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Transcriptional and Posttranscriptional Components of *psbA* Response to High Light Intensity in *Synechococcus* sp. Strain PCC 7942

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The *psbA* genes, which encode the D1 protein of photosystem II, constitute a multigene family in the cyanobacterium *Synechococcus* sp. strain PCC 7942. Levels of messages from the three *psbA* genes change rapidly when cells are shifted from low-light to high-light conditions: the *psbAI* message level drops, whereas *psbAII* and *psbAIII* message levels increase dramatically. We examined the potential contributions of transcriptional and posttranscriptional processes in these high-light responses by subjecting cells that had been grown in a turbidostat at a standard light intensity (130 microeinsteins [μE] m^{-2} s^{-1}) to either the same or a higher light intensity (500 μE m^{-2} s^{-1}) in the presence or absence of rifampin. Northern (RNA blot) analysis of RNA isolated from cells subjected to high light showed that the increases in *psbAII* and *psbAIII* transcripts were blocked by rifampin. This suggests a transcriptional induction of these genes at high light intensities. Increased mRNA stability does not contribute to their accumulation in high-light conditions, since their half-life values did not increase relative to the half-lives measured at the standard light intensity. The rate of disappearance of the *psbAI* transcript in cells shifted to high light was diminished when either transcription or translation was blocked by rifampin or chloramphenicol, suggesting that accelerated degradation of the message requires de novo synthesis of a protein factor. When rifampin was added 10 min after the shift to high light intensity rather than before the shift, *psbAI* and *psbAIII* messages, but not the *psbAII* message, decayed at a faster rate. Susceptibility of the *psbAIII* transcript to the high-light-induced factor was also demonstrated by addition of chloramphenicol prior to the shift to high light. *psbAIII* transcript levels went up more than twofold higher in chloramphenicol-treated cells than in untreated cells, whereas *psbAII* transcript levels were unaffected by the inhibitor. These experiments provide evidence that either new or increased synthesis of a degradation factor which affects a subset of *Synechococcus* transcripts occurs in cells subjected to high light intensity.

The primary reactions of photosynthesis in cyanobacteria, algae, and higher plants are carried out by two major protein-pigment complexes embedded in the thylakoid membranes, termed photosystem II (PSII) and photosystem I (PSI). The reaction center of PSII contains two structurally similar proteins, D1 and D2, which house the primary photoreactants involved in charge separation across the membrane. Together with cytochrome b_{559}, these proteins form the core of PSII (14, 23, 27, 37). D1 is encoded by the *psbA* gene, which is usually present in single copy in the chloroplast genomes of plants (38). In contrast, the prokaryotic cyanobacteria have multiple *psbA* genes, often encoding different forms of D1 (9, 12, 13, 16). The genome of the unicellular cyanobacterium *Synechococcus* sp. strain PCC 7942 contains three functional *psbA* genes, which are not closely linked on the chromosome (13). Expression from any one of these genes is sufficient to sustain photoautotrophic growth (13). Each of the genes encodes an open reading frame of 360 amino acids which is highly conserved with the 353-residue polypeptide encoded by chloroplast *psbA* genes. The *psbAI* gene encodes form I of the D1 protein, whereas both *psbAII* and *psbAIII* encode form II (13, 32). A functional difference between the two forms, which differ at 25 residues, has not been determined.

Except for a few nucleotides, the regions upstream of the coding sequences are different among the three *Synechococcus psbA* genes. Each gene is expressed as a monocistronic 1.2-kb message (13). The *psbAII* open reading frame is also present on a 1.6-kb transcript that initiates 419 bp upstream of the 1.2-kb transcript and carries an additional 342-bp open reading frame of unknown function (6). The three genes have only 7 bp of identity downstream of their stop codons, at which point the *psbAI* sequence diverges from those of the other two genes. The *psbAII* and *psbAIII* downstream flanking sequences are identical for an additional 71 bp, except for a one-nucleotide difference and a 3-bp gap in the *psbAIII* sequence relative to that of *psbAII* (13).

The three *Synechococcus psbA* genes are differentially expressed and respond to changes in light intensity (6, 34, 35). This was initially demonstrated by measuring β-galactosidase activities in strains that carry specific *psbA-lacZ* translational fusions recombined into the *Synechococcus* chromosome. The light intensity penetrating 8-liter batch cultures drops over time because of self-shading by the growing cell population; under these conditions, expression from *psbAI-lacZ* goes up 800% as the light intensity received by the culture decreases (from 600 microeinsteins to 2 [μE] m^{-2} s^{-1}), whereas expression from *psbAII-lacZ* and *psbAIII-lacZ* goes down 90% (35). Rapid transfer of the reporter strains to higher light shows that 6- to 10-fold increases in *psbAII-lacZ* and *psbAIII-lacZ* expression occur within 15 min of exposure to the new light environment (31).

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Immunoblot analysis of membrane proteins from wild-type cultures shows that the ratio of the two forms of D1 in the thylakoid changes, as predicted by reporter gene activities (34).

The differential responses of the *psbA* genes to high light that were suggested by reporter gene activities are also apparent in RNA levels, indicating that changes in transcription and/or transcript stability are occurring (6). Northern (RNA blot) analysis shows that transcripts from both *psbAII* and *psbAIII* increase to approximately 500% of control levels within 15 min in cells shifted from 125 to 500 $\mu E\ m^{-2}\ s^{-1}$ and are not detected in cells transferred to lower light intensities (6). In contrast, transcripts from *psbAI* decrease approximately 70% when cells are shifted to higher light intensities and increase in cells shifted to lower light intensities. These characteristic trends in the three *psbA* transcript levels can be detected within 5 min of transfer to new light environments (32). Increases in overall *psbA* message levels stimulated by higher light intensities have been reported for *Synechococcus* sp. strain PCC 6301 and for the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803 (22, 24, 25).

The rapid and dramatic light-responsive changes in *Synechococcus psbA* expression may be due to alterations in the transcription rates of the genes or changes in the stability of the mRNAs, or a combination of both processes. In prokaryotes, transcriptional control of gene expression has been widely demonstrated and is often the primary regulatory mechanism (28, 29). Posttranscriptional control mediated by changes in mRNA stability has also been described for prokaryotes (3). In the purple nonsulfur photosynthetic bacterium *Rhodobacter capsulatus*, segmental differences in mRNA stability within the polycistronic *pufLMX* transcript, which encodes components of the photosynthetic reaction center, result in differential expression of *pufLMX* genes. Transcription of *pufLMX* and segmental degradation of the *puf* mRNA are controlled by oxygen tension (18, 20). In *Bacillus subtilis*, the stability of specific mRNA species changes with the growth stage (30). Both transcriptional and posttranscriptional mechanisms also have been implicated in the regulation of chloroplast genes (15), which share some features with prokaryotic genes. In higher plants, *psbA* transcripts accumulate during chloroplast biogenesis and also in response to illumination (15). In spinach, it has been shown that accumulation of *psbA* mRNA is not due to increased transcriptional activity but rather to increased stability of the message (10, 17).

We examined possible transcriptional and posttranscriptional components of light-induced changes in *Synechococcus psbA* transcript levels by using the inhibitor rifampin to block transcription and measuring mRNA half-lives at different light intensities. We transferred wild-type cells that were adapted to a standard light intensity (130 $\mu E\ m^{-2}\ s^{-1}$) to either the same light intensity or a higher light intensity (500 $\mu E\ m^{-2}\ s^{-1}$) in the presence and absence of rifampin. Northern blot analysis of time-course RNA samples hybridized with gene-specific probes led us to the following conclusions: the increase in levels of transcripts from *psbAII* and *psbAIII* after a shift to high light is a transcriptional response; the rapid depletion of *psbAI* transcripts is caused by a specific degradation factor which is rapidly synthesized following a shift to high light; and the *psbAIII* transcript, but not the *psbAII* transcript, is also subject to accelerated degradation at high light intensity.

MATERIALS AND METHODS

Culture conditions and inhibitor treatments. Wild-type *Synechococcus* sp. strain PCC 7942 was grown in BG-11 medium (1) as modified by Bustos and Golden (5) and maintained as a continuous culture. The growth apparatus was a turbidostat (5), in which cells are maintained at a constant cell density and constant light intensity. All cultures were maintained at an optical density at 750 nm (OD_{750}) of 0.5 at 30°C, with aeration as well as efficient mixing of the culture achieved by bubbling with 1% CO_2 in air. The turbidostat culture served as the source of cells adapted to the standard light intensity (130 $\mu E\ m^{-2}\ s^{-1}$) for all light shift experiments. Light intensity (photosynthetic photon flux density [PPFD]) was measured by an immersible probe (Biospherical Instruments, San Diego, Calif.) and is reported as microeinsteins per square meter per second.

For light shift studies, cells were transferred to either the same (130 $\mu E\ m^{-2}\ s^{-1}$) or a higher (500 $\mu E\ m^{-2}\ s^{-1}$) light intensity in the presence or absence of rifampin. Rifampin was purchased from Sigma (St. Louis, Mo.) or Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and was used at a concentration of either 50 or 200 μg/ml. The higher concentration was used during a period in which the potency of the antibiotic stock was in question; this alteration in the protocol proved to be unnecessary and irrelevant. The time of addition of rifampin and of collection of each sample is indicated in Results and in the figure legends. Cells growing in the turbidostat were transferred to Pyrex tubes, suspended in an aquarium maintained at 30°C, illuminated at the stated PPFD, and bubbled with 1% CO_2 in air. Cells were harvested and poured over crushed ice at various time intervals after the light shift, pelleted immediately, and frozen in liquid nitrogen. All samples were stored at −90°C for RNA isolation. For the experiment shown in Fig. 3 and 5, chloramphenicol (Sigma) at a final concentration of 250 μg/ml was added to some samples prior to the shift to high light. In that experiment, cells were transferred to petri dishes and incubated at 500 $\mu E\ m^{-2}\ s^{-1}$ as described by Bustos et al. (6).

RNA methods. Total RNA was extracted from 25 ml of *Synechococcus* culture as described by Mohamed and Jansson (24). RNA samples (5 μg, based on the OD_{260} reading) were denatured, separated by electrophoresis on a 1.2% agarose gel, and blotted onto a charged nylon membrane (Magnagraph; Micron Separations Inc., Westboro, Mass.) as described by Ausubel et al. (2). Blots were probed with radioactive antisense transcripts from plasmids that contain fragments from the unique upstream untranslated regions of each of the *psbA* genes (4). Hybridization and washing conditions were as previously described by Bustos et al. (6).

Northern blots were exposed to X-ray film for various periods of time to obtain autoradiograms in the appropriate exposure ranges for photography and densitometry. Relative band intensities were determined by scanning the autoradiograms on a Bio-Rad 620 densitometer. To ensure even loading of total RNA in the lanes, the 16S RNA bands from photographic negatives of the ethidium bromide-stained gels were scanned, and small corrections were made from these values. Half-lives were calculated from the equation generated by subjecting the data to exponential regression analysis.

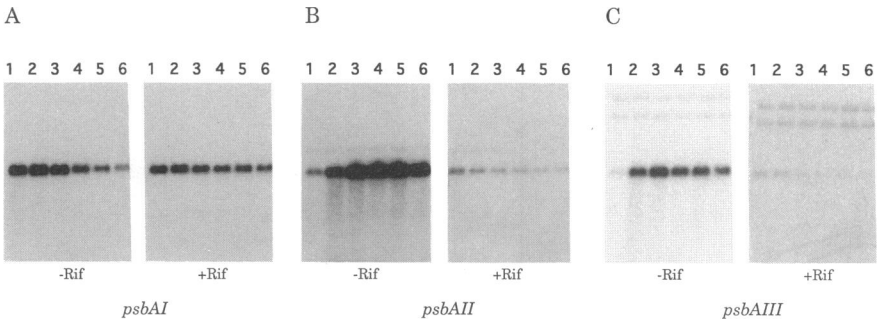

FIG. 1. Northern blot analysis of *psbA* transcripts from cells shifted to high light intensity. Wild-type *Synechococcus* sp. strain PCC 7942 cells adapted to a standard PPFD (130 μE m^{-2} s^{-1}) were transferred to a higher PPFD (500 μE m^{-2} s^{-1}) in the presence (+Rif) or absence (−Rif) of rifampin. Rifampin (200 μg/ml) was added to the cells 5 min before the light shift. Total RNA was extracted from cells harvested at various time points after the light shift. Lanes: 1, 0 min (just prior to light shift); 2, 5 min; 3, 10 min; 4, 15 min; 5, 20 min; 6, 25 min. Samples of total RNA (5 μg per lane) were denatured, separated by electrophoresis on a 1.2% agarose gel, and transferred to a charged nylon membrane. The blots were hybridized with antisense RNA probes specific for messages from *psbAI* (A), *psbAII* (B), or *psbAIII* (C). Bands corresponding to 23S rRNA appear at the top of the *psbAIII* probe panels.

RESULTS

Increase in *psbAII* and *psbAIII* transcripts at high light intensity is a transcriptional response. Turbidostatically grown *Synechococcus* cells were adapted to a standard light intensity (130 μE m^{-2} s^{-1}) and then shifted to a higher light intensity (500 μE m^{-2} s^{-1}) in the presence or absence of rifampin, an inhibitor of transcription. Total RNA extracted from samples taken at various time points was subjected to Northern blot analysis.

The levels of *psbAII* and *psbAIII* transcripts increased dramatically when cells were shifted to the high PPFD (Fig. 1B and C). This increase was completely blocked by rifampin, demonstrating that the accumulation of these transcripts is due to a transcriptional induction. For the experiment shown in Fig. 1, transcripts from *psbAII* and *psbAIII* increased 13-fold and 7-fold, respectively, within 15 min after the light shift. These conditions characteristically induced increases in the levels of *psbAII* and *psbAIII* transcripts of 10- to 20-fold and 6- to 10-fold, respectively. The level of the 1.6-kb *psbAII* transcript, which is not regulated by light intensity (6), did not change during the course of this experiment.

The levels of the *psbA* messages remained fairly constant over 40 min when cells growing in the turbidostat at a PPFD of 130 μE m^{-2} s^{-1} were shifted to the same light intensity (data not shown). The half-lives of these messages, calculated during maintenance at the standard light intensity, do not differ significantly from those at high light intensity (Table 1). These results demonstrate that the rapid increase in *psbAII* and *psbAIII* transcript levels at high light intensities is a transcriptional response and that an increased mRNA stability does not contribute to it.

Decay of the *psbAI* transcript at high light intensity is due to accelerated degradation. In contrast to *psbAII* and *psbAIII*, expression of *psbAI* decreases at high light intensity (6, 35). Figure 1A shows that the level of transcripts from *psbAI* decreased rapidly after the shift to high light intensity. This decay of the *psbAI* transcript was diminished in the set of samples treated with rifampin (Fig. 1A, Fig. 2). The shorter apparent half-life when transcription was not inhibited than when the inhibitor was present suggests that synthesis of a factor is responsible for rapid decay of the transcript. Treatment of the cells with chloramphenicol, an inhibitor of translation, also diminished the decay of the *psbAI* transcript, implying that protein synthesis is a requirement for accelerated degradation of the message (Fig. 3).

The half-lives of the *psbAI* message were calculated from an experiment in which cells growing at a PPFD of 130 μE m^{-2} s^{-1} were treated with rifampin and incubated at the same light intensity or shifted to high light intensity. No significant differences in the half-lives were observed under these two conditions (Table 1). However, the decay of the message at the high light intensity in the presence of rifampin (25 min) was considerably slower than the decay in the absence of the inhibitor (9.4 min; Table 1 and Fig. 1A). Note that the calculated values for the uninhibited control signify only the rate of disappearance of the message and not a true half-life, since transcription may be contributing to the message pool. Although half-lives were calculated from two experiments which were conducted under identical conditions, we have observed that rifampin addition prior to high-light exposure stabilized the *psbAI* transcript in nine experiments. From these experiments, it is clear that the degradation of the *psbAI* message is accelerated at high light intensity and that transcription and translation appear to be required for this response.

Evidence that a degradation factor is synthesized upon a shift to high light intensity. We performed the following

TABLE 1. Half-lives of the *psbA* messages after transfer of the cells to either standard or high-light conditions[a]

Transcript	Half-life (min) after transfer to:	
	Standard PPFD	High PPFD
psbAI	18.0 ± 3.3	25.0 ± 3.6 (9.4 ± 1.3)[b]
psbAII	39.7 ± 6.3	29.5 ± 2.7
psbAIII	9.8 ± 2.7	11.6 ± 3.7

[a] *Synechococcus* sp. strain PCC 7942 cells adapted to a standard light intensity (130 μE m^{-2} s^{-1}) were transferred to either the same light intensity or a higher light intensity (500 μE m^{-2} s^{-1}) in the presence of rifampin (50 μg/ml). Rifampin was added 5 min before the light shift, and cells were harvested for RNA isolation at various time intervals. The data were obtained by densitometry of the autoradiograms, and the half-lives were calculated from the equation computed by exponential regression of the data. Values represent a mean of at least two independent experiments, and the range between experiments is indicated.

[b] This value is a measure of the decay of the *psbAI* transcript at high light intensity in the absence of rifampin and thus may not represent the true half-life of the transcript.

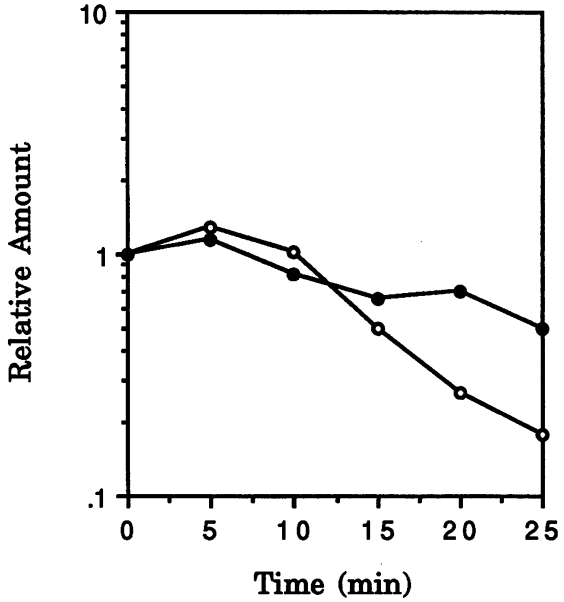

FIG. 2. Rate of disappearance of the *psbAI* transcript at high light intensity. Changes in the relative amounts of *psbAI* transcript were determined by densitometry of the autoradiograms shown in Fig. 1A. *Synechococcus* cells adapted to the standard PPFD (130 μE m^{-2} s^{-1}) were transferred to a higher PPFD (500 μE m^{-2} s^{-1}) in the presence (●) or absence (○) of rifampin.

experiment in order to determine whether a transcript degradation factor is rapidly synthesized at high light intensity or whether an indirect secondary effect of the inhibitors accounts for the data. *Synechococcus* cells were adapted to the standard light intensity and then transferred to the higher light intensity. Rifampin was added either prior to or 10 min after the shift to high light intensity. If rapid synthesis of a degradation factor is involved, the degradation of the message should be accelerated when rifampin is added after the light shift relative to the rate observed when rifampin is added before the shift. This result was observed (Fig. 4A), with the *psbAI* transcript half-life decreasing from 22.9 to 12.3 min (Table 2). This provides strong evidence that a degradation factor is indeed rapidly synthesized upon a shift to high light intensity.

The *psbAIII* transcript also decayed faster in samples that

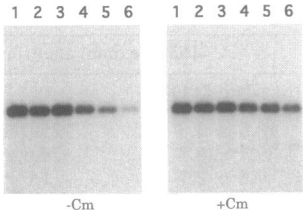

FIG. 3. Effect of chloramphenicol on high-light-induced decrease in *psbAI* message levels. Wild-type *Synechococcus* cells adapted to the standard PPFD were transferred to a higher PPFD (500 μE m^{-2} s^{-1}) in the presence or absence of chloramphenicol (Cm). Chloramphenicol (250 μg/ml) was added to the cells 5 min before the light shift. Total RNA was extracted from cells harvested at various time points after the light shift. Lanes: 1, 0 min (just prior to light shift); 2, 1 min; 3, 5 min; 4, 10 min; 5, 15 min; 6, 30 min. Northern blots were prepared as described in the legend to Fig. 1 and hybridized with a *psbAI*-specific antisense RNA probe.

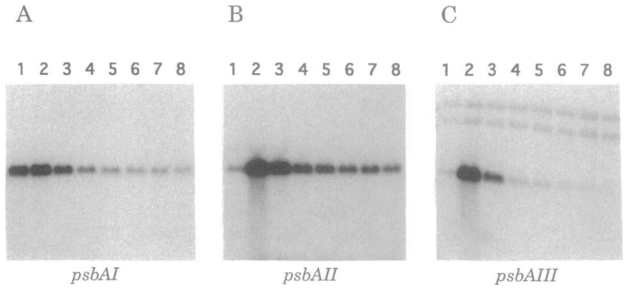

FIG. 4. Effect of rifampin on *psbA* message levels after transfer of cells to high light intensity. *Synechococcus* cells were transferred from the standard PPFD to the higher PPFD, and rifampin (200 μg/ml) was added to the cells 10 min after the light shift. Total RNA was extracted from cells harvested at various time points after the light shift. Lanes: 1, 0 min (just prior to light shift); 2, 10 min (just prior to addition of rifampin); 3, 15 min; 4, 20 min; 5, 25 min; 6, 30 min; 7, 35 min; 8, 40 min. Northern blots were prepared as described in the legend to Fig. 1 and hybridized with antisense RNA probes specific to *psbAI* (A), *psbAII* (B), and *psbAIII* (C).

were treated with rifampin after the light shift than in samples inhibited before the shift (Fig. 4C and Table 2). However, the rate of decay of the *psbAII* message was the same irrespective of the time when rifampin was added (Fig. 4B and Table 2). Although the half-life of the *psbAII* message was shorter in this experiment compared with the values reported in Table 1, this difference was not dependent on light intensity. We have observed in the majority of our experiments that *psbAII* is a very stable message.

Our finding that the *psbAIII* transcript is degraded at an accelerated rate at high light intensity means that the observed increase in the steady-state *psbAIII* message level underrepresents the transcriptional induction of the gene. If accelerated degradation of the *psbAIII* and *psbAI* messages is effected by the same factor, then blocking translation (and thus blocking the accelerated degradation) should cause a greater increase in the level of the *psbAIII* message at high light intensity. This idea was tested by using *psbAII*- and *psbAIII*-specific probes against blots of RNA samples from cells that were shifted to high light intensity in the presence or absence of chloramphenicol. Figure 5 shows the changes in transcript levels determined from these autoradiograms as well as from those shown in Fig. 3. These experiments indicated that the increase in the *psbAIII* message level at

TABLE 2. Half-lives of the *psbA* transcripts at high light intensity[a]

Transcript	Half-life (min) when rifampin added:	
	5 min before shift to high PPFD[b]	10 min after shift to high PPFD
psbAI	22.9	12.3
psbAII	13.3	13.0
psbAIII	13.0	5.5

[a] *Synechococcus* sp. strain PCC 7942 cells adapted to a standard light intensity (130 μE m^{-2} s^{-1}) were transferred to a higher light intensity (500 μE m^{-2} s^{-1}) in the presence of rifampin (200 μg/ml) added either 5 min before or 10 min after the shift. Cells were harvested at various times after the shift for RNA isolation. Data were obtained by densitometry of the autoradiograms, and half-lives were calculated from the equation computed by exponential regression of the data.

[b] Experimental conditions were similar to those for the experiments for which mean half-lives are reported in the "High PPFD" column in Table 1.

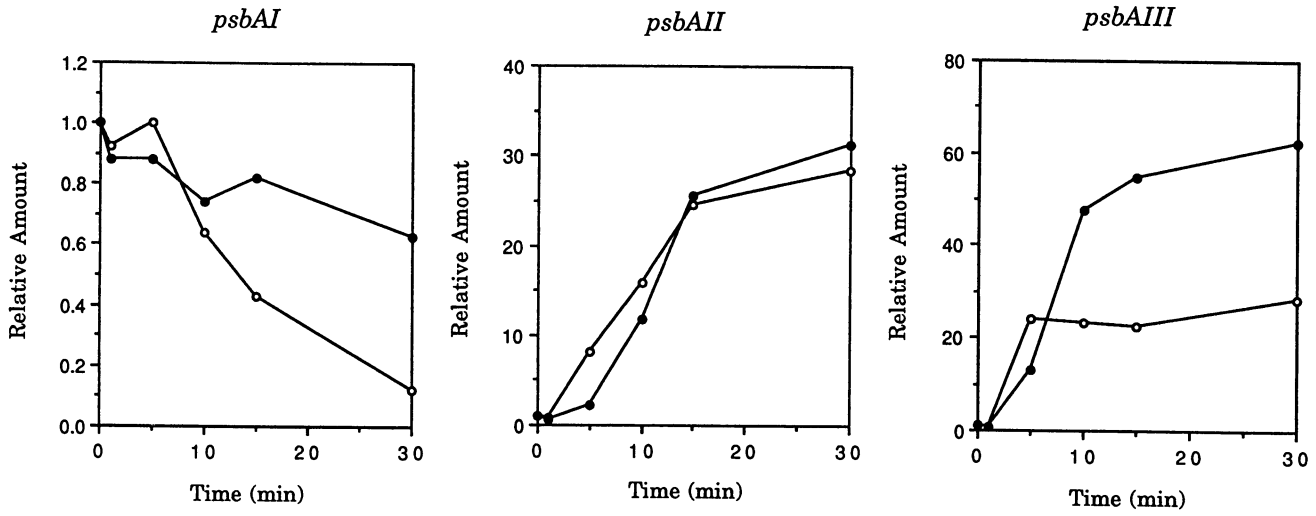

FIG. 5. Relative changes in *psbA* message levels in cells transferred to high light intensity in the presence or absence of chloramphenicol. *Synechococcus* cells were adapted to the standard light intensity and transferred to the higher PPFD in the presence (●) or absence (○) of chloramphenicol. Experimental conditions are described in the legend to Fig. 3. Northern blots were hybridized with antisense RNA probes specific for transcripts from each of the *psbA* genes. Changes in the hybridization signals of high-PPFD samples relative to that of the standard-PPFD sample were determined by densitometry of the autoradiograms. The probe used to generate the data is shown for each panel.

high light intensity was enhanced by blocking protein synthesis, suggesting that high-light-induced degradation of this transcript, like that of the *psbAI* transcript, requires protein synthesis. Furthermore, the lack of influence of chloramphenicol on *psbAII* message levels supports the results of the half-life experiments, which indicated that the *psbAII* transcript is not subject to the posttranscriptional regulation observed for those of *psbAI* and *psbAIII*.

Table 1 shows that the half-lives of all the *psbA* messages do not vary significantly between standard and high-light conditions when rifampin is added before the shift to high-light conditions. When cells are incubated briefly at high light intensity before transcription is inhibited, both the *psbAI* and *psbAIII* transcripts show a faster decay than when transcription is inhibited prior to the light shift. This argues against the possibility that rifampin has a specific transcript-stabilizing function other than inhibition of de novo synthesis.

DISCUSSION

The levels of *psbAII* and *psbAIII* transcripts increase 2- to 10-fold within minutes after cells are shifted to high light intensity (6, 32). Translational fusions to each of these genes indicate that either transcription or translation increases dramatically when the PPFD is high (35). We have shown here that rapid transcriptional induction contributes significantly to the observed increase in the transcript levels (Fig. 1B and C). The regulation of transcription of these genes may be mediated by *trans*-acting factors, which have been implicated in the regulation of both prokaryotic and eukaryotic systems (28, 29). Recent experiments in our laboratory show that proteins from soluble extracts prepared from cells adapted to 125 μE m^{-2} s^{-1} and from cells shifted to high-light intensity bind to the upstream regions of both of these genes (26). Transcriptional fusions between these DNA fragments and *lacZ* show that expression of β-galactosidase increases by 70 to 110% after a shift to high light intensity (21).

Increased mRNA stability is not involved in the accumulation of the *psbAII* and *psbAIII* transcripts at high light intensity, as shown by the similar half-lives of rifampin-treated cells incubated at the high or standard light intensity (Table 1). Surprisingly, the half-life of the *psbAIII* transcript decreased when transcription was inhibited after the cells had been exposed to high light at least for 10 min (Table 2). This indicates that the observed increase in steady-state *psbAIII* message levels at high light intensity underrepresents the transcriptional induction of the gene.

The level of *psbAI* transcripts decreased to approximately 30% of the initial level when cells were shifted to high light intensity (Fig. 2). This decrease in *psbAI* message levels could result from a decrease in the rate of transcription of this gene, accelerated degradation of the message, or a combination of these factors. Decreased transcription alone could not account for the observation that the transcript disappeared more rapidly at high light intensity in the absence of rifampin than when the drug was added before the light shift. Rifampin should mimic the effect of the complete cessation of transcription from the *psbAI* promoter. The fact that rifampin actually increased the half-life suggests that *psbAI* messages are being affected by the product of another locus that is also being blocked by rifampin. The identity of the degradation factor as a protein is suggested by the observation that chloramphenicol acted similarly to rifampin in delaying the decay of the *psbAI* message (Fig. 3 and 5). Chloramphenicol addition also blocked high-light-induced degradation of the *psbAIII* transcript, as visualized by exaggerated elevation of transcript levels at high light intensity in cells that had been treated with the inhibitor (Fig. 5).

Both *psbAI* and *psbAIII* transcripts decay at a faster rate when rifampin is added after the cells have been exposed to high light intensity for 10 min than when it is added before the light shift (Table 2). This provides direct evidence that accelerated degradation at high light intensity is due to either new or increased synthesis of a degradation factor. The half-life of the *psbAII* message does not change with the time of addition of rifampin (Table 2), indicating that the factor

shows some message specificity. The half-life of the *psbAII* message observed in this experiment was short compared with the half-lives calculated from the majority of light shift experiments (Table 1). We could not pinpoint the reason for this difference; we believe that it could be due to minor changes in the growth conditions that were not obvious to us. We have observed variations in both the magnitude of transcript level increases and decreases and the timing of these changes among experiments, even though the overall patterns are constant. These quantitative variations suggest that preexisting conditions modify the response of each of the genes to the light environment. An unexplained variation in the contribution of *psbAII* to the total *psbA* transcript pool was reported previously by Brusslan and Haselkorn (4). They found that a stock culture of wild-type *Synechococcus* sp. strain PCC 7942 that had been subcultured regularly showed a steady-state level of *psbAII* transcript higher than observed previously (13) and higher than that in RNA samples from a stock that was revived from frozen-strain archives. We did not detect elevated levels of *psbAII* transcript in the subcultured strain provided to us by Brusslan and Haselkorn, suggesting that the differences they observed were transient rather than due to a mutation (33).

Our current experiments indicate that the *psbAI* and *psbAIII* transcripts are degraded at an accelerated rate at high light intensity and that de novo transcription and protein synthesis are required to produce a degradation factor (Fig. 5 and Table 2). However, this does not necessarily mean that the degradation factor is a nuclease which is synthesized at higher light intensities. The high-light-induced factor may be a protein that modifies an existing nuclease or that binds to certain transcripts to alter their stabilities. Alternatively, the factor may be an inhibitor of translation, in which case the alteration in mRNA stability may result from the untranslated messages' being more vulnerable to attack by existing nucleases (3).

The shared property of accelerated degradation of the *psbAI* and *psbAIII* transcripts suggests that they should bear a conserved target that is not present in the *psbAII* message. Precedents exist for RNA stability determinants at different locations in bacterial messages. The degradation rates of individual segments within the polycistronic *puf* operon are determined by the stabilizing effect of the multiple hairpin loop structures and sites of nonrandom endonucleolytic cleavage within the message (7, 19). The 5′ untranslated region of the *ompA* mRNA in *Escherichia coli* serves as an mRNA-stabilizing element (8, 11). The 3′ inverted repeats found on a number of chloroplast genes, including *psbA*, have been implicated in the stabilization of the transcript (36). These 3′ inverted repeats bind gene-specific proteins, as demonstrated by gel mobility shift and UV cross-linking analyses (36), and mutations in nucleotides within a stem-loop structure of *psbA* affect the stability of the message. However, the 3′ untranslated regions of the *psbAII* and *psbAIII* transcripts are nearly identical and are not like that of the *psbAI* transcript. The 5′ untranslated leaders of the three transcripts are unique; no similarity is evident between *psbAI* and *psbAIII* in this region. Within the open reading frames, *psbAII* and *psbAIII* show more sequence identity with each other than either of these genes shows with *psbAI*, but there are eight nucleotide positions which are common only to *psbAI* and *psbAIII* (13). Two of these flank the nucleotide which was mapped as the 5′ end of a subgenic message that arises from *psbAI* (13).

The observation that *psbAI* and *psbAIII* messages are destabilized at high light intensity and that the *psbAII* message is not (Fig. 5 and Table 2) shows another component of the differential expression seen within this gene family. The accelerated decay of the *psbAI* transcript is not masked by increased transcription of this gene at high light intensity, as was observed for *psbAIII*. The rate of transcription of *psbAI* may be lowered at high light intensity. Preliminary results with transcriptional gene fusions between the *psbAI* upstream regulatory region and *lacZ* suggest that the transcription of this gene may in fact decrease upon a shift to high light intensity (21).

The qualitative result of the observed high-light-induced changes in *psbA* expression would be alteration of the ratio of the two forms of D1 protein in the thylakoid to favor incorporation of the *psbAII* and *psbAIII* product, form II (34). We still do not know whether the two forms of D1 have any distinctive physiological properties. Our experiments to date have focused on the rapid, short-term responses of the *psbA* genes within minutes of exposure to a new light environment. Recent experiments investigating the longer-term effects of high light intensity on *psbA* gene expression have indicated that the cells adapt and return to high expression of *psbAI* within 3 h. Our current efforts are aimed at characterizing the long-term changes in gene expression and determining the adaptive consequences of regulating the gene family.

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