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ORIGINAL ARTICLE

Transcript accumulation of carotenoid biosynthesis genes in the cyanobacterium *Synechocystis* sp. PCC 6803 during the dark-to-light transition is mediated by photosynthetic electron transport

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Abstract Expression of the genes for carotenoid biosynthesis (*crt*) is dependent on light, but little is known about the underlying mechanism of light sensing and signalling in the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter, *Synechocystis*). In the present study, we investigated the light-induced increase in the transcript levels of *Synechocystis crt* genes, including phytoene synthase (*crtB*), phytoene desaturase (*crtP*), ζ-carotene desaturase (*crtQ*), and β-carotene hydroxylase (*crtR*), during a dark-to-light transition period. During the dark-to-light shift, the increase in the *crt* transcript levels was not affected by mutations in cyanobacterial photoreceptors, such as phytochromes (*cph1*, *cph2* and *cph3*) and a cryptochrome-type photoreceptor (*ccry*), or respiratory electron transport components *NDH* and *Cyd/CtaI*. However, treatment with photosynthetic electron transport inhibitors significantly diminished the accumulation of *crt* gene transcripts. Therefore, the light induction of the *Synechocystis crt* gene expression is most likely mediated by photosynthetic electron transport rather than by cyanobacterial photoreceptors during the dark-to-light transition.

Keywords Carotenoid biosynthesis gene · Light induction · Photoreceptor · Photosynthetic electron transport · *Synechocystis*

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Abbreviations

Chl	Chlorophyll
CCCP	Carbonylcyanide-3-chlorophenylhydrazone
Cph	Cyanobacterial phytochrome
Ccry	Cyanobacterial cryptochrome
CtaI	Cyt aa_3-type Cyt *c* oxidase
Cyd	Cyt *bd* quinol oxidase
Cyt	Cytochrome
DBMIB	2,5-Dibromo-3-methyl-6-isopropyl-*p*-benzoquinone
DCMU	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
Fo	Instantaneous (dark) acridine yellow fluorescence
MV	Methyl viologen
NDH	NAD(P)H dehydrogenase
PQ	Plastoquinone
WT	Wild-type
Δ*F*/Fo	Relative change of acridine yellow fluorescence induced by illumination

Introduction

Carotenoids are present in all photosynthetic organisms, where they not only participate in light harvesting but also protect the photosynthetic apparatus against photo-oxidative damage (Frank and Cogdell 1996; Kirilovsky 2007). Photoreceptors and photosynthetic electron transport have been shown to be involved in the light induction of carotenoid biosynthesis (*crt*) genes. Both phytochrome (von Lintig et al. 1997; Welsch et al. 2000) and blue light photoreceptor (Bohne and Linden 2002) are critical for *crt* gene expression in higher plants. Additionally, photosynthetic electron transport seems to be responsible for eukaryotic algal *crt* gene expression (Steinbrenner and Linden 2003).

Carotenoid contents in various cyanobacteria, including *Aphanocapsa* (Nonnengeisser et al. 1996), *Microcystis aeruginosa* (Woitke et al. 1997), *Synechocystis* (Steiger et al. 1999), and *Plectonema boryanum* (Ivanov et al. 2000), are increased in a light intensity-dependent manner. Additionally, the expression of *crt* genes is also light intensity dependent, as shown in the cyanobacterium *Synechocystis* (Fernandez-Gonzalez et al. 1998). However, little is known about which photoreceptors are involved in cyanobacterial *crt* expression during the dark-to-light transition.

The Gram-negative cyanobacterium *Synechocystis* is a model organism that is widely used to study photosynthesis, as well as adaptation to various environmental stresses (Douglas 1998; Burja et al. 2003). In *Synechocystis*, there are several photoreceptors and photosynthetic electron transport components that may be candidates for light sensing and the signalling of *crt* gene expression. It has been shown that three different phytochrome homologues, Cph1 (Slr0473), Cph2 (Sll0821), and Cph3 (Sll1124), are capable of binding to chromophores and undergoing reversible photoconversion in vitro (Hughes et al. 1997; Wilde et al. 1997). The cryptochrome-type photoreceptor is also known to be encoded by the *sll1629* gene, as its deduced amino acid sequence shows a strong sequence similarity to cryptochrome (Brudler et al. 2003). Additionally, the entire *Synechocystis* photosynthetic apparatus has the potential for light sensing and signalling for *crt* gene expression. Indeed, electron transfer in the photosynthetic electron transport chain following light absorption leads to changes in the redox state of electron transport components such as PSII (Campbell et al. 1995; Hihara et al. 2003), PQ (Alfonso et al. 2000; Li and Sherman 2000), and Cyt b_6f complex (Kujat and Owttrim 2000), thereby initiating the signal transduction cascade for the expression of genes involved in photosynthesis and RNA metabolism.

Previously, we have shown that the glucose induction of *crt* genes in *Synechocystis* in the dark was mediated by increased cytosolic pH, which was generated by accelerated respiratory electron transport (Ryu et al. 2004), which in turn implies the involvement of the photosynthetic or respiratory electron transport process in *crt* gene expression, even during the dark-to-light shift period. In the present study, various *Synechocystis* mutants of phytochromes, cryptochrome, and respiratory electron transport components were used to address whether cyanobacterial photoreceptors or respiratory electron transport act as a trigger for the expression of *crt* genes upon illumination. Furthermore, specific inhibitors that modulate the redox state of photosynthetic electron transport components in the thylakoid membrane were also used to search for the initial light-sensing site. Our results indicated that the redox state of the PSI acceptor side of the photosynthetic electron transport chain is closely related to expression of the *crt* genes in *Synechocystis* during the dark-to-light transition period.

Materials and methods

Cell strains, growth conditions, and inhibitors

Cells from wild-type (WT) *Synechocystis* sp. PCC 6803 and its mutants defective in various photoreceptors (*cph1*$^-$, *cph2*$^-$, *cph3*$^-$, and *ccry*$^-$) and respiratory electron flow (M55 and Cyd/CtaI) were grown in BG-11 inorganic medium buffered with 5 mM *N*-tris(hydroxymethyl) methyl-2-aminoethane-sulfonic acid (TES)-NaOH (pH 8.0) at a photon flux density of 30 μmol m^{-2} s^{-1} unless otherwise indicated (Ryu et al. 2004). For the dark-to-light shift experiments, photoautotrophically grown cells in the exponential growth phase were incubated in the dark for 16 h and shifted to 100 μmol m^{-2} s^{-1} for 30 min. When required, electron transport inhibitors (10 μM for DCMU, DBMIB and CCCP, 0.1 mM for MV) were added to the cell cultures 5 min before illumination. The transcription inhibitor rifampicin (Rif) was added at a final concentration of 300 μg ml^{-1}. Chl content was estimated from the absorption spectra of intact cells (Myers et al. 1980).

Mutagenesis

Genomic DNA used as the PCR template was isolated as described (Porter 1988). The *cph1* (locus *slr0473* in the CyanoBase, http://www.kazusa.or.jp/cyano), *cph2* (*sll0821*), *cph3* (*sll1124*), and *ccry* (*sll1629*) genes were amplified by PCR from the genomic DNA using the following primers: *cph1*, forward, 5′-GCCAAACGCTTCA GGATAGT-3′ and reverse, 5′-TTCGTAACTGGCAA TCACATC-3′; *cph2*, forward, 5′-CGGAATTCATGAA

CCCTAATCGATCCTT-3′ and reverse, 5′-CGCGGATC CCTAAACTTCCCCATCAACAT-3′; *cph3*, forward, 5′-C GAGGTACCAGGGAAGTCACCGCCA-3′ and reverse, 5′-CGCGGATCCTGATGCCCCGACAAAAGC-3′; and *ccry*, forward, 5′-TGCCGTCTAAATCCTCCAAA-3′ and reverse, 5′-TTCCGCCAAATAAGCAGCAT-3′. The resulting fragments were then cloned into the pGEM-T Easy vector (Promega). To inactivate *cph1*, a cassette conferring spectinomycin resistance (digested with *Sma*I) was inserted by blunt end ligation into the unique *Cla*I site (position 2,439 of the insert) in the 4.2-kb *cph1* PCR product that was cloned into the pCRII vector (Invitrogen). To inactivate *cph2*, a cassette conferring spectinomycin resistance (digested with *Sma*I) was inserted by blunt end ligation into the *Eco*47III site (positions 611 and 970 of the insert) in the 3,831-bp *cph2* PCR product that was cloned into the pQE12 vector (Qiagen). To inactivate *cph3*, a cassette conferring spectinomycin resistance (digested with *Sma*I) was inserted by ligation into the *Hinc*II site (positions 172 and 3,306 of the insert) in the 3,678-bp *cph3* PCR product that was cloned into the pGEM-7zf (−) vector (Promega). To inactivate *ccry1*, a cassette conferring kanamycin resistance (digested with *Sma*I) was inserted by ligation into the *Hin*dIII and *Bst*EII sites (positions 290 and 1,366 of the insert) in the 1,821-bp *sll1629* PCR product that was cloned into pGEM-T Easy vector (Promega). Wild-type *Synechocystis* cells were transformed with these plasmid constructs, and transformants were selected on agar plates supplemented with 10 μg ml^{-1} of the antibiotics. Complete segregation was confirmed by PCR analysis (Porter 1988) using the primers for primary amplification.

RNA Isolation and RT-PCR

For analysis of the transcript levels, total RNA was isolated using the Trizol™ reagent (Life Technologies) and treated with RNase-free DNase (Promega) (Ryu et al. 2004). Reverse transcriptase-mediated PCR (RT-PCR) analysis was carried out using the total RNA and specific primers for *crtP* (forward, 5′-GGGGAGGGGAAGTACACATT-3′ and reverse, 5′-CCACGTGGGATTTAAGCAGT-3′), *crtB* (forward, 5′-CTTTACCGTTCCCGTTACCA-3′ and reverse, 5′-GGGTACATAGGCACGACGAT-3′), *crtQ* (forward, 5′-ATGGTTCCTCAGCAAAGGTG-3′ and reverse, 5′-TTCATTTCCGTAACCCAACC-3′), *crtR* (forward, 5′-CGTTTTTGGCTACTGGCAAT-3′ and reverse, 5′-AA TGGGCTTGGTGATGTGAT-3′), and *rrn16Sa* (forward, 5′-CACACTGGGACTGAGACACG-3′ and reverse, 5′-CC ACGCCTAGTATCCATCGT-3′). About 1 μg of total RNA was used for each reverse transcription reaction in a final volume of 20, and 0.8 μl of the reaction mixture was subjected to PCR amplification in an MJ Research Minicycler using the following conditions: initial denaturation at 95°C for 3 min, followed by 25 (*crtB*, *crtP*, and *crtQ*), 28 (*crtR*) or 8 (*rrn16Sa*) cycles of 95°C for 30 s, 60°C for 20 s, and 72°C for 30 s, and a final extension step at 72°C for 7 min. These RNA concentrations were within the linear response range of the PCR amplification (data not shown). The 16S rRNA genes were included as controls. To detect possible DNA contamination, control reactions were performed without reverse transcriptase, but with *Taq* polymerase. The PCR products were routinely analysed on 1.4% agarose gels and viewed with a GEL-DOC2000 densitometer and a computer-aided image analysis system (Bio-Rad Lab). The identities of all RT-PCR products were confirmed by DNA sequencing.

Chl and acridine yellow fluorescence

Measurements of the Chl fluorescence and acridine yellow fluorescence from dark-adapted intact cells suspended in culture media buffered with 5 mM TES-NaOH (pH 8.0) were conducted using a Xe-PAM fluorometer (Walz, Effeltrich, Germany) at 30°C (Ryu et al. 2004). The reduction state of PSII during steady state photosynthesis at a light intensity of 100 μmol m^{-2} s^{-1} was expressed as 1 − qP, where qP is the photochemical quenching coefficient (Campbell et al. 1995). For the excitation and detection of acridine yellow fluorescence (Teuber et al. 2001; Ryu et al. 2004), the emitter and detector were equipped with bandpass filters (UG11 and BG18 for the emitter and KV418 and BG18 for the detector; Walz). The concentrations of acridine yellow and Chl *a* were 2 and 5 μM, respectively.

Results

Expression of *crt* genes during the dark-to-light shift

Light induction of *crt* genes was achieved by illuminating dark-adapted *Synechocystis* cells for 30 min under white light (100 μmol m^{-2} s^{-1}). Dark-adapted WT cells had only a small amount of each transcript of the four *crt* genes (*crtB*, *crtP*, *crtQ*, and *crtR*), but illumination for 30 min was sufficient to significantly increase the levels of accumulated mRNAs (Fig. 1). To determine if the light induction of *crt* gene expression is a consequence of transcriptional activation, mRNA accumulation was analysed in cells in the presence of rifampicin (Rif), a transcription inhibitor. Treatment of cells with Rif for 5 min in the dark before illumination inhibited the mRNA accumulation of all four *crt* genes, suggesting that light induction is a consequence of transcriptional activation.

Redox state of photosynthetic electron transport components and the extent of cytosolic alkalisation in the presence of inhibitors and an uncoupler

In order to determine if *crt* gene expression is under the control of the thylakoid membrane redox state or cytosolic alkalisation during the light induction period, both conditions were produced by treating dark-adapted *Synechocystis* WT cells with various photosynthetic electron transport modulators: DCMU, an electron transport inhibitor at the Q_B binding site of PSII; DBMIB, an inhibitor of PQH_2 oxidation; and MV, an exogenous PSI acceptor. Additionally, CCCP (Trebst 1980), an uncoupler, was used to fully abolish light-induced alkalisation in *Synechocystis* (Ryu et al. 2004).

Chl fluorescence quenching analysis was performed to monitor redox states of the photosynthetic electron transport components (Groom et al. 1993) using photoautotrophically grown WT cells in the exponential growth. As expected, when compared to WT cells not treated with inhibitors, PSII was in a more reduced state during illumination at 100 μmol m^{-2} s^{-1} when either photosynthetic electron transport was inhibited by DCMU and DBMIB or H^+ accumulation induced by electron transport was uncoupled by CCCP (Table 1). After treatment with the PSI electron acceptor MV, PSII remained in the more oxidised state (Table 1).

In *Synechocystis*, the extent of cytosolic alkalisation was semi-quantitatively estimated by the acridine yellow (AY) fluorescence yield (Ryu et al. 2004). When dark-adapted WT cells were illuminated with 100 μmol m^{-2} s^{-1} for 30 min, the AY fluorescence yield increased immediately and remained at the high level state. When the electron input from PSII to the PQ pool was inhibited by DCMU, the extent of cytosolic alkalisation decreased to about 77% of the control (Table 1). As expected, the light-induced increase in AY fluorescence was fully inhibited when cells were treated with CCCP, but it decreased marginally by only 6–10% in the presence of MV and DBMIB. The inhibitory effect of DBMIB was somewhat lower than previously reported (Teuber et al. 2001), which is partly attributable to the low concentration of DBMIB (10 μM used in the present study), although photosynthetic oxygen evolution was almost fully inhibited at the given concentration (data not shown).

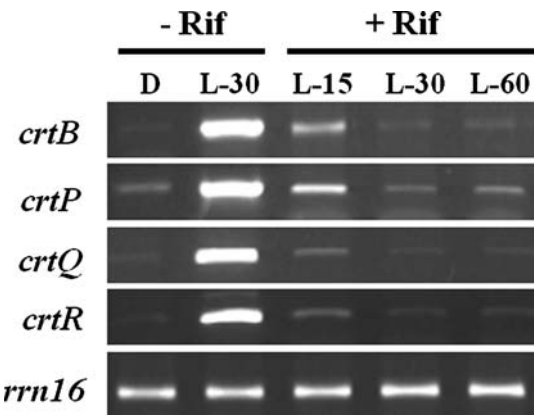

Fig. 1 Light induction of the carotenoid biosynthesis genes *crtB*, *crtP*, *crtQ*, and *crtR* in wild-type *Synechocystis* cells following a dark-to-light shift. RT-PCR was performed as described in "Materials and methods". Cells grown photoautotrophically were incubated in the dark for 16 h (*D*) and shifted to light (*L*, 100 μmol m^{-2} s^{-1}) for 15, 30, and 60 min with (+) or without (−) the transcription inhibitor rifampicin (*Rif*, 300 μg ml^{-1})

Expression of *crt* genes in the presence of photosynthesis inhibitors

To examine the involvement of photosynthetic electron flow in the transcriptional activation of *crt* genes, dark-adapted WT cells were pre-treated with various photosynthetic electron transport modulators and exposed to light conditions, which resulted in the severe alteration of the redox state of the thylakoid membrane and cytosolic alkalisation (Table 1). Upon the exposure of dark-adapted WT cells in the presence of DCMU, DBMIB, or MV to the light for 30 min, expression of the four *crt* genes was significantly inhibited (Fig. 2), the inhibitory effects on *crtB* transcript accumulation being somewhat greater than those on the *crtP*, *crtQ* and *crtR* genes. However, the extent of transcript accumulation during the dark-to-light shift was hardly influenced by treatment with the uncoupler CCCP. This is in contrast to the glucose induction of *crt* genes in the dark where transcript accumulation was almost fully abolished (Ryu et al. 2004).

Table 1 The redox state of PSII (1 − qP) and the cytosolic alkalisation (Δ*F*/Fo) in *Synechocystis* sp. PCC 6803 cells treated with various electron transport inhibitors and an uncoupler

	Control	+DCMU	+DBMIB	+MV	+CCCP
1 − qP	0.36 ± 0.04	0.99 ± 0.01	0.53 ± 0.02	0.27 ± 0.01	0.56 ± 0.04
Δ*F*/Fo	1	0.26 ± 0.05	0.91 ± 0.04	0.94 ± 0.12 0	0

The reduction state of PSII during steady state photosynthesis at a light intensity of 100 μmol m^{-2} s^{-1} was expressed as 1 − qP, where qP is the photochemical quenching coefficient, Fo is the acridine yellow fluorescence yield before illumination and Δ*F* is the increase in the steady-state fluorescence yield during illumination. The data represent mean values ± SE from three to five independent experiments

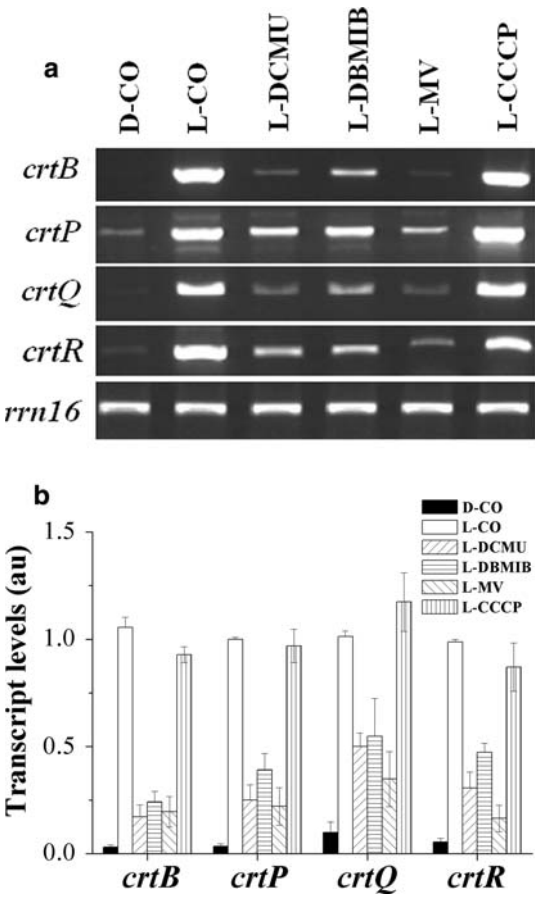

Fig. 2 Effects of photosynthetic electron transport inhibitors (*DCMU*, *DBMIB*, and *MV*) and an uncoupler (*CCCP*) on the expression of the carotenoid biosynthesis genes *crtB*, *crtP*, *crtQ*, and *crtR* in wild-type *Synechocystis* cells (**a**) and the quantification (**b**). Cells were dark-adapted for 16 h (*D*) and then illuminated for 30 min (*L*, 100 μmol m^{-2} s^{-1}) with (*DCMU*, *DBMIB*, *MV*, and *CCCP*) or without (*CO*) drug. **b** Adobe Photoshop 6.0 was used to quantify the intensity of each band. Each value is the relative intensity of the gene normalised to that of rDNA. Mean values were calculated in four independent experiments

Expression of *crt* genes in respiratory electron transport and photoreceptors mutants

Respiratory electron transport shares some of the components of photosynthetic electron transport and thus may participate in the light induction of gene expression. Compared to the wild-type, the transcript levels of three of the four genes in NDH-deficient (M55) (Ogawa 1991) and Cyd/CtaI-defective (Howitt and Vermaas 1998) strains hardly decreased, except for the *crtR* gene, which showed an approximately 50–55% decrease in the transcript level (Fig. 3), ruling out the role of respiratory electron transport in the *crt* genes expression during dark-to-light shift. In an attempt to determine whether light induction is mediated by cyanobacterial photoreceptors, we next investigated gene expression using photoreceptor mutants defective in

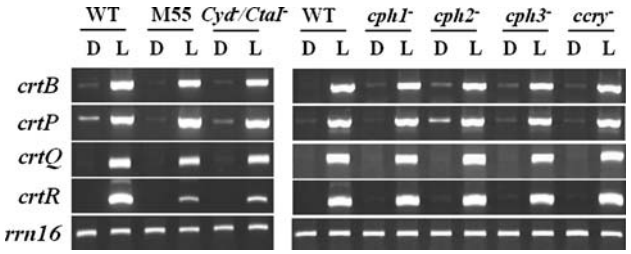

Fig. 3 Light induction of the carotenoid biosynthesis genes *crtB*, *crtP*, *crtQ*, and *crtR* in wild-type *Synechocystis* (*WT*) or respiration (*M55* and *Cyd*$^{-}$*/CtaI*$^{-}$)-, phytochrome (*cph1*$^{-}$, *cph2*$^{-}$, and *cph3*$^{-}$)- or cryptochrome (*ccry*$^{-}$)-defective mutant cells following the dark-to-light shift. Cells incubated in the dark for 16 h (*D*) were exposed to light (100 μmol m^{-2} s^{-1}) for 30 min (*L*)

the cyanobacterial phytochromes (*cph1*$^{-}$, *cph2*$^{-}$, and *cph3*$^{-}$) and cryptochrome (*ccry*$^{-}$). Compared to WT cells, the transcript levels of the four *crt* genes accumulated during the dark-to-light shift were hardly affected in all the photoreceptor-defective mutants (Fig. 3), suggesting that the currently known photoreceptors are hardly involved in the expression of *crt* genes.

Discussion

Light induction of the *crt* genes in *Synechocystis* is not likely to be mediated by known photoreceptors such as cyanobacterial phytochromes and cryptochrome-like proteins since accumulation of the transcripts of the four *crt* genes during the dark-to-light shift was not influenced by mutations of the various photoreceptor genes (Fig. 3). Although we could not exclude the presence of cross-talk and the overlap of multiple photoreceptor signalling pathways, or even the involvement of currently unidentified photoreceptors, light sensing and signalling in *Synechocystis* are different from those in higher plants and green algae where phytochrome (von Lintig et al. 1997) and blue-light receptor (Bohne and Linden 2002), respectively, are involved.

The respiration-mediated increase in cytosolic pH in *Synechocystis* is critical for the glucose induction of some *crt* genes in the dark (Ryu et al. 2004). In contrast to the dark condition, neither respiratory activity nor light-induced cytosolic alkalisation is involved in the light induction of *crt* genes, as mutations in the respiratory components in NDH and Cyd/CtaI and full inhibition of cytosolic alkalisation via CCCP treatment failed to show any strong inhibitory effect on transcript levels compared to WT or untreated control cells (Fig. 4). Furthermore, we could hardly find any positive correlation between the extent of the cytosolic pH increase, which was estimated by the change in AY fluorescence (ΔF/Fo), and the relative

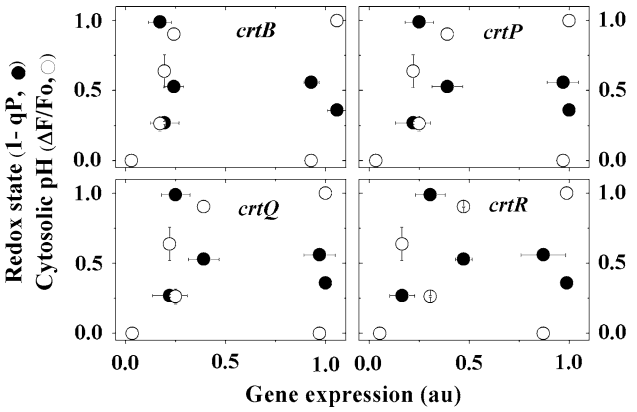

Fig. 4 Correlation between the expression levels of the carotenoid biosynthesis genes *crtB*, *crtP*, *crtQ*, and *crtR* in wild-type *Synechocystis* (taken from Fig. 2) and the redox state of PSII (1 − qP) or cytosolic pH (taken from Table 1)

gene expression (Fig. 4). Therefore, we exclude the possibility that the light accumulation of *crt* genes is mediated by changes in the cytosolic pH.

The inhibitory effect of photosynthetic electron transport inhibitors (Fig. 2) on *crt* gene expression strongly indicates that photosynthetic electron transport in the thylakoid membrane acts as a light sensor. Among the various photosynthetic electron transport components known to be light sensors, the redox state of the PSI acceptor side seems critical, since inhibition of gene expression was observed under conditions where electron input beyond the PSI was inhibited by DCMU (a PSII inhibitor), DBMIB (an inhibitor of the PQH_2 reoxidation), or MV (an inhibitor of NADPH generation), as the PSI acceptor side becomes oxidised in the presence of these inhibitors. If the reduction state of the PSI acceptor side was an important factor for regulating the expression of *crt* genes, then one would expect that the enhanced oxidation state of components on the donor side of PSI induced by DCMU or DBMIB, or on the acceptor side induced by MV, would inhibit expression of *crt* genes, regardless of the reduction state of components upstream of the point of inhibition of electron tansport. Indeed, the value of 1 − qP, a measure of the reduction state of PSII, which is also correlated with that of the PQ pool and Cytb_6f complex (Groom et al. 1993; Cleland 1998), hardly correlated with *crt* gene expression (Fig. 4).

Consistent with this view, it was shown that the redox state of PSI was highly correlated with changes in either myxoxanthophyll or the β-carotene contents in *Plectonema boryanum* (Ivanov et al. 2000; Miskiewicz et al. 2000). The importance of the redox state on the PSI acceptor side as a signal generator for gene expression that was observed in the present study is not restricted to cyanobacteria, since several genes coding for the chloroplast (Kim and Mayfield 1997) or nuclear (Karpinski et al. 1997) proteins of higher plants are also responsive to the redox state of the PSI acceptor side.

As for a redox sensor of the downstream PSI acceptor side, further systematic studies using mutants involved in cytosolic redox modulation, such as the ferredoxin–thioredoxin system, are necessary. However, by using light signalling mutants and photosynthetic electron transport inhibitors in the present study, we have demonstrated that the light induction of four *crt* genes in *Synechocystis* is dependent on the redox state of photosynthetic electron transport, specifically the redox state of the PSI acceptor side.

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