Regulation of Photosystem I Reaction Center Genes in *Synechocystis* sp. Strain PCC 6803 during Light Acclimation

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Cyanobacteria acclimate to changes in incident light by adjusting photosystem stoichiometry through regulation of PSI accumulation. To gain a deeper insight into this control mechanism in *Synechocystis* sp. strain PCC 6803, we studied the expression and regulation of the *psaAB* operon, encoding the reaction center proteins of PSI, during the initial stage of acclimation to changes in the intensity and quality of light. The *psaAB* operon was transcribed as a dicistronic transcript, which was processed into smaller, putatively monocistronic *psaA* and *psaB* transcript species. Dark treatment of the cells inhibited the *psaAB* transcription, whereas re-illumination of dark-adapted cells reactivated the transcription slowly in a process requiring de novo protein synthesis. Transfer of cells from white to orange light, favoring excitation of PSI, stimulated the *psaAB* transcription, whereas far-red light, primarily exciting PSI, down-regulated the transcription of the *psaAB* operon. These results, together with down-regulation of *psaAB* transcription upon the addition of electron transport inhibitors under constant white light illumination, suggested that the photosynthetic redox poise affects the *psaAB* transcription activity in the light. Pulse-labeling experiments demonstrated that light-induced modulations in thetranslation rate of the PsaA protein closely parallel the transcription rate of the *psaAB* operon, indicating that transcriptional regulation plays the major role in determining the content of PSI reaction center proteins and, thereby, PSI complexes, during light acclimation. The scantiness of PsaA translation in darkness despite the abundance of *psaA* transcripts demonstrated that the comprehensive regulation of PSI accumulation also involves regulation at the level of translation.

**Keywords:** Cyanobacterium — Gene regulation — Light — Photosystem I — *psaAB* operon — *Synechocystis* sp. PCC 6803

**Abbreviations:** DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone; PQ, plastoquinone pool.

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**Introduction**

Photosynthetic organisms are exposed to continuous variation in their principal source of energy, light. Accordingly, they have evolved several acclimation mechanisms in order to maintain efficient photosynthetic energy conversion while minimizing photo-oxidative damage under varying light environments. A well-documented acclimation response is the adjustment of photosystem stoichiometry, i.e. the ratio of PSI to PSII, observed widely in cyanobacteria (Kawamura et al. 1979, Fujita et al. 1985, Manodori and Melis 1986, Hihara et al. 1998), algae (Melis et al. 1996) and higher plants (Chow et al. 1990). Such adjustment has been suggested to balance the excitation of the two photosystems, containing different light-harvesting antennae, under various wavelengths of light (Manodori and Melis 1986, Murakami and Fujita 1991), or to protect the cells from photodamage under high irradiances (Hihara et al. 1998, Sonoike et al. 2001). In cyanobacteria, the changes in the PSI/PSII ratio have been shown to result mainly from a specific regulation of PSI abundance (Fujita et al. 1985, Aizawa et al. 1992, Fujita 1997, Hihara et al. 1998, Hihara 1999).

Although the structure and function of PSI are presently relatively well defined (Jordan et al. 2001, reviewed in Chittnis 2001, Fromme et al. 2001, Saenger et al. 2002), much less is known about the mechanisms regulating the biogenesis and acclimation of this multiprotein complex. In cyanobacteria, regulation of PSI synthesis during light acclimation has been suggested to occur mainly at the level of translation or assembly of the complex (Aizawa and Fujita 1997, Fujita 1997). Analyses of cyanobacterial mutants with an aberrant PSI/PSII ratio have indeed revealed several protein factors specifically involved in stable accumulation of PSI complexes (Wilde et al. 1995, Bartsevich and Pakrasi 1997, Mann et al. 2000, Wilde et al. 2001, Shen et al. 2002), and the availability of Chl *a* has also been considered as a regulatory factor due to selective suppression of PSI accumulation by inhibitors of Chl *a* biosynthesis (Fujita et al. 1990). Although required for accumulation of stable PSI complexes, the significance of these factors in regulation of PSI content has not been established as yet. Thus far, the only protein unequivocally shown to regulate the PSI content during light acclimation is PmgA, which is required to suppress the accumulation of PSI complexes under long-term high-light stress (Hihara et al. 1998).

Transcriptional regulation of PSI accumulation, on the other hand, was long neglected in cyanobacteria. Recently, however, a number of genome-wide microarray analyses of *Synechocystis* sp. strain PCC 6803 have revealed notable...
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Results

Accumulation of transcripts from the psaAB operon in response to light quantity

To study whether transcriptional regulation plays a role in PSI synthesis during light acclimation, we first determined the effect of light quantity on the expression of the psaAB operon. For this purpose, the Synechocystis sp. strain PCC 6803 cell cultures were transferred from growth light to two higher light intensities, referred to as the medium light (250 µmol of photons m⁻² s⁻¹) and high light (1,500 µmol of photons m⁻² s⁻¹), or to darkness. The Northern blot analyses of total RNA revealed three main mRNA species recognized by the psaA- and psaB-specific probes: an ∼2.6 kb psaA-specific transcript (Fig. 1A, upper panel), an ∼2.2 kb psaB-specific transcript (Fig. 1A, lower panel) and a large, 5.1 kb transcript, that was recognized by both probes and thus represents the dicistronic transcript of the psaAB operon (Fig. 1).

When Synechocystis sp. strain PCC 6803 cells were transferred from growth light to darkness, the amount of the dicistronic psaAB transcripts gradually decreased to only 5% of the original growth light level during the 2 h dark incubation (Fig. 1A). This decrease was accompanied by increasing amounts of the 2.6 kb psaA and 2.2 kb psaB transcripts during the first hour of dark incubation, and these 2.6 kb psaA and 2.2 kb psaB transcripts remained abundant even after 2 h of dark treatment. A similar dark-induced decline in the dicistronic psaAB transcripts and transient accumulation of the shorter gene-specific transcripts occurred even when the cells were grown in the presence of glucose as the carbon and energy source (Fig. 1B).

Exposure of cells to medium light intensity decreased the amount of the dicistronic psaAB transcripts by 20% within the 2 h incubation, but induced a more pronounced reduction in the 2.6 kb psaA- and 2.2 kb psaB-specific transcripts (Fig. 1A). Shift of the cells to high light intensity, in turn, induced a severe drop in the abundance of all transcripts originating from the psaAB operon; after 2 h high-light treatment, the amount of the dicistronic psaAB transcripts had dropped to only 30% of the original level, and the shorter transcript species were hardly detectable (Fig. 1A).

To clarify whether the different transcript species of the psaAB operon differ in translatability, we analyzed the distribution of the psaAB and psaA transcripts between the membrane and cytosolic fractions of Synechocystis sp. strain PCC 6803 cells, as the translation of membrane proteins for PSI and PSII reaction center complexes previously has been shown to occur on membrane-bound ribosomes (Klein et al. 1988, Tyystjärvi et al. 2001, Tyystjärvi et al. 2004). The majority of both the psaAB and psaA transcripts were found in the membrane frac-
tion under growth-light and high-light conditions, although considerable amounts were also present in the cytosolic fraction (Fig. 2). Nevertheless, the association of both the dicistronic psaAB and the 2.6 kb psaA transcripts with the membranes suggested that both transcript forms were translationally competent. In contrast, 2 h dark treatment had almost depleted the cells of the dicistronic psaAB transcripts (Fig. 2), and the 2.6 kb psaA transcripts were primarily found in the cytosolic fraction, and were thus presumed to be translationally inactive.

The steady-state amount of a given transcript is determined by two parameters: the rate of transcription and the stability of the transcript. To separate these processes from each other, we supplied the cell suspensions with the transcription inhibitor rifampicin and followed the decay of the transcripts under different illumination conditions. The half-life of the psaAB transcript was calculated from the measured decay of the transcript from three independent Northern blot experiments. Under growth light, the dicistronic psaAB transcripts were degraded rapidly with a half-life of approximately 12 min (Fig. 3). The degradation of the 2.6 kb psaA and 2.2 kb psaB transcripts was accompanied by a concomitant accumulation of smaller degradation products. The degradation pattern of all psaAB transcript species after shift to high-light irradiance was very similar to that under growth-light illumination, although a somewhat shorter half-life ($t_{1/2} \sim 10$ min) was recorded for the dicistronic psaAB transcript. In contrast, transfer of cells to darkness induced a strong stabilization of the dicistronic psaAB transcripts ($t_{1/2} \sim 45$ min) and, in particular, of the 2.6 kb psaA and 2.2 kb psaB transcripts (Fig. 3). The increase in the abundance of these 2.6 kb psaA and 2.2 kb psaB transcripts even in the presence of rifampicin led us to conclude that these short gene-specific transcripts originate via processing/degradation in the intergenic region of the dicistronic psaAB transcript that, in turn, represents the primary transcription product of the psaAB operon. Taken together, the results of transcript accumulation (Fig. 1) and stability (Fig. 3) indicated that the transcription of the psaAB operon responds to the quantity of light. Incubation of cells in darkness rapidly inhibited psaAB transcription, whereas high-light treatment down-regulated the psaAB transcription, but did not totally prevent it.

**Accumulation of transcripts from the psaAB operon with respect to photosynthetic redox poise**

To obtain further insight into the regulation of psaAB expression, we next studied the effect of light quality on the abundance and stability of the psaA/psaB transcripts. For this purpose, Synechocystis sp. strain PCC 6803 cultures were shifted from (white) growth light to orange light of the same intensity (PSII-light), which is absorbed mainly by the phycobilisome antenna and thus favors the excitation of PSII, or alternatively to far-red light (PSI-light), which is absorbed mainly by Chl a and thus favors the excitation of PSI (Fig. 4A). The preferential excitation of PSI by PSI-light was confirmed by P700 redox measurements at 820 nm. As shown in Fig. 4B, PSI-light induced an increase in 820 nm absorption, indicating stable oxidation of P700, the primary electron donor of PSI, whereas no such absorption increase was observed under PSII-light.

Exposure of cells to PSII-light doubled the amount of the dicistronic psaAB transcripts within the 2 h incubation (Fig 4C; data shown only for the psaA probe). The increase in the dicis-
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...tronc transcript pool was accompanied by increased accumulation of the 2.6 kb *psaA* transcripts as well. The decay of the different *psaAB* transcript species under PSI-light, measured in the presence of rifampicin (Fig. 4D), was similar to that under standard growth-light conditions (Fig. 3). Combined, the results in Fig. 4C and D indicate that the increase in the abundance of *psaAB* transcripts was due to enhanced transcription of the *psaAB* operon under PSI-light.

PSI-light, on the contrary, induced a net decrease in the amount of *psaAB* transcripts, although fluctuation in the transcript abundance was typical for this light treatment (Fig. 4C). The amount of the *psaAB* transcript decreased by 39 ± 6% during the first 15 min of incubation under PSI-light, then increased and decreased again, but never exceeded the control light value (Fig. 4C). Measurements of transcript stability in the presence of rifampicin showed that both *psaAB* and *psaA* transcripts became markedly more stable under PSI-light as compared with the growth-light conditions (Fig. 4D vs. Fig. 3). Collectively, the results indicated that transcription of the *psaAB* operon is down-regulated under PSI-light.

The opposite effects of PSII-light and PSI-light on *psaAB* transcription suggested that the redox poise between the two photosystems might mediate the light signal to *psaAB* transcription. To study this possibility, and to separate the photosynthetic electron flow from other possible light signals, we blocked the photosynthetic electron transfer chain under growth light illumination chemically either by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) or by 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), and studied the effects of these treatments on the accumulation and stability of the different *psaA/B* transcripts. Treatment of the cells with DBMIB, which prevents the oxidation of plastohydroquinone by the cytochrome b/f complex, induced a rapid decline in the *psaAB* transcripts, followed by a slightly slower diminution of the 2.6 kb *psaA* and 2.2 kb *psaB* transcript forms (Fig. 5A; data shown only for *psaA*). Measurements of transcript stability, on the other hand, demonstrated that DBMIB poisoning severely retarded the decay of the dicistronic *psaAB* transcripts and, particularly, of the shorter gene-specific transcripts (Fig. 5B). Thus, DBMIB treatment blocked the transcription of the *psaAB* operon completely. Treatment of the cells with DCMU, which inhibits the reduction of the plastoquinone (PQ) pool by PSII, also induced a decrease in all *psaAB*-derived transcripts (Fig. 5A), although this decrease was less prominent than that induced by DBMIB. Inhibition of the electron transfer chain by DCMU also resulted in a stabilization of the transcripts (Fig. 5B). Thus, DCMU treatment also decreased the rate of *psaAB* transcription, although it did not totally prevent it. Taken together, the inhibitor studies clearly showed that changes in the photosynthetic electron flow can modulate the transcript...
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The activity of the psaAB operon without any change in illumination conditions.

Next we studied the activation of the psaAB operon upon transfer of dark-adapted cells to different light conditions in the presence and absence of electron transfer inhibitors. After 12 h in darkness, the cells were completely depleted of the dicistronic psaAB transcripts (Fig. 6A). Return of the dark-adapted cells to the (white) growth light induced a slow reappearance of the psaAB transcripts; no psaAB transcripts were detected after the first 15 min of light incubation, but thereafter the amount of the psaAB transcripts increased throughout the 2 h experiment. It is noteworthy that activation of psaAB transcription was completely blocked by the addition of the translation inhibitor lincomycin upon shift from dark to light (Fig. 6B). PSII-light was able to induce transcription of the psaAB operon, although in a slightly lower amount than the white growth light (Fig. 6A). In contrast, activation of psaAB transcription was partially suppressed upon transfer of cells from darkness to PSI-light, or to growth light in the presence of DCMU, whereas addition of DBMIB before the light shift completely abolished psaAB transcription. These results suggest that the active electron transfer chain plays a role in regulation of psaAB transcription activity. However, the slow activation of psaAB transcription suggested the involvement of additional regulatory signals beyond the sole redox regulation in this light activation.

Fig. 5 The abundance (A) and decay (B) of psaAB and psaA transcripts in the presence of electron transport inhibitors. The cell cultures were incubated under growth-light conditions in the presence of DBMIB or DCMU for the times as indicated. In (B), the cell cultures were additionally supplemented with rifampicin to inhibit transcription. The amounts of the psaAB and psaA transcripts were determined by Northern blot analysis with the psaA probe. (0) denotes the growth light sample without any additions. All figures are representatives of three independent experiments.

Fig. 6 Activation of psaAB transcription after dark adaptation. (A) After 12 h dark adaptation (D), cell cultures were transferred to growth light (GL), to PSII-light (PSII), to PSI-light (PSI) or to growth light in the presence of DCMU or DBMIB for 15, 60 or 120 min. In (B), dark-adapted (D) cells were transferred back to growth light in the absence (−) or presence (+) of the translation inhibitor lincomycin. After the treatments, the amount of the psaAB mRNA was determined by Northern blot analysis with the psaA probe. The figures are representatives of three independent experiments.

Fig. 7 Synthesis of the PsaA protein under different light and redox conditions. Cell cultures were given a 30 min pulse with [35S]methionine in darkness (D), under growth light (GL), high light (HL; 1,500 µmol of photons m−2 s−1), PSI-light (PSI), PSII-light (PSII), or under growth light in the presence of DCMU or DBMIB. Prior to the pulse, the cells were pre-incubated under the respective labeling conditions for 1.75 h (darkness), 45 min (high light) or 10 min (other treatments). The membrane proteins were separated by SDS–PAGE, transferred to a membrane and exposed to an X-ray film. The arrowheads denote the positions of the PsaA protein, determined by immunoblotting. The figure is a representative of three independent pulse-labeling experiments.
Translation of the PSI reaction center proteins under different light and redox conditions

In order to compare how the data obtained from psaAB transcription and transcript accumulation correlates with the rate of translation, we followed the synthesis of the PsA protein under different illumination conditions by an in vivo pulse-labeling technique. The PsA protein, recognized by a protein-specific antibody, migrated as an approximately 60 kDa band in SDS–PAGE, as was shown earlier by Sun et al. (1998). Under growth-light conditions, the PsA protein was readily labeled with radioactive methionine, indicating active PsA protein synthesis (Fig. 7). When the cells were labeled after a 1.75 h pre-incubation in darkness, the synthesis of PsA protein was severely repressed (Fig. 7). Similarly, incorporation of radioactivity into the PsA protein was only marginal under high-light conditions, under PSI-light and under growth light in the presence of DCMU or DBMIB. Illumination of the cells under PSI-light, in contrast, enhanced the synthesis of the PsA protein (Fig. 7).

Discussion

Transcriptional regulation of the psaAB operon

Although the modulation of PSI content has long been recognized as the main determinant of photosystem stoichiometry during acclimation of cyanobacteria to incident light, the molecular mechanisms regulating this process are still poorly understood. Recently, a number of microarray studies on Synechocystis sp. strain PCC 6803 (e.g. Hihara et al. 2001, Gill et al. 2002, Huang et al. 2002) have suggested that modulations in transcript abundance may be involved in regulation of PSI content during acclimation. In this work, we focused on the initial phase of light acclimation (up to 2 h) and show that alterations in the intensity and quality of light induce changes in the transcriptional activity of the psaAB operon, encoding the essential PSI reaction center proteins PsaA and PsaB. The Northern blot experiments revealed that several transcript species originate from the psaAB operon: in addition to the dicistronic psaAB transcript, we observed several smaller mRNA species, which were specifically recognized either by the psaA or psaB gene probe. The largest of these transcripts, the 2.6 kb psaA and 2.2 kb psaB (sizes estimated from Northern blots), were sufficiently long to encode full-length PsA or PsB proteins. Furthermore, the light-stimulated targeting of these psaA- (Fig. 2) and psaB- (data not shown) specific transcripts to membranes where the translation takes place (Klein et al. 1988, Tyystjärvi et al. 2001, Tyystjärvi et al. 2004) indicated that they were translationally competent. Therefore, these 2.6 kb psaA and 2.2 kb psaB mRNAs are likely to represent monocistronic psaA and psaB transcripts, respectively. The smaller mRNA fragments, on the other hand, were far too short to encode full-length polypeptides, and represented the degradation products of the psaA/B transcripts. When the transcription of the psaAB operon was inhibited, either by darkness (Fig. 1) or by rifampicin treatment (Fig. 3, 4D, 5B), the amount of the dicistronic psaAB transcripts started to decline, whereas the putative monocistronic psaA and psaB transcripts first increased in abundance and only later started to decrease with concomitant appearance of smaller degradation fragments. Therefore, it is conceivable that the dicistronic psaAB transcript represents the primary transcript of the psaAB operon, whereas the monocistronic psaA and psaB transcripts originate via processing of the psaAB transcript rather than being transcribed individually from separate promoters. The extensive processing of transcripts, combined with differential stabilization of various transcript forms and degradation fragments under different environmental conditions, calls for caution when interpreting the data from microarray experiments, and emphasizes the importance of additional methods in analyzing the function of the psaAB operon.

According to our results, light was an absolute requirement for transcription of the psaAB operon. The slow activation of psaAB transcription after exposure of dark-adapted cells to the light (Fig. 6A), however, suggests that this light activation process involves regulatory components other than a direct signaling from photoreceptors or a sole redox-mediated activation, both of which are supposed to act rapidly. The growth of cyanobacteria is halted in darkness, but recovers within hours upon introduction of light (Asato 2003). As the PSI components are relatively stable under standard growth conditions, it is conceivable that the slow activation of psaAB transcription upon onset of light is coupled to activation of growth and production of new PSI complexes. Interestingly, transcripts from the sigA gene, encoding the primary sigma factor in Synechocystis sp. PCC 6803, also become depleted in darkness and the transcription of the sigA gene is only slowly activated in the light (Tuominen et al. 2003). Comparison of the light activation kinetics, however, indicates that the light activation of the sigA gene (Tuominen et al. 2003) is more rapid than that of the psaAB operon (Fig. 6). It is therefore possible that the primary sigma factor is one of the de novo-synthesized protein factors that are required for light activation of the psaAB operon. Besides the psaAB operon, similar dark-induced down-regulation and slow, lincomycin-sensitive light activation have also been observed for various other genes encoding subunits of the PSI and PSII complexes (Alfonso et al. 2000, Li and Sherman 2000, Herranen et al. 2001, Gill et al. 2002), suggesting that this growth-related transcriptional regulation upon dark–light shifts may be common to most photosynthetic genes.

Once transcription of the psaAB operon was activated by light, a change in the quantity or quality of incident light was rapidly reflected in the transcription rate of this operon. An increase in the intensity of light induced a rapid down-regulation of psaAB transcription (Fig. 1). Furthermore, the stronger the light, the more the psaAB transcription was down-regulated. The decrease in psaAB transcription, together with fast degradation of transcripts under high light (Fig. 3), resulted in a strong diminution of all psaA/B transcript species, which were
likely to become limiting for translation of the reaction center proteins (Fig. 7), eventually leading to the decrease in the PSI/PSII ratio characteristic of acclimation to high-light irradiance (Kawamura et al. 1979, Hihara et al. 1998, Hihara 1999). The maintenance of a low PSI/PSII ratio under high light was suggested to require the PmgA protein (Muramatsu and Hihara 2003).

Besides high light, the transfer of cells to PSI-light also induced down-regulation of psaAB transcription (Fig. 4). Conversely, illumination of cells by PSI-light stimulated the transcription of the psaAB operon (Fig. 4). These light quality-induced changes in psaA transcription were accompanied by parallel changes in PsaA translation (Fig. 7), and were also parallel to the well-documented modulations in the PSI/PSII ratio under different light qualities (Fujita et al. 1985, Manodori and Melis 1986, Murakami and Fujita 1991, Fujita 1997). It is therefore conceivable that the adjustment of PSI content and photosystem stoichiometry are initially regulated at the level of transcription.

Although the light quality-dependent changes in psaAB transcription could, in theory, be mediated by wavelength-specific light receptors, the down-regulation of psaAB transcription upon the addition of electron transport inhibitors, without any change in illumination conditions (Fig. 5), renders this possibility unlikely. Rather, it appears that oxidation of some photosynthetic electron carrier(s), induced both by the inhibitors and by PSI-light, mediates the down-regulation of the psaAB operon. Supporting this conclusion, a phycobilisome-less mutant of Synechocystis sp. strain PCC 6803 is unable to modulate the expression of another PSI gene in a light quality-dependent manner (El Bissati and Kirilovsky 2001).

Considering the site of redox control of psaAB transcription in Synechocystis sp. strain PCC 6803, it is important to note that DCMU and DBMIB have opposite effects on the net redox state of the PQ pool in the light: oxidation by DCMU and reduction by DBMIB. The down-regulation of psaAB transcription by both electron transfer inhibitors excludes the PQ pool as the primary redox sensor for the regulation of psaAB transcription, and suggests that the regulatory site resides rather at or after the cytochrome b/f complex. In line with this suggestion, an inhibitor of cytochrome b oxidation was shown to suppress the stimulation of PSI accumulation in Synechocystis sp. strain PCC 6714 illuminated by PSII-light (Murakami and Fujita 1993). Accordingly, it was suggested that the redox state of cytochrome b is coupled to the regulation of PSI synthesis (Murakami and Fujita 1993, Fujita 1997). A role for the cytochrome b/f complex has also been suggested in the light-regulated transcription of some other photosynthesis-related genes in cyanobacteria (El Bissati and Kirilovsky 2001, Salem and van Waasbergen 2004). In plants, the redox signal for psaAB transcription has been proposed to originate from the PQ pool (Pfannschmidt et al. 1999, Tullberg et al. 2000). The reason for different regulatory signals in plants and cyanobacteria is unclear, but recent microarray studies have indeed revealed that the PQ pool has no significant role in regulation of photosynthesis-related genes in cyanobacteria (Hihara et al. 2003), thus being consistent with our data on psaAB gene expression.

**Post-transcriptional regulation of psaAB expression**

The half-lives of transcripts originating from the psaAB operon were strongly affected by illumination conditions, being increased in darkness (Fig. 3), in PSI-light (Fig. 4D) and under white light when the photosynthetic electron flow was inhibited chemically (Fig. 5B). This created a situation where the amount of transcripts, in particular the monocistronic species, remained high even though the transcription was down-regulated. The association of both di- and monocistronic transcripts with membranes in light-treated cells (Fig. 2) suggested that both transcript species are translationally competent. In the dark, however, the abundant psaA (Fig. 2) and psaB (data not shown) mRNAs were mainly detected in the cytosolic fraction of the cells, suggesting that translation of the reaction center proteins of PSI is inhibited in the dark. This finding is confirmed by the results of pulse-labeling experiments (Fig. 7), showing that newly synthesized PSI reaction center proteins do not accumulate in the thylakoid membranes of dark-treated cells. Therefore, it is conceivable that psaAB expression is also regulated at the level of translation. The exact regulatory level of psaA/psaB mRNA translation in cyanobacteria could not be identified as yet, but, in plastids of higher plants, the translation of psaA/psaB transcripts appears to be regulated at the level of translation elongation (Klein et al. 1988). In cyanobacteria, we have shown previously that the translation of the psbA mRNA, encoding the reaction center protein of PSI, is regulated at the elongation step by membrane targeting (Tyystjärvi et al. 2001, Tyystjärvi et al. 2004). It remains to be elucidated whether a similar mechanism controls the translation of the PSI reaction center proteins.

**Conclusion**

In summary, our results indicate that the psaAB operon is transcribed as a dicistronic transcript, which is thereafter processed into smaller, putatively monocistronic psaA and psaB transcripts. The light activation of psaAB transcription after dark treatment requires de novo protein synthesis, and may be related to activation of cell growth. Once transcription is active, it is rapidly modulated by both the intensity and the quality of light in a process that is influenced by the photosynthetic redox poise at or after the cytochrome b/f complex. Our data strongly suggests that the transcription of the psaAB operon is the main regulatory level in the biogenesis and acclimation of PSI, but the comprehensive regulation of psaAB expression also involves post-transcriptional regulation at the level of transcript stability and translation.
Materials and Methods

Strain and growth conditions
A glucose-tolerant strain of *Synechocystis* sp. strain PCC 6803 (Williams 1988) was grown in batch cultures of BG-11 medium (Stanier et al. 1971) buffered with 20 mM HEPES-NaOH, pH 7.5, at 32 °C under continuous illumination of 50 µmol of photons m⁻² s⁻¹. For the experiments, the cells were harvested at logarithmic growth phase (5–7 µg of Chl a ml⁻¹) by centrifugation, and resuspended in fresh BG-11 medium to a final Chl a concentration of 10 µg ml⁻¹.

Light treatments
High-light treatments were performed by illuminating the cell suspensions (10 µg of Chl a ml⁻¹) under 250 or 1,500 µmol of photons m⁻² s⁻¹, using a 250 W slide projector as the light source. For dark treatments, the cell suspensions were wrapped in aluminum foil. Illumination of cells with far-red light, absorbed mainly by PSI (PSI-light), was accomplished by filtering white light from the slide projector through a 700 nm long-pass filter (Corion LL-700, Corion, Frankfort, USA). The preferential excitation of PSI by the PSI-light (50 µmol of photons m⁻² s⁻¹), the cell suspensions were illuminated through Balzers K-3 and K-4 filters defining a band peaking at 580 nm. The preferential excitation of PSII by the PSII-light, as compared with the PSII-light, was verified by P700 absorption measurements at 820 nm using a PAM-101 device (Walz, Effeltrich, Germany) equipped with a P700DW unit as previously described (Herranen et al. 2001). The spectra of the PSI-light and PSII-light, measured by illuminating the sensor of a calibrated diode array spectrophotometer (S2000, Ocean Optics, Dunedin, FL, USA) with the same lamp and filters as used in the experiments, are shown in Fig. 4A.

Chemical treatments
The photosynthetic electron transfer chain was completely inhibited in vivo by addition of 15 µM DCMU or 100 µM DBMIB to the cell suspension (10 µg of Chl a ml⁻¹). When the stability of transcripts was analyzed, the initiation of transcription was inhibited with rifampicin (500 µg ml⁻¹). To inhibit translation initiation, the cell suspensions were supplemented with lincomycin at the final concentration of 400 µg ml⁻¹.

RNA isolation and Northern blot analysis
Total RNA was isolated by the hot-phenol method as previously described (Tyysjärvi et al. 2001). To separate cytotoxic and membrane-bound RNAs, the cells were first fractionated into cytotoxic and membrane fractions as follows. The cell pellet containing 50 µg of Chl a was first washed with washing buffer (0.4 M sucrose, 30 mM EDTA, 10 mM MgCl₂, 50 mM Tris–HCl, pH 8.5, 0.5 mg ml⁻¹ chloramphenicol) and subsequently with isolation buffer (washing buffer without MgCl₂) by centrifugation, and resuspended in fresh BG-11 medium to a final Chl a concentration of 10 µg ml⁻¹.

The cells were washed further with isolation buffer containing 600 mM KCl to release the ribosomes (and RNA) that were associated with membranes solely by ionic interactions (Friedmann and Hachtel 1988). For RNA isolation, the membrane pellet was resuspended in RNA buffer (0.1 M sucrose, 25 mM EDTA, 10 mM sodium acetate, pH 4.5, 1.2% SDS), whereas the cytotoxic fraction was supplemented with 25 mM EDTA, 10 mM sodium acetate, pH 4.5, and 1.2% SDS. All samples were then extracted twice with phenol–chloroform (1:1) and once with chloroform, after which the RNAs were precipitated from the aqueous phase with 2 M LiCl and 70% ethanol and collected by centrifugation at 18,000 × g for 20 min at 4 °C.

The RNAs were separated on 1.2% agarose–glyoxal gels and subsequently transferred to Hybond-N membrane (Amersham, Buckinghamshire, UK) according to standard procedures (Sambrook and Russell 2001). A 4 µg aliquot of total RNA was loaded per lane. In studies of membrane and cytotoxic fractions, all RNAs rescued from each fraction (starting with a cell pellet containing 50 µg of Chl a) was loaded per lane. For size determination, RNA Marker (Promega, Southampton, UK) was run alongside the samples. Equal loading of gels and even transfer of RNAs to the membranes were verified by staining the membranes with methylene blue (Sambrook and Russell 2001) after the blotting.

The gene-specific probes were amplified from genomic DNA of *Synechocystis* sp. strain PCC 6803 by PCR. The *psaA*-probe, representing a 979 bp fragment from the 3′ end of the *psaA* coding region (1,277–2,256 bp downstream of the start codon), was amplified with the primer pair 5′-GGATCGGATGCTGCGCCAC-3′ (forward primer) and 5′-CTAGCAATGGAAAGACTGC-3′ (reverse primer). The *psaB* probe, representing a 1,113 bp fragment from the 5′ end of the *psaB* coding region (39–1,151 bp downstream of the start codon), was obtained with the forward 5′-CCAAAGCCCCACTACGGCGT-3′ and reverse 5′-GCACCAACCATCAAGATTCCAG-3′ primers. The probes were labeled with α-[32P]dCTP (3,000 Ci mmol⁻¹) using the Multiprime DNA labelling kit (Amersham, Buckinghamshire, UK). Pre-hybridization was performed in 6× SSC (1× SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 0.5% SDS, 5× Denhardt’s (1× Denhardt’s is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 200 µg ml⁻¹ denaturated herring sperm DNA, for 2 h at 60 °C. Thereafter, the labeled probe was added, and hybridization was allowed overnight at 60°C. The membranes were washed twice with 3× SSC, 0.1% SDS at 45°C for 5 min, and wrapped in saran wrap and exposed to X-ray film at -80°C. All Northern blot experiments were repeated three times with RNA isolated from independent cell cultures.

In vivo pulse labeling of membrane proteins
Synthesis of membrane proteins in vivo was analyzed by pulse-labeling experiments under the following conditions: darkness; growth light in the presence or absence of 15 µM DCMU or 25 µM DBMIB; high light (1,500 µmol of photons m⁻² s⁻¹), using a 250 W slide projector as the light source. For dark treatments, the cell suspensions were wrapped in aluminum foil. Illumination of cells with far-red light, absorbed mainly by PSI (PSI-light), was accomplished by filtering white light from the slide projector through a 700 nm long-pass filter (Corion LL-700, Corion, Frankfort, USA). The preferential excitation of PSII by the PSII-light, as compared with the PSII-light, was verified by P700 absorption measurements at 820 nm using a PAM-101 device (Walz, Effeltrich, Germany) equipped with a P700DW unit as previously described (Herranen et al. 2001). The spectra of the PSI-light and PSII-light, measured by illuminating the sensor of a calibrated diode array spectrophotometer (S2000, Ocean Optics, Dunedin, FL, USA) with the same lamp and filters as used in the experiments, are shown in Fig. 4A.
was increased to 120 V for 3 h. After electrophoresis, the proteins were electroblotted onto a PVDF membrane (Immobilon P, Millipore, Bedford, MA, USA), and the membrane was exposed to X-ray film for autoradiography. The PsA protein was immunodetected with a PsA-specific antibody (a generous gift from Professor Parag Chitnis) using a chemiluminescence kit (Bio-Rad). The pulse-labeling experiments were repeated three times with independent cell cultures.

Acknowledgments

We thank Marja Hakala for help with P700 redox measurements, and Dr. Paula Mulo for invaluable comments on the manuscript. This work was supported by the Academy of Finland, the Finnish Cultural Foundation and Biological Interactions graduate school.

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(Received March 15, 2005; Accepted June 16, 2005)