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Transcriptional regulation of CmpR, the LysR family protein involved in CO_2-responsive gene regulation in the cyanobacterium *Synechococcus elongatus*

Lu-Lu Pan[1], Kiyoshi Onai[2], Kazuma Uesaka[1], Kunio Ihara[2], Takumi Natsume[1], Nobuyuki Takatani[1], Masahiro Ishiura[2] and Tatsuo Omata[1*]

[1]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601 Japan

[2]Center for Gene Research, Nagoya University, Nagoya, 46-8602 Japan

Abstract

The *cmpR* gene of the cyanobacterium *Synechococcus elongatus* PCC 7942 encodes a LysR family protein that activates the *cmpABCD* bicarbonate transporter operon under the conditions of CO_2 limitation. Using the *luxAB* reporter genes, the promoter of the *cmpR* gene was shown to be transiently activated under the low-CO_2 conditions. Much higher levels of *luxAB* expression were observed in a *ΔcmpR* background even under high-CO_2 conditions, revealing a repressive role of *cmpR* in regulation of the *cmpR* promoter. Exposure of the *ΔcmpR* strain to the low-CO_2 conditions further increased the *luxAB* expression level, showing the involvement of an additional transcriptional activator responding to CO_2 limitation. From the *ΔcmpR* strain, two mutant strains showing slower growth, lower contents of phycocyanin and even higher levels of P_{cmpR}-*luxAB* expression than the parental strain were isolated and shown to have a partial deletion in the ORF of *cpcE*, encoding the alpha subunit of phycocyanobilin lyase. Expression of plasmid-borne *cpcE* in the mutants restored the growth and reduced the luciferase expression level to those observed in the parental strain, while partially restoring the phycocyanin content. These results indicated occurrence of multiple layers of regulation of *cmpR* expression in addition to the autoregulation by CmpR.

Abbreviations: ABC, ATP-binding cassette; CBB, Calvin-Benson-Bassham; CCD, Charge Coupled Device; CCMs, CO_2 concentrating mechanisms; HC, high-CO_2; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IPTG, Isopropyl β-D-1-thiogalactopyranoside; LC, low-CO_2; LTTRs, LysR-type transcriptional regulators; NS1, neutral site 1; OD_{730}, Optical Density at 730 nm; ORF, open reading frame; PC, phycocyanin; PCR, Polymerase Chain Reaction; 2-PG, 2-phosphoglycolate; RT-PCR, Reverse Transcription Polymerase Chain Reaction; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; UVC, ultraviolet-C

Introduction

Though the atmospheric CO_2 concentration is rising because of combustion of fossil fuels, it is not high enough to saturate ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), the enzyme responsible for photosynthetic CO_2 fixation, under ambient air *in vitro*; O_2 serves as the alternative substrate of Rubisco to CO_2 and hence interferes with the carboxylation of ribulose 1,5-bisphosphate (RuBP). To cope with this problem, photosynthetic organisms have developed various "CO_2 concentrating mechanisms" (CCMs) to raise the CO_2 concentration in the vicinity of Rubisco to enhance the carboxylation reaction.

Cyanobacteria are photoautotrophic prokaryotes capable of plant-type, oxygenic photosynthesis. They have an efficient CCM, which consists of two steps: (i) intracellular accumulation of high concentrations of HCO_3^-; and (ii) localized conversion of HCO_3^- to CO_2 in the carboxysomes, the polyhedral inclusion bodies to which Rubisco is sequestered. Intracellular accumulation of HCO_3^- is mediated by active HCO_3^- transporters and specialized NADPH dehydrogenase complexes that convert CO_2 to HCO_3^- in an energy-dependent manner [1-5]. The intracellular conversion of CO_2 to HCO_3^- lowers the intracellular CO_2 level low, allowing for uptake of CO_2 from the external medium via passive diffusion. For both the HCO_3^- and CO_2 uptake mechanisms, those having high-affinity for the respective substrates are distinguished from those having low-affinity for the substrates. The genes related to the high-affinity HCO_3^- and CO_2 uptake mechanisms are generally repressed under high-CO_2 (HC) conditions, i.e., when cells are incubated under air supplemented with 1-5% (v/v) of CO_2. Since transcription of these genes are sharply induced by incubation of the HC-grown cells under low-CO_2 (LC) conditions, i.e., under air containing 0.04% or lower CO_2 concentrations, they are thought to be LC-inducible genes [6-8].

LysR-type transcriptional regulators (LTTRs) of CbbR subfamily were first identified in chemoautotrophic bacteria and phototrophic bacteria as the regulator of the genes encoding the enzymes of the Calvin-Benson-Bassham (CBB) cycle [9]. In cyanobacteria, CbbR homologs were shown to be the major player in regulation of the LC-induced expression of the CCM-related genes; *Synechocystis* sp. PCC 6803 has two closely-related CbbR homologs, one of which (CmpR) was identified as the activator of the *cmp* operon, which encodes the

Correspondence to: Tatsuo Omata, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya-464-8601, Japan, Tel: +81 52 789 4106; Fax: +81 52 789 4107; E-mail: omata@agr.nagoya-u.ac.jp

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subunits of an ABC-type high-affinity HCO_3^- transporter [10], while the other (NdhR or CcmR) was shown to serve as a repressor under HC conditions, inhibiting expression of several CCM-related genes including its own gene, the *sbtA* gene encoding the Na^+-dependent high-affinity HCO_3^- transporter, and the *ndhF3* operon encoding the components of the high-affinity CO_2 uptake mechanism [6,7,11]. *Synechococcus elongatus* PCC 7942, on the other hand, has only one CbbR homolog designated CmpR, which was shown to be the activator of the *cmpABCD* operon [10]. The CmpR protein of *S. elongatus* PCC 7942 was shown by *in vitro* experiments to bind to the promoter of the *cmpABCD* operon in the presence of 2-phosphoglycolate (2-PG), one of the products of the RuBP oxygenation reaction [12]. It is therefore supposed that 2-PG serves as the signal of CO_2 deficiency *in vivo* to activate transcription of the *cmpABCD* operon. It has remained unclear whether the *S. elongatus* CmpR protein regulates its own expression as many of the LTTR proteins do. Using a *luxAB* reporter gene transcriptionally fused to the *cmpR* promoter, it is shown in this study that the protein not only activates the *cmp* operon under LC conditions, but represses its own expression under HC conditions. Studies on the reporter strain reveal an additional layer(s) of regulation of the promoter, involving CmpR-independent mechanism(s) responding to the LC conditions. Genetic studies on the strain further point to a novel relationship between the integrity of the light harvesting system and the activity of the promoter.

Materials and methods

Strains and growth conditions

A derivative of *S.elongatus* strain PCC7942 (SPc), which is cured of the resident pUH24 plasmid [13], was used as the parental strain of all the mutants used in this study. For construction of a *ΔcmpR* mutant, a 0.8-kbp DNA region upstream of *cmpR* (nucleotides -798 to +3 with respect to the translation start site) and a 0.9-kbp region downstream of *cmpR* (nucleotides +1 to +888 with respect to the termination codon) were amplified by PCR using the primer pairs P1/P2 and P3/P4, respectively (Table 1). The chloramphenicol resistance gene was also amplified by PCR using the primers P5 and P6, which respectively included the sequences complementary to the primers P2 and P3. The three DNA fragments were joined by overlap extension PCR using the primers P1 and P4. The resulting DNA fragment was used to transform the SPc strain to construct a chloramphenicol-resistant *ΔccmR* mutant (MR5) via replacement of the *cmpR* gene with the chloramphenicol resistance gene (Figure 1A). After three serial streak purifications of single colonies, successful genome segregation was confirmed by PCR using the primers P9 and P10 (Figure 1B).

For construction of a transcriptional fusion of the promoter of *cmpR* (P_{cmpR}) and the *luxAB* ORFs encoding a bacterial luciferase, the 115-bp DNA region upstream of *cmpR* (nucleotides -115 to -1 with respect to the translation start site, Figure 1C), including 45 bases of the 3' portion of the *synpcc7942_1309* ORF, was amplified by PCR, using the primers P7 and P8 carrying added NotI and BamHI recognition sequences, respectively (Table 1), and cloned between the NotI and BamHI sites of the pAM1414 plasmid carrying a promoter-less *luxAB* gene cluster [14]. The P_{cmpR}::*luxAB* fusion thus constructed was transferred into the neutral site 1 (NS1) locus on the chromosome of the SPc and MR5 strains to construct the CW and CT strains, respectively.

The liquid medium used for cultivation of the cyanobacterial strains was a modification of the BG11 medium containing 15 mM KNO_3 and 20 mM HEPES-KOH (pH 8.2) [15]. Cells were grown at 30℃ under continuous illumination provided by fluorescent lamps at a light intensity of 50 μE m^{-2} s^{-1}. Cultures were aerated with ambient air (low-CO_2 conditions) or air supplemented with 2% (v/v) CO_2 (high-CO_2 conditions). Solid medium was prepared by addition of 1.5 % (w/v) Bactoagar (Difco) to the liquid medium. Where appropriate, chloramphenicol (5 μg ml^{-1}), spectinomycin (20 μg ml^{-1}) and kanamycin (15 μg ml^{-1}) were added to the media.

For random mutagenesis in the CT strain, cells in liquid medium were irradiated with a 15W UVC lamp (254 nm) for 4 min (survival rate ~ 0.2%) and then treated for 24 h with 40 μg ml^{-1} of ampicillin in the light under LC conditions. The cells were then spread onto solid medium prepared in rectangular plates of 128 mm × 86 mm in size and incubated under the HC conditions for 10 days. The agar plates, which contained a total of 17,000 colonies, were subsequently incubated under low CO_2 conditions and screened for mutant colonies showing altered expression levels of bioluminescence.

Bioluminescence measurements

Time-course measurements of bioluminescence from colonies of the P_{cmpR}::*luxAB* reporter strains were performed essentially as described previously [16,17] using an automated bioluminescence

Table 1. Primers used in this study.

No.	Name	Sequence (5'-3')
P1	*ccmR*up798F	AACAGGGGATTGAACCGATCTGC
P2	*ccmR*up17R	CACAACTCAAATCACCATTC
P3	*ccmR*down1F	GCAGATGGATAGGCTCCACTCC
P4	*ccmR*down888R	CCGGGCTCTCTATCGCCGTGCCTCCG
P5	*ccmR*inCm^{r}F	GAATGGTGATTTGAGTTGTGGCGATCGCTCGACTAGAGTCGATCTCAA
P6	*ccmR*inCm^{r}R	GGAGTGGAGCCTATCCATCTGCGCGATCGCGTTTAAGGGCACCA
P7	*proccmR*115NotIF	CGATCAGGCAGGCATTGCGCGGCCGCAACCCTCCAGCGAT
P8	*proccmR*BamHIR	TGCAGCGTTAGATTTTTGGATCCAACTCAAATCACCATTC
P9	*cmpR*up30F	ACTTAAATCTATTGAATGGTGATTTGAGTT
P10	*cmpR*down28R	TTGCATGGAGTGGAGCCTATCCATCTGC
P11	*cpcE*-NcoIupF	TGAGCCGACTTCCATGGAATGAGTGAAGCG
P12	*cpcE*-XbaIdownR	GTGCTCATCTAGAAGGTGCCTACAGCAAACC
P13	*cpcF*F	ATGAGCACGGAATTGATCGCTGCTGTTGAAGC
P14	*cpcF*R	TTATACACCAGCGGCTAGGCGTCGCTCG
P15	*rnpB*F	GAGGAAAGTCCGGGCTCCCA
P16	*rnpB*R	TAAGCCGGGTTCTGTTCTCT
P17	*luxA*F	CAGCCGAGCCTTAATGGACT
P18	*luxA*R	ATTCGGCGATTGGTGTCTTTGTG

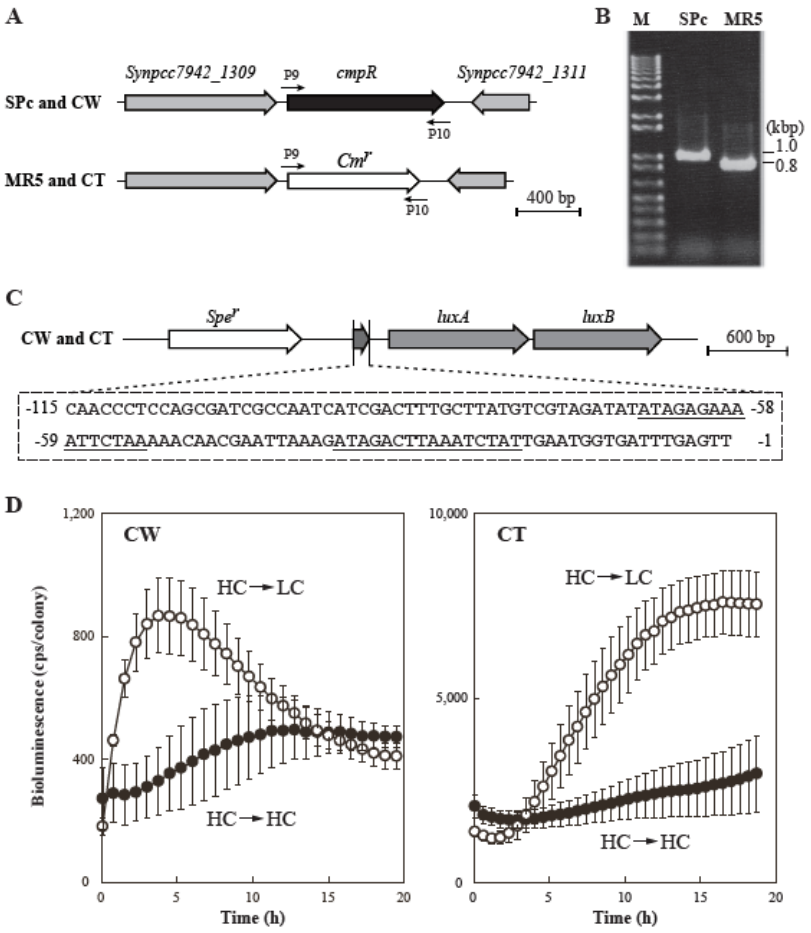

Figure 1. Analysis of the effects of CmpR deficiency on regulation of the *cmpR* promoter, using the transcriptional fusion of the *cmpR* promoter and the *luxAB* ORFs encoding a bacterial luciferase. A. The map of the *cmpR* genomic region of the SPc and CW strains, as compared with that of the Δ*cmpR* strains MR5 and CT. Arrows show the primers used for the analysis shown in panel B. B. PCR analysis of the structure of the *cmpR* genomic region in SPc and MR5. C. Organization of the genes inserted into the NS1 genomic region of the reporter strains CW and CT. The nucleotide sequence of the 115-bp DNA fragment carrying the *cmpR* promoter is indicated below the map, with the numbers showing the position of the nucleotides relative to the *cmpR* translation start site. The underlines show the sequences conforming to the proposed consensus sequence for binding of cyanobacterial CbbR homologs. D. Induction of bioluminescence from the CW and CT strains after transfer of the colonies grown under HC conditions to LC conditions. Data shown are the means ± SE from biological triplicates.

monitoring apparatus (model LL04-1,Churitsu Electric Co., Nagoya, Japan); Cells of CT and CW strains were plated on solid medium and incubated under HC conditions for 7 days. Agar pieces carrying 10 to 20 colonies were sealed in 35-mm petri dishes together with 30 µl of 3% *n*-decanal solution in cooking oil and 100 µl $NaHCO_3$ (10%, w/v), and incubated under illumination for 16 h. Expression of P_{cmpR}::*luxAB* was induced by removing the $NaHCO_3$ solution from the petri dish, and bioluminescence was measured at 30-min intervals. The data were analyzed using the SL00-01 bioluminescence-analyzing software (SL00-01,Churitsu Electric Co.) [18]. Bioluminescence intensity was expressed as cps per colony.

Identification of the mutant colonies derived from the UVC-irradiated population of the *luxAB* reporter strain CT was carried out using a high-throughput real-time bioluminescence monitoring system equipped with a CCD image sensor (Churitsu Electric Corp., Nagoya, Japan; http://www.churitsu.co.jp/products/bio/highthroughput.html) essentially as described by Kondo and Ishiura [19].

For measurements of bioluminescence from the E3 and E11 mutants, which form only tiny colonies on solid medium, cells grown in liquid medium under HC conditions were collected by centrifugation, re-suspended in fresh medium to give an OD_{730} value of 0.5, and incubated under illumination with aeration with ambient air. 0.5 ml aliquots of the cell suspension were occasionally withdrawn from the culture, mixed with 20 µl of 3% *n*-decanal solution, incubated for 5 min and then subjected to photon counting for 30 s using an AQUACOSMOS/VIM system (Hamamatsu Photonics, Hamamatsu, Japan).

Re-sequencing analysis of genomic DNA

Genomic DNA was extracted from *Synechococcus* cells using a Wizard Genomic DNA Purification Kit (Promega), after treatment of the cells with lysozme as follows; Cells were collected and re-suspended in 450 µl of a buffer containing 50 mM Tris-HCl (pH 8.0), 50 mM NaCl and 5 mM EDTA and after mixing with 50 µl of a lysozyme solution (50 mg ml^{-1}), incubated at 37°C for 1 h. The cells were subsequently collected by centrifugation at 15,000 g at RT for 2 min and lysed by mixing with 0.6 ml of the Nuclei Lysis Solution of the Promega kit. Re-sequencing of the genomic DNA was performed using a SOLiD 5500 system (Life Technologies) as described previously [20].

Expression of the plasmid-borne *cpcE* in *Syenchococcus*.

An 859-bp DNA fragment carrying the coding region of *cpcE* was amplified from the genomic DNA of S. *elongatus* PCC 7942 by PCR using the primers P11 and P12 carrying added NcoI and XbaI recognition sequences, respectively (Table 1). The amplified DNA

fragment was digested with NcoI and XbaI and ligated between the NcoI and XbaI sites of the shuttle expression vector pSE1 [21]. The resulting plasmid was transformed into the cells of E3 and E11 to yield the strains E3R and E11R.

RNA extraction and semi-quantitative RT-PCR

25 ml aliquots of the cultures of *S. elongatus* (OD_{730}~0.5) were used for RNA extraction, following the protocol reported by Ruffing [22]. From the lysate/ethanol mixture thus obtained, RNA was purified using a SV Total RNA Isolation System (Promega). The isolated total RNA was used for the synthesis of cDNA using a SuperScript III First-Strand Synthesis System (Life Technologies). The obtained cDNA was used as the template for semi-quantitative RT-PCR analysis using the primer pairs specific to *luxA*, *cpcF* and *rnpB* (P13-P18; Table 1). The cycle numbers for PCR amplification was 30 for *luxA*, 30 for *cpcF*, and 26 for *rnpB*.

Results

CmpR-dependent and independent mechanisms of *cmpR* regulation

As previously reported for the *cmpR* insertional mutant MR4 [10], cells of the *cmpR* deletion mutant MR5 grew as fast as the parental SPc strain both under LC and HC conditions (data not shown). Accordingly, the P_{cmpR}::*luxAB* strains CW and CT, which were constructed from SPc and MR5 respectively, formed colonies of essentially the same size (data not shown). Low CO_2 response of the CW and CT strains were therefore compared by measuring the bioluminescence emitted from colonies on the surface of agar plates, using a real-time bioluminescence monitoring system (Figure 1D). When HC-grown cells of the CW strain, which carries the wild-type *cmpR* gene, was transferred to LC conditions, there was a sharp induction of bioluminescence (Figure 1D, CW, open symbols). After reaching a peak at t ~ 4 h, the intensity of bioluminescence declined to a lower level. These results indicated that transcription of *cmpR* is activated by CO_2 limitation. Unlike the CW strain, the reporter strain deficient in *cmpR* (CT) showed high level of bioluminescence even when grown under the HC conditions (Figure 1D, CT, t = 0), showing that CmpR autoregulates its own expression as many other LTTRs do. After transfer of CT cells to LC conditions, further increase in the luciferase expression level was observed (Figure 1D, CT, open symbols); The bioluminescence level increased for about 15 h after the transfer and remained at the high level thereafter. The results indicated the presence of a CmpR-independent mechanism(s) for LC-responsive activation of *cmpR*.

Characterization of the mutants with altered P_{cmpR} regulation

To gain insight into the CmpR-independent mechanism(s) of *cmpR* regulation, CT cells were treated with UVC irradiation, subjected to an ampicillin enrichment procedure under LC conditions, plated on solid medium and screened for colonies showing altered levels of bioluminescence, using a high-throughput bioluminescence monitoring system equipped with a CCD image sensor. Among the mutant candidates, two (designated E3 and E11) were found to reproducibly show slow growth compared to the parental CT strain irrespective of the CO_2 conditions (Figure 2). Since these mutants formed only tiny colonies, their bioluminescence intensity was compared with that of the parental CT strain using cells grown in liquid cultures and re-suspended in fresh growth medium to give an OD_{730} value of 0.5 (Figure 4). The results confirmed that the expression level of luciferase was much higher in the E3 and E11 strains than in the parental CT strain (Figure 4).

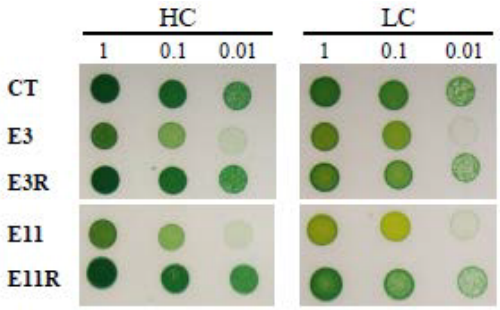

Figure 2. Growth of the CT strain, the E3 and E11 mutants generated from CT, and the E3 and E11 derivatives expressing a plasmid-borne copy of *cpcE*(E3R and E11R). Cells grown under HC conditions in liquid medium were collected by centrifugation and re-suspended in fresh medium to give an OD_{730} value of 1. Seven µl of the cell suspensions and their 10-fold serial dilutions were spotted on solid medium supplemented with 1 mM IPTG and incubated under the LC or HC conditions for 4 days. Numbers on the top indicate the dilution factor.

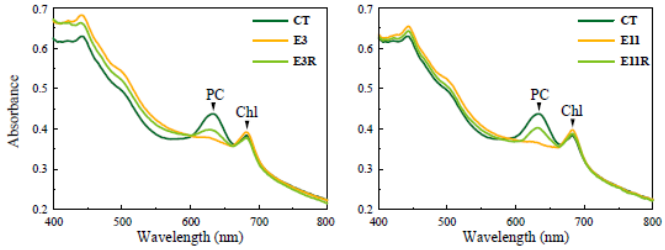

Figure 3. Comparison of the whole cell absorption spectra of the CT strain with those of the *cpcE*-defective mutants (E3 and E11) and their derivatives expressing the plasmid-borne *cpcE*(E3R and E11R). Cells grown in liquid medium under the HC conditions were used for the measurements. Absorption peaks of PC and chlorophyll (Chl) in the red region are indicated.

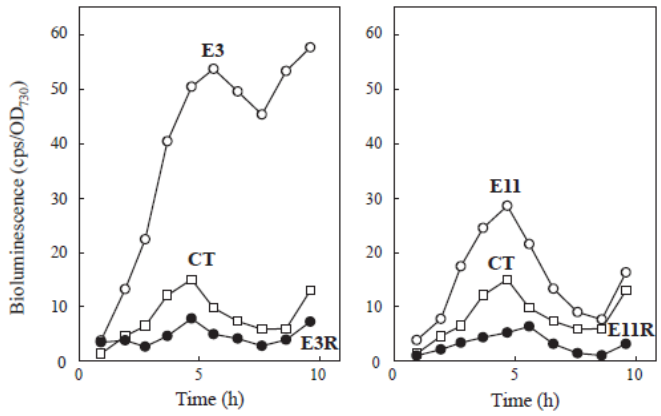

Figure 4. Induction of bioluminescence from the CT strain and its derivatives. Cells grown under the HC conditions in liquid medium were collected by centrifugation, re-suspended in fresh liquid medium to give an OD_{730} value of 0.5 at t = 0, and aerated with ambient air containing 0.05% CO_2. 500 µl aliquots from the cultures were withdrawn at the time points indicated, mixed with 20 µl of 3% *n*-decanal and subjected to photon counting. Data shown are from one of the three sets.

To identify the mutations that caused activation of the *cmpR* promoter in the E3 and E11 mutants, genome re-sequencing analysis of the CT, E3 and E11 mutants was performed (Table 2). The CT strain had no mutation as compared with the parental SPc strain except for the replacement of the *cmpR* gene with the chloramphenicol resistance gene and the insertion of a spectinomycin resistance gene and the *PcmpR::luxAB* reporter construct into the NS1 site. E3 and

Table 2. List of mutations found in the E3 and E11 strains compared to the CT genomic sequence.

Position	GENE ID	CT[a]	E3[b]	E11[b]	Amino acid change	Gene product
463280-466710	0479-0481	-	3431-bp deletion (100%)	-	-	GTP-binding protein LepA, GAF sensor signal transduction histidine kinase, Protease
924881	0918	G	T (100%)	G	G268V	Acyl-ACP synthetase
1061288	1048	C	C	T (100%)	A133V	Phycocyanin α subunit CpcA1
1065043	1053	C	C	T (100%)	A133V	Phycocyanin α subunit CpcA2
1065485-1065605	1054	-	121-bp deletion (100%)	121-bp deletion (100%)	41-AA deletion, frame shift	Phycocyanin alpha subunit phycocyanobilin lyase CpcE subunit
2660395	Upstream of R0052	G	A (50%)	G	-	16S ribosomal RNA

[a]This column shows the nucleotide in the genome sequence of the CT strain.

[b]These columns show the mutations and their frequency in E3 and E11.

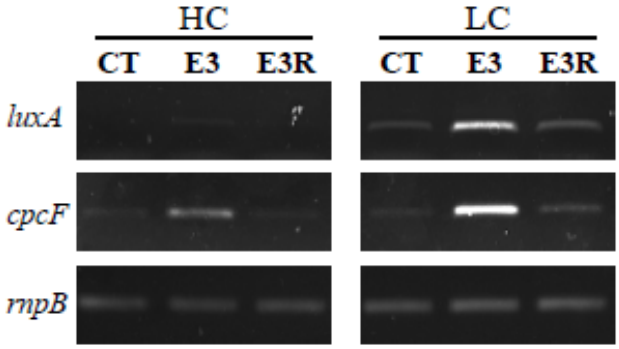

Figure 5. Semi-quantitative RT-PCR analysis of expression of the *cpcF*, *luxA*, and *rnpB* genes in the CT, E3 and E3R strains under the HC and LC conditions. HC-grown cells collected by centrifugation and re-suspended into fresh liquid medium were separated into two portions and incubated under HC or LC conditions. RNA samples extracted from the cells after 25 min of incubation were used for the analysis.

E11 respectively had several mutations on the genome, but they had a common deletion of 121-bp in the *cpcE* gene, which forms an operon with the *cpcF* gene located downstream. The *cpcE-cpcF* operon encodes the subunits of the heterodimeric phycocyanin-α-subunit phycocyanobilin lyase, which mediates attachment of phycocyanobilin to the α subunit (CpcA) of phycocyanin (PC) [23], a blue-colored light-harvesting protein consisting of the α and β subunits encoded by the highly conserved *cpcA* and *cpcB* genes. In accordance with the previously reported properties of the *cpcE* deletion mutant of *S. elongates* [24], both E3 and E11 showed significantly reduced amounts of PC as revealed by their absorption spectra, showing lower absorption peak at 625 nm as compared with the parental CT strain (Figure 3).

To determine whether the CpcE deficiency was the cause of the growth defect and the enhanced activation of P_{cmpR} under LC condition, a plasmid carrying the *cpcE* ORF downstream of the P_{trc} promoter (see methods) was introduced into E3 and E11 to construct the E3R and E11R strains. Expression of *cpcE* from the P_{trc} promoter could only partially restore the PC content (Figure 3), presumably due to the polar effect of the 121-bp deletion in the chromosomal *cpcE* gene on the expression of the *cpcF* gene. Nevertheless, growth of the E3R and E11R strains were comparable to that of CT, indicating that the amount of PC was sufficient to support normal photosynthetic growth under the given conditions (Figure 2). The level of bioluminescence was also reduced to that observed in the parental CT strain by expression of *cpcE*.

Semi-quantitative RT-PCR analysis of the transcripts from the *luxA* gene showed that the gene is expressed at a much higher level in E3 than in CT under the LC conditions. Also, expression of plasmid-borne *cpcE* reduced the transcript level to a level comparable to that in CT. These results confirmed that it was the activity of the P_{cmpR} promoter that responded to the presence or absence of the *cpcE* gene (Figure 5).

Discussion

The CmpR protein of *S. elongatus* PCC 7942 activates transcription of the *cmpABCD* operon, which encodes the subunits of a high-affinity ABC HCO_3^- transporter, under the conditions of CO_2 limitation [10]. Micromolar concentrations of 2-PG were shown to enhance the binding of the protein to the promoter of the *cmp* operon *in vitro*, suggesting that the product of the RuBP oxygenation reaction acts as a co-inducer of CmpR under the LC conditions [12]. In addition to its role as an activator of gene expression, the present results show that CmpR acts as a repressor of its own expression under the HC conditions (Figure 1). Thus, CmpR has two distinct modes of interaction with DNA; the one involving 2-PG as a co-activator and the other independent of 2-PG. The CcmR (NdhR) protein of *Synechocystis* sp. PCC 6803, the homolog of CmpR, is involved in repression of various CCM related genes under HC conditions [6,7,11]. By comparison of the promoters of these genes, a sequence ATAG-N_8-CTAT has been proposed as the consensus binding motif for CcmR [6]. Since the *cmpR* promoter of *S. elongatus* has two of this sequence (Figure 1C), CmpR presumably binds to these sites under HC in a 2-PG independent manner to repress transcription.

Since the P_{cmpR}::*luxAB* fusion is activated under LC conditions even in the CT strain deficient in CmpR (Figure 1D, CT), it is deduced that *S. elongatus* has a CmpR-independent mechanism for LC-responsive activation of P_{cmpR}. In an attempt to identify the genes relevant to this mechanism, we aimed at isolation of mutants that are defective in growth under LC conditions because of impaired LC response. The UVC-treated cells were hence subjected to an ampicillin enrichment procedure under LC conditions. The two mutant strains that showed a slow-growth phenotype were, however, found to grow slowly irrespective of the CO_2 conditions (Figure 2) and to show stronger P_{cmpR} induction than the parental CT strain under LC conditions (Figures 4 and 5). The *cpcE* mutation in the E3 and E11 strains is clearly responsible for the slow-growth phenotype and hyper-induction of P_{cmpR} (Figures 2 and 5). The slow growth is presumed to be due to the reduced capacity of the cells to absorb light energy (Figure 3), but the underlying molecular mechanism that links CpcE and P_{cmpR} activity

is currently unclear. It is commonly supposed that the function of CCM becomes more important under high-light conditions, where the energy supply tends to exceed the supply of CO_2 to Rubisco. The activation of P_{cmpR} in the *cpcE* mutant cells having smaller capacity of light absorption than the parental strain is therefore novel. Possible involvement of CpcE, a heat repeat protein, in direct interaction with P_{cmpR} cannot be excluded, but it seems more likely that the reduced photosynthetic electron transport and the resulting metabolic change have relevance to the activation of P_{cmpR} in the CmpR-deficient *cpcE* mutants. Although further study is required, the present results reveal a novel link between the light-harvesting capacity of the cells and regulation of the key regulator of CCM.

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