinomycin resistance cassette (Hihara et al., 1998), and the $psaAB$-OX strain
(see below) were grown under the same condition, except that spectinomycin
(20 μg ml$^{-1}$) and chloramphenicol (25 μg ml$^{-1}$) were added to the medium,
respectively. Cell density was estimated by optical density at 730 nm using a
spectrophotometer (model UV-160A; Shimadzu). Cultures containing 1.0 x 10$^8$
cells ml$^{-1}$ give OD$_{730}$ of 1.0 with this spectrophotometer. HL shift experiments
were performed by transferring cells at the exponential growth phase (OD$_{730}$=0.1
- 0.2) from low light (LL) (20 μmol photons m$^{-2}$ s$^{-1}$) to HL conditions (250 μmol
photons m$^{-2}$ s$^{-1}$).

13 **Determination of chlorophyll content**

*In vivo* absorption spectra of whole cells suspended in BG-11 medium
were measured at room temperature using a spectrophotometer (model 557;
Hitachi) with an end-on photomultiplier. Chlorophyll content was calculated
from the peak height of absorption spectra using the equations of Arnon *et al.*
(1974).

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20 **Escherichia coli and DNA manipulation**

An *E. coli* strain, XL1-Blue MRF’ (Stratagene), was the host for all
plasmids constructed in this study. Procedures for the growth of *E. coli* strains and for the manipulation of DNA were carried out as described in Sambrook *et al.* (1989). When required, ampicillin (100 μg ml⁻¹), spectinomycin (20 μg ml⁻¹) or chloramphenicol (25 μg ml⁻¹) was added to Terrific Broth medium for selection of plasmids in *E. coli*. Plasmids were sequenced by dideoxy-chain termination method using dye terminator cycle sequencing ready reaction kit (ABI PRISM; Applied Biosystems).

**Generation of the psaAB overexpressing strain (psaAB-OX)**

The full intergenic region between sll1730 and psaA (slr1834) genes (415 bp in length) was amplified by PCR using the primer pair, PpsaA-F (5’-AACGTACGTGTCAAAAATCCGCTTCTT-3’) and XhoI-PpsaA-R (5’-AACTCGAGCAGGTTCCTCTCGT-3’). The underlined sequences correspond to the restriction site of BsiWI and XhoI, respectively. The amplified PCR products were cloned into the TA cloning site of pT7Blue vector (Novagen) and cut out by digestion with SphI (a restriction site within the multiple cloning site of pT7Blue vector) and XhoI. A fragment including the chloramphenicol resistance cassette and the promoter region of psbA2 gene (slr1311) was cut out from pTCP2031V by digestion with XhoI and NdeI. pTCP2031V vector is a kind gift from Prof. M. Ikeuchi (The University of Tokyo, Japan). The coding region, 501 bp fragment from the start codon of psaA
gene, was amplified by PCR using the primer pair, NdeI-psaAcod-F (5’-AAGTTCCCTCAGGCATATGACAATTAGTCCACCC-3’) and psaAcod-R (5’-AACCAAACCGCCAATGGCG-3’). The underlined sequence corresponds to the restriction site of NdeI. The amplified PCR products were cloned into the TA cloning site of pT7Blue vector (Novagen) and the resultant plasmid was digested with SphI (a restriction site within the multiple cloning site of pT7Blue vector) and NdeI. Then, the SphI/XhoI fragment containing the psaA upstream region and the XhoI/NdeI fragment containing the chloramphenicol resistance cassette and the psbA2 promoter were cloned with specific restriction sites into the SphI/NdeI-digested pT7Blue vector containing the psaA coding region to yield an overexpression plasmid for psaAB. The WT strain of Synechocystis sp. PCC 6803 was transformed with this construct, and transformants were selected on plates containing 25 μg ml⁻¹ chloramphenicol.

**RNA isolation and Northern blot analysis**

RNA isolation and Northern blot analysis were performed as described previously (Muramatsu & Hihara, 2003).

**Immunoblot Analysis**

LL or HL exposed *Synechocystis* cells at the exponential growth phase (OD₇₃₀=0.2) were harvested by centrifugation. The pellet was resuspended in
100 μl of TBS buffer (20 mM Tris-HCl, pH 7.5, 0.15 M NaCl). The cell suspension was mixed with approximately 50 μl volume of glass beads (diameter 0.1 mm; BioSpec Products) and cells were disrupted by three times of vigorous agitation for 2 min, each followed by cooling on ice for 1 min. After removal of unbroken cells and debris by centrifugation at 700 g for 3 min, cell extracts were treated with 5% (w/v) lithium dodecyl sulfate, 60 mM DTT, and 60 mM Tris-HCl (pH 8.0) for 2 h at room temperature. Then, samples corresponding to 5 x 10^5 cells per a lane for the detection of PsaAB, 1 x 10^7 cells for that of PedR (Nakamura & Hihara, 2006) and 2 x 10^7 cells for that of HemA were loaded onto SDS polyacrilamide gel. SDS gel electrophoresis was performed by the procedure of Laemmli (1970). After electroblotting onto PVDF membranes (Immobilon-P; Millipore), samples were probed with polyclonal antibodies. The antiserum against PsaAB from *Thermosynechococcus elongatus* (Kashino *et al.*, 1990) was kindly provided by Prof. I. Enami (Science University of Tokyo, Japan). The antiserum against HemA from cucumber was a kind gift from Prof. A. Tanaka (Hokkaido University, Japan). Goat anti-rabbit IgG conjugated to alkaline phosphatase was used for secondary antibodies. PedR, a transcriptional regulator whose amount was reported to remain unchanged during HL acclimation (Nakamura & Hihara, 2006), was used for the loading control. The PedR gives single monomer band at around 12 kDa when reduced. The results of immunoblot analysis were
digitized by a scanner and the band intensity was quantified by using Scion Image software (Scion Corporation).

**Determination of the activity of ALA synthesis**

Activity of 5-aminolevulinic acid (ALA) synthesis was determined according to Goslings et al. (2004) with some modifications. Cells were incubated under LL or HL in growth medium with levulinic acid (LA) for 3 h to accumulate ALA. 3 mM of LA could completely inhibit chlorophyll synthesis both in WT and in the pmgA mutant. Exceptionally, 4 mM of LA was required for the complete inhibition of chlorophyll synthesis of the pmgA mutant after 9 h of HL incubation. 50 ml of LL-grown cultures (OD$_{730}$=0.4) or 100 ml of HL-grown cultures (OD$_{730}$=0.2) were used for the analysis. After 3 h of LA treatment, cells were harvested by centrifugation. The pellet was resuspended in 150 μl of 4% (w/v) TCA and cells were disrupted by four times of vigorous agitation with glass beads (diameter 0.1 mm; BioSpec Products) for 2 min with intervals of 2 min. After cell debris and glass beads were removed by centrifugation, 100 μl of supernatant was mixed with 1 ml of 50 mM NaH$_2$PO$_4$ (pH 7.5) for neutralization. 100 μl of ethylacetocetate was added to the aliquot (500 μl) of reaction mixture and the sample was boiled at 100°C for 10 min to yield porphobilinogen by condensation of ALA. After cooling on ice for 5 min, 600 μl of freshly made Ehrlich’s reagent (consisting of 0.2 g
p-dimethylaminobenzaldehyde; 8.4 ml acetic acid; 1.6 ml 70% (v/v) perchloric acid) was added and incubated for 15 min. The sample mixed with Ehrlich’s reagent without p-dimethylaminobenzaldehyde was used as a control. The produced 2-methyl-3-acetyl-4-(3-propionic acid) pyrrole derivatives were spectroscopically quantified by the absorbance at 553 nm. The concentration was estimated using absorption coefficient of 74.5 mM$^{-1}$ cm$^{-1}$ (Harel & Klein, 1972). The activity of ALA synthesis was shown in terms of $\mu$mol of ALA accumulated (g fresh weight of cells)$^{-1}$ (3 h)$^{-1}$.

**Results and Discussion**

**Time course of the change in PSI content after the transfer to HL conditions**

Previous observation told us that the decrease in the $psaAB$ transcripts and of chlorophyll content in HL-illuminated *Synechocystis* cells is achieved by the two-phase mechanism in terms of the involvement of PmgA (Hihara et al., 1998, Muramatsu & Hihara, 2003). In the initial phase, designated Phase 1 (i.e. during first 6 h of HL exposure), drastic decrease of the levels of $psaAB$ transcripts and of chlorophyll occurs, owing to the strong repression of their *de novo* synthesis. In contrast, in the second phase, designated Phase 2 (i.e. from 6 h onward following HL shift), significant re-accumulation of the $psaAB$ transcripts and of chlorophylls occurred in the *pmgA* mutant, while WT cells
maintained those at low levels.

To know if the levels of PsaAB proteins are regulated in the two phase manner, the change of their amount after the shift to HL conditions was followed by immunoblot analysis. Amount of PsaAB decreased similarly in WT and the pmgA mutant until 6 h of HL exposure while pmgA-mutant specific re-accumulation was observed after 6 h (Fig. 1a, b). Equal loading of samples was verified by detection of PedR (Fig. 1a), a transcriptional regulator whose amount was not affected by the changes in environmental photon flux densities (Nakamura & Hihara, 2006). These data clearly indicate that the decrease of PSI content under HL is also accomplished in two phases as in the amount of psaAB transcript and chlorophyll. Considering that phenotypic differences between the WT and the pmgA mutant appeared only in Phase 2, decrease of psaAB transcripts, chlorophyll and PSI complex observed in Phase 1 should be achieved by some factors other than PmgA, while the regulation of their amount in Phase 2 should be under the control of PmgA. Since the amount of PsaAB proteins (Fig. 1) changes in parallel with that of psaAB transcript (Muramatsu & Hihara, 2003) and of chlorophyll (Hihara et al., 1998) through both Phase 1 and Phase 2, either the level of psaAB transcription or that of chlorophyll synthesis can be the determinant of PSI content.

Does the amount of psaAB transcripts determine PSI content under HL?
We overexpressed the endogenous psaAB genes by inserting the HL-inducible psbA2 promoter to just upstream of the psaA coding region (psaAB-OX strain) and examined if PSI content under HL would be affected. Upon the shift to HL, psaAB, psaA, and psaB transcripts drastically decreased and were kept at low levels in WT cells, while they were highly accumulated in the psaAB-OX strain as expected (Fig. 2a). However, the amount of PsaAB proteins on a per cell basis in the psaAB-OX strain still decreased under such conditions albeit at a slower rate compared with WT (Fig. 2b,c). Concomitantly, chlorophyll content on a per cell basis decreased more slowly in the psaAB-OX strain than in WT upon the shift to HL condition (Fig. 2g). Since the total chlorophyll content remains unchanged during the first 9 h after the shift to HL both in WT and in the psaAB-OX strain (Fig. 2f), the apparent decrease of chlorophyll content on a per cell basis should be the consequence of the dilution effect during cell proliferation. Thus, the difference between WT and the psaAB-OX strain in the decrease rate of the apparent chlorophyll content (Fig. 2g) as well as of the PsaAB protein content (Fig. 2b,c), could be ascribed to the slower growth of the psaAB-OX strain than that of WT (Fig. 2d, also see below). After 9 h of HL exposure, WT cells resume chlorophyll synthesis (Fig. 2f) to maintain a certain level of cellular chlorophyll concentration (Fig. 2g). In contrast, the psaAB-OX strain did not show the increase of chlorophyll synthesis
even after 9 h (Fig. 2f). It took more than 24 h for the psaAB-OX strain to
decrease the chlorophyll content to the steady state levels for HL-acclimated
cells (Fig. 2g).

We assume that the lack of the over-accumulation of PsaAB protein in
the psaAB-OX strain would be due to the degradation of the newly synthesized
PsaAB polypeptide without proper insertion of chlorophylls, rather than the
suppressed translation of psaAB messengers. Enhancement of chlorophyll
synthesis as well as overexpression of psaAB genes might be required for the
high accumulation of PSI complexes. The results indicate that the high level of
the psaAB transcripts alone is not sufficient for the aberrant accumulation of PSI
complex under continuous HL conditions.

Unexpectedly, growth of the psaAB-OX strain was strongly inhibited
under HL conditions (Fig. 2d), while that under LL condition was normal (Fig.
2e). Although we cannot exclude the possibility that growth inhibition of the
psaAB-OX strain is due to the polar effect originated from the interruption of
5’-UTR region of the psaAB genes, keeping the psaAB transcripts at low level
may be essential for the normal cell growth under HL conditions. Upon the shift
to HL conditions, many stress responsive genes were induced to support cell
growth in WT cells (Hihara et al., 2001). Repression of psaAB transcription
under HL may be important to recruit the transcriptional machinery for such
stress inducible genes.
Does the amount of chlorophyll $a$ determine PSI content under HL conditions?

The possibility that chlorophyll content determines PSI content under HL conditions could be tested through the inhibition of chlorophyll synthesis by the addition of levulinic acid (LA), an inhibitor of 5-amino levulinic acid dehydratase. When 2 mM LA was added 6 h after HL shift, cellular chlorophyll content of both WT and the $pmgA$ mutant after 24 h of HL incubation was suppressed to 70% compared with the respective cultures without LA addition. Chlorophyll content of the LA-treated mutant decreased to the levels of the untreated WT cells (Fig. 3a). Concomitantly, the amount of PsaAB in LA-treated mutant decreased nearly to the level of the untreated WT after 24 h of HL exposure (Fig. 3b, c), although $psaAB$ transcripts remained at a high level (Fig. 3d). This clearly indicates that the regulation of chlorophyll content plays a critical role in the modulation of PSI content under HL conditions. To date, the influence of chlorophyll availability on photosystem content has been studied under LL or in darkness. For example, Xu et al. (2004) reported a decrease in the PSI/PSII ratio following the addition of gabaculin, an inhibitor of chlorophyll synthesis, to WT cells of *Synechocystis* sp. PCC 6803. Kada et al. (2003) reported a decrease in PSI subunits (PsaAB, PsaC) but not in PSII subunits (D1 and CP47) in a *chlL* mutant of *Plectonema boryanum* incubated in the dark where chlorophyll synthesis in the mutant was arrested. All of these
data suggest that biogenesis of PSI is more closely linked to chlorophyll availability compared to that of PSII under LL conditions or in darkness. The same seems to be true under HL conditions, judging from the observation on the PSI content that showed more drastic decrease than PSII content (Hihara et al., 1998).

We observed that the suppression of chlorophyll content in the pmgA mutant by the addition of 2 mM LA could not result in the down-regulation of psaAB transcript level (Fig. 3d). Moreover, we observed that the higher rate of psaAB transcription in the psaAB-OX strain could not bring about over-accumulation of chlorophyll (Fig. 2f). Thus, there seems to be no causal relationship between the synthesis of chlorophyll and psaAB transcripts. During Phase 2 of HL acclimation, levels of chlorophyll and of psaAB transcripts should be independently repressed by the PmgA-mediated regulatory mechanism.

What is the cause of the high chlorophyll content in the pmgA mutant under HL conditions?

In plants and cyanobacteria, the tetrapyrrole biosynthesis pathway starts from glutamate, which is converted to 5-aminolevulinic acid (ALA). It is widely accepted that chlorophyll synthesis is regulated at three steps, that is, ALA synthesis, branching point of heme and chlorophyll, and reduction step of
protochlorophylide to chlorophylide. Among them, ALA synthesis is the major regulatory step (Vavilin & Vermaas, 2002). We found that the activity of ALA synthesis drastically decreased in WT cells upon the shift from LL to HL conditions during Phase 1 and the activity was maintained at relatively low level after 6 h (Phase 2) (Fig. 4). The time-course change of the activity in ALA synthesis upon the shift to HL is similar to that in chlorophyll content, showing that decrease of the chlorophyll content under HL conditions is mainly achieved by the block of ALA synthesis. The regulation of tetrapyrrole biosynthesis at a step preceding the formation of ALA seems reasonable, since accumulation of any tetrapyrrole intermediates having visible light absorption could cause photodamage to cells under HL conditions.

Unlike WT, ALA synthesis in the pmgA mutant started again at Phase 2, although ALA synthesis decreased both in WT and the pmgA mutant at Phase 1. This result strongly suggests that the increase of the chlorophyll content in the pmgA mutant at Phase 2 was due to the loss of the repression of ALA synthesis.

ALA is synthesized from glutamate in three steps catalyzed by GltX (GTS; glutamyl-tRNA synthetase), HemA (GTR; glutamyl-tRNA reductase), and HemL (GSA; glutamate-1-semialdehyde aminotransferase). ALA synthesis is suggested to be regulated mainly at the step catalyzed by HemA (Reinbothe & Reinbothe, 1996; Vavilin & Vermaas, 2002). When we examined the amount of HemA protein under HL conditions by immunoblot analysis, an increase in
HemA levels after the shift to HL conditions was observed both in WT and in the pmgA mutant (Fig. 5a, b). The extent of the increase in HemA was almost the same between WT and pmgA mutant, which is apparently inconsistent with the observed change in the activity of ALA synthesis after the HL shift (Fig. 4). Availability of the substrate is also not the cause of the difference in ALA synthetic activity between the two strains, since the measurement using capillary electrophoresis mass spectrometry (CE/MS) revealed that the contents of glutamate, the substrate of ALA synthesis, in WT and the pmgA mutant after 12 h of HL incubation are comparable, i.e. 9.2 and 9.7 μmol·(g fresh weight)⁻¹, respectively. Thus, we assume that HemA activity is under posttranslational regulation under HL conditions.

In higher plants and algae, HemA activity is known to be subject to feedback inhibition by intermediates of the tetrapyrrole biosynthesis pathway (Reinbothe & Reinbothe, 1996; Vavilin & Vermaas, 2002). In Synechocystis sp. PCC 6803, HemA activity is inhibited by the addition of (proto)heme in vitro (Rieble & Beale, 1991). However, the contribution of feedback inhibition can be excluded in this case because we measured the activity of ALA synthesis in the presence of LA, i.e. without accumulation of intermediates in tetrapyrrole biosynthesis. There have been several studies reporting the isolation of regulatory factors for ALA synthesis. These proteinous factors, such as FLU in higher plants (Meskauskiene et al., 2001; Goslings et al., 2004), FLP in green
algae (Falciatore et al., 2005) and SCPs in cyanobacteria (Xu et al., 2002), are all assumed to work in response to the availability of chlorophylls or its intermediates. PmgA seems to be an unusual factor in the point that it regulates the activity of ALA synthesis independently of the levels of tetrapyrrole intermediates. The mechanism of such regulation, directly or indirectly, is yet to be elucidated.

Conclusion

During the first 6 h following the shift to HL (Phase 1), levels of chlorophyll and psaAB transcripts are strictly down-regulated by mechanisms independent of PmgA. Repression of chlorophyll synthesis is mainly achieved at the level of ALA synthesis, which is crucial for the down-regulation of PSI content under HL conditions. Selective repression of PSI content is observed since PSI is more sensitive to chlorophyll availability than PSII. Although the mechanism of the repression of psaAB transcription during Phase 1 was not assessed in this report, we recently revealed that an AT-rich light-responsive element located just upstream of the basal promoter region is responsible for the coordinated and rapid down-regulation of PSI genes upon the shift to HL conditions during Phase 1 (Muramatsu & Hihara, 2006, 2007). The stability of psaAB and psaA mRNA was lower under HL than under LL, suggesting mRNA degradation also contributes to the dramatic loss of psaAB transcript following
HL shift (Muramatus and Hihara 2003). Normal PSI content in the psaAB-OX strain suggests that psaAB transcription is not a rate-limiting step of PSI content under HL conditions, though its down-regulation appeared to be indispensable for growth under HL conditions.

After 6 h of HL incubation (Phase 2), the PmgA-mediated regulatory mechanism becomes active to maintain the amounts of chlorophyll and psaAB transcripts at a low level. Lack of this regulation causes aberrant accumulation of PSI under HL conditions and finally results in cell death (Hihara et al., 1998; Sonoike et al., 2001). It is likely that ALA synthesis and psaAB transcription are independent targets for the PmgA-mediated regulatory mechanism. Coordinated synthesis of chlorophyll and chlorophyll-binding protein is essential for photosynthetic organisms, since accumulation of free chlorophylls or chlorophyll intermediates causes severe photooxidative damage to cellular components. However, to date, the regulatory mechanism common to the synthesis of chlorophyll and chlorophyll-binding protein has not been identified. PmgA should be a key for the elucidation of such a regulatory mechanism.

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**Figure legends**

**Fig. 1.** The change in the level of PsaAB after the shift to HL conditions.

(a) Immunoblot analysis of PsaAB and PedR in WT and the *pmgA* mutant. Cells grown under LL conditions were transferred to HL conditions and sampled at the indicated time. Total proteins corresponding to 5 x 10^5 cells for PsaAB detection and 1 x 10^7 cells for PedR detection were loaded in each lane.

(b) The intensity of PsaAB bands in the immunoblot analysis were quantified
densitometrically by Scion Image software. Values are presented relative to that in LL-grown WT cells.

**Fig. 2.** Characterization of the *psaAB*-OX strain.

(a) The change in the levels of the *psaA*, *psaB* and *psaAB* transcripts after the shift to HL conditions in WT and the *psaAB*-OX strain. Cells grown under LL conditions were transferred to HL conditions and sampled at the indicated time. The *psaA*, *psaB* and *psaAB* transcripts were analyzed by Northern blot using gene specific RNA probes of *psaA* (the upper panel) or *psaB* (the lower panel). An aliquot of total RNA (2 μg) was loaded in each lane. rRNA was visualized with methylene blue staining (lower panel).

(b) Immunoblot analysis of PsaAB in WT and the *psaAB*-OX strain grown under LL or HL conditions for 6 h and 24 h. Total proteins corresponding to 5 x 10^5 cells were loaded in each lane.

(c) Quantification of the band intensity of PsaAB in the immunoblot by Scion Image software. Values are presented relative to that in LL-grown WT cells.

(d) Growth curves of the WT (open circle) and the *psaAB*-OX strain (closed circle) under HL and (e) those under LL conditions. LL-grown cells were shifted to HL conditions at time 0. The data represent the mean ± SD of three independent experiments.

(f) The changes in chlorophyll content on a per liquid culture and (g) those on a
per cell basis after the shift to HL conditions in WT (open circle) and the
psaAB-OX strain (closed circle). LL-grown cells were shifted to HL conditions
at time 0. The data represent the mean ± SD of three independent experiments.

Fig. 3. Effect of the inhibition of chlorophyll synthesis.

2 mM of LA was added to the culture 6 h after the shift to HL conditions to keep
the chlorophyll content of the mutant to the level of WT cells.

(a) Chlorophyll content on a per cell basis in WT (white bars), the pmgA mutant
(gray bars), WT with LA (hatched bars) and the pmgA mutant with LA (gray
bars with hatches) grown under LL or HL conditions for 24 h. The data
represent the mean ± SD of four independent experiments.

(b) Immunoblot analysis of PsaAB in WT, WT with LA, the pmgA mutant (Δ)
and the pmgA mutant with LA grown under LL or HL conditions for 24 h. Total
proteins corresponding to 5 x 10^5 cells were loaded in each lane.

(c) Quantification of the bands of PsaAB in the immunoblot by Scion Image
software. Values are presented relative to that in LL-grown WT cells.

(d) The changes in the level of the psaA and psaAB transcripts after the shift to
HL conditions in WT, the pmgA mutant and the pmgA mutant with LA. Cells
grown under LL conditions were transferred to HL conditions and sampled at
the indicated time. The psaA and psaAB transcripts were analyzed by Northern
blot using psaA gene specific RNA probe. An aliquot of total RNA (2 μg) was
loaded in each lane. rRNA was visualized with methylene blue staining (lower panel).

Fig. 4. The changes in the activity of ALA synthesis in WT (open circle) and the \textit{pmgA} mutant cells (closed circle) after the shift to HL conditions. 3 h prior to the harvesting of cells at the indicated time point, LA was added to the culture and the amount of the accumulated ALA during the 3 h period was regarded as the activity of ALA synthesis. The data represent the mean ± SD of three independent experiments.

Fig. 5. The changes in HemA levels in WT and the \textit{pmgA} mutant after the shift to HL conditions.
(a) Immunoblot analysis of HemA in WT and the \textit{pmgA} mutant. Cells grown under LL conditions were transferred to HL conditions and sampled at the indicated time. Total protein corresponding to 2 x 10^7 cells for HemA detection and 1 x 10^7 cells for PedR detection were loaded in each lane.
(b) Quantification of the bands of HemA in the immunoblot by Scion Image software. Values are presented relative to that in LL-grown WT cells.
(a) | WT | ΔpmgA |
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<td>0</td>
<td>6</td>
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HemA |

PedR

(b) Levels of the amount of HemA (relative) over time (h).

WT | ΔpmgA
---|---
0 | 6 | 24 | 0 | 6 | 24

Levels (relative):

0 1 2