Photosynthetic Electron Transport Controls Expression of the High Light Inducible Gene in the Cyanobacterium *Synechococcus elongatus* Strain PCC 7942

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The *hliA* gene of *Synechococcus*, encoding a photoprotective high light inducible polypeptide, is up-regulated by high light (HL) or low intensity blue/UV-A light (BL). *hliA* expression was found to be up-regulated by KCN in low light (LL) (but not in the dark), and up-regulation in HL, BL, and LL (with KCN) was inhibited by 2,5-dibromo-3-methyl-6-isopropyl-benzoquinone. A working hypothesis is proposed whereby up-regulation is in response to the reduced state of cytochrome *b*$_6$*f* or a carrier beyond in photosynthesis. Modest up-regulation occurs in LL by treatment with 3-(3,4-dichlorophenyl)-1,1-dimethylurea, but this is related to effects on *hliA* mRNA stability rather than on transcription.

Keywords: Blue/UV-A light — Cyanobacteria — Electron transport control — High intensity light — High light inducible gene — *Synechococcus elongatus* PCC 7942.

Abbreviations: BL, blue/UV-A light; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-benzoquinone; HL, high light; LL, low light.

Because of their dependence on light as an energy source, cyanobacteria, like all photosynthetic organisms, have naturally evolved mechanisms that acutely attenuate them to the light incident in their environment. Processes within the photosynthetic cell, including changes in gene expression, may be triggered through sensing of changes in light color (quality) (through the use of photoreceptors that respond to specific light wavelengths) or in response to changes in light intensity (through the activity of redox-sensitive regulators sensing changes in the electron flow through the photosynthetic electron transport chain) (Anderson 1986, Fankhauser and Chory 1997, Golden 1995, Mullineaux 2001, Pfannschmidt 2003, Quail 2002, Thompson and White 1991).

There is growing evidence that demonstrates that, in all organisms, changes in gene expression may be regulated in response to aspects of the redox state of their photosynthetic or respiratory electron transport systems (Allen 1993a, Allen 1993b). In photosynthetic organisms, there are a number of examples of genes that are regulated by light through changes in the oxidation–reduction potential of components of the photosynthetic electron transport chain (Pfannschmidt et al. 2001, Pfannschmidt 2003). For instance, in green algae it has been shown that transcription of the *cab* gene (encoding the light-harvesting protein) is regulated in response to the redox state of the plastoquinone pool in photosynthesis (Escoubas et al. 1995). Another study in green algae showed that translation of the *psbA* gene (which encodes the D1 protein of photosystem II) was regulated in response to the redox state of photosynthesis via changing the redox state of thioredoxin (Danon and Mayfield 1994). A number of studies have linked changes in gene expression in cyanobacteria to changes in the redox state of carriers in photosynthetic electron transport (Alfonso et al. 2000, Alfonso et al. 2001, El Bissati and Kirilovsky 2001, Garcia-Dominguez and Florencio 1997, Glatz et al. 1997, Hihara et al. 2001, Hihara et al. 2003, Kis et al. 1998, Li and Sherman 2000, Mohamed and Jansson 1991, Navarro et al. 2000, Reyes and Florencio 1995, Sippola and Aro 2000).

Indeed, in the significant paper by Hihara et al. (2003), by examining differential gene expression in the presence of the photosynthetic inhibitors 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-benzoquinone (DBMIB) using microarrays, the authors found that the expression of 140 different genes in *Synechocystis* sp. strain PCC 6803 were significantly altered under normal growth conditions in response to changes in the redox state of the photosynthetic electron transport chain. This included two of the four *hli* genes present in the genome of that species (both of which showed induction by DCMU and DBMIB, the induction by DBMIB being greater than that by DCMU) (Hihara et al. 2003).

The family of *hli* genes [also called *scp* genes (Funk and Vermaas 1999)] of cyanobacteria encode small polypeptides termed high light inducible proteins (HLIPs) (Bhya et al. 2002, Dolganov and Grossman 1995). HLIPs represent single-helix members of the light harvesting complex (Lhc) extended gene family (Green et al. 1991, Montané and Kloppstech 2000). They exhibit close sequence similarity to the early light
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The ELIPs have recently been demonstrated to function in photoprotection [as has long been proposed (Adamska and Kloppstech 1994)], likely by functioning as transient pigment carriers during light stress-induced turnover of chlorophyll binding proteins (Hutin et al. 2003). Like the ELIPs, the HLIPs are localized to the thylakoid membranes and are important for photoacclimation during high light (HL) exposure (Havaux et al. 2003, He et al. 2001). It has been proposed that the HLIPs function directly or indirectly in the dissipation of excess absorbed light energy (Havaux et al. 2003, Jansson et al. 2000, Montané and Kloppstech 2000). It has also been proposed that the HLIPs could serve as transient carriers of chlorophyll (Funk and Vermaas 1999) and that they may play a role in the regulation tetrapyrrole biosynthesis (Xu et al. 2002). The hliA gene was first identified in the cyanobacterium Synechococcus elongatus PCC 7942 (Dolganov and Grossman 1995) where, like the ELIP genes, it was found to be induced in response to exposure to high-intensity white light (HL) or low intensity blue/UV-A light (BL) by as-of-yet unidentified mechanisms, presumably involving, in part, a blue/UV-A photoreceptor. The HLIPs in Synechocystis PCC 6803 have been found to be synthesized in response to chilling or deprivation for nitrogen or sulfur as well as HL (He et al. 2001). Since HL, chilling, and

Fig. 1  (A) Diagram of linear photosynthesis (solid arrows), respiration (dashed and dotted arrows), and cyclic electron transfer (dashed arrow) in cyanobacteria showing where DCMU and DBMIB block electron flow. In the thylakoid membrane of cyanobacteria photosynthesis and respiration are intermingled, while in the cytoplasmic membrane, only a respiratory pathway is present. Fd, ferredoxin; FNR, ferredoxin-NADP(+) reductase; PC, plastocyanin; PQ, plastoquinone pool. (B) Fluorescence induction in cells in the presence of various inhibitors. The actinic light was turned on for 1 s at the zero time point. Measurements were done for each of the treatments on three different days with the same results; a representative set is shown. To help distinguish among the curves the +DCMU sample is shown in boldface and the +DBMIB is shown in gray.
nutrient deprivation are stresses that generate excitation energy beyond that which can be used in photosynthesis and thus generate an overreduced photosynthetic electron transport chain (Demmig-Adams and Adams 1992), one might expect the expression of the hli genes to be partially controlled through changes in the redox state or photosynthetic electron transport components.

In order to determine whether hliA expression is controlled in response to photosynthetic electron flow, we conducted Northern analysis of hliA transcript levels in cells treated with various inhibitors. DCMU blocks photosynthetic electron flow from photosystem II to the PQ pool. DBMIB blocks photosynthetic and respiratory electron flow [which are intermingled in the thylakoid membrane of cyanobacteria (Schmetterer 1994)] from the PQ pool to cytochrome b$_{6}$f (Fig. 1A). High concentrations of KCN can block photosynthetic electron flow between cytochrome b$_{6}$f and PSI at plastocyanin [by removing copper from plastocyanin (Berg and Krogmann 1975)]. However, it should be noted that many cyanobacteria are also known to have cytochrome c$_{553}$ (also called cytochrome c$_{6}$) as an electron carrier between cytochrome b$_{6}$f and PSI (including Synechococcus PCC 7942, Clarke and Campbell 1996). KCN is also a classic, potent inhibitor of the cytochrome oxidase in many respiratory chains and can affect a number of other enzymes. The concentrations of inhibitors used in the experiments presented herein were those that were found to inhibit oxygen evolution (data not shown), which would suggest that they were inhibiting photosynthesis. (Moreover, the small amount of oxygen uptake observed in cells in the dark was eliminated by KCN treatment, indicating that KCN was inhibiting respiration.)

Fluorescence induction kinetics were used to explore the effect that the inhibitors had on photosynthetic electron transport in our experiments. Fig. 1B shows that the variable fluorescence yield upon illumination was much higher in the presence of DCMU and DBMIB than it is in the presence of KCN or in the absence of inhibitors indicating that, although both DCMU and DBMIB were inhibiting electron flow between the two photosystems, KCN was not. [Note that the slow rise seen after ~0.2 s in DCMU-treated samples (Fig. 1B) is probably not related to Q$_{A}$ reduction since that occurs much more rapidly.] Moreover, the area over the induction curve for samples containing DBMIB were larger than those containing DCMU (Fig. 1B). Since the area over the fluorescence induction curve is proportional to the number of electrons that can be transported through PSII, one would expect that it would take more electrons to get to the fluorescence maximum in the presence of DBMIB than in the presence of DCMU. Thus, this result indicates that these inhibitors are acting at the expected places, with DCMU blocking electron flow between Q and the PQ pool and DBMIB inhibiting electron flow out of the PQ pool.

Fig. 2A shows the effects that treatment of cells with the various inhibitors had on hliA transcript levels. The normally very low level of the hliA transcript in low light (LL)-adapted cells was increased dramatically by treatment with KCN (even though KCN was not inhibiting photosynthetic electron flow between the photosystems) and the LL levels were increased somewhat by treatment with DCMU. The HL- and BL-mediated increases in levels of hliA mRNA were effectively inhibited by treatment with DBMIB, but were not inhibited by treatment with DCMU or KCN. To obtain more information we examined hliA expression in LL in the presence of two inhibitors at once. The results, seen in Fig. 2B, show that DBMIB inhibits both the LL up-regulation of hliA by KCN and DCMU, but that DCMU and KCN together still result in significant LL hliA up-regulation.

The fact that DBMIB (which blocks electron flow after the PQ pool) inhibits the light-induced up-regulation and DCMU (which blocks electron flow into the PQ pool from PSII) causes up-regulation in LL (data in Fig. 2A) could indicate that hliA is up-regulated by a system sensing the oxidized state of the PQ pool. However, in the presence of DCMU and DBMIB together (in which case, as with DCMU alone, the PQ...
pool would be expected to be in a fairly oxidized state in this obligate phototroph), there is no up-regulation of hliA (Fig. 2B). This prompted us to explore other reasons for DCMU up-regulation in LL.

In other studies (Salem and van Waasbergen 2004) we have found that the level of hliA mRNA transcripts can be dramatically increased by treatment with chloramphenicol. We hypothesized that chloramphenicol was acting as been found for some other transcripts (Petersen 1993) by inhibiting translational elongation, stalling ribosomes on the transcripts, and protecting the message from decay. Since DCMU is known to inhibit translational elongation in chloroplasts (Mühlbauer and Eichacker 1998), DCMU could be acting in a manner similar to that proposed for chloramphenicol: inhibiting translational elongation in Synechococcus, stalling ribosomes, and protecting low, normally undetectable levels of hliA transcripts in LL from normal turnover. The fact that DBMIB inhibits LL DCMU up-regulation (Fig. 2B) is consistent with this idea. We examined the half-life of transcripts in LL up-regulated by DCMU or KCN. The results in Fig. 3A show that the half-life...
of messages generated by DCMU treatment in LL are significantly longer (13 min) than those generated by light treatment (both HL and UV-A light treatments generate transcripts with half-lives of 7 min; UV-A data is not shown) or by KCN in LL (8 min), indicating that DCMU treatment is acting to stabilize hliA transcripts. In *E. coli*, treatment with puromycin appears to cause premature release of ribosomes from mRNA and destabilize messages that are protected by ribosomes as evidenced by the shorter half-life of messages believed to be protected by stalled ribosomes in the presence of puromycin than in its absence (Cremer et al. 1974, Pato et al. 1973, Petersen 1993). The addition of puromycin to *Synechococcus* cultures treated with DCMU in LL resulted in a decrease in the half-life of hliA messages to 9 min, close to the half-lives of hliA messages generated by light treatment or by KCN treatment in LL (Fig. 3A). Moreover, analysis of cells treated with puromycin for 5 min prior to the addition of photosynthetic inhibitors showed a dramatic reduction in the LL, DCMU-mediated increase in hliA mRNA levels while having much less effect on the LL, KCN-mediated increase (Fig. 3B). These data suggest that DCMU is acting to stall ribosomes and protect hliA mRNA and that treatment with puromycin is causing the release of ribosomes from the mRNA stalled by DCMU and preventing the DCMU-mediated increase in hliA mRNA levels seen in LL.

The distinct inhibitory effect of DBMIB upon hliA up-regulation by light (Fig. 2A) strongly suggests that photosynthetic/respiratory electron flow through cytochrome b₆f is important for hliA expression. On the other hand, KCN treatment (albeit at levels that did not inhibit photosynthetic electron flow) caused dramatic up-regulation of hliA in LL (Fig. 2A). Fig. 2B shows that co-treatment with DBMIB blocks KCN-mediated LL up-regulation indicating that KCN is affecting hliA expression through its effects on electron transport. These results indicate that the hliA gene expression is controlled through the activities of a KCN-sensitive factor that is affected by electron flow through the cytochrome b₆f complex.

To see if the KCN-mediated up-regulation was primarily due to its effects on the photosynthetic/respiratory electron transport chain (in the thylakoid membrane) or to its effects on respiration alone (in the cytoplasmic membrane), we tested to see the influence of the presence or absence of light on KCN-mediated up-regulation. Fig. 4 shows that the increase in hliA transcript levels seen in the presence of KCN in LL does not occur in the dark, but that it does occur in low-intensity red light. The fact that light is required and low-level light is sufficient for up-regulation by KCN indicates that control of hliA up-regulation is related to photosynthesis and helps to further generate a model in which hliA is up-regulated in response to the activities of a KCN-sensitive factor beyond cytochrome b₆f in the photosynthesis/respiratory electron transport chains of the thylakoid membrane. Since the activity of a number of enzymes can be affected by KCN it is difficult to pinpoint with certainty what this factor may be. KCN is an inhibitor of ribulose-1,5-bisphosphate carboxylase (Ishida et al. 1998). Inhibition of this Calvin cycle enzyme would prevent recovery of the terminal electron acceptor in photosynthetic electron transport, NADP⁺. This would cause an overreduction of the photosynthetic electron transport chain as one might see during light stress. [Inhibition of ribulose-1,5-bisphosphate carboxylase, which is inhibited by reactive oxygen species (Ishida et al. 1998), could also explain our observation that treatment of cells with hydrogen peroxide results in a weak up-regulation of hliA (data not shown).] As the photosynthetic electron transport carriers would be expected to become naturally overreduced by exposure to light stress conditions [i.e. during HL, chilling, or nutrient stress; stresses that are known to up-regulate the hli genes (Dolganov and Grossman 1995, He et al. 2001)], regardless of what KCN-responsive factor is responsible for hliA up-regulation, one hypothesis would be that hliA up-regulation, in general, is in response to the reduced state of one of the electron transport carriers in photosynthesis. Given that hliA up-regulation by light or by KCN is inhibited by DBMIB (Fig. 2A, B), the regulatory system may be responding to the reduced state of cytochrome b₆f itself or a carrier beyond cytochrome b₆f in the photosynthetic electron transport chain. The fact that DCMU does not inhibit up-regulation by KCN in LL (Fig. 2B) would suggest that this reduced state can be attained by electron flow through cyclic electron transport around PSI. However, we should emphasize that our data do not eliminate certain other possibilities for the redox sensory mechanism such as the possibility that hliA is up-regulated in response to its effects on cytochrome c oxidase, which itself is located in the respiratory/photosynthetic chain of the thylakoid membrane beyond cytochrome b₆f, or that a sensory system is involved that is sensitive to KCN itself (e.g. it bears a KCN-sensitive heme cofactor) and is responsive to electron flow through cytochrome b₆f.

The fact that hliA induction by BL is also blocked by DBMIB suggests that BL-mediated hliA expression involves a redox component as well as a blue light component. Other
processes in cyanobacteria are affected by exposure to blue light and by changes in the photosynthetic redox, including phototaxis in the motile strain of *Synechocystis* PCC 6803 (Wilde et al. 2002) and state transitions and changes in photosystem stoichiometry, both of which are controlled through changes in light quality (including blue light) and light intensity, likely through a control system monitoring the redox state of cytochrome *b*₅ (Fujita et al. 1994, Fujita 1997, Mao et al. 2002). As blue light is unlikely to generate a significant reduction of the photosynthetic electron transport chain [indeed, a pulse of low intensity BL as short as 3 min is enough to cause up-regulation of *hliA* (Salem and van Waasbergen 2004)], it is likely that there are separate but interrelated sensory systems that are responding to BL and to photosynthetic redox. The increase in message levels upon transfer from LL to HL or BL in the presence of DCMU (Fig. 2A) may reflect a combination of a blue light signal and an increase in electron flow through cyclic electron transport and/or respiration combined with protection of the transcript due to treatment with DCMU.

It remains to be shown for *hliA* what sensory system is responsive to photosynthetic redox. Recently, a photosynthetic redox-responsive two component regulatory system, RppB and RppA, was found to control expression of photosynthesis-related genes in *Synechocystis* PCC 6803 (Li and Sherman 2000). However, this system apparently controls genes in response to the redox state of the plasquinone pool. The NblS sensor kinase that is involved in *hliA* gene control during HL and UV-A light may be a redox-responsive regulator (van Waasbergen et al. 2002) and is a good candidate for the redox sensor controlling *hliA* expression. *hliA* is not up-regulated by KCN and DCMU in LL in an *nblS* mutant [**nblS-1** (van Waasbergen et al. 2002)], and this mutant shows a drastic reduction in the ability to up-regulate *hliA* in HL and BL, indicating that normal NblS activity is necessary for up-regulation of the gene (data not shown). NblS [and the related *DspA* (Hik33) protein from *Synechocystis* PCC 6803] regulate genes involved in a variety of different stresses, including HL, nutrient stress, and chilling (Suzuki et al. 2001, van Waasbergen et al. 2002), all of which can generate an overexcited photosynthetic electron transport chain (Demming-Adams and Adams 1992). Moreover, sequence similarity of NblS with the ResE sensor kinase, which is involved in respiratory gene control in *Bacillus subtilis* during aerobic/anaerobic shifts, is consistent with the idea of NblS’s involvement in gene control through electron transport (van Waasbergen et al. 2002).

*Synechococcus elongatus* PCC 7942 was grown at 30°C in BG-11 medium (Laudenbach and Grossman 1991) at 50 µmol photon m⁻² s⁻¹ incandescent light, and cultures were bubbled with 3% CO₂ in air during growth and during light and inhibitor treatments. HL at 800 µmol photon m⁻² s⁻¹ was obtained using incandescent white light bulbs. Blue/UV-A light (BL) (from 320 to 410 nm with a peak at 366 nm) was supplied from black-light blue bulbs at 27 µmol photon m⁻² s⁻¹. Red light (RL) was obtained by wrapping fluorescent light bulbs with a deep golden amber cut-off filter (Lee Filters; λₘₚₓ, 640 nm) and supplied at 10 µmol photon m⁻² s⁻¹. Prior to treatments, cultures were grown to an A₅₇₀ of approximately 1.0, diluted to an A₅₇₀ of 0.2 with fresh BG-11 medium (to avoid self-shading of cells during light exposure), and adapted to LL for 18 h in LL, 10 µmol photon m⁻² s⁻¹. The photosynthesis inhibitor concentrations used were the minimum found necessary to inhibit oxygen evolution under the light conditions used in the various experiments (determined using an oxygen electrode (Oxygraph System; Hansatech, Norfolk, U.K.) with bicarbonate as an electron acceptor; DCMU, 5 µM; DBMIB, 20 µM; and KCN, 5 mM). Rifampin was used at a concentration of 200 µg ml⁻¹ and puromycin, at 500 µg ml⁻¹. Following the various treatments, cell suspensions were swirled on liquid nitrogen and harvested by centrifugation at 4°C and cell pellets were stored at −80°C. RNA was isolated from cells as previously described (Bhaya et al. 1999). 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 AUG start codon of the *hliA* gene to 3 bp downstream of the translation termination codon) was PCR-amplified and cloned into the pGEM-T Easy vector (Promega) to form the plasmid pTHL. Transcription of *Ncol*-digested pTHL with SP6 RNA polymerase (using a Strip-EZ™ RNA probe synthesis kit (Ambion) with [α-³²P]UTP) generated the riboprobe used to detect *hliA*-encoding transcripts. A 303-bp internal fragment of the *rnpB* gene, which encodes the constitutively expressed RNA component of ribonuclease P, of *Synechococcus* PCC 7942 was PCR amplified (using primers 5'-AAAGTCGGGCCGTCCCAAAAGAC and 5'-CGGGTTCTGTTCCTCTGCGAAG), 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 *rnpB* DNA probe prepared using the *rnpB*-bearing NorI fragment of pTRP. Northern blot hybridizations were performed using standard protocols (Sambrook et al. 1989). Fluorescence measurements were performed on intact cells using a commercial fluorometer (Walz, Effeltrich, Germany). The cells were grown and low light adapted as above. Cultures were concentrated by centrifugation to an OD₇₅₀ of 3 in 10 mM HEPES buffer (pH 7.4) and dark adapted for 30 min prior to addition of an inhibitor. The fluorescence level, F₀, was then measured for 1 s with a non-actinic measuring light after which the sample was exposed to 1 s of actinic light (the time constant for all measurements was 24 ms).

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References


Photosynthetic regulation of hliA in Synechococcus


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