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Influence of light on accumulation of photosynthesis-specific transcripts in the cyanobacterium *Synechocystis* 6803

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Abstract

Transcript accumulation for the *psbA*, *psbD*, *psbD-C*, *rbcL-S* and *rrn* genes in *Synechocystis* 6803 was followed under different light conditions. *psbA*, *psbD*, *psbD-C* and *rbcL-S* transcripts required light to accumulate and the relative abundance of these transcripts differed between high and low light conditions. Under high light conditions, steady-state levels of *psbA*, *psbD* and *psbD-C* transcripts were higher while levels of *rbcL-S* transcripts were lower than under low light conditions. *rrn* transcripts accumulated in the dark and the transcript levels were the same under illuminated conditions. Analyses of constructed *Synechocystis* 6803 mutants showed that both *psbA*-2 and *psbA*-3 could produce high levels of transcripts under illuminated conditions. No *psbA*-1 transcripts were detected.

Introduction

Biogenesis of Photosystem (PS) II displays an intricate pattern of light regulation. The reaction center polypeptide D1 is influenced by light at different levels (for a review, see [19]). Synthesis of this polypeptide as a precursor form is stimulated by light [7, 14, 17]. A light-induced acylation of the D1 polypeptide was reported from studies on the aquatic plant *Spirodella* [18]. Furthermore, degradation of the D1 polypeptide is substantially accelerated by light [10, 19, 29]. The mechanisms behind these different light responses are largely unknown.

The D1 polypeptide is encoded by the *psbA* gene. In plants and algae this gene is located on the plastid genome. Expression of the *psbA* gene seems to be light-regulated mainly at the level of translation [14, 17]. Whether there is light regulation also at the level of transcription is not clear. Analyses of plastid RNA have provided support both for [12, 15] and against [14, 17] strict transcriptional light regulation of the *psbA* gene. Little has been reported on the effects of light on *psbA* transcript accumulation in the prokaryotic cyanobacteria. It was shown in a recent study that the amount of *psbA* transcripts in *Anacystis nidulans* was considerably higher in cells grown under high than under low light conditions [16].

We are studying the influence of light on synthesis and degradation of the D1 polypeptide in the cyanobacterium *Synechocystis* 6803. In the present work we looked at steady-state levels of *psbA* transcripts in *Synechocystis* 6803 cells incubated in the dark or in two different light regimes. As a comparison to the *psbA* gene we also studied transcript levels for the *psbD*, *psbC*, *rbcL*, *rbcS* and *rrn* genes. The *psbD* gene encodes the PSII

reaction center polypeptide D2, the *psbC* gene the PSII polypeptide CP43, the *rbcL* and *rbcS* genes the large and small subunit, respectively, of the Calvin-Benson cycle enzyme ribulose-bisphosphate carboxylase (Rubisco), and the *rrn* genes the 23, 16 and 5 S ribosomal RNAs. Since *Synechocystis* 6803 [13], like other cyanobacteria [4, 8, 20], has multiple *psbA* genes we studied the individual *psbA* copies by looking at transcript levels in constructed *Synechocystis* 6803 mutants with only one intact *psbA* gene.

Material and methods

Strains and growth conditions

Synechocystis 6803-G [30] was used. The cells were grown in BG 11 medium as previously described [13] with the following exceptions. An orbital shaking incubator (Gallenkamp) was used for liquid cultures. Constant illumination was provided by a mixture of warm fluorescent and daylight-simulating light tubes, with a total photon flux density of 70 μE m^{-2} s^{-1} for wild-type and 50 μE m^{-2} s^{-1} for mutants. For the different *psbA* mutants the medium was supplemented with antibiotics (Sigma) and glucose as follows: A3: 5 μg ml^{-1} kanamycin, 10 μg ml^{-1} streptomycin, 20 μg ml^{-1} spectinomycin; A2: 5 μg ml^{-1} kanamycin, 5 μg ml^{-1} chloramphenicol; A1: 10 μg ml^{-1} streptomycin, 20 μg ml^{-1} spectinomycin, 5 μg ml^{-1} chloramphenicol, 10 mM glucose.

Dark and light incubations

Cells were grown to mid-log phase ($A_{730nm} \approx 0.6$) under different growth conditions. For dark incubation, cultures were wrapped in aluminium foil and incubated further for 48 hours under otherwise normal growth conditions. For low-light incubations, 120 ml aliquots were taken from dark cultures and incubated for 20 hours at room temperature (25 °C) under an average incident photon flux density of 50 and 1500 μE m^{-2} s^{-1}, respectively. Illumination was provided by a 250 W projector lamp. During illumination, cells were stirred continuously and the temperature was maintained at 25 °C. The doubling time for low-light and high-light cultures was 8.9 and 8.3 hours, respectively.

Construction of mutants

Construction of *Synechocystis* 6803 mutants with only one intact *psbA* gene was performed by insertional mutagenesis, following the protocol outlined previously [13].

RNA extraction

RNA was extracted from 100 ml of *Synechocystis* 6803 cultures at $A_{730nm} \approx 0.5$. The cells were harvested by centrifugation at 6000 × ***g*** for 10 min together with 50 ml crushed ice in a 250 ml centrifuge bottle. The cell pellet was frozen in liquid nitrogen and thawed on ice. The freeze-thaw cycle was repeated once. The cells were suspended in resuspension buffer (0.3 M sucrose, 10 mM sodium acetate, pH 4.5), transferred to an Eppendorf tube and pelleted at 12000 × ***g*** for 5 min. The pellet was suspended in 250 μl resuspension buffer with 75 μl 250 mM Na_2-EDTA, and the suspension was incubated on ice for 5 min. 375 μl lysis buffer (2% (w/v) SDS, 10 mM sodium acetate, pH 4.5) was added, followed by incubation at 65 °C for 3 min. 700 μl hot (65 °C) phenol (redistilled, molecular biology grade, IBI) was added to the lysed cells, followed by incubation at 65 °C for 3 min and then at −70 °C for 15 s. The suspension was centrifuged at 12000 × ***g*** for 5 min, the upper phase was collected and the hot phenol treatment was repeated twice, followed by an extraction with hot phenol : chloroform (1 : 1). 1/5 volume of 10 M LiCl and 2.5 volumes of 99% EtOH was added and the RNA was precipitated at −20 °C for 30 min. The pellet was washed with 80% EtOH, suspended in H_2O and stored in aliquots at −70 °C. If necessary, the RNA preparation was

treated with RNase-free DNase (Promega). Solutions used for RNA extraction were treated with 0.1% (v/v) diethyl pyrocarbonate (Sigma) according to instructions from the manufacturer. Glassware was treated with 0.1% diethyl pyrocarbonate and baked. The average RNA yield per 100 ml culture at $A_{730nm} \approx 0.5$ was 130 μg for dark cultures and 150 μg for low-light and high-light cultures. The cell size was similar for all three cultures and, as an approximation, we can therefore assume that the RNA content per cell did not vary significantly among the different cultures.

RNA electrophoresis and Northern blot analysis

Separation of RNA on formaldehyde gels, transfer to nylon membranes (Hybond N, Amersham), prehybridization and hybridization conditions were mainly according to instruction manuals from Amersham. The formamide (IBI) was deionized with Amberlite MB3 (Rohm and Haas). Prehybridization was done at 42 °C for 4 h and hybridization at 42 °C for 18 h. After hybridization the filters were washed four times for 10 min each in 2 × SSPE (20 × SSPE = 3.6 M NaCl, 0.2 M NaH_2PO_4 pH 7.7, 20 mM Na_2-EDTA) with 0.1% (w/v) SDS at room temperature, followed by two washes for 45 min each in 1 × SSPE with 0.1% SDS at 66 °C. Filters were air-dried and exposed to X-ray films (Fuji) with intensifying screens at − 70 °C for 6 h (*psbA* and *psbD* probes), 18 h (*rbcL* probe) or 16 h (*rrn* probe).

The same filter was used for different probes. Prior to rehybridizing with a new probe the old probe was washed off by pouring boiling H_2O containing 0.1% SDS over the filter and leaving it at room temperature for 30 min. This treatment was repeated as required. Removal of label was confirmed by exposing the washed filter to an X-ray film for 6–24 h depending on the probe.

The following DNA probes were used: An equal mixture (on a molar basis) of internal fragments of the *psbA*-1 and *psbA*-2 genes from *Synechocystis* 6803 (the latter fragment covering a region with high homology to *psbA*-3), isolated from plasmids pKW 1214 and pKW 1266, respectively [13], provided by John Williams and Lee McIntosh, Michigan State University; an internal fragment of the *psbD*-2 gene from *Synechocystis* 6803, isolated from plasmid pRD 655 [5], provided by Richard Debus and Lee McIntosh, Michigan State University; a fragment containing 5′ coding and non-coding regions of the *rbcL* gene from *Synechocystis* 6803, isolated from plasmid pSF 2.6, provided by Thomas Reich and Lee McIntosh, Michigan State University; the pAN4 plasmid [28] containing the *rrn* genes from *Anacystis nidulans* provided by Petter Gustavsson and Jonas Lidholm, University of Umeå. The probes were radioactively labelled using a multiprime DNA labelling kit from Amersham. A total activity of around 10^8 cpm was added per hybridization.

Additional conditions are given in the legend to Fig. 1.

Results

Transcript levels in different light regimes in Synechocystis *6803 wild-type cells*

RNA isolated from dark, low-light and high-light cultures was subjected to Northern blot analyses. The results are shown in Fig. 1.

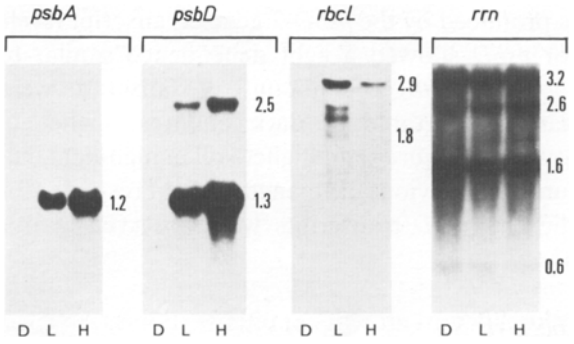

Fig. 1. Steady-state levels of transcripts in *Synechocystis* 6803 wild-type cells. RNA was isolated from cultures incubated either in the dark (D) for 48 hours or in low light (L) (50 $\mu E\, m^{-2}\, s^{-1}$) or high light (H) (1500 $\mu E\, m^{-2\ -1}$) for 20 hours. The RNA was separated on formaldehyde gels, transferred onto nylon membranes and hybridized with ^{32}P-labelled *psbA*, *psbD*, *rbcL* or *rrn* probes. 15 μg of total RNA was loaded per lane. Sizes in kb are indicated.

psbA.
Illuminated cultures of *Synechocystis* 6803 accumulated high levels of a 1.2 kb *psbA* transcript. This size corresponds to a full-length *psbA* transcript. The amount of *psbA* transcripts was considerably higher in high-light cultures than in low-light cultures. In contrast, no *psbA* transcripts were detected in dark cultures.

psbD.
In plant chloroplasts the *psbD* gene overlaps the open reading frame of the *psbC* gene, and the two genes are cotranscribed [1, 11]. In *Synechocystis* 6803 there are two different copies of the *psbD* gene, *psbD-1* and *psbD-2* [30]. Analogous to the situation in plants, the *psbD*-1 gene in *Synechocystis* 6803 overlaps the *psbC* gene [3]. The same arrangement was found for one of the two *psbD* genes in *A. nidulans* [9]. Furthermore, it was demonstrated that the overlapping *psbD* and *psbC* genes in *A. nidulans* were transcribed as a dicistronic message as in plant chloroplasts, while the other *psbD* gene was transcribed as a monocistronic message [9].

The Northern blot analyses in Fig. 1, show two *psbD* transcripts, of 2.5 and 1.3 kb. The transcript sizes are similar to those reported for *A. nidulans* [9]. This suggests that the 2.5 kb transcript in *Synechocystis* 6803 is produced by the overlapping *psbD*-1/*psbC* genes and that the 1.3 kb transcript is produced by the *psbD*-2 gene. Transcript levels for *psbD* showed a light dependence similar to those for *psbA*. The amounts of transcripts were barely detectable in dark cultures, higher in low-light cultures and higher still in high-light cultures. No obvious differences in light response for the two *psbD* transcripts were observed in this assay.

rbcL, *rbcS*.
Plant and chlorophytic algal *rbcL* and *rbcS* genes are located in different compartments, *rbcL* in the plastid genome and *rbcS* in the nuclear genome. In the two cyanobacteria studied to date, *A. nidulans* [27] and *Anabaena* 7120 [21], the *rbcL* and *rbcS* genes are organized in a single operon with *rbcS* located downstream of *rbcL*. In Northern blot analyses of *Synechocystis* 6803 RNA, four transcripts, ranging in size from 2.9 to 1.8 kb, hybridized to a 5′ fragment of the *rbcL* gene (Fig. 1). The major transcript of 2.9 kb corresponds in size to an *rbcL-S* dicistronic message [21]. This suggests that the *rbcL* and *rbcS* genes form an operon also in *Synechocystis* 6803. The identity of the smaller transcripts are uncertain, but they may be cleavage products of the full-length transcript.

As for the *psbA*, *psbD* and *psbD-C* genes, transcripts of the *rbcL-S* genes required light to accumulate (Fig. 1). A notable difference, however, is that *rbcL-S* transcript levels were lower in high-light cultures than in low-light cultures.

rrn.
A DNA fragment containing the *rrn* operon from *A. nidulans* recognized four transcripts in RNA isolated from *Synechocystis* 6803 (Fig. 1). The 3.2, 1.6 and 0.6 kb transcripts correspond to 23S, 16S and 5S rRNA, respectively. The 2.6 kb transcript is a cleavage product of the 23S rRNA [6].

In contrast to the photosynthesis genes, transcripts of the *rrn* genes were present in dark cultures and the transcript levels did not change significantly in cultures transferred to low-light or high-light environments. Since rRNA accounts for $>80\%$ of the total RNA in each preparation it is important to emphasize that the RNA yield per cell was approximately the same for all three treatments.

Transcript levels in mutants with only one intact psbA *gene*

In cyanobacteria, *psbA* belongs to multigene families with two or more copies [4, 8, 13, 20]. In *Synechocystis* 6803 there are three different *psbA* genes, *psbA*-1, *psbA*-2 and *psbA*-3 [13]. The DNA sequences for the *psbA*-1 and *psbA*-2 genes have been published [22, 25]. Ongoing sequencing of the *psbA*-3 gene shows that *psbA*-2 and *psbA*-3 are very homologous and may encode identical D1 polypeptides, whereas the *psbA*-1 gene is more divergent. To assess the capacity for each of these *psbA* genes to produce transcripts under different

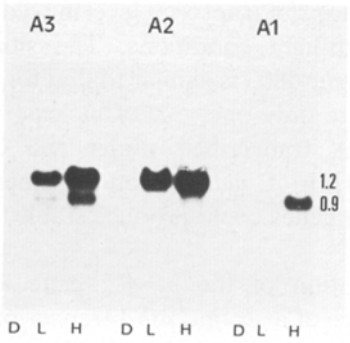

Fig. 2. Steady-state levels of *psbA* transcripts in *Synechocystis* 6803 mutants. Conditions are described in Fig. 1.

light regimes, Northern blot analyses were conducted using RNA isolated from *Synechocystis* 6803 mutants with only one intact *psbA* gene. The mutants were constructed by *in vitro* mutagenesis of two of the three *psbA* genes. The following terminology is used, stating the mutants and the remaining, intact *psbA* gene in parenthesis: A1 (*psbA*-1), A2 (*psbA*-2), A3 (*psbA*-3).

Our initial characterization showed that mutants A2 and A3 grew photoautotrophically, with growth rates comparable to those of the wild-type. In contrast, mutant A1 lacked oxygen-evolving activity and had to be grown photoheterotrophically.

Mutants A2 and A2 showed a similar light-stimulated accumulation of the 1.2 kb *psbA* transcript as did the wild-type, whereas no full-length *psbA* transcript could be detected in mutant A1 (Fig. 2). In mutants A3 and A1 a smaller transcript of 0.9 kb was seen in addition to the full-length *psbA* transcript. This is a truncated transcript originating from the inactivated *psbA*-2 gene (see Discussion).

All mutants, including A1, produced *psbD*, *psbD-C*, *rbcL-S* and *rrn* transcript levels comparable to those of wild type, under dark and illuminated conditions, respectively (not shown).

Discussion

Synechocystis 6803 can grow both photoautotrophically and photoheterotrophically, in the latter case with glucose as reduced carbon source. The photoheterotrophic growth requires PSI but not PSII activity. Why PSI is needed and what parts of this photosystem are actually involved is not known. *Synechocystis* 6803 can also utilize glucose in the dark but this chemoheterotrophic growth is usually very slow with a generation time of more than 80 h [30]. Chemoheterotrophic growth of *Synechocystis* 6803 with a generation time as short as 19 h has been reported, however [2]. These discrepant results may be due to different varieties of the *Synechocystis* 6803 strain used in different laboratories, or to differences in growth conditions. In the present work, *Synechocystis* 6803 cells (with the exception of mutant A1) were incubated in the dark without glucose. Under such conditions the cells rely on internal polyglucose stores (probably glycogen) for their dark respiration. It should be mentioned that cells incubated in the dark for 48 hours were fully viable when they were returned to illuminated conditions. The growth rate for such cells was comparable to that for cells grown continuously in the light.

Influence of light on transcript levels

No *psbA*, *psbD*, *psbD-C* or *rbcL-S* transcripts were detected in dark-incubated cells, despite high levels of *rrn* transcripts (Fig. 1). We do not know the turnover rates for *rrn* transcripts in *Synechocystis* 6803, and hence cannot rule out that these transcripts are stable during 48 h in the dark. In *Anacystis nidulans* the half-life for the 23S RNA was found to be 10 h in the dark [6]. If the value is similar for *Synechocystis* 6803, then the data in Fig. 1 suggest that the *rrn* genes were transcribed throughout the dark incubation. A massive increase in total RNA upon illumination would invalidate this conclusion. However, as suggested earlier, the yield of isolated RNA from dark cultures was ca. 85% of that from illuminated cultures, on a per cell basis.

Cells grown under illuminated conditions accumulated high levels of *psbA*, *psbD*, *psbD-C* and *rbcL-S* transcripts (Figs. 1). The light

intensity used for our high-light conditions (1500 μE m^{-2} s^{-1}) was chosen to represent an intermediate light irradiance at the water surface under natural conditions. Such high irradiances by far exceeds the optimum light intensities (50–100 μE m^{-2} s^{-1}) for temperate freshwater cyanobacteria under laboratory conditions [31]. It has been demonstrated for cyanobacteria [23, 26], as well as for plants and algae [23], that exposure to high light intensities results in photoinhibition of photosynthesis (for a review, see [23]). Such photoinhibition causes loss of oxygen-evolving activity and rapid degradation of the D1 polypeptide [19, 29], and with increasing light intensities also of the D2 polypeptide [19]. Concomitant with photoinhibition is an adaptation to high light intensities [26]. The nature of this adaptation is unknown but it may involve intensified synthesis of the D1 polypeptide and possibly other PSII polypeptides [26].

Thus, it is conceivable that the higher levels of *psbA*, *psbD* and *psbD-C* transcripts under high-light as compared to low-light conditions were required to meet an accelerated turnover of the D1 and D2 polypeptides. A similar requirement would not be expected for *rbcL-S* transcripts. Why the transcript levels for *rbcL-S* were lower under high light than under low light conditions (Fig. 1) is not obvious, but it could be due to an increased degradation rate at higher light intensities. It has been shown previously that *rbcL* transcript levels in barley seedlings declined with time much more rapidly in light than in the dark, while *psbA* transcript levels were maintained under both conditions [14].

Transcript accumulation in psbA *mutants*

Analyses of the *psbA* mutants (Fig. 2) demonstrated that both *psbA*-2 and *psbA*-3 could produce high levels of transcripts. In contrast, no transcripts from the *psbA*-1 gene could be detected. Either this gene was not transcribed or the transcripts were rapidly degraded. Our detection limit, based on densitometric measurements of the autoradiograms, was approximately 0.2% of the *psbA* transcript level in wild-type cells under high-light conditions. The situation for *Synechocystis* 6803 is similar to that for *Anabaena* 7120 where only one, out of at least two, *psbA* genes was transcribed under the conditions studied [4]. In *A. nidulans*, on the other hand, all three *psbA* genes could produce stable transcripts [8].

Inactivation of the *psbA*-2 gene led to the appearance of a second *psbA* transcript of 0.9 kb in mutants A3 and A1 (Fig. 2). The *psbA*-2 gene was inactivated *in vitro* by insertional mutagenesis at a unique *Nco* I site, around 200 nucleotides from the 3′ end of the gene [13]. The insertional element was the Ω fragment, which contains antibiotic resistance genes flanked by transcription and translation termination signals [24]. In Northern blot analyses of RNA from mutant A3 the 0.9 kb transcript hybridized to an internal fragment of the *psbA*-2 gene specific for the region 5′ of the *Nco* I site but not to a fragment specific for the region 3′ of the *Nco* I site (not shown). We infer that the 0.9 kb transcript is a truncated *psbA*-2 transcript terminated and stabilized by the transcription termination signals in the Ω fragment. This conclusion is strengthened by the observation that insertional mutagenesis of the *psbA*-3 gene at the conserved *Nco* I site, using an element that lacks transcription termination signals [13], did not result in a second *psbA* transcript in mutant A2 (Fig. 2). Also, the Ω fragment was used to construct a truncated *psbD* transcript in *A. nidulans* [9].

It is worth noting that the abundance of truncated *psbA*-2 transcripts fluctuated with light, with a pattern similar to that for full-length *psbA* transcripts (Figs. 1 and 2). The foreign 3′ end of the truncated *psbA*-2 transcript is unlikely to have any regulatory sequences in common with the 3′ end of the full-length *psbA* transcript. We suggest, therefore, that the light-mediated changes in *psbA* transcript levels observed in this work were caused by differences related to the 5′ end of the gene (i.e. differences in transcript production) rather than the 3′ end (i.e. differences in transcript stability).

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Note added in proof

For *Synechococcus* PCC 7942 it has been demonstrated that expression of one of the three *psbA* genes increased with decreased light intensity while expression of the other two *psbA* genes decreased. (Schaefer MR, Golden SS: Differential expression of members of a cyanobacterial *psbA* gene family in response to light. J Bact 171: 3973–3981 (1989)).