Light Control of *hliA* Transcription and Transcript Stability in the Cyanobacterium *Synechococcus elongatus* Strain PCC 7942

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The high-light-inducible proteins (HLIPs) of cyanobacteria are polypeptides involved in protecting the cells from high-intensity light (HL). The *hliA* gene encoding the HLIP from *Synechococcus elongatus* strain PCC 7942 is expressed in response to HL or low-intensity blue or UV-A light. In this study, we explore via Northern analysis details of the transcriptional regulation and transcript stability of the *hliA* gene under various light conditions. Transcript levels of the *hliA* gene increased dramatically upon a shift to HL or UV-A light to similar levels, followed by a rapid decrease in UV-A light, but not in HL, consistent with blue/UV-A light involvement in early stages of HL-mediated expression. A 3-min pulse of low-intensity UV-A light was enough to trigger *hliA* mRNA accumulation, indicating that a blue/UV-A photoreceptor is involved in upregulation of the gene. Low-intensity red light was found to cause a slight, transient increase in transcript levels (raising the possibility of red-light photoreceptor involvement), while light of other qualities had no apparent effect. No evidence was found for wavelength-specific attenuation of *hliA* transcript levels induced by HL or UV-A light. Transcript decay was slowed somewhat in darkness, and when photosynthetic electron transport was inhibited by darkness or treatment with DCMU, there appeared a smaller mRNA species that may represent a decay intermediate that accumulates when mRNA decay is slowed. Evidence suggests that upregulation of *hliA* by light is primarily a transcriptional response but conditions that cause ribosomes to stall on the transcript (e.g., a shift to darkness) can help stabilize *hliA* mRNA and affect expression levels.

Like all photosynthetic organisms, cyanobacteria must carefully balance their absorption and utilization of light energy with the ambient light conditions. Absorption of light energy beyond what can be used in photosynthesis, such as during periods of high-intensity light (HL) exposure at midday, can be extremely damaging to a photosynthetic cell. This can lead to overexcitation of the photosynthetic apparatus, the generation of dangerous triplet chlorophyll molecules, and damaging oxygen radicals that can ultimately lead to photoinhibitory oxidative damage. Therefore, acclimation mechanisms have evolved to allow an organism to avoid, compensate for, dissipate, or repair damage caused by excess light energy, including producing protective pigments or altering the activity and composition of the photosynthetic apparatus (for a recent review, see reference 33). Many of the acclimation responses to light stress necessitate alterations in gene expression and are controlled through mechanisms that allow the cell to sense changes in the light environment.

One way that plants can perceive changes in their light environment is through sensing shifts in light color (quality). Light quality controls development and many other processes in plants and is perceived through the use of photoreceptors that respond to specific light wavelengths (9, 36). Arguably the best-characterized photoreceptors are the phytochromes, with photointerconvertible red-light and far-red-light forms. Although best studied with higher plants, phytochrome-like proteins have been found in cyanobacteria, photosynthetic bacte-

ria, and recently in nonphotosynthetic bacteria (29, 44). For cyanobacterial phytochromes, the target genes and processes that are controlled remain largely unknown (31). Two blue/UV-A light photoreceptors have been identified in higher plants, the cryptochromes and phototropin. Despite the presence of blue-light-triggered processes in cyanobacteria and the presence of a gene with similarity to cryptochrome in the *Synechocystis* strain PCC 6803 genome sequence (20), the cyanobacterial cryptochrome has not yet been demonstrated to act as a blue-light photoreceptor and no blue-light photoreceptor has yet been definitively identified in cyanobacteria (31).

Control of gene expression can take place at the transcriptional and posttranscriptional levels. One posttranscriptional process is the decay of mRNA, the detailed mechanism of which has only relatively recently become understood, and it has become well accepted that mRNA stability plays an important role in the expression of many genes in all organisms, including bacteria (37). There are numerous examples of transcriptional control of gene expression by light in photosynthetic organisms (e.g., see references 14 and 41). In cyanobacteria, as in other photosynthetic organisms, there are also examples of genes that are regulated by light at the posttranscriptional level, including through alterations in mRNA stability (13, 23, 24, 27, 38). In bacteria, mRNA decay proceeds through a combination of endonuclease and exonuclease activity, and it can be controlled by altering the ability of degradation factors to access mRNA sites by such means as the presence of mRNA secondary structures, protection by translating ribosomes, and polyadenylation of transcripts (37).

One response in cyanobacteria that is triggered by light stress is the production of small polypeptides termed high-light-inducible proteins (HLIPs) that are encoded by the *hli*

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genes (also called *scp* genes). The HLIPs are localized in the thylakoid membranes of cyanobacteria (12, 19). They have been shown to be important in photoprotection during exposure to HL (18, 19), and it has been proposed that they function directly or indirectly in the dissipation of excess absorbed light energy (18, 21, 28). It has also been proposed that the HLIPs could serve as transient carriers of chlorophyll (12) and that they may play a role in the regulation of tetrapyrrole biosynthesis (47). HLIPs are single-helix members of the Lhc (light-harvesting complex) extended gene family (16, 28). They exhibit close sequence similarity to the early light-inducible protein (ELIP) members of the family in higher plants. The transcription of *ELIP* genes is induced in response to light stress (3, 35). In addition, in etiolated pea seedlings, *ELIP* transcription is induced by blue or red light (1), while in adult tissues it is induced by blue or UV-A light (2, 3). In *Arabidopsis*, phytochromes were found to mediate the red-light and far-red-light regulation of *ELIP* gene expression, while blue-light control of the *ELIP* genes in this organism appeared to be mediated by an as of yet unidentified blue-light photoreceptor (not cryptochromes or phototropin) (17). Through the use of a GUS reporter fusion, expression of the *hliA* gene from *Synechococcus* strain PCC 7942 was found to increase in response to high-intensity white-light or low-intensity blue- or UV-A-light exposure, with little expression in low light (LL) and red light (8). In a previous study, the sensor kinase NblS that controls *hliA* upregulation by HL and UV-A light was identified (43). NblS was found to control the expression of a number of other photosynthesis-related genes in HL and UV-A light and also to control, during starvation for nitrogen or sulfur, expression of the *nblA* gene, the product of which is involved in degradation of the light-harvesting phycobilisome complex (43). This study was undertaken in order to understand the transcriptional and posttranscriptional aspects of the light control of *hliA* expression towards a further understanding of the signal transduction mechanisms involved in controlling light-mediated gene expression in cyanobacteria.

MATERIALS AND METHODS

Culture conditions and light treatments. *Synechococcus elongatus* strain PCC 7942 was grown (25) at 30°C in BG-11 medium under incandescent light (50 μmol of photons $\text{m}^{-2} \text{s}^{-1}$), and the cultures were bubbled with 3% CO_2 in air during growth and during treatments with light and inhibitors. HL (800 μmol of photons $\text{m}^{-2} \text{s}^{-1}$) was obtained by using incandescent white-light bulbs. UV-A light (350 to 400 nm with a peak at 366 nm) was supplied from black-light blue bulbs at 27 μmol of photons $\text{m}^{-2} \text{s}^{-1}$. Other light qualities, all delivered to the cultures at 30 μmol of photons $\text{m}^{-2} \text{s}^{-1}$, were obtained by wrapping fluorescent light bulbs with the following Lee Filters: blue (bright blue narrow-band filter [λ_{max} , 440 nm]); green (dark green narrow-band filter [λ_{max} , 520 nm]); red (deep golden amber cutoff filter [λ_{max} , 640 nm]); and far red (medium red cutoff filter [λ_{max} , 750 nm]). Prior to the various light treatments, cultures were grown to an A_{750} of approximately 1.0, diluted to an A_{750} of 0.2 with fresh BG-11 medium (to avoid self-shading of cells during exposure to light), and adapted to LL (10 μmol of photons $\text{m}^{-2} \text{s}^{-1}$) for 18 h. Inhibitors, when used, were added in the last 5 min of LL adaptation (to allow time for the cells to absorb the inhibitor), and then the exposure to the light conditions proceeded for the designated amounts of time before cells were harvested for RNA. Rifampin was used at a concentration of 200 $\mu\text{g}/\text{ml}$; chloramphenicol was used at 250 $\mu\text{g}/\text{ml}$; puromycin was used at 500 $\mu\text{g}/\text{ml}$; and DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] was used at 5 μM .

RNA isolation and RNA blot hybridizations. Following the various treatments, cell suspensions were harvested in darkness, samples were swirled in flasks on liquid nitrogen, cells were placed in centrifuge tubes on ice and immediately centrifuged for 10 min at 4°C, and cell pellets were stored at -80°C . RNA was isolated from cells as previously described (6). For RNA blot hybridizations,

equal amounts of RNA (determined spectroscopically) were resolved by electrophoresis in formaldehyde gels. A fragment of *hliA* (extending from 26 bp upstream of the ATG start codon of the *hliA* gene to 3 bp downstream of the translation termination codon) was amplified by PCR and cloned into the pGEM-T Easy vector (Promega) to form the plasmid pTHL. Transcription of *Nco*I-digested pTHL with SP6 RNA polymerase and the Strip-EZ RNA probe synthesis kit (Ambion) with [α - ^{32}P]UTP generated the riboprobe used to detect *hliA*-encoding transcripts. A 303-bp internal fragment of the *mpB* gene, which encodes the constitutively expressed RNA component of RNase P, of *Synechococcus* strain PCC 7942 was amplified by PCR (by use of primers 5'-AAAGTC CGGGCTCCAAAAGAC and 5'-CGGGTCTGTCTCTCGAAG) and cloned into pGEM-T Easy (Promega) to form the plasmid pTRP. As a control to confirm equal loading of RNA samples, Northern blots were stripped of the *hliA* probe and hybridized with an *mpB* DNA probe prepared by using the *mpB*-bearing *Not*I fragment of pTRP and labeled by using the Strip-EZ DNA probe synthesis kit (Ambion) with [α - ^{32}P]dATP. Gel electrophoresis of RNA was performed by using standard protocols (39). Northern hybridizations were done by using ULTRAhyb hybridization buffer (Ambion) per the manufacturer's protocol with hybridizations and washes at 60°C for RNA probes and at 42°C for DNA probes.

RESULTS

Evidence for early blue/UV-A-light photoperception triggering increases in *hliA* mRNA levels. *hliA* expression is upregulated by low-intensity blue or UV-A light and by HL; the sensory mechanism that controls the HL-mediated increase in *hliA* transcript levels may wholly or partially operate through the same system as the blue-light response. In order to help explore the relationship between the two responses, we monitored *hliA* mRNA levels in HL and UV-A light. We used UV-A light in these studies rather than blue light because previous studies had shown that *hliA* promoter-driven GUS activity was somewhat higher after several hours in UV-A light than it was in blue light of the same intensity (8). Figure 1A shows that levels of the *hliA* transcript, 300 nt in size, over time rapidly increase in both HL and UV-A light (with both showing peak expression at the first time point, 10 min, at similarly high levels) but drop off dramatically after 30 min with further UV-A exposure, whereas transcript levels decrease at a much slower rate in HL. This timing would be consistent with a model in which a sensory system responds to the blue/UV-A light component of HL in the early stages of HL-mediated *hliA* expression, whereas other factors are involved in maintaining high levels of expression upon longer-term exposure to HL. In order to help determine the nature of the blue/UV-A photoperceptive event involved in *hliA* expression, we monitored transcript levels following exposure to short pulses of UV-A light. Figure 1B shows that a pulse of low-intensity UV-A light (27 μmol of photons $\text{m}^{-2} \text{s}^{-1}$) as short as 3 min is enough to cause a visible increase in *hliA* transcript levels. (A spectrum of the UV-A light source used is shown in Fig. 1C.)

The increase in *hliA* transcript levels is a transcriptional response, and transcript stability can be important in determining final *hliA* mRNA levels. To see if *hliA* mRNA upregulation in HL and UV-A was a result of an increase in transcription or a result of a light-mediated increase in stability of the message, we treated cells with the transcriptional inhibitor rifampin prior to exposure to HL or UV-A. Rifampin blocked the HL- and UV-A-mediated increases in *hliA* mRNA levels, indicating that accumulation of the message is due to transcriptional induction (Fig. 2A). To determine if *hliA* induction involved the production of a protein factor that is upregulated in

FIG. 1. (A) Northern hybridization analysis of *hliA* transcripts over time during HL or UV-A light exposure. LL ($10 \mu\text{mol of photons m}^{-2} \text{s}^{-1}$)-adapted cells were exposed to HL ($800 \mu\text{mol of photons m}^{-2} \text{s}^{-1}$) or UV-A light ($27 \mu\text{mol of photons m}^{-2} \text{s}^{-1}$) for the indicated amounts of time before being harvested for RNA. A densitometric analysis of the signal from the *hliA* hybridization is presented. Hybridization of RNA blots with an *rnpB*-specific probe serves as a loading control for all the Northern hybridization analyses presented in this work. (B) Effects of short pulses of UV-A light on *hliA* transcript levels. LL-adapted cultures were transferred to UV-A light at $27 \mu\text{mol of photons m}^{-2} \text{s}^{-1}$ for the indicated amounts of time. (C) Light spectrum from the UV-A light source used in this study.

HL and UV-A light, we treated cells with the translational inhibitor chloramphenicol before shifting cells to HL or UV-A light. Figure 2A shows that, rather than blocking *hliA* induction, as would be the case if a light-induced protein transcription factor were involved in *hliA* upregulation, treatment with

chloramphenicol resulted in dramatically elevated *hliA* mRNA levels. Densitometry analysis of the RNA blot in Fig. 2A shows that *hliA* mRNA levels in chloramphenicol-treated samples are 2, 4, and 7.5 times greater than in nontreated samples in LL, HL, and UV-A light, respectively. This suggests that the factors responsible for *hliA* induction are already present in the cells (and their activity is altered in response to HL and UV-A light), and they do not have to be synthesized de novo in response to these light conditions. Another explanation for the results with chloramphenicol is that, rather than a transcription factor being synthesized in response to light, a protein degradation factor involved in *hliA* mRNA turnover is upregulated by light. However, treatment with another inhibitor of translation, puromycin (which blocks the initiation of translation), did not cause an increase in *hliA* mRNA (Fig. 2B). Thus, the increase in *hliA* mRNA in the presence of chloramphenicol is not likely due to lack of translation of a light-induced degradation factor; nor is it due to some indirect effect caused by a general inhibition of protein synthesis. Some transcripts have been found to be stabilized by antibiotics that block translational elongation, chloramphenicol in particular, by stalling ribosomes on the transcripts and protecting them from degradation (34). The effect seen with chloramphenicol may be due to this phenomenon, and the dramatic difference in transcript levels observed in the presence and absence of chloramphenicol suggests then that factors that perturb RNA stability can

FIG. 2. Effects of inhibitors of transcription and translation on the HL- and UV-A light-mediated changes in *hliA* expression. LL-adapted cultures were exposed to chloramphenicol (Cm) or rifampin (Rif) (panel A) or puromycin (P) (panel B) or no inhibitor (–) before shifting to HL or UV-A or maintenance in LL. Light exposures proceeded for 30 min before cultures were harvested for RNA. (Neither ethanol nor methanol, in which Cm or Rif, respectively, was dissolved, was found to affect *hliA* induction [data not shown].)

FIG. 3. Effects of spectral quality on *hliA* expression. LL-adapted cells were exposed for 20 min to LL of various wavelengths. A densitometric analysis of the signal from the *hliA* hybridization is presented. BL, blue light; GL, green light; RL, red light; FR, far-red light.

have a significant effect on the ultimate, steady-state levels of *hliA* mRNA.

Red light triggers a low-level, transient increase in *hliA* mRNA levels. We tested to what extent various qualities of light affect expression of *hliA*. Figure 3 shows analysis of RNA from samples exposed to 20 min of light of different qualities (all presented at $30 \mu\text{mol of photons m}^{-2} \text{ s}^{-1}$). UV-A causes the largest increase in transcript levels, followed closely by blue light. Red light caused a very slight increase in message levels, while green light and far-red light caused no significant increase over LL levels. We then monitored transcript levels over time in red light (Fig. 4). A red-light-induced transcript was seen only at 10 min of exposure and dropped to approximately LL levels thereafter.

The HL and UV-A light responses are not apparently reversible by any particular wavelength of light. As phytochrome is red and far-red reversible, some other wavelength-specific photoreceptive events are specifically reversible by light of another wavelength. For example, certain blue-light-mediated events are specifically attenuated by another wavelength of light, including the blue-light-induced opening of plant stomata, which is reversed by green light (11), and the blue-light induction of the *psbAII* and *psbAIII* genes in *Synechococcus* strain PCC 7942, which is specifically attenuated by red light (42). Therefore, we next tested to see if upregulation of *hliA* by UV-A or HL would be reversed by subsequent exposure of cells to any other specific light wavelength. Cells were exposed to 10 min of HL or UV-A light and then shifted to light of various qualities or left in HL or UV-A light for 20 min (Fig. 5). *hliA* mRNA levels were lower in all samples shifted to a different wavelength (rather than left in HL or UV-A or

FIG. 4. Time course of *hliA* mRNA levels in red light. LL-adapted cells were exposed to red light (RL) for the indicated amounts of time. Samples exposed to 10 min of UV-A light or HL were included on the blot for comparison. A densitometric analysis of the signal from the *hliA* hybridization is presented.

shifted to the dark). These results suggest that the induction by HL and UV-A is attenuated by light in general but is not photoreversible by any other specific light quality in particular. (However, we did not test shorter times after the light shift and thus would not be able to detect more rapid changes.)

***hliA* mRNA turnover is slowed in the dark, and two mRNA species appear when photosynthesis is inhibited.** As shown in Fig. 5, detectable levels of transcript were observed only in

FIG. 5. Effects of various light wavelengths in attenuation of the HL- and UV-A-mediated increase in *hliA* mRNA levels. Cells were exposed to 10 min of HL or UV-A light and then either harvested for RNA (HL and UV-A) or shifted to light of various qualities or the dark (D) for 20 min before being harvested for RNA. (Other abbreviations are the same as in the legend to Fig. 4.) The sizes of the two mRNA bands (the usual, full-length 300-nt transcript and a smaller, 265-nt fragment) seen in samples that have been shifted to the dark are indicated.

FIG. 6. Effects of darkness on the stability of *hliA* transcripts. LL-adapted cells were treated for 10 min with HL (A) or UV-A (B) light, and then rifampin was added. Incubation was continued under the same light conditions for an additional 5 min, at which time zero time point samples for RNA were taken, and cultures were either maintained under the same light conditions or shifted to the dark (D samples) or to red light (R samples). Subsequent time point samples for RNA were taken at the indicated times following the light shift. RNA extracted from each of the samples was subjected to Northern hybridization analysis with an *hliA*-specific probe. Data were obtained by densitometry of the autoradiograms. (In the dark-shifted samples, which contain both the 300-nt transcripts and small amounts of the 265-nt transcripts, only the 300-nt transcripts have been included in the quantitation, since we view the 265-nt transcript as being inactive. The half-lives calculated for these samples with or without both transcripts included are very similar.) The average values of two independent repetitions of the experiments were subjected to exponential regression analysis and are plotted; the range between repetitions is indicated.

those samples shifted to red light or to the dark. Likely what is visualized in the samples shifted to red light is the result of a general decay of transcripts that have been removed from HL- or UV-A light-inducing conditions superimposed with a low-level induction by red light.

Regarding samples shifted to the dark, since darkness does not induce *hliA* expression (data not shown), the increase in *hliA* transcripts in dark-shifted samples over those exposed to noninducing light qualities could be due to increased stability of the mRNA. Thus, the *hliA* transcript half-life was examined under different light conditions to determine the contribution of light-induced changes in mRNA stability to *hliA* transcript accumulation and to quantitate the stability of transcripts in the light versus the dark (Fig. 6). Cells were exposed to HL or UV-A light for 10 min, and then rifampin was added to block transcription and samples were exposed to different light conditions, with samples taken at various time points after the shift for Northern blot hybridization analysis. Modest but significant

differences were found between half-lives calculated for messages maintained under inducing conditions and those shifted to the dark, with the half-lives as follows: UV-A light, 7 min, versus UV-A light to the dark, 10 min; and HL, 7 min, versus HL to the dark, 14 min. We also explored whether the relative instability of the transcripts in HL and UV-A light versus the dark was specific to the light conditions under which the transcripts were induced or if light in general might cause the same phenomenon by calculating the half-lives of induced samples shifted to red light ($30 \mu\text{mol}$ of photons $\text{m}^{-2} \text{s}^{-1}$). The half-lives calculated were as follows: UV-A to red light, 5 min, and HL to red light, 8 min. The half-life of *hliA* is longer in the dark than in continued HL or UV-A or red light, indicating that some amount of light in general is required for optimal *hliA* mRNA turnover. Another phenomenon that occurred in induced samples that have been shifted to the dark is the appearance of two *hliA* probe-hybridizing mRNA bands on RNA blots (Fig. 5A)—a typical, full-length transcript of approximately 300 nt and a smaller, approximately 265-nt species. Since the *hliA* probe used is a riboprobe, both mRNA species are transcripts off the coding strand of *hliA*. Examination of the *hliA* sequence (8) shows that the 265-nt species, even if starting from the usual transcriptional start site of *hliA*, would end before the termination codon of this small polypeptide sequence. Alternatively, if the smaller species ends at the predicted transcriptional termination site for *hliA*, its beginning would be within the coding region of the gene. Therefore, this species is not likely to be a transcript starting from a second, downstream initiation site. Thus, the smaller species is not likely a message in and of itself but instead may be an mRNA processing intermediate of the 300-nt transcript. Decay of the *hliA* message is slowed in the dark versus in various light conditions (Fig. 5A and 6), and exposure to darkness causes the appearance of two fragments. These results suggest that it is the absence of light that causes the *hliA* mRNA decay to slow, and this results in the appearance of a decay intermediate. To test this idea, we treated samples with the photosynthesis inhibitor DCMU before shifting them to light-inducing conditions. Figure 7 shows that both fragments appear in the presence of DCMU in both HL and UV-A light and supports this hypothesis.

DISCUSSION

Possible photoperceptive mechanisms involved in *hliA* expression. This work explores details of the transcriptional and posttranscriptional response of *hliA* to light. By comparing the timing of *hliA* induction under inducing light conditions, it appears that blue/UV-A light and perhaps RL photoperception are involved in the early stages (the first 10 to 30 min) of HL-induced *hliA* expression. If this is the case, what maintains high *hliA* transcript levels in HL and causes *hliA* transcript levels to drop in UV-A light after the first 30 min is not clear. One possibility is the action of a sensory system monitoring the photosynthetic redox state, as we have found that exposure to photosynthetic inhibitors perturbs *hliA* expression in both HL and UV-A light (K. Salem and L. G. van Waasbergen, submitted for publication). Moreover, the NblS sensor kinase that controls *hliA* expression also controls expression of the *nblA* gene under nutrient stress—a condition that, like HL, can

FIG. 7. Effects of treatment with DCMU on the appearance of two *hliA* mRNA fragments. LL-adapted cells were exposed to DCMU (C) and then shifted to HL or UV-A light for 25 min before being harvested for RNA. The sizes of the two *hliA*-hybridizing fragments (the usual, full-length 300-nt transcript and a smaller, 265-nt fragment) seen in samples that have been exposed to the photosynthesis inhibitor DCMU are indicated. A densitometric analysis of the signal from each of the resulting fragments in the *hliA* hybridization is presented.

result in overexcitation of the photosynthetic apparatus—raising the possibility that NblS may be sensing some aspect of photosynthetic redox in the control of these genes (43). A number of other genes in cyanobacteria and plants are regulated by both HL and blue light, including the *ELIP* (2), chalcone synthase (10), and *psbA* (42) genes, further supporting a role for blue light in HL gene control.

A 3-min pulse of low-intensity UV-A light at $27 \mu\text{mol}$ of photons $\text{m}^{-2} \text{s}^{-1}$ was enough to cause *hliA* transcript accumulation. This result is consistent with blue/UV-A photoreceptor involvement in *hliA* induction. A number of other processes in cyanobacteria are known to be affected by blue light. These include such activities as phototaxis in the motile strain of *Synechocystis* strain PCC 6803 (32, 45); light-activated heterotrophic growth of *Synechocystis* strain PCC 6803 (5); and, as mentioned above, the regulation of specific genes. Similar to our findings with *hliA*, the *psbAII* and *psbAIII* genes of *Synechococcus* were found to be induced by a 5-min pulse of blue light and may involve the action of a blue-light photoreceptor (42). Like *hliA*, the *psbAII* and *psbAIII* genes are transcriptionally upregulated by HL and blue light (24, 42). The *psbAIII* gene (but not the *psbAII* gene), like *hliA*, is upregulated somewhat by red light, but unlike *hliA*, is also upregulated somewhat

by far-red light (42). Moreover, red light was found to attenuate blue-light induction of *psbAII* and *psbAIII* (42). We have not found *hliA* induction to be attenuated by any specific wavelength of light. Thus, the *hliA* and *psbA* genes appear to be regulated by somewhat different photocontrol systems that may partially overlap. It should be noted that the NblS sensor kinase that controls *hliA* expression also controls expression of the *psbA* genes in HL and UV-A light (43). It is not clear if NblS itself is a redox sensor and/or a blue/UV-A photoreceptor or if it acts in conjunction with a separate blue/UV-A photoreceptor in controlling the expression of the genes.

A survey of various light qualities showed that blue light and UV-A light cause the most profound increase in *hliA* mRNA levels. The so-called blue-light photoreceptors are typically responsive to UV-A light as well (22). Red light also caused a slight increase in transcript levels. It may be that both a red light and a blue/UV-A light photoreceptor are involved in *hliA* expression, as has been seen for the ELIPs (17). Another possibility is that the red-light and blue-light responses in *hliA* upregulation involve the same photoreceptor. Such may be the case for the cyanobacterial phytochrome Cph2 in *Synechocystis* strain PCC 6803, which, when inactivated, generated a phototaxis toward blue light (45). The authors suggest that this phytochrome may be involved in blue-light as well as red-light photoperception, since one of the two bilin lyase domains had been found to exhibit a high blue spectral absorption maximum (46), while the other shows typical phytochrome red and far-red photoconversion in vitro (46). Even so, as the strain inactivated for Cph2 is able to sense blue light for taxis, the involvement of Cph in taxis likely involves interaction with a separate blue-light photoreceptor (45). An intriguing third possibility for *hliA* light quality control, given that photosynthetic redox is involved in *hliA* regulation during UV-A light as well as HL exposure (Salem and van Waasbergen, submitted), is that photosynthetic pigments are involved as photoreceptors in mediating the blue- and red-light responses. However, it is equally possible that *hliA* is controlled by the activities of separate but interacting blue or UV-A photoreceptor and photosynthetic redox-monitoring systems. It should be noted, nevertheless, that the fluence of red light in which we observed slight *hliA* upregulation ($18,000 \mu\text{mol}$ of photons m^{-2}) was approximately fourfold higher than the fluence of UV-A light that we found to cause *hliA* induction ($4,860 \mu\text{mol}$ of photons m^{-2}). Such a small (and transient) response to red light suggests that red light does not play a major role in HL-mediated *hliA* induction by itself; but further studies are necessary to determine how the red-light response interacts with the blue and UV-A light and photosynthetic redox responses.

Posttranscriptional events involved in *hliA* expression. We have found that light affects *hliA* expression mainly at the transcriptional level. However, the results showing that *hliA* expression is greatly upregulated by treatment with chloramphenicol suggest that mRNA stability can have a significant effect on the ultimate mRNA level; as has been seen for some other transcripts (34), chloramphenicol (which blocks translational elongation) may act to stall ribosomes on the *hliA* transcript and protect it from decay, thus providing a level of control linking transcription with translation.

We found the half-life of the *hliA* transcript to be slightly greater in the dark than in the light. Likewise, in another study,

transcripts of the *psbA* genes of *Synechocystis* strain PCC 6803 were found to be highly stable in the dark; this was linked to the lack of photosynthetic electron transport (27). It could be that light energy is required directly for some step of *hliA* mRNA decay and that there simply is not as much energy for efficient processing of *hliA* transcripts in the dark. However, most of the enzymes involved in RNA decay do not require energy input. Alternatively, it may be that in cyanobacteria, as in chloroplasts (30), light-dependent formation of a photosynthetic proton gradient across the thylakoid membrane is required for translational elongation (the process being slowed in the dark or by DCMU treatment); thus, in the dark, translational elongation is slowed and ribosomes stall and protect the *hliA* transcript, in a process similar to that which may be happening upon the addition of chloramphenicol (above).

In darkness the half-life of *hliA* approximately doubles (at most) over that seen under light conditions (Fig. 6). In the study mentioned above, in *Synechocystis* strain PCC 6803 the half-life of *psbA* transcripts in darkness was found to be dramatically higher in the dark (7 h) than in the light (15 min), and it was theorized that stabilization of the mRNA in the dark would allow its ready availability upon transition to a light period and would benefit the cells during dark-to-light cycles in the natural environment (27). It is not clear whether the relatively modest increase in *hliA* mRNA stability observed in the dark over that in the light has a similarly meaningful ecological or physiological relevance.

A 265-nt mRNA fragment appeared in samples that had been shifted to the dark or treated with DCMU. This fragment may represent a decay intermediate that is visible when mRNA turnover is slowed. A similar result was seen for *Synechocystis* strain PCC 6803 *psbA* transcripts, for which darkness or treatment with DCMU slowed their turnover and led to the appearance of a specific degradation product (4, 26, 27). mRNA fragments that represent decay intermediates have also been observed for the *psbA* genes of *Synechococcus* strain PCC 7942 (15, 40) and photoinhibited *Synechocystis* strain PCC 6714 (7). The present model for RNA degradation in prokaryotes is that it involves a series of 5'-to-3' endonuclease cleavages that generate 3' ends that are degraded 3' to 5', resulting in decay in a net 5'-to-3' direction (37). The 265-nt *hliA* mRNA fragment may be the result of an initial 5' cleavage of the 300-nt transcript at a site apparently unprotected by ribosomes as translation is slowed by darkness or DCMU. The smaller 35-nt product either may be completely degraded or is too small to visualize by Northern hybridization analysis, while the 265-nt fragment is protected by stalled ribosomes and the 3' terminator hairpin from subsequent endonuclease cleavages and degradation. The 265-nt fragment is not as prominently visible in samples treated with chloramphenicol (Fig. 2A). It may be that chloramphenicol, perhaps by having a more pronounced effect on translational elongation and ribosomal stalling than darkness or DCMU, allows the entire transcript to be protected. The fact that inhibition of translational elongation by chloramphenicol takes place under conditions where *hliA* induction is still occurring (i.e., light) could explain the much higher increase in message levels caused by the presence of chloramphenicol than by darkness or treatment with DCMU (compare Fig. 2A, 5, and 7). Future analysis of this cleavage site in the *hliA* transcript and sites in other, similarly cleaved

transcripts (e.g., *psbA*) may yield insight into site specificity for cyanobacterial (and possibly chloroplast) endonucleases and other events involved in the processing of light-regulated messages.

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