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Light-regulation of photosystem I reaction centre genes in the cyanobacterium *Synechocystis* sp. PCC 6803

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Introduction

Adjustment of photosystem stoichiometry in response to changes in the light environment is a well-documented phenomenon in cyanobacteria (Fujita, 1997). This adjustment, manifested as a change of photosystem I to photosystem II ratio (PSI/PSII), operates to optimise the efficiency of photosynthesis according to the prevailing light conditions (Murakami and Fujita, 1988) and to protect the cells from photodamage under high irradiance (Sonoike et al., 2001). The change of the PSI/PSII ratio has been reported to occur by modulation of the synthesis of PSI at translational and/or post-translational levels (Aizawa and Fujita, 1997; Fujita, 1997). Transcriptional regulation, on the other hand, has long been neglected. The *psaA* and *psaB* genes, encoding the PSI reaction centre proteins PsaA and PsaB, respectively, are cotranscribed as a dicistronic transcript, which is further processed into monocistonic *psaA* and *psaB* transcripts (Smart and McIntosh, 1991). Here we have addressed the role of transcriptional regulation in the process of PSI synthesis and studied the modulation in *psaAB* transcription and transcript stability under various light and redox conditions in the cyanobacterium *Synechocystis* sp. PCC 6803.

Materials and methods

A glucose-tolerant strain of *Synechocystis* sp. PCC 6803 was grown in BG-11 medium under continuous illumination of 50 μmol photons m^{-2} s^{-1} at 32 ºC. High-light treatments were performed by illuminating the cell cultures under 250 (medium light) or 1500 μmol photons m^{-2} s^{-1} (high light). Illumination of the cells with light exciting preferentially PSI (PSI-light, >700 nm) or PSII (PSII-light, 15-nm HWHM band peaking at 580 nm) was carried out as described earlier (Herranen et al., 2001). The photosynthetic electron flow was inhibited *in vivo* by either 15 μM DCMU or 100 μM DBMIB in the presence of 5mM glucose. When analysing the stability of the transcripts, the initiation of transcription was inhibited with rifampicin (500 μg/ml).

Isolation of total RNA and Northern blot analysis were performed as described in Tyystjärvi et al. (2001).

Results

Light intensity modulates the expression of the psaAB operon

Transfer of *Synechocystis* cells from growth light to darkness induced a gradual loss of the dicistronic *psaAB* transcripts (Fig. 1A). The monocistronic *psaA* transcripts, on the contrary, accumulated in high amounts during the dark incubation. When the cells were transferred

from growth light to higher irradiances, the amounts of both the *psaAB* and *psaA* transcripts decreased.

The stability of the dicistronic and particularly of the monocistronic transcripts increased remarkably upon transfer of the cells from light to darkness (Fig. 1B). The half-lives of the transcripts were practically the same under the growth-light and high-light intensity. Taken together, these results demonstrate transcriptional down-regulation of the *psaAB* operon in the dark and under high irradiance.

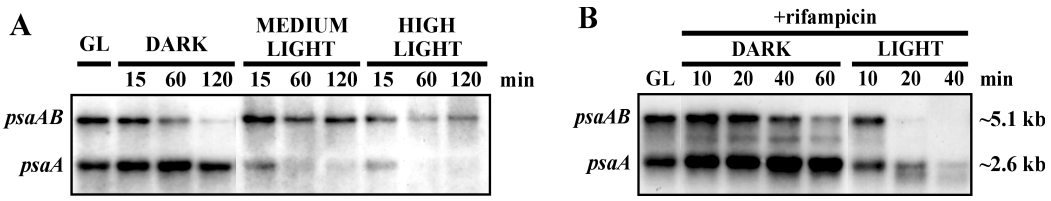

Fig. 1. Changes in the abundance (A) and stability (B) of the *psaAB* and *psaA* transcripts with respect to changes in light intensity. GL, growth light, 50 µmol photons m^{-2} s^{-1}; medium light, 250 µmol photons m^{-2} s^{-1}; high light, 1500 µmol photons m^{-2} s^{-1}.

Light quality modulates the expression of the psaAB operon

Illumination of the cells under PSI-light induced a decrease in the amount of *psaAB* transcripts (and hence also in the amount of the *psaA* transcripts; Fig. 2A) which, however, became more stable. Collectively, these results thus indicate that the transcription of the *psaAB* operon is down-regulated in the light favouring PSI excitation. Conversely, under PSII-light the amount of the *psaAB* transcripts increased (Fig. 2B) despite of rapid degradation of the transcripts, indicating transcriptional up-regulation of the *psaAB* operon in PSII-light. Following the increase in *psaAB* transcripts, also the amount of *psaA* transcripts increased.

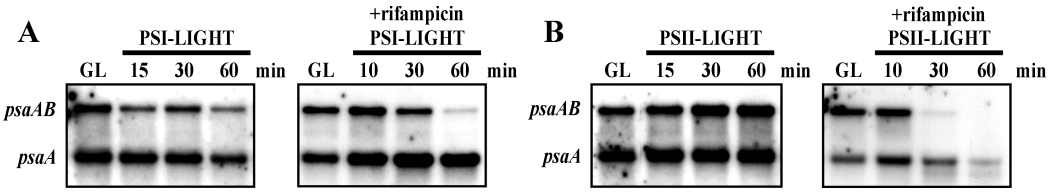

Fig. 2. The abundance and decay (+rifampicin) of *psaAB* and *psaA* transcripts under PSI-light (A) and PSII-light (B). GL denotes the growth light conditions.

Inhibition of photosynthetic electron flow down-regulates psaAB transcription

Addition of photosynthetic electron transfer inhibitor, DCMU or DBMIB, under growth light conditions induced a decrease in *psaAB* transcripts but also stabilized the transcripts (Fig. 3). This suggests that the photosynthetic electron flow plays a role in modulation of *psaAB* transcription and mRNA stability.

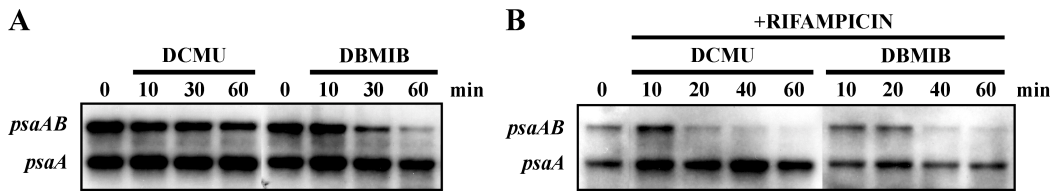

Fig. 3. Effect of inhibition of photosynthetic electron transport under growth light conditions on the amount (A) and stability (B) of *psaAB* and *psaA* transcripts.

Light activates the psaAB transcription only slowly

Next, to study how rapidly the *psaAB* transcription is activated in the light, we followed the accumulation of *psaAB* transcripts in cells transferred back to the growth light after a 12-hour dark-incubation. No transcripts were detected after a 15-min light-treatment (Fig. 4). Thereafter, the *psaAB* transcripts started to accumulate rather slowly during the 2-hour experiment. Inhibition of translation with lincomycin totally prevented the light-induced transcriptional activation, suggesting an involvement of transcriptional activator proteins.

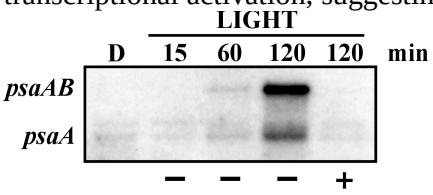

Fig. 4. Light-activation of *psaAB* gene transcription. Cells were transferred back to the light after a 12-hour dark-incubation (D) in the absence (-, below the figure) and presence (+, below the figure) of lincomycin.

Discussion

Biosynthesis of PSI was long believed to be regulated solely at the level of translation and/or assembly (Fujita, 1997). Our results, however, clearly demonstrate the involvement of transcriptional regulation in the biosynthesis of PSI complexes during acclimation. In line with our results, recent microarray analysis has demonstrated changes in the steady-state *psaA* and *psaB* mRNA levels in response to changes in the light intensity (Hihara et al., 2001). We extend these results further by showing that the changes observed in the steady-state transcript level are due to changes in the transcription of the *psaAB* operon which, in turn, occurred parallel to the well-known modulations in the accumulation of PSI complexes under various light conditions (Fujita, 1997). Moreover, regulation of transcript stability by the intersystem redox poise appears to be an important factor in determining the steady-state transcript levels in *Synechocystis* cells under particular environmental conditions.

Transcription of the *psaAB* operon is strictly dependent on light. The dark-induced down-regulation of *psaAB* transcription could not be counteracted by addition of glucose to the growth medium (data not shown), thus excluding starvation as a potential cause for inhibition of *psaAB* transcription in the dark. Transcription of the *psaAB* operon was likewise down-regulated when the light intensity increased above the growth light intensity. Such decrease in transcription occurs parallel to the decrease in the accumulation of PSI complexes (Hihara et al., 1998), which has been proposed to play a role in protection of the cells against photodamage (Sonoike et al., 2001).

The opposing effects of PSI- and PSII-light on the transcription of the *psaAB* operon suggested that the redox state of an intersystem electron carrier plays a role in regulation of the *psaAB* operon. Experiments employing electron transfer inhibitors further suggested that such redox component resides at or after the cytochrome b_6f complex, as has been suggested also for the regulation of PSI synthesis at the (post)translational level (Fujita, 1997). Recently, a two-component regulatory system RppB-RppA was proposed to mediate the signal of the

intersystem redox status to the *psaAB* transcription in *Synechocystis* (Li and Sherman, 2000). On the other hand, the slow light-activation of *psaAB* transcription after a long dark-treatment implies that the redox state of the photosynthetic electron transfer chain is not the sole determinant for the transcription of the *psaAB* operon. It is thus conceivable that two distinct mechanisms are involved in the regulation of *psaAB* transcription. One system, mediated via transcriptional activators, may be responsible for the production of new PSI centres during the cell growth. The other system, responding to the redox state of the photosynthetic electron transfer chain, may operate to balance the synthesis of PSI upon rapid changes in the light environment.

Acknowledgments

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