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Different and Rapid Responses of Four Cyanobacterial *psbA* Transcripts to Changes in Light Intensity

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The genome of the cyanobacterium *Synechococcus* sp. strain PCC 7942 contains three *psbA* genes which encode two forms of the D1 protein of photosystem II. Experiments using *psbA-lacZ* translational fusions and Western blot (immunoblot) analysis have shown that the *psbA* genes respond differently to changes in light intensity, altering the ratio of the two forms of D1 in the thylakoid membrane. Each gene produces a 1.2-kilobase (kb) mRNA. A probe specific for *psbAII* transcripts also identified a 1.6-kb mRNA which starts 419 base pairs upstream of the 5′ end of the 1.2-kb species and overlaps the entire 1.2-kb transcript. This 419-base-pair region includes an open reading frame (ORF1) of 114 amino acids. We investigated the effects of changes in light intensity on *psbAII* transcript levels in a series of light shift experiments in the wild-type *Synechococcus* sp. and in AMC084, a mutant which does not produce the 1.6-kb transcript. After exposure to high light intensities for 15 min, the level of the 1.2-kb *psbAII* transcript increased in both strains. This transcript was not detected in either strain after transfer to low light intensity. The *psbAIII* transcript showed the same pattern of response as the 1.2-kb *psbAII* transcript, whereas the 1.6-kb *psbAII* transcript was unaffected by different light intensities. The *psbAI* transcript levels responded oppositely to those of *psbAII* and *psbAIII*. These data, considered along with previous results obtained by using *lacZ* translational gene fusions, indicate that the response of *psbA* genes to changes in light intensity is controlled primarily at the transcriptional level.

The genome of the cyanobacterium *Synechococcus* sp. strain PCC 7942 contains a family of three unique *psbA* genes which encode two different forms of D1 (6). The three genes predict that D1 form I (encoded by *psbAI*) differs from form II (encoded by both *psbAII* and *psbAIII*) at 25 of 360 amino acids (6). D1 and a structurally similar protein, D2, are integral thylakoid proteins that constitute the reaction center of photosystem II, the site at which chlorophyll gives up an electron in response to excitation by light (12, 21). *Synechococcus* strains with different combinations of inactivated *psbA* genes have been used to demonstrate that each of the *psbA* genes is transcriptionally active and individually capable of supporting photoautotrophic growth (6).

All three of the *psbA* genes give rise to 1.2-kilobase (kb) transcripts that have 5′ ends 49 to 52 bases upstream of their open reading frames (6). Because the 5′-untranslated regions are unique, it is possible to produce gene-specific probes for each of the *psbA* messages (3). These radioactive antisense RNA probes can detect low-abundance transcripts not detected with either nick-translated or random-primer-labeled DNA probes (J. A. Brusslan, Ph.D. dissertation, The University of Chicago, Chicago, Ill., 1988). A second transcript from *psbAII* that is present in low abundance was identified with the *psbAII*-specific probe (19; Brusslan, Ph.D. dissertation). In this study, we characterized this 1.6-kb transcript, which originates 419 base pairs (bp) upstream of the start site for the 1.2-kb *psbAII* mRNA and overlaps the complete *psbAII* open reading frame. The 1.6-kb transcript is a dicistronic message containing a 342-bp (114-amino-acid) open reading frame, designated ORF1, immediately upstream of the *psbAII* open reading frame. Northern (RNA) blot analysis of RNA from a strain blocked for transcription of the 1.6-kb *psbAII* species showed that the 1.2-kb *psbAII* mRNA was still expressed normally.

We have shown previously that the three *psbA* genes in *Synechococcus* sp. strain PCC 7942 are differentially regulated and respond to changes in light intensity (18, 19). Translational gene fusions between each of the *psbA* genes and the *Escherichia coli lacZ* gene were recombined into the chromosome of otherwise wild-type *Synechococcus* sp. strain PCC 7942 cells to serve as in vivo reporters of *psbA* expression (19). As light intensity decreased because of growth of the culture, expression from the *psbAI-lacZ* gene fusion increased, whereas expression from both the *psbAII-lacZ* and *psbAIII-lacZ* reporters decreased (19). Western blot (immunoblot) analysis of thylakoid membrane proteins using D1 form-specific antisera showed that in wild-type cells harvested at different light intensities, the ratio of the forms changed as predicted by the activities of the reporter genes (18).

These studies did not address whether altered expression of *psbA* genes is controlled at the transcriptional, posttranscriptional, or translational level. To investigate whether rapid changes in light intensity would affect *psbA* transcript levels and to study the possible function of ORF1 in light regulation of the 1.2-kb *psbAII* transcript, wild-type and ORF1-inactivated mutant cells were subjected to a series of light shift experiments. Wild-type cells adapted to medium light intensity were transferred to high and low light intensities for 15 or 30 min and harvested for isolation of total RNA. Northern blot analysis with gene-specific probes revealed three different response patterns of the *psbA* transcripts. Transfer to high light intensity significantly increased the levels of the 1.2-kb *psbAII* and *psbAIII* transcripts, whereas the same transcripts were not detected in cells transferred to low light intensity. This result was not affected by absence of the *psbAII* 1.6-kb transcript and

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TABLE 1. Strains and plasmids used

Strain or plasmid	Description	Reference or source
Synechococcus strains		
PCC 7942	Wild type (also called *Anacystis nidulans* R2)	Laboratory collection
AMC084	*psbAII* 1.6-kb message inactivated by Ω cassette at *Apa*I site 285 bp downstream from transcription start site	This study
Plasmids		
pUC19	Cloning vector, encodes ampicillin resistance	2
pBluescript SK(+)	Cloning vector, encodes ampicillin resistance, used to transcribe radiolabeled antisense RNA probes	Stratagene
pSG201	3.6-kb *Nco*I-*Bam*HI fragment including part of the *psbAII* open reading frame and 5′-untranslated region; vector pBR328	6
pAM087	Plasmid carrying 90-bp fragment of downstream region of *psbAII*	J. A. Brusslan[a]
pAM088	Plasmid used to transcribe *psbAI*-specific RNA probe (also called U1)	3
pAM089	Plasmid used to transcribe *psbAII*-specific RNA probe (also called U2)	3
pAM090	Plasmid used to transcribe *psbAIII*-specific RNA probe (also called U3)	3
pAM165	Source of Ω cassette; modification of pHP45Ω	5, 13
pAM593	1.1-kb *Apa*I-*Pst*I fragment carrying 5′ upstream region of *psbAII*; vector pBluescript SK(+)	This study
pAM595	1.7-kb *Pst*I-*Pst*I fragment carrying part of the *psbAII* open reading frame and 5′-untranslated region; vector pUC19	This study
pAM596	Derivative of pAM595 which carries a spectinomycin resistance cassette inserted at the *Apa*I site upstream of the *psbAII* gene; recombination substrate used to produce AMC084	This study
pJC1.6	1.6-kb *Pvu*II-*Sal*I fragment carrying all of the *glnA* gene from *Synechococcus* sp. strain PCC 7942; vector pBluescript KS(+)	J. Curry and S. Robinson, unpublished results
pAN4	Plasmid containing rRNA operon from *Synechococcus* sp. strain PCC 6301; vector pBR322	20a

[a] Ph.D. dissertation.

interruption of ORF1 in the mutant cells. In contrast, transcripts from *psbAI* decreased at high light intensity and increased slightly at low light intensity. Levels of the *psbAII* 1.6-kb transcript remained relatively constant under all light conditions.

MATERIALS AND METHODS

Strains and plasmids. The strains and plasmids used in this work are described in Table 1. Wild-type *Synechococcus* sp. strain PCC 7942 (previously referred to as *Anacystis nidulans* R2, Pasteur Culture Collection no. 7942) and mutant strain AMC084 were grown in liquid BG-11 medium (1) or on solid BG-11 agar as previously described (7).

A detailed procedure for transformation and gene inactivation of *Synechococcus* sp. strain PCC 7942 has already been published (7). Antibiotics were added to BG-11 plates (as 400 μl of a 100× stock) beneath the agar at 4 h postinoculation (7, 8) to provide the following final concentrations: spectinomycin, 40 μg/ml; streptomycin, 5 μg/ml. Cyanobacterial transformants containing a modified Ω cassette (5) were initially plated on a combination of spectinomycin and streptomycin to select against spontaneous spectinomycin-resistant mutants. Thereafter, transformed strains were maintained on spectinomycin alone.

Escherichia coli DH5α (*mcrA mcrB*) (Bethesda Research Laboratories, Inc., Gaithersburg, Md.), used as the host for all plasmids, was propagated on LB (10) and Terrific Broth (20) media with antibiotics at standard concentrations (10).

***Synechococcus* culture conditions.** For controlled light studies, wild-type *Synechococcus* sp. strain PCC 7942 and mutant strain AMC084 were cultured in 8-liter carboys as previously described (19). An ethanol-sterilized immersible probe (Biospherical Instruments) was used to measure the photosynthetic photon flux densities (PPFD) at the centers of the cultures. In light shift experiments, 8-liter cultures were grown until the PPFD reached approximately 125 microeinsteins $\cdot m^{-2} \cdot s^{-1}$, at which time a portion of the culture was harvested for RNA isolation. Portions (250 ml) of the remainder were transferred to petri dishes (150 by 25 mm; Becton Dickinson Labware, Oxnard, Calif.) and incubated at different light intensities. Cells were placed at 4, 17, 40, and 118 cm from 18-in. (1 in. = 2.54 cm) 20-W fluorescent tubes to provide PPFD of 500, 250, 125, and 50 $\mu E \cdot m^{-2} \cdot s^{-1}$, respectively. The time of exposure to different light intensities for each round of experiments was 15 or 30 min, after which the cells were harvested for RNA isolation.

DNA and RNA methods. Most of the restriction and modifying enzymes used were purchased from Bethesda Research Laboratories, Inc., or Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and used as directed by the manufacturers.

The total-DNA minipreparation procedure used to screen cyanobacterial transformants was as previously described (7), with the following modification. After proteinase K treatment, 55 μl of 5 M NaCl and 45 μl of a solution of 10% (wt/vol) hexadecyltrimethylammonium bromide in 0.7 M NaCl were added to the lysate, which was incubated for 10 min at 65°C; the suspension was extracted with 500 μl of a 24:1 mixture of chloroform and isoamyl alcohol and then with equilibrated phenol, as in the published protocol.

Total RNA was isolated from 500-ml cultures as previously described by Golden et al. (7) and modified by Schaefer and Golden (19). All samples were kept on ice, and reagents were chilled during the extraction. For Northern blot analysis, RNA was denatured with formaldehyde, sep-

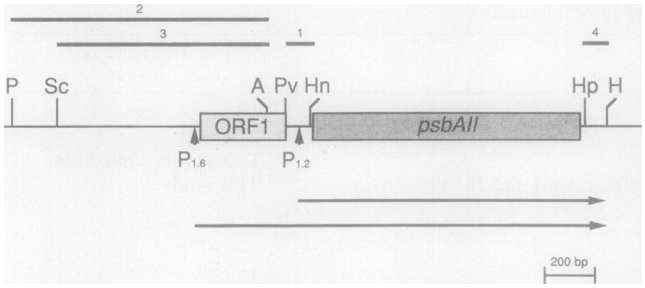

FIG. 1. Schematic representation of the *Synechococcus* sp. strain PCC 7942 *psbAII* locus. Boxes denote locations of a 342-bp potential open reading frame (ORF1) and the 1,080-bp *psbAII* open reading frame that encodes D1 form II. Upward-pointing arrows indicate apparent transcription start sites for the 1.6- and 1.2-kb *psbAII* transcripts (horizontal arrows). Restriction sites are shown as thin vertical bars for enzymes used to clone specific DNA fragments and construct gene-specific antisense RNA probe plasmids. Numbered horizontal bars identify regions that correspond to gene-specific antisense RNA probes used in characterization of the *psbAII* transcripts. Abbreviations used for restriction enzyme recognition sites: A, *Apa*I; H, *Hin*dIII; Hn, *Hin*fI; Hp, *Hpa*I; P, *Pst*I; Pv, *Pvu*I; Sc, *Sca*I. Other sites may be present for some of these enzymes.

arated by electrophoresis on 1.2% agarose gels, and transferred to a charged nylon membrane as described by Ausubel et al. (2). Radioactive antisense RNA probes were synthesized from restriction fragments subcloned into either the pT3T7 (Bethesda Research Laboratories) or pBluescript (Stratagene) vector by using T3 or T7 RNA polymerase and [α-^{32}P]UTP. A fragment from pAN4 containing the rRNA genes was radiolabeled by the random primer method (2).

Northern blots were hybridized to RNA probes at 50°C in a mixture of 50% (vol/vol) formamide–5× SSPE (10)–1% (wt/vol) sodium dodecyl sulfate–denatured salmon sperm DNA (100 μg/ml). Unhybridized probe was removed by washing the filters in a mixture of 0.5× SSPE and 0.1% sodium dodecyl sulfate at 65°C. To quantitate the relative transcript levels, Northern blots from duplicate RNA isolations were performed, and the autoradiographic bands were read in a Bio-Rad 620 video densitometer. The same RNA blots were stripped and reprobed with 16S rRNA; the 16S rRNA signals were scanned densitometrically, and these values were used as internal standards to ensure even loading of total RNA in each lane.

S1 protection and primer extension analyses. The 5′ end of the *psbAII* 1.6-kb RNA was identified by primer extension and S1 nuclease protection. Each reaction contained 100 μg of total RNA from wild-type *Synechococcus* sp. strain PCC 7942 cells. For primer extension, two 17-bp primers were synthesized; these were complementary to the sense strand between positions 290 and 306 and 493 and 509 of the sequence (see Fig. 2). The extended product generated from each primer was measured against a sequencing ladder generated by the dideoxynucleotide chain termination reaction of the same primer on a DNA template (17).

Probe 3 (see Fig. 1) was 5′ end labeled at an *Apa*I site and cleaved at an upstream *Sca*I site. The labeled strand was isolated and used for S1 nuclease protection experiments to map the 5′ end of the low-abundance 1.6-kb transcript (10). To determine the size of the protected fragment, the same labeled fragment was used to generate a sequencing ladder by chemical cleavage reactions (11).

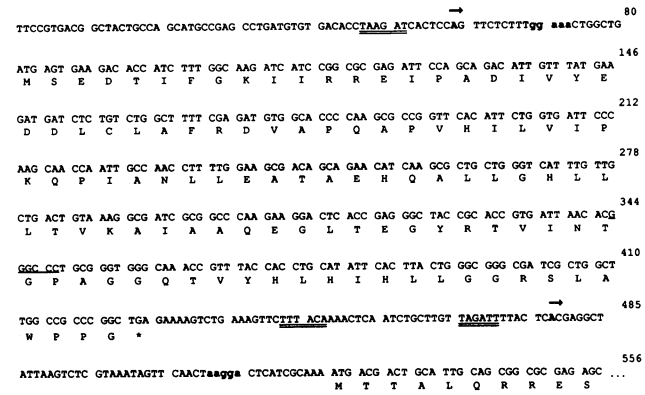

FIG. 2. *Synechococcus* sp. strain PCC 7942 nucleotide sequence upstream of the *psbAII* gene. The sequence contains two transcription start sites (arrows above boldface letters). The 1.6-kb dicistronic transcript begins at position 59 and encodes both a 342-bp open reading frame (ORF1) and the *psbAII* gene; the more abundant *psbAII* transcript begins at position 478. Putative −10 and −35 promoter sequences are doubly underscored. Potential ribosome-binding sites are depicted with boldface lowercase letters. Predicted amino acid sequences for the protein encoded by ORF1 and the amino terminus for that encoded by *psbAII* are shown by single-letter code below the corresponding line of the nucleotide sequence. ORF1 was interrupted in strain AMC084 by insertion of the Ω cassette (13) at the *Apa*I site (underscored; positions 344 to 349). The ellipsis indicates omission of the remainder of the previously published *psbAII* sequence.

RESULTS

Characterization of the 1.6-kb *psbAII* transcript. Northern blot analysis of total RNA from *Synechococcus* sp. strain PCC 7942 indicates that all three *psbA* genes give rise to 1.2-kb messages (6, 19). In earlier studies, a *psbAII*-specific probe (Fig. 1, probe 1) identified a longer low-abundance message (19; Brusslan, Ph.D. dissertation) that we determined to be approximately 1.6 kb long. To characterize the longer *psbAII* transcript without interference from the stronger signal from the 1.2-kb species, we constructed a probe that covered a region upstream of the putative transcription start site for the 1.2-kb *psbAII* message (Fig. 1, probe 2). This probe detected only the 1.6-kb *psbAII* transcript. The 5′ end of the longer message was mapped by primer extension and by S1 nuclease protection analysis by using probe 3 (Fig. 1) and was determined to be 419 bp upstream from the 5′ end of the 1.2-kb *psbAII* mRNA (Fig. 2). A probe specific for the nearly identical 3′ ends of the 1.2-kb *psbAII* and *psbAIII* transcripts (Fig. 1, probe 4) also hybridized to the 1.6-kb *psbAII* message, indicating that the longer transcript overlaps the complete *psbAII* open reading frame (data not shown).

The sequence of the region from which the 1.6-kb transcript originates and the presumptive transcription start site are shown in Fig. 2. Sequences centered around position −10 relative to the 5′ end of the 1.6-kb mRNA show sequence similarity with the 1.2-kb message upstream region (6) and with *E. coli* −10 consensus sequences. However, the 1.6-kb message appears to lack an *E. coli*-like −35 consensus sequence. The longer message contains a possible ribosome-binding site and a 342-bp open reading frame, ORF1; the stop codon for ORF1 is 17 bp upstream of the presumptive promoter for the 1.2-kb *psbAII* mRNA.

Relationship between the two *psbAII* messages. To investigate whether the 1.2-kb *psbAII* message is a processed

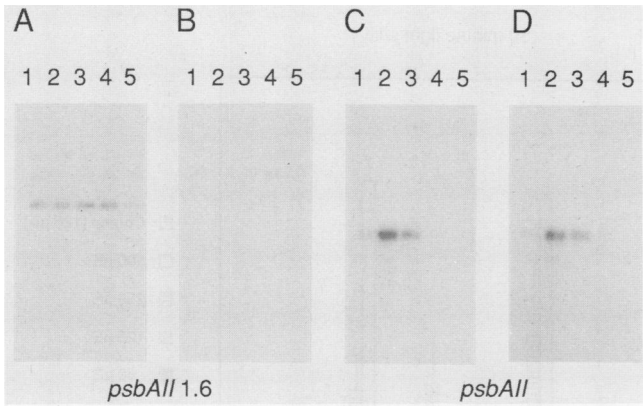

FIG. 3. Northern blot analysis of *psbAII* transcripts in the wild-type *Synechococcus* strain and strain AMC084. Total RNA was isolated from cells transferred to different PPFD for 15 min in a light shift experiment. Duplicate RNA samples were denatured with formaldehyde, separated by agarose gel electrophoresis, and transferred to nylon filters. Each blot was hybridized with a different *psbAII*-specific RNA probe (Fig. 1). Northern blots containing RNAs from the wild type (A and C) and strain AMC084 (B and D) were hybridized with probe 2 (A and B) or 1 (C and D). The lanes contained 5 μg of total RNA harvested from cells incubated at the following PPFD. Lanes: 1, control; 2, 500 $\mu E \cdot m^{-2} \cdot s^{-1}$; 3, 250 $\mu E \cdot m^{-2} \cdot s^{-1}$; 4, 125 $\mu E \cdot m^{-2} \cdot s^{-1}$; 5, 50 $\mu E \cdot m^{-2} \cdot s^{-1}$.

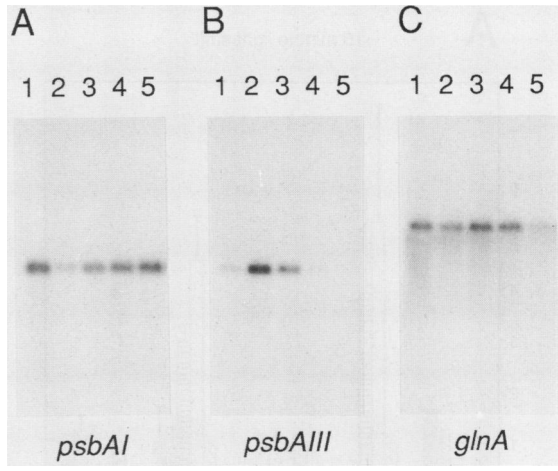

FIG. 4. Northern blot analysis of *psbAI*, *psbAIII*, and *glnA* transcripts in cells transferred to different PPFD in a 15-min light shift experiment. Triplicate blots containing RNA samples isolated from wild-type cells were prepared as described in the legend to Fig. 3, and each blot was hybridized with an antisense RNA probe specific for transcripts from *psbAI* (A), *psbAIII* (B), or *glnA* (C). The lanes contained 5 μg of total RNA harvested from cells incubated at the following PPFD. Lanes: 1, control; 2, 500 $\mu E \cdot m^{-2} \cdot s^{-1}$; 3, 250 $\mu E \cdot m^{-2} \cdot s^{-1}$; 4, 125 $\mu E \cdot m^{-2} \cdot s^{-1}$; 5, 50 $\mu E \cdot m^{-2} \cdot s^{-1}$.

product of the 1.6-kb transcript or a primary transcript, mutant strain AMC084 (Table 1) was constructed. This strain contains the Ω cassette (5, 13) inserted between the two putative promoters. The Ω cassette, which contains transcriptional terminators and a spectinomycin-streptomycin resistance (Sp^r) marker, was recombined into the *Synechococcus* sp. strain PCC 7942 chromosome at an *Apa*I site located 129 bp upstream of the presumptive transcription start site for the 1.2-kb message (Fig. 1). Southern analysis (16) of DNA from the transformants produced the predicted restriction fragments and confirmed that the expected recombination event had occurred (data not shown). If the 1.2-kb *psbAII* message is a processed product of the 1.6-kb message, neither of the transcripts should be present in cells that exhibit an Sp^r phenotype. RNA blots hybridized with probe 2 (Fig. 1) revealed complete absence of the 1.6-kb species in total RNA from strain AMC084 compared with that from wild-type cells (Fig. 3A and B). The presence of the 1.2-kb message in RNAs from both wild-type cells and strain AMC084 (Fig. 3C and D) indicates that the 1.2-kb message is transcribed independently and provides evidence that the predicted promoter region is functional.

Effects of changes in the light environment on *psbA* transcripts. Having identified all of the *psbA* transcripts, we investigated the effects of changes in light intensity on *psbA* mRNA levels. Wild-type cells were grown in 8-liter carboys to a cell density sufficient to decrease the PPFD measured in the centers of the vessels to approximately 125 $\mu E \cdot m^{-2} \cdot s^{-1}$. At that point, 250-ml samples were rapidly transferred to different light intensities and incubated for 15 or 30 min. Following incubation, these samples, as well as a control sample from the original culture, were rapidly frozen for RNA isolation.

Figure 3C shows a blot containing RNA isolated from wild-type cells subjected to a 15-min light shift experiment and hybridized with a *psbAII*-specific probe (probe 1). Relative to the amount detected in the control cells (lane 1), the 1.2-kb *psbAII* message increased dramatically in cells transferred to 500 and 250 $\mu E \cdot m^{-2} \cdot s^{-1}$ (lanes 2 and 3), remained constant at 125 $\mu E \cdot m^{-2} \cdot s^{-1}$ (lane 4), and was not detected in cells transferred to 50 $\mu E \cdot m^{-2} \cdot s^{-1}$ (lane 5). Longer exposure of the autoradiogram shown in Fig. 3 showed the less abundant 1.6-kb message, which is also recognized by the probe (data not shown). When the same RNA samples were hybridized with the 1.6-kb *psbAII* probe (probe 2), no significant changes were observed, suggesting that the level of this message is not rapidly affected by changes in light intensity (Fig. 3A).

To investigate whether the absence of the 1.6-kb message affected light regulation of the 1.2-kb *psbAII* transcript, RNA isolated from strain AMC084 in a light shift experiment was hybridized with probe 1. The same pattern was observed in RNA from mutant cells as in RNA from wild-type cells, indicating that the effect of changes in light intensity on *psbAII* 1.2-kb transcript levels is independent of the presence of the longer species (Fig. 3C and D). Additionally, this result demonstrates that the small open reading frame (ORF1) does not encode a regulator of *psbAII* expression.

The responses of the other members of the *psbA* gene family to changes in the light environment were also investigated. Similar RNA blots were hybridized with *psbAI* and *psbAIII* gene-specific probes. The pattern of expression seen for *psbAII* was also observed for *psbAIII* (Fig. 4B); the highest transcript level was detected at 500 $\mu E \cdot m^{-2} \cdot s^{-1}$, with a gradual decrease at lower light intensities. Conversely, the level of *psbAI* mRNA dramatically decreased in cells transferred to 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ and was highest in cells transferred to low light intensity (Fig. 4A). RNA blots from the same light shift experiments also were hybridized with a glutamine synthetase gene (*glnA*) probe to examine the effects of the light shifts on a gene not expected to be light regulated. *glnA* mRNA levels did not change, except for a decrease at 50 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Fig. 4C), which was sometimes observed for the 1.6-kb *psbAII* transcript as well. This result may indicate a general decrease in cellular mRNA levels at low light intensities.

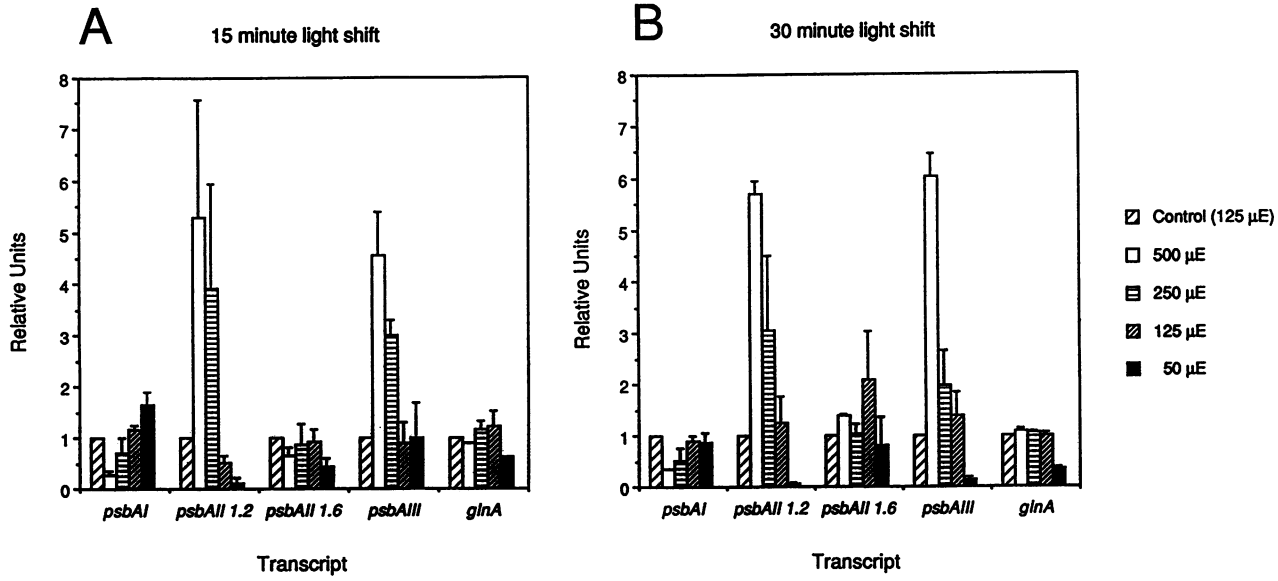

FIG. 5. Changes in *psbA* transcript levels in response to changes in light intensity. The histograms show levels of individual *psbA* and *glnA* transcripts in light-shifted samples relative to the unshifted control. Wild-type cells were transferred to different PPFD for 15 (A) or 30 (B) min. µE, Microeinsteins per square meter per second. Data were obtained by densitometric scanning of Northern blot autoradiograms and normalized to 16S rRNA signals in each lane. The data do not reflect the abundance of the individual transcripts compared with transcripts of the other genes; rather, the amount of each transcript at different PPFD is shown relative to the amount of the same transcript in a nonshifted control sample. The characteristic 1.2- and 1.6-kb transcripts for *psbAII* are identified as *psbAII* 1.2 and *psbAII* 1.6, respectively. The heights of the histogram bars show mean values from two identical experiments, and the upper ranges of the values are indicated by the vertical lines.

The magnitudes of changes in transcript levels were estimated by densitometric scans of autoradiograms from light shift experiments. Figure 5 shows histograms that express the amount of a transcript in light-shifted cells relative to the amount detected in unshifted control cells. Each bar shows the mean of two light shift RNA isolation experiments, and ranges of values are indicated. After cells were transferred to 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 15 min, transcripts from *psbAII* and *psbAIII* were 530 and 455% of the respective control levels, whereas the *psbAI* transcript decreased to approximately 27% of the control level (panel A). At 50 $\mu E \cdot m^{-2} \cdot s^{-1}$, the amount of mRNA from *psbAI* increased to 165% of the control, whereas mRNA from *psbAII* decreased to 12% of the control levels. RNA from *psbAIII* appeared unchanged at 50 $\mu E \cdot m^{-2} \cdot s^{-1}$, but this value was influenced by a single gel lane in which a high value was measured (indicated by a range bar). Levels of both the 1.6-kb *psbAII* transcript and the *glnA* transcript were unresponsive to changes in light intensity, except in cells transferred to 50 $\mu E \cdot m^{-2} \cdot s^{-1}$, in which both decreased to approximately 55% of the control level.

When light shift experiments were performed in which cells were incubated at the experimental light intensities for 30 min, all of the trends seen in the previous experiments were reproduced. However, the ranges of values were smaller between experiments with the longer incubation time (Fig. 5B). When cells were shifted to 50 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 30 min, mRNA from *psbAIII* dropped significantly in duplicate experiments.

DISCUSSION

Each of the three members of the *psbA* gene family of *Synechococcus* sp. strain PCC 7942 produces a major transcript with an apparent size of 1.2 kb (6). Here we report the characterization of an additional *psbAII* transcript of 1.6 kb. The 1.6-kb message is not a precursor of the 1.2-kb message; insertion of the Ω cassette between the two presumptive *psbAII* promoters and within ORF1 had no effect on 1.2-kb transcript levels, indicating that the smaller message originates from its own promoter. This experiment also eliminates a major role for the ORF1 gene product in expression of the 1.2-kb *psbAII* transcript.

The function of the 1.6-kb *psbAII* message or the ORF1 gene product remains unknown. A search of the National Biomedical Research Foundation protein sequence data base (4) revealed no significant matches with the putative ORF1 product. However, strain AMC084, in which the 1.6-kb transcript and ORF1 have been inactivated, grows more slowly than the wild-type *Synechococcus* strain. In mutant *Synechococcus* strains in which *psbAI*, *psbAIII*, or both have been inactivated, levels of both *psbAII* transcripts are elevated relative to their levels in wild-type cells (M. R. Schaefer and S. S. Golden, unpublished data), suggesting that under those conditions the 1.6-kb transcript contributes to a basal level of the *psbAII* transcript for form II of the D1 protein.

The relationship between light intensity and expression of the three *Synechococcus* sp. strain PCC 7942 *psbA* genes has been investigated previously by using *psbA-lacZ* translational gene fusions (19) and Western blot analysis of the two forms of D1 in the thylakoid membrane (18). These studies demonstrate that *psbA* expression is influenced by light intensity, yet they do not differentiate between regulation at the transcriptional level and regulation at the translational level. Northern blot analysis and light shift experiments showed that changes in light intensity rapidly and differentially modify the levels of the individual *psbA* transcripts. The most dramatic light-induced response was the increase

in the levels of *psbAII* and *psbAIII* transcripts shifted from 125 to 500 $\mu E \cdot m^{-2} \cdot s^{-1}$. These changes were also observed in a light shift experiment in which the incubation time at 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ was only 5 min (M. R. Schaefer, S. A. Bustos, and S. S. Golden, *Progress in Photosynthesis Research, vol. V,* in press). The similarity of the effects on the *psbAII* and *psbAIII* transcripts is consistent with the response of the *psbA-lacZ* reporters in previous experiments. These results suggest that *psbAII* and *psbAIII* share the same regulatory mechanism, which differs from that of *psbAI*.

The rapid changes in transcript levels could reflect altered rates of transcription or mRNA degradation. We did not determine the half-life of each of the *psbA* transcripts. However, others have reported that combined *psbA* transcript levels remain constant for up to 90 min postaddition of rifampin to *Synechococcus* sp. strain PCC 7942 cells cultured at 10 and 25 $\mu E \cdot m^{-2} \cdot s^{-1}$ (9). Our results suggest that the half-life of the *psbAII* transcript is shorter than 15 min at, and presumably below, 50 $\mu E \cdot m^{-2} \cdot s^{-1}$, since the message level dropped more than 50% during that time of incubation at low light intensity. The amount of the *psbAIII* transcript decreased to 14% of the control after 30 min of incubation at 50 $\mu E \cdot m^{-2} \cdot s^{-1}$, and one measurement taken after 15 min showed that the transcript level decreased to 68% of the control level. These values provide maximal estimates of half-lives, since the message levels reflect both transcription and degradation of transcripts. These experiments provide no information about *psbAII* and *psbAIII* transcript stabilities at high light intensities, since both message levels were elevated under those conditions.

The *psbAI* transcript level dropped to 27% of the control level after transfer from 125 to 500 $\mu E \cdot m^{-2} \cdot s^{-1}$, indicating that the message half-life is less than 15 min under high light intensity. However, the *psbAI* transcript may be much more stable at low light intensity. In cells cultured below 100 $\mu E \cdot m^{-2} \cdot s^{-1}$, *psbAI* transcripts account for 94 to 98% of total *psbA* messages (3, 19) and would make up virtually all of the *psbA* signal detected by Lonneborg et al. (9). We saw *psbAI* transcript levels increase after exposure to 50 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 15 min and remain constant after 30 min. These results are consistent with, although not indicative of, a long half-life for this message at low light intensity, especially since messages that do not appear to be otherwise light regulated decrease significantly under these conditions. The same result would also be obtained if *psbAI* were transcribed at a high rate at low light intensity, as suggested by levels of β-galactosidase from *psbAI-lacZ* fusions (19).

When considered together, the previous translational *lacZ* fusion experiments (19) and the changes in transcript levels reported here indicate that the major response of the *psbA* genes to changes in light intensity occurs at the transcriptional level. The *lacZ* fusions showed changes in expression that could have occurred at the transcriptional or translational level but did not reflect the half-lives of the native *psbA* transcripts (19). The current study shows only transcriptional or transcript stability changes; only transcriptional responses would have been detected by both methods.

Regulation of *Synechococcus* sp. strain PCC 7942 *psbA* expression by light intensity may involve *trans*-acting repressors or activators of promoter activity. Extensive genetic and molecular analyses of procaryotic regulatory systems have identified several classes of positive and negative control elements that influence transcription via *trans*-acting factors. Positive factors include the *araC*, *malT*, and *cpr* gene products of *E. coli* (15); negative factors include repressor proteins for the *gal*, *ara*, and *lac* operons (14). In both cases, the regulatory factor is a DNA-binding protein with high affinity for specific sites located near the promoter (14). Recent experiments in our laboratory suggest that proteins bind to the upstream region of *psbAII* (U. W. Mueller and S. S. Golden, unpublished data). Deletion mutagenesis upstream of *psbAI* to within 216 bp of the translational start site had no effect on light regulation of a *psbAI-lacZ* translational gene fusion (E. M. Neumann and S. S. Golden, unpublished data). Here we show that insertion of the Ω cassette 129 bp upstream of the promoter for the 1.2-kb *psbAII* transcript did not alter light regulation of that species. These experiments limit the region of potential binding by regulatory factors to within 129 bp of the *psbAII* and *psbAI* promoters.

Plants possess photoreceptor molecules that detect changes in either light quality or intensity and, subsequently, influence expression of various photosynthesis genes. Whether cyanobacteria possess a primary photosensor capable of detecting changes in light intensity has not been determined. However, indirect evidence suggests that light-regulated expression of *Synechococcus* sp. strain PCC 7942 *psbA* genes is modulated by photosynthetic activity and not directly by a photoreceptor molecule. Inactivation of one of the *psbA* genes affects the light response of the other members of the gene family, although the promoter and presumptive regulatory regions of the inactivated gene are still present (19; M. S. Schaefer and S. S. Golden, unpublished data). This idea is consistent with the observation of Brusslan and Haselkorn that inhibition of photosystem II by the herbicide Diuron increases the steady-state levels of transcripts from the *psbDII* and *psbA* genes (3). These experiments suggest that changes in photosynthetic capacity lead to altered expression of specific photosynthesis genes.

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