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Expression of photosynthesis genes in the cyanobacterium *Synechocystis* sp. PCC 6803: *psaA-psaB* and *psbA* transcripts accumulate in dark-grown cells

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Abstract

We have cloned and sequenced the *psaA* and *psaB* genes from the unicellular cyanobacterium *Synechocystis* sp. PCC 6803. These genes are arranged in tandem, are co-transcribed, and are highly homologous to the *psaA* and *psaB* genes previously characterized. RNA was isolated from light-grown cells, from cells put in total darkness with and without glucose, and from cells grown under light-activated heterotrophic growth (LAHG) conditions. Quantitation of hybridization to northern blots revealed only a slight decrease in the accumulation of the *psaA-psaB* transcript in cells grown in complete darkness with glucose and in LAHG cells, relative to light-grown cells. Accumulation of the *psbA* transcript steadily declines through dark incubation, with a steady-state level in LAHG cells 28% of that in light-grown cells. Transcripts from *psbD*, *psaD*, and *rbcLS* accumulate in cells grown in complete darkness and in LAHG cells to approximately the same levels as in light-grown cells. Photosynthesis gene transcripts in cells grown in the dark without glucose were detected, but were highly degraded. Our data prove that transcripts from photosynthesis genes do accumulate in dark-grown *Synechocystis* 6803, which may allow for synthesis and assembly of photosystem (PS) I and PS II in the dark.

Introduction

The study of oxygenic photosynthesis has been expedited by the use of the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 as a model system. The photosynthetic apparatus in cyanobacteria is highly homologous to that of higher plants, including subunit composition, functional components, and primary sequence [23]. *Synechocystis* 6803 is very amenable to molecular genetic manipulation. It is naturally competent, is readily transformable, has an active homologous recombination mechanism, expresses bacterial drug resistance genes, and can be grown photoheterotrophically [50] or heterotrophically [1]. Considerable progress has been made in the study

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X58825.

of PS II in *Synechocystis* 6803, including cloning and mutagenesis of the genes for the D1 and D2 core polypeptides, *psbA* (present in three copies) and *psbD* (present in two copies) [11, 25]. In addition, mutagenesis of two peripheral proteins of PS I has been accomplished in *Synechocystis* 6803 [7, 8]. The recent discovery of heterotrophic growth conditions for *Synechocystis* 6803, which negate the selective advantage of wild-type PS I, make it an excellent system for molecular analysis of the core of PS I [1]. *Synechocystis* 6803 grows in the dark if supplied with glucose and 5 min of light every 24 h [1]. Anderson and McIntosh have termed this light-activated heterotrophic growth (LAHG) [1].

The biochemistry of PS I from plants and cyanobacteria has been extensively characterized [2, 15, 31]. Electron transfer components have been defined by various spectroscopic techniques and have been shown to be associated with three polypeptides: the two P_{700} apoproteins (PSA-A and PSA-B) and the 9 kDa iron-sulfur (Fe-S) protein (PSA-C) [14]. PSA-A and PSA-B bind P_{700}, A_0, A_1, and F_X, while F_A and F_B are bound to PSA-C [16, 24]. PSA-A and PSA-B also bind approximately 100 chlorophyll molecules for each P_{700} [14]. The electron transfer component F_X is most likely a [4Fe-4S] center bound by four cysteine residues, two in PSA-A and two in PSA-B [15]. Immediately adjacent to those conserved cysteines in both PSA-A and PSA-B are series of conserved leucines, spaced seven residues apart, in regions predicted to form α-helices. It has been proposed that these helices may interact to form a leucine zipper [27, 49].

The *psaA* and *psaB* genes have been cloned and sequenced from many different organisms, including: maize [13], spinach [26], pea [29], tobacco [46], rice [22], *Marchantia polymorpha* [36], *Euglena gracilis* [10], *Chlamydomonas reinhardtii* [28], and *Synechococcus* sp. PCC 7002 [5]. In plants and algae, these genes are located in the plastid genome. The *psaA* genes cloned from algae contain introns, and in *C. reinhardtii* the exons are joined by *trans*-splicing [9, 18]. In plants and *Synechococcus* 7002, the genes are arranged in tandem and have been shown to be co-transcribed [5, 6, 32, 39]. All the *psaA* and *psaB* genes sequenced so far show a very high degree of conservation [5].

The expression of photosynthesis genes in the process of plastid differentiation and chloroplast maturation has been the subject of intense research and has revealed the significant role of post-transcriptional regulation (for a recent review, see [35]). The expression of photosynthesis genes has also been studied in cyanobacteria. Lönneborg *et al.* [30] showed there is greater accumulation of *psbA* transcript under high-light conditions relative to low-light conditions in *Synechococcus* sp. PCC 6301. Greater accumulation of *psbA* and *psbD* mRNA in high-light relative to low-light conditions was also seen in *Synechocystis* 6803 [34]. However, no hybridization to mRNA from *psbA*, *psbD*, or *rbcL* (encoding the large subunit of ribulose-bisphosphate carboxylase/oxygenase [Rubisco]) could be detected from *Synechocystis* 6803 grown in the dark without glucose [34]. In *Synechocystis* 6803 copies 2 and 3 of *psbA* are nearly identical, while copy 1 is more divergent [33, 37]. By mutagenesis of two of the three copies of *psbA*, Mohamed and Jansson demonstrated that copy 1 is not expressed, while copies 2 and 3 are expressed in *Synechocystis* 6803 [34]. *Synechococcus* sp. PCC 7942 has three copies of *psbA* as well. However, copy 1, which encodes form I of the D1 protein, is expressed at much higher levels than copies 2 or 3, which encode form II of D1 [4, 43, 44]. In *Synechococcus* 7942 the accumulation of mRNA from copies 2 and 3 decreases with decreasing light intensity, while the accumulation of mRNA from copy 1 increases with decreasing light intensity [4, 44].

This paper describes the cloning and sequencing of the *psaA* and *psaB* genes from *Synechocystis* sp. PCC 6803, and their use, together with other photosynthesis genes, as probes in the analysis of photosynthesis gene expression. Quantitation of transcript accumulation for *psaA-psaB* and *psbA* in light-grown cells, cells put in total darkness with glucose, and LAHG cells will be presented.

Materials and methods

Materials

All chemicals and reagents used were of the highest grade available and were obtained from Sigma (St. Louis, MO), Boehringer Mannheim Biochemicals (Indianapolis, IN), or Research Organics (Cleveland, OH). Restriction and modification enzymes were purchased from New England Biolabs (Beverly, MA) or Gibco BRL (Gaithersburg, MD). Nitrocellulose was obtained from Schleicher and Schuell (Keene, NH). α^{-32}P-dATP was obtained from Amersham (Arlington Heights, IL).

Strain and growth conditions

A glucose-tolerant [50], dark-growth-adapted [1] strain of *Synechocystis* sp. PCC 6803 was grown in BG-11 medium supplemented with 5 mM TES (*N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid) pH 8.0, as previously described [25]. Glucose was added to a final concentration of 5 mM. Cell growth was measured by absorbance at 730 nm (OD_{730}). Light-grown cells received 20 μmol m^{-2} s^{-1} of continuous white light. LAHG conditions were: total darkness except for 5 min of white light (40 μmol m^{-2} s^{-1}) every 24 h as previously described [1]. For dark incubations, cells were grown to early exponential phase (OD_{730} = 0.2–0.5), then wrapped in aluminum foil and put in a light-tight box in a darkroom accessed by a darkened hallway for 24, 30, or 48 h. Cells grown without glucose were incubated for 48 h only in complete darkness as described [34].

Cloning

All nucleic acid manipulations were performed using standard techniques [41], except where otherwise noted. All DNA fragments used as hybridization probes (see Table 1) were purified by agarose gel electrophoresis and were random primer-labelled with ^{32}P [12]. Genomic DNA isolated from *Synechocystis* 6803 was purified on cesium chloride gradients as described [50]. Conditions for hybridization with the *Synechococcus* 7002 probes were: 1 × Denhardt's solution (0.2 mg/ml Ficoll, 0.2 mg/ml polyvinylpyrrolidone, 0.2 mg/ml bovine serum albumin), 3 × SSC,

Table 1. DNA probes used for screening, Southern, and northern hybridizations.

Source organism	Genes encoded	Fragment
Synechococcus 7002	*psaA, psaB*	pAQPR80 7.8 kb *Eco* RI-*Bgl* II-pUC9[a] [5]
Synechococcus 7002	85% of *psaA*	1.9 kb *Hind* III (from pAQPR80)
Synechococcus 7002	60% of *psaB*	1.4 kb *Hind* III (from pAQPR80)
Synechococcus 7002	5′ end of *psaA*	0.5 kb *Eco* RI-*Hind* III (from pAQPR80)
Synechocystis 6803	80% of *psaA*	1.8 kb *Kpn* I (from pLS18)
Synechocystis 6803	half of *psaB*	0.9 kb *Kpn* I (from pLS19)
Synechocystis 6803	3′half of *psaB*	1.2 kb *Hind* III (from pLS15)
Synechocystis 6803	3′ half of *psbA2*	0.5 kb *Kpn* I-*Hinc* II (from pKW1266) [25]
Synechocystis 6803	3′ half of *psbD2*	0.6 kb *Kpn* I-*Sma* I (from pRD655) [11]
Synechocystis 6803	3′ half of *rbcL*	0.7 kb *Bam* HI (from pSF2.6)[b]
Synechocystis 6803	*psaD*	0.4 kb *Bst* EII-*Bgl* I (from pPR8)[c] [38]
Synechocystis 6803	3′ flanking *psaB*	1.0 kb *Eco* RI-*Kpn* I (from pLS31)
Synechococcus 7942	16S rRNA	2.0 kb *Xba* I (from pAN4)[d] [47]

[a] Kind gift of D.A. Bryant, Dept. of Molecular and Cell Biology, Pennsylvania State University.
[b] Reich and L. McIntosh (unpublished).
[c] Kind gift of N. Nelson, Roche Institute, Nutley, NJ
[d] Kind gift of S.S. Golden, Dept. of Biology, Texas A & M University.

35% v/v formamide, 0.1% w/v sodium dodecyl sulfate (SDS), 40 mg/l salmon sperm DNA, 37 °C. Hybridization conditions for *Synechocystis* 6803 probes were the same except 50% v/v formamide was used. Blots were washed with 6 × SSC; two times at 25 °C, then once at 37 °C. A mini-library was constructed by ligating size-fractionated *Synechocystis* 6803 *Kpn* I fragments purified from an agarose gel into pUC119 [48]. This was transformed into *Escherichia coli* HB101 [3] and screened by colony hybridization [19]. A λEMBL3 library of *Synechocystis* 6803 DNA was previously described [25].

Sequencing and sequence analysis

DNA sequence was determined from either single-stranded or double-stranded templates generated from pUC118 or pUC119 [48] by the chain-termination method [42] using Sequenase version 2 (U.S. Biochemical, Cleveland, OH) as described by the manufacturer. Nested deletions were generated using exonuclease III and mung bean nuclease [21]. Oligonucleotides for use as sequencing primers were synthesized using an Applied Biosystems DNA synthesizer. These primers were used to sequence those regions not recovered in the series of exonuclease III deletions. The polymerase chain reaction (PCR) was performed using genomic *Synechocystis* 6803 DNA, two of the aforementioned oligonucleotides, and AmpliTaq polymerase (Perkin-Elmer Cetus, Norwalk, CT), as described by the manufacturer. The amplified fragment was purified, digested, and subcloned into pUC118 for sequencing. DNA sequence analysis was performed using Editbase (courtesy of N. Neilson, Purdue University), MCF and Amphi (A.R. Crofts, Univ. of Illinois) and the programs of the University of Wisconsin Genetics Computer Group.

RNA isolation and northern blots

RNA was isolated from cells in late exponential phase ($OD_{730} = 0.7–0.9$) using the technique of Golden *et al.* [17], except that immediately after harvest, the cells were frozen at −70 °C in BG-11, then thawed. Great care was taken to perform manipulations in complete darkness or very dim light until the cells were exposed to chloroform. RNA was denatured, fractionated on agarose/formaldehyde gels, and transferred to nitrocellulose. Molecular sizes were estimated by comparison to an ethidium bromide stained lane of a 9.5–0.24 kb RNA ladder (Gibco-BRL). Northern blots were hybridized (1 × Denhardt's solution, 3 × SSC, 30 mM Tris pH 8.0, 0.1 mM EDTA, 50% v/v formamide, 0.1% w/v SDS, 40 mg/l salmon sperm DNA, 37 °C) with ^{32}P random primer-labelled probes (see Table 1), washed with 6 × SSC, then quantitated using a Betascope machine (Betagen, Waltham, MA) and/or exposed to X-ray film. The blots were then stripped using boiling water with 0.2% w/v SDS (three times, 10 min each), verified to be non-radioactive, and reprobed.

Results

Cloning

Southern blots of genomic *Synechocystis* 6803 DNA digested with *Kpn* I were probed with the plasmid pAQPR80 (the kind gift of D.A. Bryant), which contains the *psaA* and *psaB* genes from *Synechococcus* sp. PCC 7002 [5]. Four hybridizing fragments of approximately 1.8, 1.7, 1.4, and 0.9 kb were detected (data not shown). The sum length of these fragments corresponds to the expected length of the *psaA-psaB* operon [5]. *Kpn* I-digested genomic *Synechocystis* 6803 DNA was fractionated on an agarose gel, and the fragments corresponding in size to the hybridizing bands were purified and ligated into pUC119. This mini-library was screened by colony hybridization using two *Hin*d III fragments from pAQPR80 (Table 1): a 1.9 kb fragment (containing most of *psaA*) and a 1.4 kb fragment (containing the 3′ half of *psaB*). Two hybridizing clones were isolated: one contained a 1.8 kb *Kpn* I fragment (pLS18) and the other contained a 0.9 kb *Kpn* I

fragment (pLS19) (Fig. 1). Plasmids pLS18 and pLS19 were partially sequenced and, by comparison to the *Synechococcus* 7002 sequence, were verified to encode portions of *psaA* and *psaB*. Inserts from pLS18 and pLS19 and a 0.5 kb *Eco* RI-*Hin*d III fragment from pAQPR80 (encoding the 5′ end of *psaA*) (Table 1) were then used as probes in screening a λEMBL3 library of *Synechocystis* 6803 DNA to recover full-length clones. Several hybridizing λ clones were isolated and their DNA purified, mapped, and subcloned into pUC118 or pUC119. The maps of two of the λ clones, 311 and 121, and their subclones are shown in Fig. 1. Southern analysis of digested genomic *Synechocystis* 6803 DNA, probed with the inserts from pLS18 and pLS19, revealed only one large or a few small hybridizing fragments, as with pAQPR80, indicating *psaA* and *psaB* are present in single copy in the *Synechocystis* 6803 genome (data not shown).

DNA sequence

The sequencing strategy is shown in Fig. 1. Sequencing revealed a single base-pair deletion in one of the original clones from the mini-library (pLS18), evidenced by a disruption of the reading frame in a highly conserved region of *psaA*. Unfortunately, this region was not overlapped by any of the λ clones. Therefore, the PCR technique was utilized to amplify the region between two of the sequencing primers from genomic *Synechocystis* 6803 DNA. The PCR product was purified, subcloned into pUC118, and sequenced, verifying the true sequence and our hypothesis of a deletion in pLS18.

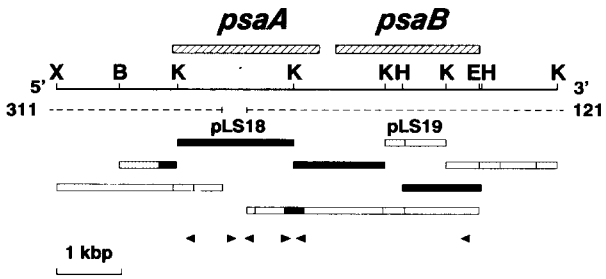

Fig. 1. Restriction map and sequencing strategy of the *Synechocystis* 6803 *psaA* and *psaB* operon. Hatched boxes represent the protein coding regions of *psaA* and *psaB*. A partial restriction map is shown on the solid line: X, *Xba* I; B, *Bgl* II; K, *Kpn* I; H, *Hin*d III; E, *Eco* RI. Dashed lines represent the insert regions from two of the λ clones (311 and 121). Boxes below the dashed lines represent clones from the mini-library or sub-clones of the λ clones: black regions were sequenced from both strands, stippled regions were sequenced from one strand. Inserts from the plasmids pLS18 and pLS19 are labelled above the boxes. Triangles represent oligonucleotides used as sequencing primers and in PCR.

The DNA and deduced amino acid sequences of the *Synechocystis* sp. PCC 6803 *psaA* and *psaB* genes and flanking regions are shown in Fig. 2. The genes are arranged in tandem with *psaA* upstream of *psaB*. The *psaA* gene consists of 2256 nucleotides, which would encode a protein 751 amino acids long with a predicted molecular mass of 82.9 kDa. The *psaB* gene is 2196 nucleotides long and would encode a protein with 731 amino acids and a predicted molecular mass of 81.3 kDa. The region between the genes is 245 nucleotides long, with no apparent open reading frame or inverted repeat sequences. The *Synechocystis* 6803 *psaA* gene shows 78.0% DNA sequence identity to the *Synechococcus* 7002 *psaA* gene [5] and 69.4% DNA identity to the spinach *psaA* gene [26]. The *Synechocystis* 6803 *psaB* gene has 81.1% DNA sequence identity to the *Synechococcus* 7002 *psaB* gene [5] and 69.4% DNA identity to the spinach *psaB* gene [26]. Seven nucleotides upstream from the start of *psaA* and fifteen nucleotides upstream from the start of *psaB* is the sequence AGGAGAA, which has similarity to the Shine-Dalgarno sequence and may constitute a ribosome binding site [45]. A sequence (TATTAT) similar to the *E. coli* consensus −10 sequence (TATAAT) lies 154 nucleotides upstream of the start of *psaA* gene, while a sequence (TTGCCT) similar to the *E. coli* −35 sequence (TTGACA) lies 17 nucleotides upstream from the potential −10 sequence [20]. Partial nucleotide sequence obtained for the region 3′ to the *psaB* gene includes G-C-rich inverted repeats that may form a stem-loop structure followed by a series of thymines. This type of structure is involved in transcription termination in *E. coli* [40]. The par-

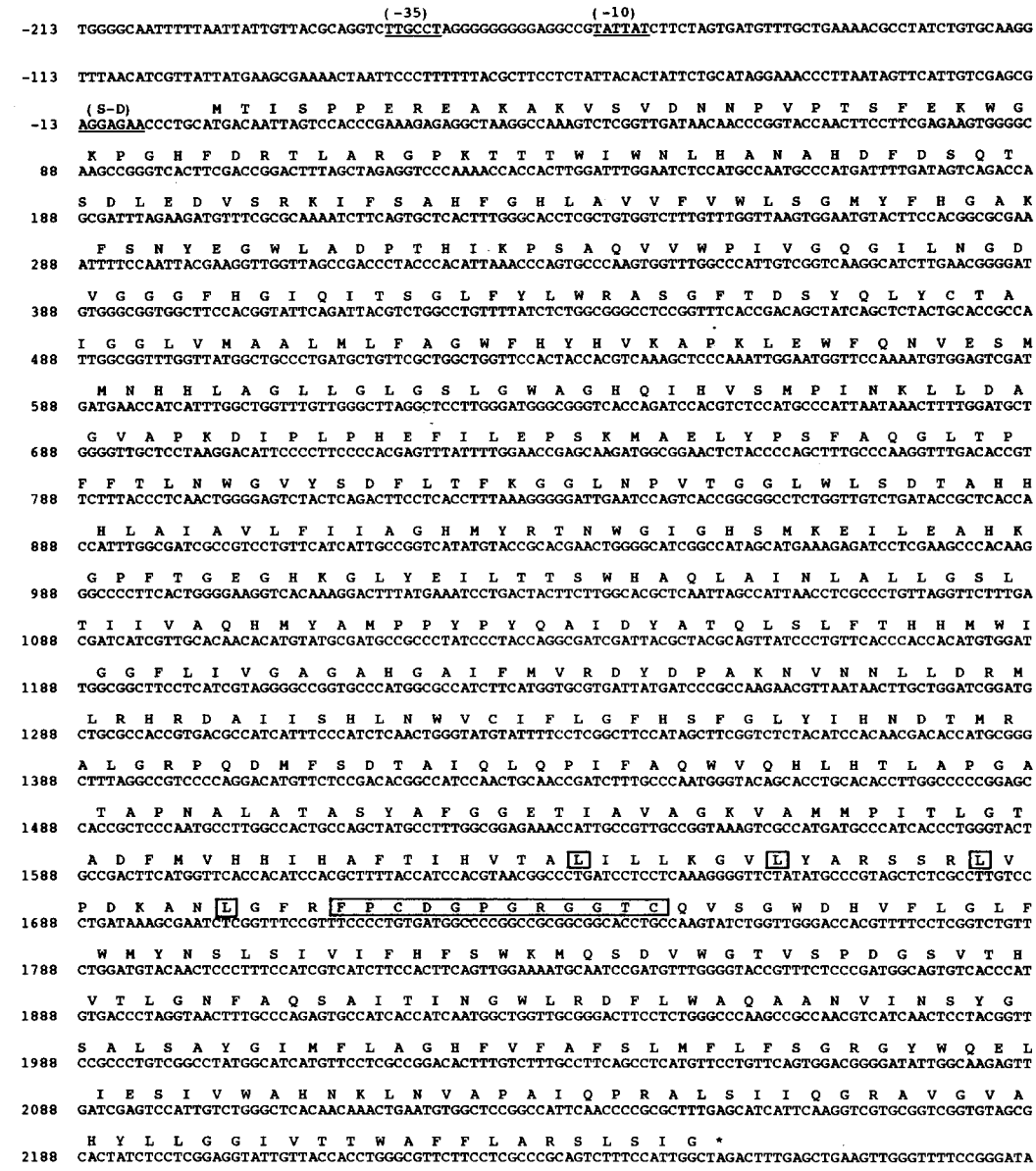

Fig. 2. Nucleotide and deduced amino acid sequences of the *Synechocystis* 6803 *psaA* and *psaB* operon. The DNA sequence is numbered on the left; the deduced amino acid sequence is numbered on the right. Sequences showing similarity to *E. coli* promoters and ribosome binding sites are underlined. The *psaA* gene extends from nucleotide 1 to 2256. The *psaB* gene extends from nucleotide 2502 to 4697. The amino acid sequence FPCDGPGRGGTC, conserved in PSA-A and PSA-B and including cysteines thought to bind F_x, as well as the conserved leucines which may form a leucine zipper, upstream of those cysteines, are enclosed in boxes.

tial sequence data from the region 3′ to *psaB* show no homology to *rps14* from spinach [26] or to ORF128 from *Synechococcus* 7002 [5], which have been found downstream of *psaB* in those organisms.

psaA-psaB *deduced amino acid sequence*

Specific residues as well as predicted secondary structures are conserved in the *Synechocystis* 6803 *psaA-psaB* deduced protein sequences. The pre-

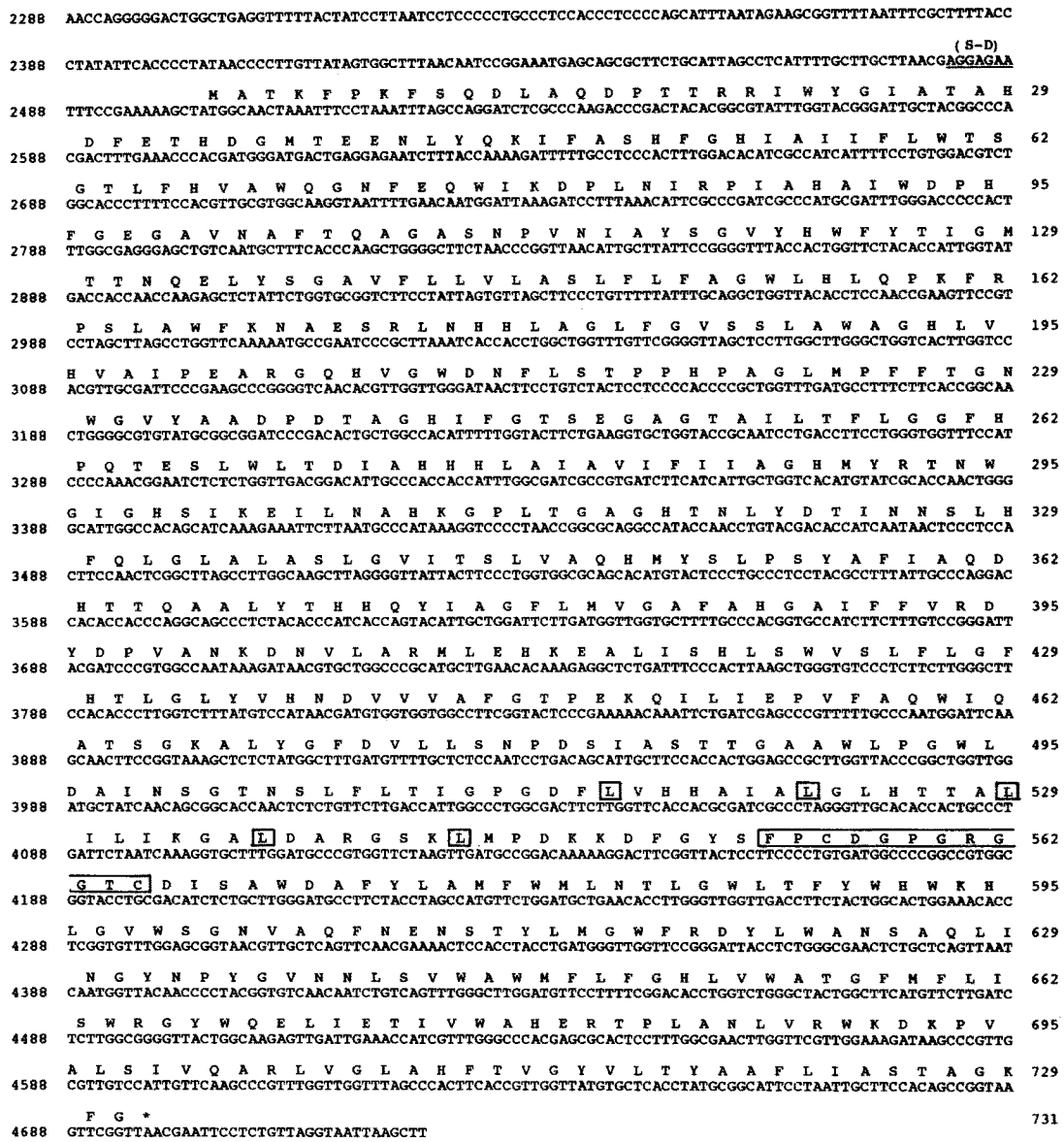

Fig. 2. (continued).

dicted amino acid sequence of the *psaA* gene (PSA-A) is 88.4% identical to the *Synechococcus* 7002 PSA-A [5] and 80.9% identical to the spinach PSA-A [26]. The *Synechocystis* 6803 *psaB* deduced amino acid sequence (PSA-B) is 92.8% identical to the *Synechococcus* 7002 PSA-B [5] and 79.6% identical to the spinach PSA-B [26]. Eleven membrane-spanning α-helices are predicted for both PSA-A and PSA-B, as has been predicted for previously characterized sequences [5, 13, 26, 29]. Also conserved in both deduced protein sequences is the sequence FPCDGPGRGGTC (residues 572 to 583 in PSA-A, 554 to 565 in PSA-B), which is absolutely conserved in the deduced amino acid sequences of all of the *psaA* and *psaB* genes sequenced to date. On the immediate amino-terminal side of this sequence in PSA-A and PSA-B are conserved series of

leucine residues (four leucines in PSA-A, five in PSA-B), each leucine spaced seven amino acids apart. These regions are predicted to form α-helices, each with a string of leucines, that would have the potential to interact with each other, forming a leucine zipper [27, 49].

mRNA accumulation in cells grown with glucose

Northern blots of total RNA from *Synechocystis* 6803 were hybridized with probes encoding *psaA*, *psaB*, *psbA2*, 16S rRNA, *psaD*, *psbD2*, and *rbcL*, as well as a probe encoding 1.0 kb 3′ to *psaB* (Table 1). RNA was isolated from cells grown in continuous light; cells grown in the light with glucose, then put in total darkness for 24, 30, or 48 h; and from cells grown under LAHG conditions [1]. Autoradiographs from hybridizations to a representative northern blot with samples from each of the above conditions are shown in Fig. 3. Both the *psaA* and *psaB* probes hybridized to a 5.0 kb transcript, indicating they are co-transcribed. The *psaA* probe also hybridized to a 2.4 kb message, while the *psaB* probe also hybridized to a 2.1 kb message. Both probes hybridized to a smear of bands smaller than 5.0 kb. The fragment encoding *psbA2* hybridized to a 1.2 kb message that may be a transcript from one or both of *psbA2* or *psbA3*. A fragment of the *rrn* operon from *Synechococcus* 7942 specific for 16S rRNA hybridized to a 1.5 kb transcript. The *psaD* probe from *Synechocystis* 6803, encoding subunit II of PS I, hybridized to a single message of 0.5 kb. The *psbD2* probe hybridized strongly to the 1.2 kb transcript from *psbD2*, but also hybridized weakly to the 2.5 kb transcript from the overlapping *psbD1-psbC* genes. The probe for *rbcL* from *Synechocystis* 6803 hybridized to a 2.6 kb transcript, representing the full-length message from the *rbcLS* operon, which encodes the large and small subunits of Rubisco, a 2.0 kb message, and a smear of transcripts smaller than 2.6 kb.

In order to quantitate the steady-state levels of mRNA accumulation for *psaA-psaB* and *psbA*, radioactivity in the region of the bands was measured directly from the blots using a Betascope

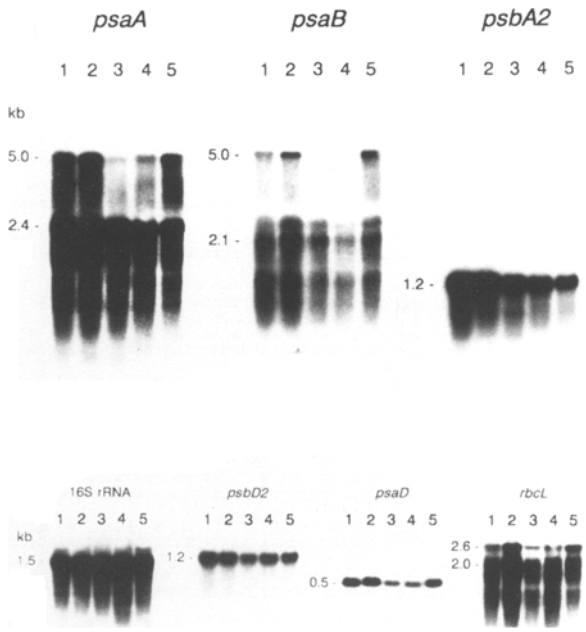

Fig. 3. Autoradiographs of a single northern blot, stripped and reprobed. Probes used are indicated above each panel and are described in Table 1. The upper three panels are aligned in the same scale. The lower four panels are not in alignment. Sizes of bands are in kb and were determined by comparison to an ethidium bromide-stained RNA ladder. Lanes are: 1, light-brown control; 2, 24 h dark with glucose; 3, 30 h dark with glucose; 4, 48 h dark with glucose; 5, LAHG conditions. Exposure times were different for each autoradiograph.

machine. These values were then standardized to the quantitation of hybridization of the 16S rRNA probe. Quantitation of hybridization of the *psaA* probe was measured in the regions of the 5.0 kb full-length *psaA-psaB* message and in the region of the 2.4 kb *psaA*-specific message. Values for at least one quantitation from at least two preps for each condition were averaged and are expressed as a percentage of the accumulation in the light-grown samples (Fig. 4). Accumulation of the *psaA-psaB* transcript drops to 87% of the level in the light after 24 h in the dark, 47% after 30 h, then back up to 82% after 48 h, while the steady-state level in LAHG cells is 80% of light-grown cells. However, the accumulation of the 2.4 kb *psaA* message increases to 152% of the level in the light after 24 h, but drops to 97% after 30 h,

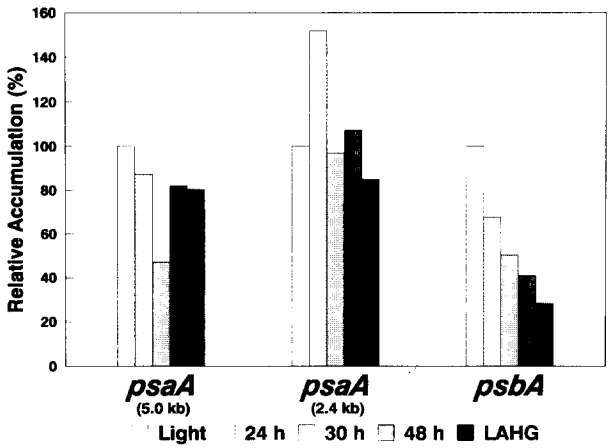

Fig. 4. Relative levels of mRNA accumulation in *Synechocystis* 6803 cells. Values are averages of at least one quantitation of hybridization to at least two preps from each condition and are expressed as a percentage of the light-grown control. mRNA quantitations were standardized to quantitations of 16S rRNA for each lane. The left group is quantitation of the 5.0 kb *psaA-psaB* transcript. The middle group is quantitation of the 2.4 kb *psaA*-specific transcript. The right group is quantitation of the 1.2 kb *psbA* transcript. Bars are: Light, light-grown control; 24 h, 24 h dark with glucose; 30 h, 30 h dark with glucose; 48 h, 48 h dark with glucose; LAHG, light-activated heterotrophic growth conditions.

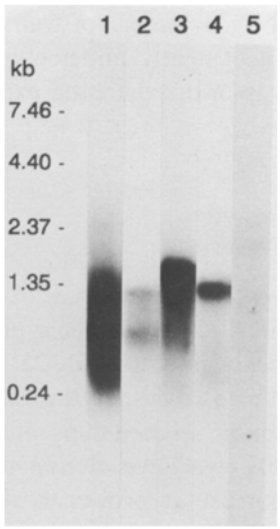

Fig. 5. Autoradiographs of a single lane from a northern blot (48 h dark without glucose), stripped and reprobed. Size labels (in kb) are from an ethidium bromide stained RNA ladder. Probes used: 1, *psaA*; 2, *psaA2*; 3, 16S rRNA; 4, *psbD2*; 5, *rbcL*. Probes used are described in Table 1. Exposure times were different for each autoradiograph.

107% after 48 h, while the steady-state level in LAHG cells is 85% of the level in light-grown cells. The accumulation of the *psbA* message steadily decreases through the dark incubation: 68% after 24 h, 50% after 30 h, and 41% after 48 h, while the steady-state level of *psbA* message in LAHG cells is 28% of that in light-grown cells. Although the hybridization of the other probes used was not quantitated, none showed a significant and repeatable change upon growth in the dark with glucose.

mRNA accumulation in cells grown without glucose

RNA was isolated from duplicate cultures of *Synechocystis* 6803 grown to early exponential phase in the light without glucose, then put in complete darkness for 48 h. The final OD_{730} of the culture was lower than when the dark incubation started, and the yield of RNA from these preps was significantly lower than for cells grown with glucose. When the RNA in the formaldehyde gel was stained with ethidium bromide and visualized under UV light, the samples from cells grown without glucose were indistinguishable from those from cells grown with glucose (data not shown). Autoradiographs of a representative lane from a northern blot with this RNA hybridized to the *psaA*, *psbA2*, *psbD2*, *rbcL*, and 16S rRNA probes are shown in Fig. 5. By inspection of autoradiographs, the level of hybridization to the mRNA from cells lacking glucose was significantly lower than the level of hybridization to mRNA from either the 48 h dark with glucose sample or the LAHG sample, while the hybridization of the 16S rRNA probe appeared to be approximately equal. The *psaA* probe hybridized to a smear of transcripts of 3.0 kb and smaller, with bands of 2.4, 1.6, 1.1, and 0.7 kb. The *psbA2* probe hybridized to the expected 1.2 kb message and a 0.6 kb transcript. The *psbD2* probe hybridized strongly to the 1.2 kb *psbD2* transcript and very weakly to

the 2.5 kb *psbD1-psbC* message. Hybridization of the *rbcL* probe to a smear of transcripts 2.5 kb and smaller was nearly undetectable. The 16S rRNA probe hybridized to the expected 1.5 kb transcript.

Discussion

The use of *Synechocystis* sp. PCC 6803 as a model system for the study of oxygenic photosynthesis has been well documented [11, 25, 50]. In order to broaden the analysis of the biogenesis and structure/function relationships of PS I in *Synechocystis* 6803, we have cloned the *psaA* and *psaB* genes for use as probes to study gene expression and for targeted mutagenesis. As a part of a study to determine whether the photosystems assemble in the dark, a quantitative analysis of the expression of photosynthesis genes in the dark was performed. Although the *psaA* and *psaB* genes have been cloned and sequenced from a cyanobacterium as well as multiple plant and algal species, the recent discovery of heterotrophic growth conditions for *Synechocystis* 6803 [1] makes mutagenesis of the PS I core tractable in this organism.

The *psaA* and *psaB* genes from *Synechocystis* 6803 are highly homologous to the genes from *Synechococcus* 7002 and are only slightly less homologous to the genes cloned from higher-plant chloroplasts. The *psaA* gene is homologous to the *psaB* gene, with long stretches of absolute amino acid conservation and 56.9% DNA sequence identity in *Synechocystis* 6803. This suggests one of these genes arose from a duplication of the other prior to the evolution of chloroplasts. Fundamental differences in the *psaA-psaB* operon structure from cyanobacteria and higher-plant chloroplasts do exist. In the plant sequences, there are only 25 bp between the *psaA* and *psaB* genes, while in *Synechococcus* 7002 this region is 173 bp, and in *Synechocystis* 6803 it is 245 bp. There is no apparent open reading frame in the intergenic region of *Synechocystis* 6803, while there is a short open reading frame in *Synechococcus* 7002. Also, in tobacco, rice, and spinach, the gene for ribosomal protein S14 (*rps14*) is found downstream of *psaB* and is co-transcribed with *psaA* and *psaB*. A fragment encoding 1.0 kb immediately downstream of *psaB* was used as a probe of northern blots, but no hybridization was detected (data not shown). Neither *Synechocystis* 6803 nor *Synechococcus* 7002 have *rps14* immediately downstream of *psaB*, and in *Synechocystis* 6803, this region may not be transcribed or, if so, the message accumulates to undetectable levels.

The predicted amino acid sequences of *psaA* and *psaB* from *Synechocystis* 6803 are highly homologous to those from *Synechococcus* 7002 and higher plants. Eleven membrane-spanning α-helices are predicted for both PSA-A and PSA-B. An interesting feature of the eighth helix in both PSA-A and PSA-B is the presence of conserved leucine residues, spaced seven amino acids apart. It has been proposed that these helices may interact to form a leucine zipper, pulling PSA-A and PSA-B together [27, 49]. These helices are immediately adjacent to highly conserved regions containing the four cysteines thought to bind the 4Fe-4S center F_X [15]. We plan to use site-directed mutagenesis of the leucines described above to address the leucine zipper hypothesis and to assess its role in the assembly of F_X and of the PS I core. In addition, we plan to mutate one or more of the conserved cysteines to confirm their role as ligands to F_X, to assess the importance of F_X in maintaining the integrity of the core heterodimer, and to answer questions about the role of F_X in electron transport.

Hybridization patterns of *psaA* and *psaB* to northern blots indicate these genes are co-transcribed, as both genes hybridize to a 5.0 kb transcript. However both genes also hybridize to smaller transcripts that may represent the products of processing in the intergenic region, yielding transcripts that only encode *psaA* or *psaB*. The lack of an open reading frame in the relatively long intergenic region may leave the transcript susceptible to processing, as this region apparently would not be bound to ribosomes. Probes for *psaA* and *psaB* hybridize to a smear of transcripts smaller than 5.0 kb. Smearing was not seen in hybridizations with other probes, suggest-

ing this is not the result of general degradation of the RNA sample, but perhaps due to rapid turnover of this message. A similar complex pattern of hybridization has been seen in maize [39] and in tobacco [32]. The half-life of the *psaA-psaB* transcript has not been measured in cyanobacteria or chloroplasts.

The accumulation of transcripts from genes encoding core proteins of PS I and PS II was quantitated for three time points of incubation in total darkness with glucose, for LAHG conditions, and for light-grown cells. These quantitations were averaged from multiple trials from at least two preparations of each type. The doubling time for cells in continuous light is about 12 h [50], while the doubling time for cells in LAHG conditions is 36 h [1]. When cells were transferred from the light to total darkness, their growth rate slowed to approach that for LAHG conditions, presumably due to a change in energy status. After approximately 48 h without a light pulse, the cells would stop dividing. The accumulation of the *psaA-psaB* transcript drops only slightly through the dark incubation, with a steady-state level of 80% of light-grown cells in the LAHG sample. The accumulation of the *psaA*-specific transcript generally shows the same pattern as the full-length transcript. The accumulation of *psbA* transcript shows a consistent decline through the dark incubation to a level in LAHG cells 28% of that in light-grown cells. These changes in message accumulation may not be due to transcriptional regulation, but rather to changes in mRNA stability. Also, any changes in message accumulation seen under these conditions cannot be attributed solely to light regulation, but may be the result of the difference in energy status when cells are grown in the dark. It is clear that transcripts of the photosynthesis genes assayed in this experiment do accumulate in cells grown in the dark.

Mohamed and Jansson [34] published results of an experiment using *Synechocystis* 6803 in which they saw no accumulation of *psbA*, *psbD*, or *rbcL* mRNA in cells put in the dark for 48 h without glucose. Our results from a duplication of this experiment indicate that transcripts from these genes and *psaA-psaB* do accumulate in cells grown in the dark without glucose, but the mRNA is degraded (Fig. 5). The OD_{730} of the cultures actually decreased after the 48 h incubation, and the yield of RNA was much lower than from cultures grown in complete darkness with glucose. Anderson and McIntosh showed that *Synechocystis* 6803 does not divide in the dark without supplemental glucose [1]. We would conclude that the mRNA of cells grown under these conditions has been degraded and is not satisfactory for the study of gene expression.

The presence of transcripts from photosynthesis genes in cells grown in total darkness indicates that either transcription continues in the dark or that the transcripts are stable for as long as 48 h. In either case, the mRNA is available for the synthesis and assembly of photosynthesis proteins. The pattern of hybridization to *psaA-psaB* suggests this message is rapidly turned over, thus transcription of this operon probably continues in the dark at near light-grown rates. The half-life of *psbA* in *Synechococcus* 6301 has been estimated to be approximately 120 min [30]. If this is similar in *Synechocystis* 6803, the rate of transcription of *psbA* may drop shortly after the shift to the dark, but the *psbA* transcript pool decreases gradually. Changes in the *psbA* transcript pool have been observed when *Synechocystis* 6803 [34] or *Synechococcus* 6301 [30] are grown in high light. Transcriptional regulation of *psbA* may be one of the ways this organism alters its photosynthetic apparatus to adjust to available light conditions.

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