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Interference of an *apcA* insertion with complementary chromatic adaptation in the diazotrophic *Synechocystis* sp. strain BO 8402

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Abstract

Complementary chromatic adaptation was studied in two unicellular diazotrophic *Synechocystis*-type cyanobacteria, strains BO 8402 and BO 9201. Strain BO 8402 was isolated from Lake Constance as a mutant lacking phycobilisomes due to an insertion sequence element in the gene *apcA*, encoding α-allophycocyanin. Strain BO 9201 recovered the ability to assemble functional phycobilisomes after a spontaneous excision of the insertion sequence element in *apcA*. Simultaneously, the strain became able to perform group II complementary chromatic adaptation by regulating the synthesis of phycoerythrin. The two strains had identical phycoerythrin operons, *cpeBA*, and similar-sized transcripts were formed upon induction by green light. However, in strain BO 8402 the *cpeBA* transcript level was approx. 20-fold lower than in strain BO 9201. Because strain BO 8402 cannot synthesize allophycocyanin and phycocyanin is sequestered in paracrystalline inclusion bodies, non-assembled phycoerythrin may accumulate inside the cells. It was examined whether non-assembled phycoerythrin or other effects caused by the absence of phycobilisomes, such as a permanently oxidized redox status of the photosynthetic electron transport chain or a distorted ratio of C and N assimilation mediated the repression of *cpeBA* transcription in strain BO 8402. No such links could be established. We therefore concluded that in these diazotrophic *Synechocystis*-type cyanobacteria the green light-induced transcription of the *cpe* operon directly required a functional *apc* operon.

Keywords: Nitrogen metabolism; Phycoerythrin; Complementary chromatic adaptation; Internal signaling; Cyanobacterium; *Synechocystis*

1. Introduction

Cyanobacteria are oxygen-evolving photosynthetic prokaryotes that use phycobilisomes as major light harvesting complexes to absorb visible light in the range from 500 nm to 700 nm. Phycobilisomes are

Abbreviations: *apc*, *cpc*, *cpe*, genes encoding allophycocyanin-, phycocyanin- and phycoerythrin-related proteins, respectively; AP, allophycocyanin; CCA, complementary chromatic adaptation; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DIG, digoxigenin; GL, green light; LiDS–PAGE, lithium dodecyl sulphate–polyacrylamide gel electrophoresis; MSX, methionine sulphoximine; PC, phycocyanin; PE, phycoerythrin; p*I*, isoelectric point; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid; PS, photosystem; RL, red light; WL, white light

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large, water-soluble multiprotein structures that are attached to the stromal side of thylakoid membranes [1,2]. Two structural domains can be distinguished: a central core complex, connecting the phycobilisome to the photosystems (PS) I and II in the thylakoids, and peripheral rods radiating from the core complex. The core complex consists of mostly three cylinders, each containing four trimeric $(\alpha\beta)_3$ complexes composed of allophycocyanin (AP) that are stabilized by pigmented and non-pigmented linker polypeptides. In the peripheral rods, hexameric phycocyanin (PC)–linker complexes $(\alpha\beta PC)_6 \times L_R^{PC}$ and, if present, hexameric phycoerythrin (PE)–linker complexes $(\alpha\beta PE)_6 \times L_R^{PE}$ are linearly arranged. The arrangement of hexamers in the rods is organized by the non-coloured linker polypeptides L_R^{PC}, L_R^{PE} and L_{RC}^{PC}, which determine the specific assembly of hexamers. The different spectral characteristics of the phycobiliprotein–linker complexes allow a directed radiation-less energy transfer from the phycobilisome periphery to the core and further to the photosystems. In cyanobacteria, phycobilisomes transfer light energy primarily to PSII, which has less accessory chlorophylls than PSI, but also to the latter. A rapid adaptation mechanism, called state transition, allows adjusting the energy transfer from phycobilisomes to the two photosystems according to the energetic requirements of the cell [3–5].

Some cyanobacteria can perform complementary chromatic adaptation (CCA) by altering the phycobiliprotein composition of the rods for maximal absorbance of available light. The most striking change in pigment protein composition is observed when cells are shifted between red light (RL) and green light (GL). Cyanobacteria have been assigned to three light acclimation groups [6]. Acclimation group I comprises strains that can alter phycobilisome size and number but are incapable to adjust their phycobiliprotein composition to changes in spectral quality. Cyanobacteria of group II perform a 'unidirectional' CCA by expressing PE in GL conditions and repressing its synthesis in RL, while strains of group III can independently modulate both, the PE and PC levels performing a bidirectional regulation. Genes encoding structural components of phycobilisomes have been sequenced from many cyanobacteria. The genes encoding the subunits of the phycobiliproteins AP and PC, *apcAB* and *cpcBA*, respectively, are located on operons that additionally contain corresponding linker polypeptide genes downstream. However, in all CCA performing cyanobacteria the genes encoding the subunits of PE, *cpeBA*, and the corresponding linker polypeptide genes are located on two discrete operons.

In contrast to the relatively broad knowledge about the gene structure, we still have limited information about the regulatory mechanisms of gene transcription during CCA. Furthermore, this information is largely derived from the study of two very similar cyanobacteria, *Calothrix* sp. strain PCC 7601 and *Fremyella diplosiphon*. They belong to CCA acclimation group III and contain one AP operon, two PE operons (one encoding PE subunits and one encoding PE linker polypeptides) and three PC operons. At least three operons, one for PC and the two for PE and its linkers, are involved in CCA. It is believed that regulation of CCA occurs mainly on the transcriptional level by a four-step phosphorelay signal transduction system [7]. Two proteins, designated RcaA and RcaB, which are detected in extracts from *Calothrix* sp. PCC 7601 cells grown under GL, have been shown to interact with the *cpeBA* promoter in mobility shift and footprinting assays [8]. The RcaA protein binds to a region of the *cpeBA* promoter, which contains a direct repeat separated by 4 bp, $TTGTTA(N_4)TTGTTA$. The RcaB protein binding site is less clear but it appears to map to a region between the RcaA binding site and the region bound by the RNA polymerase. Recently, a gene, *cpeR*, has been identified [9,10] which is required for GL-stimulated synthesis of PE, but its co-identity with RcaA (or RcaB) remains to be shown. Several environmental factors, in particular the supply with nutrients, are known to interfere with phycobiliprotein synthesis [1,11,12], but this has not been studied in detail in CCA performing strains.

In this study we examined CCA in two diazotrophic unicellular cyanobacteria, *Synechocystis* spp. strain BO 8402 and strain BO 9201. *Synechocystis* sp. strain BO 8402 was originally isolated from the pelagic zone of Lake Constance as a natural mutant lacking the phycobiliprotein AP [13]. Cells produce large amounts of PC sequestered in highly fluorescent inclusion bodies [14–16] as well as traces of PE [17], but no vinylated chlorophyll *b* (Ernst, unpublished). Therefore, strain BO 8402 is not a prochlo-

rophyte. In the *apcA* gene, encoding α-AP, the insertion sequence element *IS*8402 is found, which prevents the synthesis of core complex proteins (phycobiliproteins as well as linker polypeptides) and, hence, the assembly of functional phycobilisomes [18]. The strain does not show significant shifts in whole cell absorption spectra when grown under RL or GL and was therefore previously assigned to CCA acclimation group I. Nevertheless, in the supernatant of cell extracts of white light (WL)-grown cells, which were prepared in the presence of medium strength phosphate buffer (0.65 M), traces of PE were detected by its characteristic fluorescence (emission maximum at ≈580 nm) [17]. Later it was discovered that a higher molarity of phosphate was required to maintain the integrity of paracrystalline PC aggregates during preparation [15] so that the cellular location of this PE remained ambiguous. Strain BO 9201 is a revertant of strain BO 8402, which emerged after spontaneous, precise excision of the IS element in *apcA* [18]. Transcription of genes encoding core complex proteins is restored and the strain assembles functional phycobilisomes. Coincidentally, the strain regained the ability to perform CCA by synthesizing large amounts of PE under GL and repressing this synthesis under RL conditions. It was not clear whether this coincidence was due to a second mutation or whether synthesis of PE was suppressed in the original isolate by a pleiotrophic effect of the mutation in *apcA*.

In this study we analysed and compared the sequences of the *cpeBA* operon, its transcription and the PE synthesis and assembly under different light conditions in both strains. As there is currently no protocol to genetically manipulate these *Synechocystis*-type cyanobacteria, we tried to influence the physiology of strain BO 9201 using different growth conditions and inhibitors in the hope to mimic the effect of phycobilisome loss and to reproduce the CCA phenotype of strain BO 8402.

2. Materials and methods

2.1. Bacterial strains and culture conditions

For maintenance, *Synechocystis* sp. BO 8402 and BO 9201 were grown in BG11 medium containing 15 mM nitrate at low light intensity (20 μE m^{-2} s^{-1}) in a light–dark regime of 16 h:8 h or on plates containing BG11 medium solidified with 2% agar. Experimental cultures of *Synechocystis* sp. BO 8402 and BO 9201 were grown in continuous light at 30°C in Arnon's medium [19] supplemented with 15 mM nitrate. Culture vessels (Edward-Kniese, Marburg, Germany) were bubbled up with air enriched with 1.6% CO_2 (v/v). Light intensity was 130 μE m^{-2} s^{-1} under WL, 25 μE m^{-2} s^{-1} under RL and 50 μE m^{-2} s^{-1} under GL conditions. Specifications of fluorescent tubes used for RL and GL illumination were previously reported [20]. *Escherichia coli* strains HB101 and DH5α, harbouring pACYC184- and pUC57-derived plasmids, respectively, were used for cloning experiments. Plasmid containing transformants were maintained in LB medium supplemented with the appropriate antibiotics (for pACYC184-derived plasmids: chloramphenicol (25 μg ml^{-1}) or tetracycline (12 μg ml^{-1}); for pUC57-derived plasmids: ampicillin (100 μg ml^{-1})).

2.2. Isolation and characterization of phycobiliproteins

Paracrystalline PC–linker complexes from strain BO 8402 and phycobilisomes from strain BO 9201 were isolated and analysed by lithium dodecyl sulphate–polyacrylamide gel electrophoresis (LiDS–PAGE) as reported previously [14]. UV/Vis spectroscopy and phycobilin detection were essentially performed as described in [15,21]. Room temperature in vivo fluorescence of phycobiliproteins was induced at 540 ± 10 nm and emission spectra were recorded between 550 and 700 nm with a Hitachi F 2000 fluorescence spectrophotometer using a spectral correction for loss of sensitivity of the detector at high wavelengths (see [13]). The fluorescence maximum of free PE (F_{max} = 582 nm) was determined after dissociation of phycobilisomes from GL-grown *Synechocystis* sp. BO 9201 in low molarity buffer. Chlorophyll was determined in duplicate using methanol extraction [22] and protein concentration was measured using a modified Lowry method [23].

2.3. Immunodetection of P_{II} protein

A polyclonal antibody, raised against the P_{II} pro-

tein from *Synechococcus* sp. PCC 7942 and kindly provided by Dr. K. Forchhammer, University of Giessen, Germany, was used. Cell extracts were prepared and separated by native PAGE according to [24], including the following changes: cells were acclimated for 2 h under the appropriate conditions instead of only for 1 h, and the non-ionic detergent Nonidet P-40 was replaced in gel electrophoresis by IGEPAL CA 630 (Sigma). Following electrophoresis, Western blotting was performed as previously described [25]. A horseradish peroxidase-conjugated rabbit antibody (Bio-Rad) was used as second antibody, and the ECL Western blot chemiluminescence detection kit together with Hyperfilm ECL films (both Amersham) were applied for detection.

2.4. Southern blot analysis and cpeA probe preparation

For Southern hybridization analysis 6 μg of total DNA of strain BO 8402 and BO 9201, respectively, were digested with 10 units of several restriction endonucleases for 4 h and subsequently separated on a 0.8% agarose gel together with digoxigenin (DIG)-labelled marker fragments (Boehringer Mannheim, Germany). Standard procedures were used for fragment blotting onto nylon (Hybond N^+, Amersham) membranes [26]. The blots were analysed with a DIG-labelled *cpeA* probe prepared from the *Synechocystis* sp. BO 8402 gene by PCR, using 40 ng genomic DNA, 0.1 vol. of DIG-DNA labelling mixture (Boehringer Mannheim) and degenerate primers P1 and P2, previously reported for the amplification of a *cpeA* fragment from *Prochlorococcus marinus* CCMP 1375 [27]. A 231 bp *cpeA* fragment was amplified from BO 8402 DNA by 35 cycles of 1 min 52°C, 40 s 72°C and 30 s 96°C in a PTC 100 thermocycler (MJ Research, Watertown, MA, USA) and purified by agarose gel electrophoresis. Hybridization to blotted DNA was performed at 68°C according to standard protocols for high stringency [26].

2.5. Cloning of the cpe operon

50 μg of genomic DNA of BO 8402 and BO 9201, respectively, were digested using 80 units of endonuclease *Mfe*I (New England Biolabs) and separated on a 0.8% (w/v) agarose gel. Fragments of 1700–2500 bp length were excised, purified and ligated to a dephosphorylated vector pACYC184 linearized with *Eco*RI (*Mfe*I and *Eco*RI produce compatible DNA ends). Ligation products were electroporated into *E. coli* strain HB101. Colonies of transformants were analysed by colony hybridization [26] with the DIG-*cpeA* probe. Two 2112 bp *Mfe*I fragments containing *cpe* genes were cloned, one originating from BO 8402-DNA in plasmid pNRPE8 and one from BO 9201-DNA in plasmid pNRPE9. For sequence analysis, subcloning in high copy vector pUC57 was performed. Both recombinant plasmids were cleaved by the endonuclease *Dra*I (New England Biolabs), and restriction fragments were ligated to *Eco*RV-linearized and dephosphorylated high copy vector pUC57. The generated plasmids pNR92/1 respectively pNR84/1 contained a 1404 bp insertion including a 256 bp *Dra*I-*Eco*RI/*Mfe*I fragment from parental pACYC184, plasmid pNR92/4 respectively pNR84/4 a 591 bp *Dra*I-*Dra*I insertion and plasmid pNR92/2 respectively pNR84/2 a 456 bp insertion including a 83 bp *Mfe*I/*Eco*RI-*Dra*I fragment from parental pACYC184 (see Fig. 3). The sequence of *cpeBAY* from *Synechocystis* sp. BO 9201 was deposited in GenBank under accession No. AF268001.

2.6. RNA isolation, Northern and dot blot analyses

RNA was isolated as described previously [14], either from 20 ml samples collected during a time course of GL illumination, or from 100 ml samples of RL-grown cells or cells induced for 24 h under GL. The chlorophyll content of all samples was less than 10 μg ml^{-1}. For Northern blot analyses total RNA (5 or 15 μg per lane) were applied to denaturing agarose gel electrophoresis and transferred to a nylon membrane (Hybond N^+; Amersham, UK). High stringency hybridization was performed at 50°C in 'High SDS hybridization buffer' (DIG System User's Guide for Filter Hybridization, Boehringer Mannheim) and *cpe* containing transcripts were detected using the same *cpeA* probe as in Southern hybridization. Additionally, a DIG-labelled 430 bp *Sty*I fragment of the cloned *cpcB* gene from strain BO 8402 was used for detection of phycocyanin transcripts [14]. The turnover of *cpeBA* transcripts was examined after addition of rifampicin (200 μg ml^{-1}) to 24 h GL-exposed RL-grown cells.

RNA was isolated from 10 ml samples, taken 0, 5, 10, 15, 30 and 60 min after addition of the inhibitor. Using different exposure times of the blots, the approximate half-life of *cpeBA* transcripts was calculated for both strains.

2.7. Determination of cpeBA transcription start position(s)

Primer extension reactions (25 μl) were performed as previously reported [14], using 25 pmol of the 5′-biotinylated oligonucleotide pcpeBbio (5′-GCAGCAGTGCTAGAATCTGCCG-3′, complementary to the sequence +52 bp downstream from the first translated codon of *cpeB*), 75 μg of total RNA and 200 units of MMLV reverse transcriptase RNase H− (Promega).

2.8. Reverse transcriptase (RT)-PCR assays

For a more sensitive detection of *cpeBA* containing transcripts, total RNA was examined by RT reaction and subsequent PCR: 20 μl RT reactions contained 1 μg of total RNA from both strains, respectively, 10 pmol of the antisense primer p921minus (5′-CTGCGGCTACGGCAG-3′, complementary to +683 bp downstream from the first translated codon of *cpeB*, Fig. 3, primer 1), 200 units of MMLV reverse transcriptase RNase H− (Promega) and 10 units of RNase inhibitor (MBI Fermentas, Lituana). The reactions were incubated for 30 min at 37°C, products were purified by isopropanol precipitation and finally dissolved in 20 μl water. 2 μl of each RT reaction were used as template in subsequent PCR reactions (35 cycles of 30 s 52°C, 30 s 72°C and 30 s 96°C after initial denaturation for 1 min at 96°C) with the following primer pairs (location of primers relative to the first translated codon of *cpeB*): (i) p921plus (5′-CGCTGGTTAAATTACAAGTCC-3′; −46 to −25, Fig. 3, primer 2) and p921minus2 (5′-CCTTCATGATTTGCACGGCG-3′; +399 to +380, Fig. 3, primer 3); (ii) p921plus2 (5′-GGTAACTGCTATCCCAACCGTCG-3′; +204 to +226, Fig. 3, primer 4) and p921minus2; (+399 to +380); (iii) p921plus2 (+204 to +226) and p921minus (5′-CTGCGGCTACGGCAG-3′; +683 to +669, Fig. 3, primer 1).

5 μl aliquots were analysed on a 2% agarose gel. As a control for the absence of contaminating DNA the RT reactions were also performed without RT enzyme.

2.9. Sequencing of DNA

DNA cloned in the high copy vector pUC57 was sequenced using the non-radioactive GATC 1500 direct blotting electrophoresis system (GATC, Konstanz, Germany) with colorimetric detection of fragments on nylon membranes [28] as described previously [14].

3. Results

3.1. Growth, CCA and phycobiliprotein content in Synechocystis sp. strains BO 8402 and BO 9201

The growth rate of strain BO 8402 was slower in RL than in WL (approx. 0.5 day^{-1} and 1 day^{-1}, respectively) due to the lowered light intensity under RL conditions. But although GL was admitted at twice the intensity of RL, growth under GL conditions was even slower (approx. 0.3 day^{-1}). Cells grown under the different light conditions exhibited almost identical absorbance spectra (RL and GL spectra, see Fig. 1B). Strain BO 9201 grew at similar growth rates under the various light conditions (approx. 3 day^{-1}) and the cultures showed the typical colours of chromatically adapting cyanobacteria: blue–green under RL and brownish–green under GL conditions. The absorbance spectra of BO 9201 cells from RL and GL cultures are shown in Fig. 1A.

LiDS–PAGE analysis of paracrystalline PC–linker complexes isolated from RL- and GL-grown cells of strain BO 8402 (Fig. 2, lanes 8402) revealed the phycobiliprotein subunits αPC and βPC (18 and 20 kDa, respectively), a single PC rod-linker polypeptide L_R^{35PC} (35 kDa) and a special protein, L^{55} (55 kDa), which is formed by a posttranslational fusion of a βPC subunit and a rod-linker polypeptide L_R^{35PC} [14]. In GL-grown cells of BO 8402 (Fig. 2, lane 8402 GL) traces of PE (22 kDa, visible as red coloured traces in non-stained gels) and of a 33 kDa protein, the PE rod-linker polypeptide L_R^{33PE}, were found in these aggregates. The phycobilisomes of RL-grown cells of strain BO 9201 (Fig. 2, lane 9201 RL) contained

the α and β subunits of PC and AP (not resolved in Fig. 2), two PC-associated linker polypeptides, $L_R^{28.5PC}$ (28.5 kDa) and L_R^{35PC} (35 kDa), two coloured core linker polypeptides, a dominant L_{CM}^{95} (95 kDa) and traces of L_{CM}^{105} (L_{CM} species with 105 kDa), and two smaller linker polypeptides (L_R^{14PC} and L_C^{10AP}). The two L_{CM} linker polypeptides, which were absent in strain BO 8402, and faint bands (in the range of 40–70 kDa) representing L_{CM} degradation fragments exhibited similar cross-reactivity with an antibody produced against the homologous L_{CM} protein from *Nostoc* sp. ([15]; Neuschaefer-Rube, unpublished data). In addition to the polypeptides mentioned above, phycobilisomes isolated from GL-grown BO 9201 cells (Fig. 2, lane 9201 GL) contained substantial amounts of PE and the PE-associated rod-linker polypeptide L_R^{33PE}, and the two coloured L_{CM} linker polypeptide species L_{CM}^{95} and L_{CM}^{105} were present in about equal amounts (compare Fig. 2, lanes 9201 RL and GL).

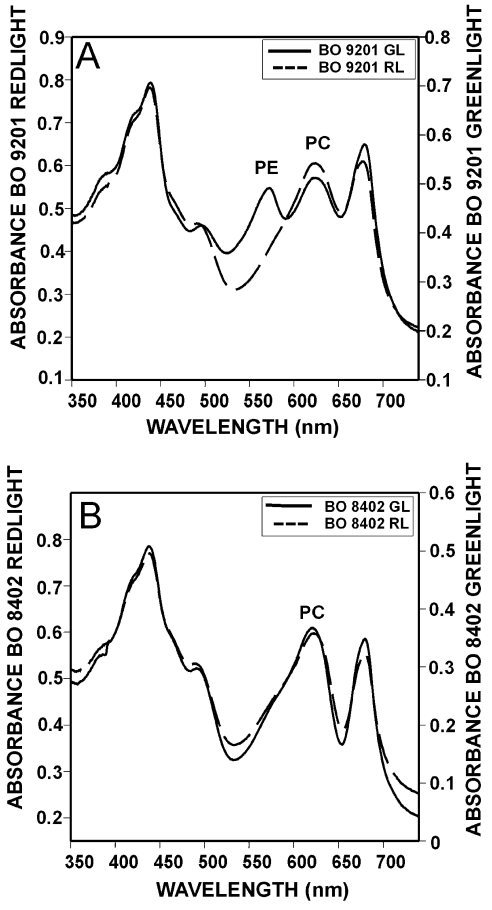

Fig. 1. Absorbance spectra of cultures of *Synechocystis* sp. BO 9201 (A) and BO 8402 (B), grown for 7 days either under red light (– – –) or green light conditions (———). Note that no PE absorbance could be observed spectroscopically in BO 8402 cells grown for 7 days under green light, while this phycobiliprotein formed a distinct peak at 570 nm in GL-grown cells of strain BO 9201.

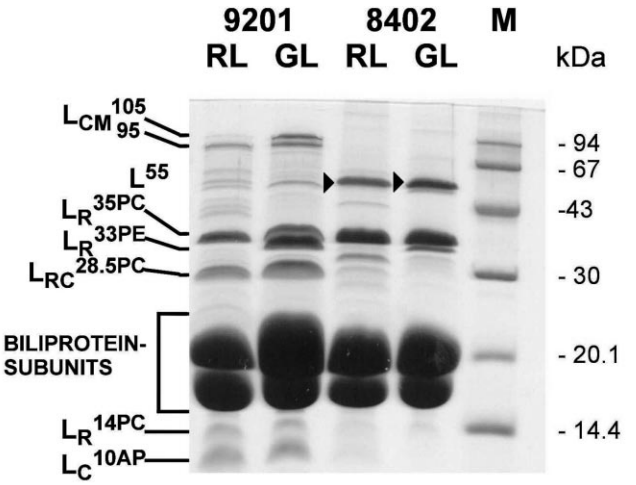

Fig. 2. 15% LiDS–PAGE analysis of phycobilisomes and paracrystalline biliprotein inclusion bodies. Phycobiliprotein–linker complexes were isolated from *Synechocystis* sp. strain BO 9201 (lanes 9201) or from *Synechocystis* sp. strain BO 8402 (lanes 8402), grown for 7 days either under red light (lanes RL) or green light conditions (lanes GL). L_x^x, phycobiliprotein linker polypeptides; L^{55}, coloured βPC-L_R^{35PC} fusion protein only found in BO 8402 biliprotein paracrystals [14]; lane M, protein molecular mass marker ranging from 14.4 kDa to 94 kDa; separated proteins were stained with Serva Blue-R-250. The different phycobiliprotein subunits between 17 and 22 kDa are not resolved as singular bands in this gel.

3.2. Characterization of the cpeBA operons

It was not obvious why the regulation of PE synthesis differed between the original isolate, strain BO 8402 defective in AP synthesis, and the revertant, strain BO 9201. We wanted to know whether the rearrangement, which led to the formation of the revertant strain BO 9201, had also affected genes required for PE synthesis. Therefore, we analysed the primary structure of the *cpeBA* operons in both strains. Southern blots of genomic DNA, restricted by the endonucleases *Eco*RI, *Eco*RV, *Hin*dIII, *Mfe*I

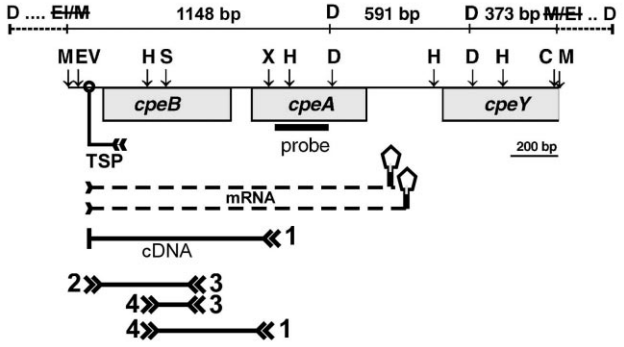

Fig. 3. Endonuclease restriction map of the 2112 bp genomic *Mfe*I fragment encoding the *cpeBA* operon of *Synechocystis* sp. strains BO 8402 and BO 9201. Location of the PCR-generated *cpeA* probe (bold line) and primers used in the determination of the transcription start position (TSP) and in RT-PCR (primers 1–4) are indicated (see Section 2). The dashed lines represent the approximate length of the proposed *cpeBA* transcripts in both strains after determination of the putative transcription start position TSP (see Fig. 4) and putative transcription terminating 3′ hairpin structures. *Dra*I fragments, subcloned for sequencing from the initially cloned genomic *Mfe*I fragments, are shown at the top. The label '*Mfe*I/*Eco*RI' represents the two ligation sites, that were formed after the cloning of the genomic *Mfe*I fragments into the single *Eco*RI restriction site of plasmid pACYC184. Concerning the resulting recombinant plasmids, this ligation led to destroyed *Eco*RI and *Mfe*I restriction sites. C, *Cla*I; D, *Dra*I; EI, *Eco*RI; EV, *Eco*RV; H, *Hin*dIII; M, *Mfe*I; S, *Sty*I; X, *Xba*I.

and *Xba*I and probed with a DIG-labelled *cpeA* fragment, revealed single, similar-sized fragments in both strains for every endonuclease examined (not shown) and none of these fragments seemed to cross-react with the *apcA* probe described in [18] (not shown). A 2112 bp genomic *Mfe*I fragment was cloned from both strains and sequenced. The sequences of the two cloned fragments were identical and both carried a typical cyanobacterial promoter sequence followed by the putative genes *cpeB* and *cpeA*, encoding the apoproteins of βPE and αPE, respectively, two putative hairpin structures (3′ of the gene *cpeA*) and the 5′-part of *cpeY*, a subunit of a proposed phycoerythrin α or β subunit lyase [29] (Fig. 3). The predicted masses for the apo-βPE and apo-αPE proteins were 19 453 Da and 17 780 Da, respectively. Calculation of the isoelectric point p*I* of both PE subunits showed almost neutral p*I* values of 7.64 for the βPE and 6.94 for the αPE subunit.

3.3. Mapping of the transcription initiation site of *cpeBA* by primer extension reaction and RT-PCR

The start site of the proposed *cpeBA* transcript was mapped using total RNA of BO 8402 and BO 9201, isolated after 24 h GL induction. A single transcription start position was found for strain BO 9201, located 46 bp upstream from the first translated methionine codon of the putative *cpeB* gene (Fig. 4, lane P_{92}). Unfortunately, the amount of *cpeBA* specific mRNA was to low in BO 8402 to detect a product with this method (Fig. 4, lane P_{84}). Therefore, RT-PCR was applied to verify the presence of similar *cpeBA* transcripts in strain BO 8402. RT reactions were performed with total RNA of both strains and with the upstream directed primer p921minus (for the location of primers, see Fig. 3). In the subsequent PCRs, three different primer combinations were used to amplify fragments containing the 5′-region of the *cpeBA* transcript. Expected fragments of 445 bp, 480 bp and 195 bp length were generated using cDNA generated from total RNA of both strains (not shown). These signals were only detected after reverse transcription proving the absence of contaminating DNA in the RNA samples. This assay showed that strain BO 8402 produced a *cpeBA* transcript with a 5′-untranslated sequence of at least 46 bp (the 5′-binding position of primer p921plus) relative to the putative translation start site of *cpeB*.

3.4. Synthesis and turnover of *cpeBA*-derived mRNA

To study the expression of the *cpeBA* operon, Northern and slot-blot hybridization experiments were performed with the DIG-labelled *cpeA* probe and, as a control, with the DIG-labelled *cpcB* probe. In semi-quantitative slot-blot assays, we used transcripts containing *cpcB* to correct for variations in mRNA loading and for changes in cellular content of phycobilisomes that may have been induced by shifts in light intensity, which occurred together with shift in light quality. In analysing mRNA from cells grown under the different conditions we addressed only changes in the ratio of *cpeA*/*cpcB* transcripts as a transcriptional response in CCA. Strains BO 8402 and BO 9201, grown for prolonged periods in RL, did not contain detectable amounts of

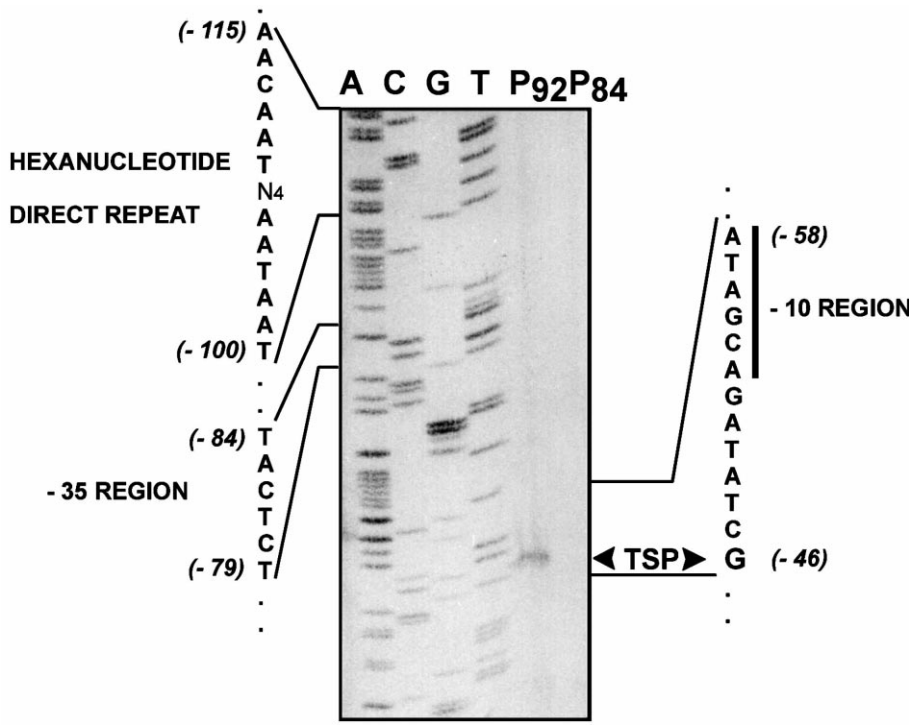

Fig. 4. Determination of the transcription initiation site of *cpeBA* transcripts. Total RNA (75 μg) of 24 h GL-illuminated cells of *Synechocystis* sp. strain BO 9201 (lane P_{92}) or *Synechocystis* sp. strain BO 8402 (lane P_{84}) was annealed to the biotinylated primer pcpeBbio and extended by MMLV reverse transcriptase (see Section 2). Lanes A, C, G and T show sequencing reactions of the sense strand of the cloned *cpeBA* of BO 9201 carried out with an identical, but non-biotinylated primer. A single transcription start position labelled as TSP, 46 nucleotides upstream from the first translated methionine codon of *cpeB*, is indicated. No labelled fragment was observed with RNA from BO 8402, due to the low amount of PE specific mRNA present in the RNA preparations. The marked sequences represent the proposed cyanobacterial −10 region, the proposed −35 region and the conserved hexanucleotide direct repeat, respectively (sense strand sequenced, compare to Fig. 10). The numbering reflects the distances to the first translated nucleotide of *cpeB*.

cpeBA transcripts. After the transfer to GL, two transcripts of approx. 1400 and 1450 nucleotides length became detectable after 1 h in strain BO 9201 and after 2 h in strain BO 8402, and reached a maximum after approx. 8 h in GL (Fig. 5A,B). The steady-state level of the *cpeBA* transcripts in strain BO 9201 was approx. 20-fold higher than in strain BO 8402. The turnover of *cpeBA* transcripts was examined after addition of rifampicin (200 μg ml^{-1}) to cultures exposed for 24 h to GL. Using different exposure times of the blots, a half-life of *cpeBA* transcripts of approx. 15 min was calculated for both strains (Fig. 5C,D). It should be mentioned here that cells transferred from GL to RL exhibited a significantly slower degradation rate of *cpeBA* transcripts (data not shown) compared to the mRNA turnover results of the rifampicin experiment, indicating that transcription of *cpeBA* was not immediately stopped after transfer from GL to RL conditions.

3.5. Detection of autofluorescent, free phycoerythrin

The strong autofluorescence of phycobiliproteins provides a very sensitive detection method for the analysis of isolated pigmented proteins. However, phycobiliproteins emit their characteristic fluorescence also in situ, if the energy transfer between phycobiliproteins or from phycobiliproteins to the photosynthetic reaction centres is disturbed inside the cell. Strain BO 8402, which lacks the core components of phycobilisomes, exhibits naturally a very strong autofluorescence at 648 nm, originating from paracrystalline PC [15] (Fig. 6A). There is no energy transfer between the excited PC–linker complexes in the paracrystals and chlorophylls of the photosys-

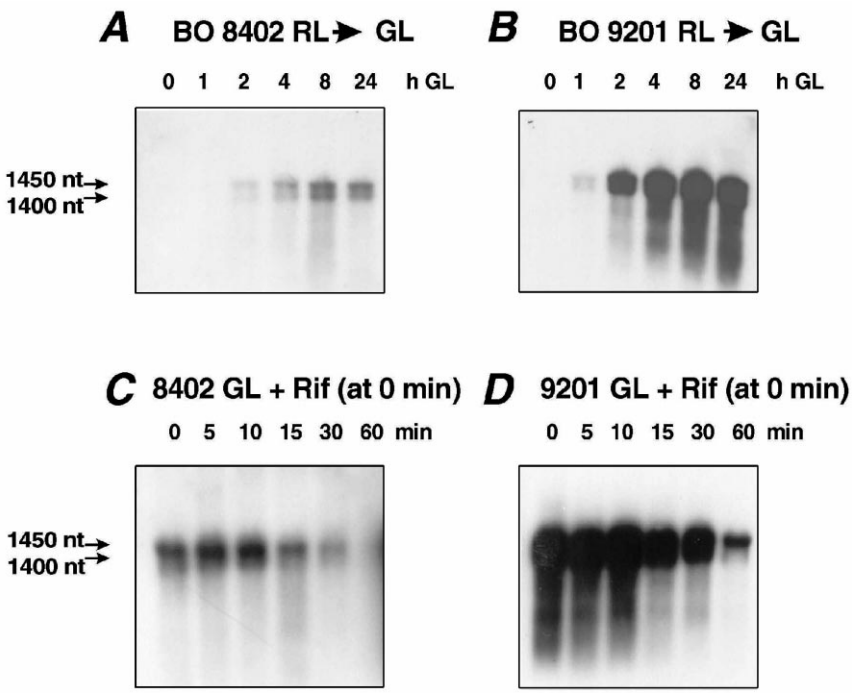

Fig. 5. Northern blot analysis of *cpeBA* mRNA from *Synechocystis* sp. BO 8402 (A,C) and *Synechocystis* sp. BO 9201 (B,D). Total RNA was separated on a 2 M formaldehyde–1.2% (wt/v)–agarose gel. Blots were hybridized with a DIG-labelled *cpeA* probe. (A,B) Induction of *cpeBA* transcription after GL illumination for the indicated time (hours) in *Synechocystis* sp. BO 8402 (A) and *Synechocystis* sp. BO 9201 (B). 15 μg of total RNA were applied per lane. Two transcripts with approx. 1400 and 1450 nucleotides length were detected for both strains. (C,D) Kinetics of *cpeBA* mRNA decay of *Synechocystis* sp. BO 8402 (C) and *Synechocystis* sp. BO 9201 (D). After addition of 200 μg ml^{-1} rifampicin (Rif) to 24 h GL-illuminated RL-grown cells RNA was purified from 10 ml samples, taken at the indicated time after rifampicin application (in minutes). 15 μg of total RNA from BO 8402 and 5 μg of total RNA from BO 9201 per lane were applied. Different scans of similar assays where used to determine the half-life of transcripts, which was at about 15 min for both strains.

tems. In BO 8402 cells, which were transferred from PE inducing GL conditions to non-inducing RL conditions, the characteristic autofluorescence of free PE (at 582 nm) became transiently apparent (Fig. 6A). However, in long-term RL-adapted cells of BO 8402, no PE protein was detectable, and GL induction experiments performed with these cells showed an approx. 3 h lag phase before a slow increase of the fluorescence signal at 582 nm became detectable (Fig. 7A, insert). In long-term GL-adapted cells of BO 8402 the fluorescence signal at 582 nm reached only about 3 times the background fluorescence value measured in RL cells (Fig. 7A), indicating the presence of only low amounts of free PE under continuous GL conditions. In contrast to the mutant strain, cells of strain BO 9201 exhibited fluorescence emission signals at 582 nm, 654 nm and 682 nm originating from uncoupled PE, whole phycobilisomes, and PSII complexes, respectively (Fig. 6B). Fluorescence of PSII complexes and the ratio of phycobilisome/PSII fluorescence changed during the light shift experiments indicating the occurrence of state transitions. In phycobilisomes isolated from RL-grown BO 9201 cells no PE proteins were detected by LiDS–PAGE (Fig. 2), but, nevertheless, a low-level fluorescence at 582 nm was still observed in whole cells, probably originating from free PE (Fig. 7B). This low-level fluorescence decreased rapidly upon transfer from RL to GL conditions (Fig. 7B, insert), but started to recover after 2.5 h and increased further to reach an about 10-fold higher value under continuous GL compared to RL conditions (Fig. 7B). Just as observed in strain BO 8402, strain BO 9201 exhibited a transient accumulation of free PE after transfer from GL to RL (visible as a rapid increase in fluorescence at 582 nm, see Fig. 6B). When chlorophyll was chosen as the reference, the autofluorescence of free PE in cells grown under pro-

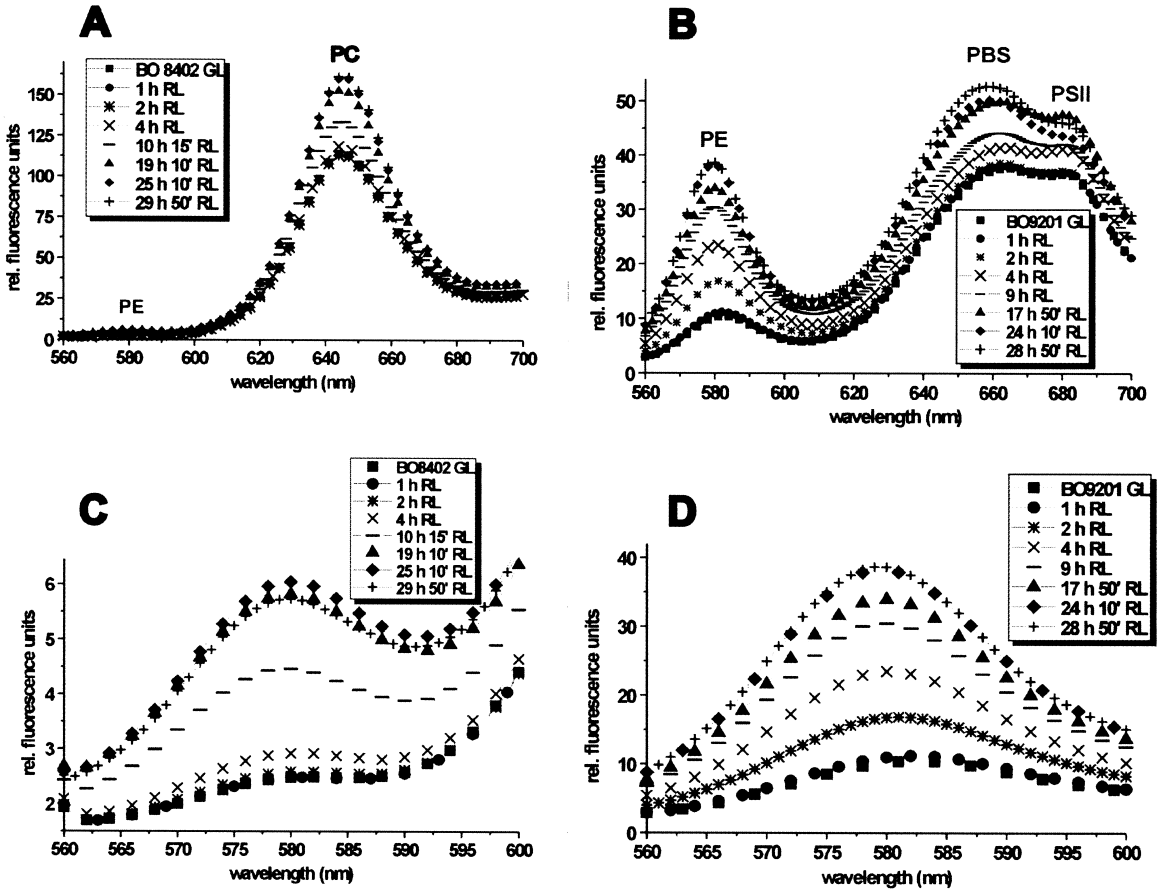

Fig. 6. Demonstration of transient changes in room temperature fluorescence spectra of whole cells of BO 8402 (A,C) and BO 9201 (B,D) after transfer from GL to RL conditions. C and D show blowups of the fluorescence emission originating from PE. Notice the large differences in arbitrary fluorescence units originating from different phycobiliproteins in both strains. Note also that the experimental conditions led to a relative increase in fluorescence at 582 nm, indicating a transient accumulation of non-assembled PE. Assembled as well as non-assembled PE disappeared upon prolonged growth under RL conditions (compare to Fig. 7). Fluorescence was induced at 540 ± 10 nm, and initial chlorophyll content was 1–2 μg ml^{-1}.

longed GL condition and in cells shifted from GL to RL (both conditions leading to a transient accumulation of free PE) was about 3 times higher in strain BO 9201 than in strain BO 8402 (compare Fig. 7A,B).

3.6. *The effects of photosynthetic electron transport inhibitors on cpeBA transcription*

The pleiotrophic effect of the insertion in *apcA* on the PE expression in strain BO 8402 may be caused by internal signals that were changed due to the absence of phycobilisomes. Strain BO 8402 appeared to be fixed in a 'RL status', in which the PE protein is absent and the PSII/PSI ratio is high [16,30]. However, despite the increased PSII/PSI ratio the intersystem chain components of the photosystems may remain in an oxidized state due to the lack of phycobilisomes. Therefore, we examined whether in the revertant strain BO 9201 an artificial oxidation of the plastoquinone pool by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of the photosynthetic electron transport, would lead to an inhibition of the GL induction of *cpeBA* transcription. DCMU was added in different concentrations to RL cultures, which were, 1 h later, transferred to GL conditions. The amount of *cpeBA* and *cpcBA* transcripts was examined by slot-blot analysis after a 6 h GL induction period. As demonstrated in Fig. 8, even high concentrations of DCMU, leading to a decline in

growth of up to 70% (as determined as the difference in chlorophyll concentration), did not prevent the GL-induced expression of *cpeBA* transcripts in BO 9201.

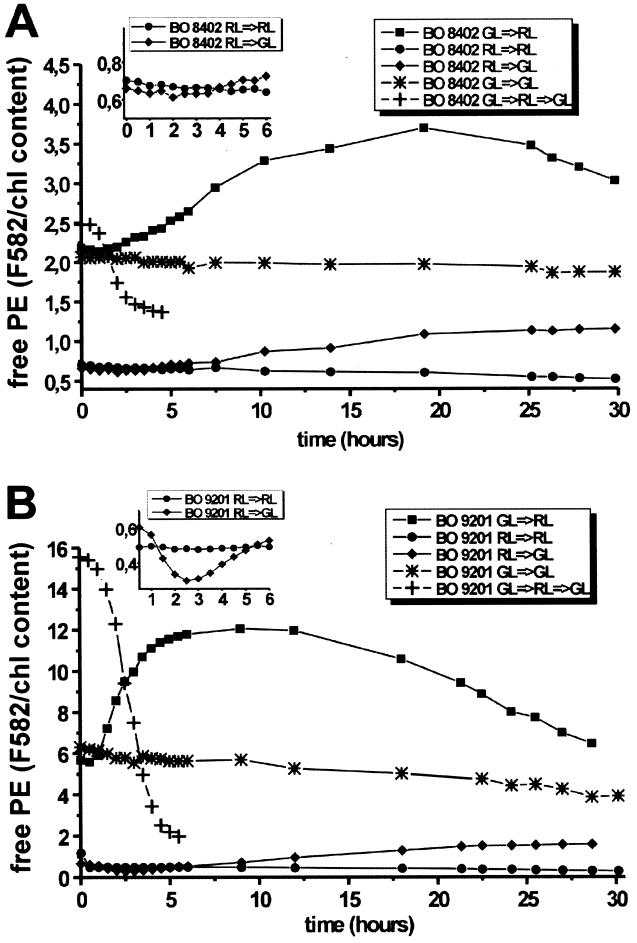

Fig. 7. Detection of autofluorescent, free PE in cells of strain BO 8402 (A) and strain BO 9201 (B) under different light conditions. Cultures were or were not (controls) exposed to shifts in light quality (RL⇔GL). Fluorescence excitation wavelength was 540±10 nm. Fluorescence emission of free PE (at 582 nm, 'F582') was calibrated using a chlorophyll-derived exponential growth function as external standard, calculated to fit the chlorophyll concentrations measured four times during the experiment (used software: Origin 5.0). (Inserts in A,B) Comparison of changes in free fluorescent PE in cells grown either continuously under RL or in cells grown under RL and shifted to GL for the indicated time (hours). Note that BO 9201 cells differed from BO 8402 cells relative to a transient decline in the fluorescence signal of free PE (reaching a minimum at about 2 h after transfer to GL) that coincided with an increase in *cpeBA* transcription in BO 9201 (compare Fig. 7B (insert)Fig. 5B).

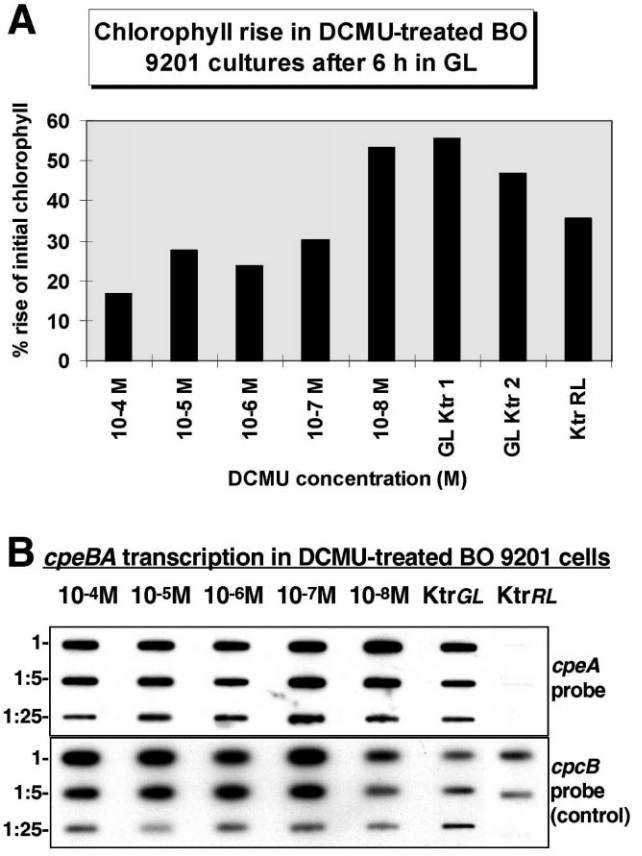

Fig. 8. Effect of DCMU treatment on cellular growth (A), shown as increase in chlorophyll concentration, and on transcription of phycobiliprotein genes (B). RL-grown cells of BO 9201 (10 ml cultures) were exposed to different concentrations of DCMU (10^{-2} M stock and successive dilutions in ethanol, applied each in a volume of 100 μl), before these were transferred to GL conditions 1 h later. Control cultures in RL or GL were treated only with ethanol. Extraction of total RNA and chlorophyll was performed after 6 h under GL. (A) Transfer from RL to GL led to an increased growth, when compared to a culture that remained under RL. Addition of 10^{-8} M DCMU did not affect this acceleration, but it was abolished by addition of higher DCMU concentrations ($\geq 10^{-7}$ M), that retarded growth up to about 70% (10^{-4} M). (B) For the slot-blot analysis of phycobiliprotein transcripts 3.2 μg RNA were applied to the first row of slots, 0.64 μg RNA to the second row (1/5 dilution) and 0.128 μg RNA to third row (1/25 dilution). Transcripts were either hybridized with a 231 bp *cpeA* probe (three top rows) or with a 430 bp *Sty*I fragment of *cpcB* (three lower rows) as loading control.

3.7. Effects of N-source on P_{II} protein phosphorylation and CCA

Lack of phycobilisomes may also distort the C:N balance in strain BO 8402 by reducing the rate of

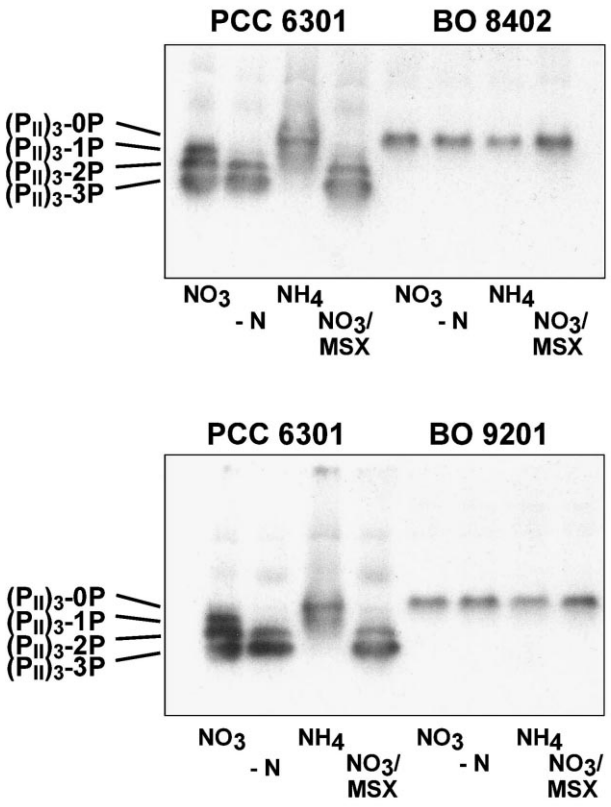

Fig. 9. Western blot analysis of trimeric P_{II} protein in cell extracts from *Synechococcus* sp. PCC 6301, *Synechocystis* sp. BO 8402 or BO 9201. Cells were grown under WL and concentrated to produce experimental cultures with an OD_{750} of 2.5. Incubation as 10 ml cultures was performed for 2 h in media supplemented with different N-sources or inhibitors (see below) in 15 ml culture vessels (Kniese) that were placed at the same WL conditions and aerated as mentioned for general experimental cultures (see Section 2). Total proteins were extracted from 2 ml samples as mentioned in [24], and 10 μl of the extracts were mixed with 10 μl loading buffer (50 mM Tris–Cl (pH 6.8), 20% (v/v) glycerol, 0.005% (w/v) bromophenol blue) and subjected to 10% PAGE under non-denaturing conditions. Applied culture conditions: NO_3, Arnon medium [19] supplemented with 15 mM NO_3; −N, cells, grown with 15 mM NO_3, were washed twice with N-free Arnon medium and further incubated in N-free medium; NH_4, N-free Arnon medium supplemented with 6 mM NH_4Cl (buffered after NH_4Cl addition with 12.5 mM TES pH 8); NO_3/MSX, addition of the glutamine synthetase inhibitor MSX (final concentration 10 μM) to NO_3 cultures to inhibit further N-assimilation.

NO_3 reduction due to limiting the amount of reductive power. However, growth on ammonia as N-source (6 mM NH_4Cl; culture buffered with 12.5 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid (TES) pH 8) did not enhance the GL-induced *cpeBA* transcription and the PE biosynthesis in strain BO 8402 (data not shown). On the other hand, two types of treatments known to cause a severe N-limitation and to induce the accumulation of glycogen in strain BO 9201, i.e. the growth in medium without bound nitrogen (in these *Synechocystis* strains the synthesis of nitrogenase is prevented by the presence of O_2, see [31]) or the addition of methionine sulphoximine (MSX; an inhibitor of NH_3 assimilation), did not lead to a repression of the GL-induced transcription of *cpeBA* in BO 9201 (data not shown).

In several non-diazotrophic and diazotrophic cyanobacteria the trimeric P_{II} protein was shown to change electrophoretic mobility under N-starvation due to its phosphorylation state [24,32,33]. This protein is proposed to be involved in the internal signalling of the C:N status of cyanobacteria [24,32,34–36]. In Fig. 9 the different phosphorylation states of the trimeric P_{II} protein in the non-diazotrophic strain *Synechococcus* sp. PCC 6301 are demonstrated by Western hybridization after native PAGE. Analysis of cell extracts of similarly treated *Synechocystis* sp. strains BO 8402 and BO 9201 showed that the P_{II} protein in the two *Synechocystis* strains did not change the electrophoretic mobility in response to the N-source or to the MSX treatment.

4. Discussion

4.1. Phycobilisome structure and acclimation type of Synechocystis sp. BO 9201

Analyses of isolated phycobilisomes of strain BO 9201 showed a constitutive expression of two PC-associated linker polypeptides, $L_{RC}^{28.5PC}$ and L_{R}^{35PC}, and a single PE-associated linker polypeptide, L_{R}^{33PE}, which is present under GL conditions only (Fig. 2). The differences in the non-coloured linker polypeptides observed under RL and GL conditions as well as the changes in phycobiliproteins and in spectral characteristics (Fig. 1A) identified *Synechocystis* sp. strain BO 9201 as a typical representative of CCA light acclimation group II, as defined by Tandeau de Marsac [6]. *Synechocystis* sp. strain BO 9201 showed a more simple structure of CCA group

II regulation than other strains examined to date. *Synechocystis* sp. strain PCC 6701, which also belongs to this acclimation group, exhibits two PE-associated rod-linker polypeptides, $L_R^{30.5PE}$ and $L_R^{31.5PE}$, that are both induced under GL [37–39], and *F. diplosiphon*, a representative of the acclimation group III, responds to GL by repression of two PC rod linkers and induction of three PE linkers [40–42]. However, in strain BO 9201 light quality not only affected the phycobilisome rod structure but also that of the phycobilisome core complex: in phycobilisome preparations of GL-grown cells two coloured core linker polypeptides, L_{CM}^{95} (95 kDa) and L_{CM}^{105} (105 kDa), were present in about equal amounts, while in phycobilisomes from RL-grown cells the linker polypeptide L_{CM}^{95} prevailed. The ratio of (L_{CM}^{95}+L_{CM}^{105}) per AP of GL phycobilisomes was almost identical to the ratio of L_{CM}^{95} per AP of RL phycobilisomes; this may indicate a special structural role of the larger L_{CM} polypeptide in phycobilisomes isolated from GL-grown cells. The two L_{CM} polypeptides showed similar cross-reaction to an anti-L_{CM} antibody prepared against the homologous *Nostoc* sp. polypeptide (data not shown), but the corresponding gene(s) (*apcE*) were not yet identified in BO 9201. In *Calothrix* sp. PCC 7601 and in *Mastigocladus laminosus*, a single *apcE* gene product is posttranslationally processed resulting in two different-sized L_{CM} polypeptides. In both cases processing of ApcE appears not to be regulated by light [43–45]. Thus, it remains to be shown whether the formation of two L_{CM} polypeptides in GL in *Synechocystis* sp. strain BO 9201 was caused by a partial inhibition of L_{CM} processing or by another mechanism.

4.2. Analysis of cpeBA promoter sequence

Strains BO 8402 and BO 9201 did not differ in the sequence of the *cpeBA* operon, in 150 bp of adjacent upstream sequence or in the downstream sequence including the 5′-region of the following open reading frame, *cpeY*. An *E. coli*-like −35 region (5′-ATGAGA-3′) and a proposed cyanobacterial −10 consensus sequence (5′-**TA**TCG**T**-3′, consensus in bold) were identified at positions −33 to −38 and −7 to −12 relative to the putative transcriptional start position. The proposed *cpeBA* promoter region was compared to that of *Calothrix* sp. PCC 7601 [8], *Pseudanabaena* sp. PCC 7409 [46] and *Synechocystis* sp. PCC 6701 [37] (Fig. 10). All sequences contained a sequence similar to the motif 5′-TTGTTA (N_4)TTGTTA-3′, centred at position −62 relative to the putative transcription start of *cpeBA*. This motive is the proposed binding site for a transcriptional activator, RcaA, which is regulated by GL-induced phosphorylation [8,47], and for PepB, a protein, which is present in both RL- and GL-grown cells of *F. diplosiphon* [48]. In our *Synechocystis* strains the tandemly arranged hexanucleotide motives contained only one deviant position (5′-TTGTTA(N_4)TTaTTA-3′). Thus, the promoter structure of *cpeBA* in the two diazotrophic *Synechocystis* spp. was very similar to that of other cyano-

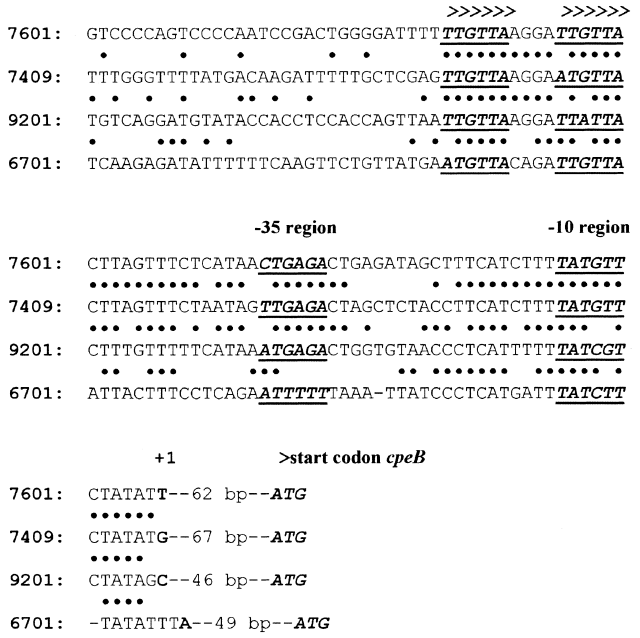

Fig. 10. Analysis of the *cpeBA* promoter sequence of the cyanobacteria *Calothrix* sp. PCC 7601 (termed 7601; accession No. X04592), *Pseudanabaena* sp. PCC 7409 (termed 7409; accession No. X63073), *Synechocystis* sp. BO 9201/BO 8402 (termed 9201; accession No. AF268001) and *Synechocystis* sp. PCC 6701 (termed 6701; accession No. M33812). The nucleotide (+1) of each sequence (only sense strand shown) represents the transcription start position TSP. Two regions with homology to the cyanobacterial −10 and −35 region, respectively, are indicated. The dashed marks highlight a conserved repeated hexanucleotide motive 5′-TTGTTA-3′ that occurs in all shown cyanobacterial sequences. Identical nucleotides between sequences are indicated by dots. Hyphens represent arbitrary shifting of nucleotides to maximize homology.

bacteria exhibiting CCA and provided no explanation for a differential expression of the *cpe* operon in the two strains.

4.3. Regulation of cpeBA transcription

BO 8402 and BO 9201 cells grown for prolonged time under RL contained no detectable *cpeBA*-derived mRNA. In both strains transcription of *cpeBA* was inducible by GL, but reached an approx. 20-fold higher steady-state transcript level in strain BO 9201 compared to strain BO 8402. This difference was shown to be due to differential transcription initiation and not to differential turnover rates of the *cpeBA* transcripts (Fig. 5). In strain BO 9201 *cpeBA* transcripts became detectable after 1 h GL illumination, while in strain BO 8402 only after 2 h under GL, possibly due to the lower growth rate of BO 8402 and/or detection problems caused by the much lower rate of transcription. As neither promoter structure nor Southern blot analysis indicated any differences in the vicinity of *cpeBA*, which could explain a difference in activator binding, the reduced GL induction of *cpeBA* in strain BO 8402 must be caused by an effect on the transcription activator itself. This activator is not known, but evidence was presented above that it could be a homologue of the phosphorylated activator RcaA. We therefore hypothesize that the interruption of the *apc* operon in strain BO 8402 results in a disturbance of the synthesis or phosphorylation of this activator. In the following we will discuss our results with respect to this possibility.

4.4. Influence of internal signals on cpeBA transcription

Our first hypothesis concerned the accumulation of free PE as the repressing element of its own biosynthesis. In strain BO 8402, PC is sequestered in large paracrystalline structures consisting of bundles of unusually long rods [15,16]. Hence, in this strain most of the PC seems to be unavailable for binding new synthesized PE–linker complexes. We examined whether due to this scarcity of acceptor sites free PE accumulated inside the cell, thereby initiating a feedback signal to suppress its own synthesis (either directly or via the proposed activator RcaA). Free PE was detected in situ by its characteristic fluorescence at 582 nm and this signal was particularly prominent a few hours after a transfer from GL to RL, the condition which led – over a longer period – to a repression of PE synthesis (Fig. 7). However, when transferred from RL to GL, thus to a condition that induced the transcription of *cpeBA*, strain BO 8402 did not accumulate free PE (Fig. 7, compare to Fig. 5). Only after a lag phase of 3 h traces of free PE became detectable. In strain BO 9201, which produced much more PE protein (see Figs. 1 and 2), free PE also accumulated slowly in GL after an initial 2.5 h lag phase; but as a remarkable difference this autofluorescence became transiently quenched during this lag phase, probably due to an immediate incorporation of free PE into existing phycobilisomes of RL-grown cells (Fig. 7B, insert). On a chlorophyll basis strain BO 9201 accumulated about 3 times more free PE than strain BO 8402, after a shift from GL to RL as well as under continuous GL (Fig. 7). The facts that strain BO 9201, which is able to synthesize large amounts of PE, accumulated more free PE under GL conditions than strain BO 8402 as well as the absence of free PE in BO 8402 after transfer from RL to GL precluded a function of free PE in the 20-fold reduction in *cpeBA* transcription in strain BO 8402.

The synthesis of phycobiliproteins is known to be affected by light and nutrient supply. This requires an integration of signals derived from light receptors and from endogenous parameters reflecting the nutrient status. Little is known about internal signalling in cyanobacteria (see [11,12]). We examined the potential of inhibitors to shift the metabolism of strain BO 9201 into a metabolic state, which may characterize that of the *apcA* mutant, strain BO 8402. We used inhibitors whose effects on the metabolism are well known, such as an inhibitor of photosynthetic electron transport (DCMU) or an inhibitor of NH_3 assimilation (MSX). DCMU, which blocks the electron transport at the reducing site of PSII and leads to an artificial oxidation of the intersystem chain, did not prevent the GL-induced expression of PE in this strain (Fig. 8). Also forcing changes in the cellular C:N ratio, either by addition of ammonia to facilitate an easier N-uptake and a decrease in the C:N ratio or by addition of MSX to hinder N-uptake and to increase the C:N ratio, did not lead to a change in

the differential regulation of the GL-induced expression of *cpeBA*.

Moreover, the trimeric P_{II} protein, which in some non-diazotrophic as well as in some diazotrophic cyanobacteria changes electrophoretic mobility upon phosphorylation, thus signalling the C:N status via the cellular concentration of 2-oxoglutarate [35,36], was not responsive in both *Synechocystis* strains (Fig. 9).

4.5. Concluding remarks

This study gave some surprising insights into CCA of cyanobacteria, of which the most surprising one, the interference of an insertion in *apcA* with the GL-activated transcription of *cpeBA*, remained disclosed. However, during this study it became clear that in our *Synechocystis* strains small amounts of free PE accumulate in GL whether phycobilisomes, which can bind PE, are present or not. This pool of free PE does not interfere with the transcription of its own genes or with the proposed transcriptional activator. A transient increase of free PE after a shift from GL to RL indicated that the regulation of the PE-linker polypeptide, which is required for assembly of PE into phycobilisomes, responds much faster to suppressing RL conditions than the synthesis of PE; furthermore, this pool of free PE seems to be the primary source of PE incorporation into phycobilisomes, once the cell is exposed to GL again. Our results further showed that the strong, but incomplete suppression of the GL activation of *cpeBA* transcription was not mediated via a more oxidized cellular redox status or a distorted C:N status. Therefore, we conclude that the lack of the functional *apc* operon in BO 8402, resulting in the loss of phycobilisomes, must have a more direct effect on the GL-stimulated activation of *cpeBA* transcription.

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