Cyclic nucleotides, the Photosynthetic Apparatus and Response to a UV-B Stress in the Cyanobacterium Synechocystis sp. PCC 6803*

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Cyclic nucleotides cAMP and cGMP are ubiquitous signaling molecules that mediate many adaptive responses in eukaryotic cells. Cyanobacteria present the peculiarity among the prokaryotes of having the two types of cyclic nucleotide. Cellular homeostasis requires both cyclases (adenylyl/guanylyl, for their synthesis) and phosphodiesterases (for their degradation). Fully segregated null mutants have been obtained for the two genes, slr2100 and sll1624, which encode putative cNMP phosphodiesterases. We present physiological evidence that the Synechocystis PCC 6803 open reading frame slr2100 could be a cGMP phosphodiesterase. In addition, we show that Slr2100, but not Sll1624, is required for the adaptation of the cells to a UV-B stress. UV-B radiation has deleterious effects for photosynthetic organisms, in particular on the photosystem II, through damaging the protein structure of the reaction center. Using biophysical and biochemical approaches, it was found that Slr2100 is involved in the signal transduction events which permit the repair of the UV-B-damaged photosystem II. This was confirmed by quantitative reverse transcriptase-PCR analyses. Altogether, the data point to an important role for cGMP in signal transduction and photoacclimation processes during a UV-B stress.

Light provides to photosynthetic organisms the energy for life and, thus, is a key environmental factor. However, it may also produce important damages when the incoming flux of excitation energy overwhelms the metabolic capacities of the cells. A variety of cellular and molecular responses exist that allow the organisms, among which are cyanobacteria, to cope with and adapt to changes in the intensity and/or spectral quality of light (1, 2). Extensive research efforts have focused on the elucidation of the molecular mechanisms that regulate acclimation and adaptation of photosynthetic organisms to high light and UV radiations. The latter, through UV-B-generated radicals in particular, have a number of negative impacts on cell physiology, damaging nucleic acids, proteins, and/or lipids (3–5). Among their effects, UV-B photons are known to cause important damages to the photosynthetic apparatus, leading to decreased oxygen evolution and CO2 fixation (6–8) and decreases in biomass production, secondary sugars, and chlorophyll content as well as to inactivation of ATPase (9).

In cyanobacteria more than 99% of the UV-B is absorbed by chlorophyll; PC, phycocyanin. Thus, a cAMP-mediated osmotic regulation strategy developed to cope with UV-B, cyanobacteria increase their de novo synthesis of the D1 and D2 subunits to repair PSII (20, 21).

To adapt to new conditions, cells must first perceive the environmental signals and then transduce them to the response apparatus so as to modify their metabolism accordingly. Second messengers such as cyclic nucleotides (cNMPs) play key roles in the transduction steps (22). Cyanobacteria are the only prokaryotes that, like eukaryotes, possess both cAMP and cGMP, their precise role being, however, poorly documented at present (23, 24). Information has been obtained from different strains, and currently only an incomplete picture of the role of cNMPs is available. For Spirulina cells, which excrete cAMP under standard growth conditions, cAMP stimulates respiration and gliding motility for Synchocystis PCC 7120 (27). In Anabaena flos-aquae, the extracellular cAMP concentration was shown to be 10-fold higher in stationary than in exponential phase (25). In Anabaena cylindrica, light-off and light-on signals modulate the intracellular cAMP concentration (26). cAMP-mediated photosignaling through phytochrome-like proteins has now been demonstrated in Anabaena PCC 7120 (27). In contrast to what happens in Anabaena cylindrica, the cellular cAMP concentration increases upon a shift from dark to light, especially blue light, in Synchocystis PCC 6803 (24, 28). We also know that in that strain, both cAMP and its receptor protein (Sycrp1) are required for motility. Mutants in either the adenyl cyclase or Sycrp1 are indeed sessile, motility for cyan mutants being recovered by the addition of exogenous cAMP (29, 30). Finally, in that same strain the cGMP concentration increases when cells are grown phototrophically and starved for nitrogen (31).

Cyclic nucleotide homeostasis requires both cyclases to achieve their synthesis from ATP or GTP and phosphodiesterases for their degradation to AMP or GMP. By complementation of Escherichia coli cya mutants, a few cyanobacterial genes coding for adenyl cyclases have been cloned (32). The recent availability of complete genome sequences for 13 cyanobacterial strains has revealed that many putative adenyl...
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cyclases may exist within a single species, with at least 6 present in Anabaena/Nostoc PCC 7120 (33) and up to 13 in Trichodesmium erythraeum.4 Both an adenylyl and a putative guanylyl cyclase have been described for Synechocystis PCC 6803 (29, 34). Although phosphodies- terase enzymatic activities have been measured for cAMP degradation in a few species (35), no orthologue of bacterial-type phosphodiesterases could, however, be recognized by in silico approaches in Synechocystis PCC 6803 (36).

Two Synechocystis PCC 6803 open reading frames (slr2100 and slr1624) carry a so-called HD (or phosphohydrolase) domain. Belonging to the HD protein family, they were proposed to be putative cNMP phosphodiesterases (36). The questions we addressed were: do these molecules regulate the intracellular level of cAMP and/or cGMP, and what is their in vivo function? To get an answer, the two genes were inactivated, and the phenotype of the corresponding mutants was studied. Under steady-state standard conditions, both grow at a rate similar to that of the wild type strain. However, the slr2100 mutant contains slightly lesser amounts of the photosynthetic apparatus components and higher amounts of cGMP and is more sensitive to a UV-B stress than the wild type. We found that it is impaired in its ability to repair the damaged PSIIIs under UV-B radiation. Altogether, the data show that cGMP could play an important role in the adaptation, regulation, and functioning of the photosynthetic apparatus in Synechocystis PCC 6803.

MATERIALS AND METHODS

Strains and Growth Conditions—Synechocystis sp. PCC 6803 was obtained from the Pasteur Culture Collection. Liquid cultures were routinely grown photoautotrophically at 30 °C under white light (30 μmol of photon·m−2·s−1), cool-white Philips fluorescent tubes in BG11 medium (37) supplemented with 10 mM NaHCO3 and buffered with 10 mM HEPES-NaOH, pH 8.0, under a 1% (v/v) CO2-enriched atmosphere in an illuminated rotary shaker (120 rpm). For growth on plates, 1% of separately autoclaved agar was added. Whenever appropriate, antibiotics (spectinomycin (30 μg·ml−1), kanamycin (75 μg·ml−1), or chloramphenicol (7 μg·ml−1)) were added to the plates.

Cyanobacterial growth was followed by recording optical densities at 750 nm. Cyanobacterial cell mass was estimated by measuring the Chla concentration of the cultures. Chla was determined in 90% acetone extracts (38) or using the Chlorophyll 1.03 program (bilbo.bio.purdue.edu/www-cyanosite/protocols/chl103.html). Whole cell spectra were recorded from 400 to 750 nm with an Aminco DW-2 spectrophotometer. The E. coli strain routinely used as host, DH5α, was grown at 37 °C in Luria broth medium with appropriate antibiotics whenever necessary (ampicillin and/or chloramphenicol at 50 or 30 μg·ml−1, respectively).

UV-B Treatment—UV-B irradiation was performed at 30 °C in open, rectangular glass containers in which 11-mm-height layers of cells were maintained in suspension by magnetic agitation. UV-B light was provided by a Vilbert-Lourmat VL-215M lamp in combination with a 0.1-mm cellulose acetate filter (Clarfoil, Courthalds Chemicals, UK), yielding an intensity of 6 μmol of photons·m−2·s−1 at the surface of the samples. Incident wavelengths range from 290 to 340 nm, with a maximum at 312 nm. Cells were used at a concentration of 6.5 μg Chl·ml−1.

Oxygen Evolution Measurements—Steady-state rates of oxygen evolution were measured using a Hansatech DW2 O2 electrode at a light intensity of 1000 μmol of photons·m−2·s−1 of photosynthetically active radiation (400–700 nm) in the presence of 0.5 mM 2,5-dimethyl-p- benzoquinone as electron acceptor. Light was provided by a Halogen-Bel-

| Oligonucleotides used for mutant constructions | 2100Fw1 | 5′-cggagtccatacgctgatc-3′ |
|-----------------------------------------------|---------|-------------------------|
| 2100-Rev1                                      | 5′-tttctgcagaattccatcgccttt-3′ |
| 1624-Fw                                        | 5′-ggacctctgcactcatcagacatca-3′ |
| 1624-Rev                                       | 5′-tttctgcagaagggctgatcactcaca-3′ |
| Del2100-Fw                                     | 5′-tttccggccatgaatgtatgactaatc-3′ |
| Del2100-Rev                                    | 5′-tttccggccatgaatgtatgactaatcaagaa-3′ |
| Flank1624-Rev                                 | 5′-tttccggccatgaatgtatgactaatcgc-3′ |
| Flank1624-Fw                                   | 5′-tttccggccatgaatgtatgactaatcaca-3′ |

Fluorescence Relaxation Decay—Flash-induced increase and subsequent decay of chlorophyll fluorescence yield was measured with the P.S.I. double-modulated fluorometer FL-100 (P.S.I., Brno, Czech Republic) and was performed in the 150 μs to 100 s range as described (19).

Plasmid Constructions—Standard cloning procedures were used according to Sambrook et al. (39). A 2680-bp DNA fragment containing the entire slr2100 (rre20) open reading frame and two flanking regions (704 bp upstream and 870 bp downstream, i.e. from nucleotides 1567613 to 1570293 in Cyanobase; www.kazusa.or.jp/cyanobase) was amplified by PCR from genomic DNA using two specific primers that were designed from the sequences available in Cyanobase (TABLE ONE). For orientation cloned into pBluescriptSK+, BambHI and PstI restriction sites were added at the 5′- and 3′-ends of the sequence, respectively. The PCR product was obtained using 0.1 μg of purified Synechocystis genomic DNA after 35 cycles of amplification with the Expand High Fidelity system (Roche Applied Science), with cycles set up as 94 °C (30 s), 63 °C (30 s), and 68 °C (3 min). The PCR fragment was digested by BambHI and PstI and cloned into pBluescript SK vector (Stratagene) digested by the same enzymes, producing pSlr2100. A similar protocol was used to construct pSlr1624, the BamHI-PstI insert (2427 bp) containing the entire slr1624 (rre18) open reading frame and two flanking regions (782 bp upstream and 640 bp downstream, i.e. from nucleotides 1320195 to 1322621 in Cyanobase).

The pΔSlr2100 derivative was constructed by substituting the entire slr2100-coding sequence with the Ω cassette (aadA gene), which confers resistance to both spectinomycin and streptomycin. The slr2100-coding region was removed by performing a reverse PCR on pSlr2100 using primers specific for the slr2100-flanking regions Del2100-Fw and Del2100-Rev and the above-described protocol. Both primers were designed to generate a XmaI site at the extremity of the PCR product site used to insert the Ω cassette (2-kilobase-long XmaI fragment from pHP45) after dephosphorylation with shrimp alkaline phosphatase.

The pΔSlr1624 derivative was constructed by substituting the entire slr1624-coding sequence with the cassette from pUC4K, which confers resistance to kanamycin. The two flanking sequences were prepared by PCR and ligated after restriction by Smal to pBluescript SK+. Then the kanamycin cassette was inserted into the Smal site.

Construction of the Synechocystis Mutants—Wild type Synechocystis PCC 6803 cells were transformed separately with plasmids pΔSlr2100 and pΔSlr1624, which do not replicate in the cyanobacterium, according to Golden et al. (40). Cells were incubated for 48 h on nitrocellulose filters (Nuclepore REC-85) without any selection, and the transformants were selected after transfer to antibiotic-containing BG11 plates.

4 J. Ochoa de Alda, personal communication.
Cells were repeatedly subcultured until full segregation of the mutation was obtained. Total segregation was ascertained by PCRs performed using the flanking region oligonucleotides.

**cGMP Determination**—Cells (100 ml) between 6 and 6.5 μg of Chl/mg were harvested by filtration under vacuum on glass fiber prefilters (Millipore: APF B04700). Filters were immediately transferred to a tube containing 10 ml of 1 mM NaH₂PO₄ buffer at pH 6.5 preheated at 100°C. After 5 min at 100°C, a known volume of the filtrate was filtered through a 0.45-μm polyvinylidene difluoride membrane (Millipore) before transfer to a Vivaspin M, 50,000 column (Vivascience) and centrifugation for 30 min at 5000 × g. An aliquot of the eluate was freeze-dried, and the lyophilisate was resuspended in 600 μl of 30 mM, pH 6.5, of NaH₂PO₄ buffer. After centrifugation for 5 min at 12,000 × g, 100 μl were injected onto a high performance liquid chromatography XTerra rp18 (3.5-μm 4.6 × 150 column, Waters) equipped with a 20-mm-long precolumn. The column was developed at a flow rate of 0.8 ml/min⁻¹ with solvent A (30 mM sodium phosphate buffer at pH 6.5) and solvent B (acetonitrile/H₂O (99:1, v/v)). Quantification was achieved by calibrating the system with precisely known quantities of the different cyclic nucleotides.

**Thylakoid Preparation and Protein Analyses**—Thylakoid membranes were prepared by breakage of the cell wall with glass beads (150–200 μm in diameter) at 4°C followed by differential centrifugations according to Komenda et al. (41). Protein composition was assessed by electrophoresis in a denaturing 12–20% linear gradient polyacrylamide gel containing 6 M urea. After solubilization (41), thylakoid extracts containing 150–200 μg of Chl were separated by the apparatus for the different genes to that for the RNAs. Membranes were incubated with an antibody raised against the C terminus of an antibody-alkaline phosphatase conjugate. The antigen–antibody complexes were visualized by colorimetric reaction using a BCP–MP–NBT system. Membranes were scanned, and the bands were quantified using the Image program (a public domain image processing and analysis program provided by the National Institutes of Health).

**Macroarray and Quantitative PCR**—Total RNAs were isolated by the hot-phenol method adapted from Mohamed and Jansson (42) and treated with DNase I (Invitrogen, 1 unit/μg of RNA) according to the manufacturer’s instructions before use. The absence of DNA products from these RNA preparations in standard PCR assays was checked to ascertain the removal of any DNA contaminants.

Probes for the macroarray analyses were prepared as follows. RNA was reverse-transcribed with a set of hexanucleotides (CGATCG, GGGCAT, CAAAAT, CAATGG, GGCAAT, AAATCC, CTTTTT, ACCAAT, GGCCAC, AAAACC, CCAGCA) kindly designed by Anne Ciof (Millipore) before transfer to a Vivaspin M, 50,000 column (Vivascience) and centrifugation for 30 min at 5000 × g. An aliquot of the eluate was freeze-dried, and the lyophilisate was resuspended in 600 μl of 30 mM, pH 6.5, of NaH₂PO₄ buffer. After centrifugation for 5 min at 12,000 × g, 100 μl were injected onto a high performance liquid chromatography XTerra rp18 (3.5-μm 4.6 × 150 column, Waters) equipped with a 20-mm-long precolumn. The column was developed at a flow rate of 0.8 ml/min⁻¹ with solvent A (30 mM sodium phosphate buffer at pH 6.5) and solvent B (acetonitrile/H₂O (99:1, v/v)). Quantification was achieved by calibrating the system with precisely known quantities of the different cyclic nucleotides.

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For quantitative PCRs, primers were designed using the LightCycler software (Roche Applied Science) so as to get products with size about 150-bp long with a Tₘ of 60°C. The cDNA synthesis was achieved in a final volume of 20 μl containing 1 μg of RNA, 2 pmol of the primers for each of the genes to be further amplified, 0.5 mM concentrations of each dNTP, 200 units of SuperScript II reverse transcriptase, and 20 units of RNasOUT in 1× manufacturer’s reaction buffer (Invitrogen). Real-time PCRs were performed according to the manufacturer’s instructions using the QuantiTect SYBR Green kit (Qiagen) and a Roche LightCycler system on 0.4 μl of the cDNA solution prepared above. Amplifications were done by incubating the reaction mixtures at 95°C for 30 s before 45 cycles of 30 s at 95°C (melting), 30 s at 60°C (annealing), and 30 s at 72°C (extension). At the end of the runs, a melting curve was generated and analyzed to ascertain that the recorded data correspond to only one PCR product of expected Tₘ. The relative abundance of each transcript was determined by comparison of the threshold cycle values (Cₜ) recorded by the apparatus for the different genes to that for the rnpB product. The ratio of expression (R) of the gene of interest (goi) to rnpB was calculated using the simple equation: \[ R_{goi/rnpB} = 2^{(C_{T_{goi}} - C_{T_{rnpB}})} \].

**RESULTS**

**Isolation and Growth Properties of the Mutants**

Targeted substitution of the sll1624 and slr2100 genes by the antibiotic resistance cassettes was achieved by transforming wild type *Synechocystis* PCC 6803 cells with the non-replicative plasmid pΔsll1624 and pΔslr2100, respectively. Full segregation was easily obtained for the two mutations and ascertained by Southern blot experiments using slr2100, sll1624, and antibiotic resistance genes as hybridizing probes (data not shown). Under standard conditions, no significant differences in terms of growth rate could be observed between the null mutants (gene deletions) and wild type strain, with doubling times ~14 h at 30°C and 30 μmol of photon·m⁻²·s⁻¹.

**Characterization of the Mutants**

**Pigment Composition**—Because of an apparent color difference between cultures, whole cell absorption spectra were recorded (Fig. 1A). The phycocyanin over chlorophyll ratio (PC/Chl) was consistently 10% lower for the slr2100 mutant (1.01 ± 0.004) compared with the sll1624 and wild type strains (1.12 ± 0.013 and 1.09 ± 0.007, respectively). No significant difference was, however, observed in the carotenoid absorbance bands under standard growth conditions, and the three strains exhibit similar rates of oxygen evolution.

**Effect of the Light Regime on Oxygen Evolution**—Transfer of wild type *Synechocystis* PCC 6803 cells from a moderate photon flux density (30 μmol of photon·m⁻²·s⁻¹) to >300 μmol of photon·m⁻²·s⁻¹ produces photoinhibition. Oxygen evolution is then reduced as a consequence of partial PSII inactivation. The two mutants behave similarly to the wild type upon a shift to 1500 μmol of photon·m⁻²·s⁻¹ (only slr2100 is shown, Fig. 1B). In contrast, differences were observed in the response to a UV-B stress. After a 2-h exposure to UV-B (at an intensity in the range found in natural environments), oxygen evolution decreased by ~29% for the wild type and ~40% for slr2100 (Fig. 2A). The sll1624 mutant, however, does not differ from the wild type (Fig. 2B).

To determine the origin of this increased sensitivity of the slr2100 mutant to UV-B radiation, the same experiment was repeated in the presence of lincomycin, a translation inhibitor. For the wild type the
The simplest explanation is that the mutation has an effect on the cas- 
de novo protein synthesis (43). Upon transfer back to medium intensity visible light (50 \( \mu \text{mol m}^{-2}\text{s}^{-1} \)) after the 2-h UV exposure, both wild type and mutant strains recover the same level of \( \text{O}_2 \) evolution (90–100% of the initial value within 1 h). PSII inactivation is, thus, fully reversible for the two strains (Fig. 2A).

**Effect of UV-B on Fluorescence Relaxation**—To ascertain the previous results, the relaxation of the fluorescence induced by a saturating flash delivered to dark-adapted cells in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea was monitored for both the wild type and \( \text{slr2100} \) strains. Recordings were made with cells sampled before the treatment, 2 h after the UV-B exposure, and after 1 h of recovery under white light conditions (Fig. 3A). As previously described (19), sigmoidal type curves are obtained when the fluorescence intensity was plotted against the log of the time. This decay was assigned to the back recombination of \( \text{Q}_A^- \) with the \( S_2 \) state of the water oxidation complex. Such experiments, thus, give information on the functional integrity of the redox components on the donor side of PSII.

The half-life for relaxation is 0.6 s for the two strains when grown under standard conditions (Fig. 3A). After 2 h of a UV-B treatment, the decay was faster for the wild type (half-life, 0.4 s), indicating that \( \text{Q}_A^- \) cannot recombine with the \( S_2 \) state in part of the centers and exchanges with redox components closer to \( \text{Q}_A^- \) like the redox-active tyrosine (Tyr-Z) (19, 44). The difference was even more pronounced for the \( \text{slr2100} \) mutant (half-life, 0.3 s). Thus, the mutation likely affects the PSII repair process that takes place during the UV-B exposure.

**D1 Contents of the Thylakoid Membrane**—The similarity in the \( \text{O}_2 \) evolution profile found between the \( \text{slr2100} \) mutant and the lincomy-
Cin-treated wild type strain (Fig. 2A) pointed to a defect in the PSII repair process. The content in mature D1 protein of the thylakoid membrane was, thus, determined by immuno-reaction with a specific antiserum. As shown in Fig. 4, there is a significant difference between the two strains, showing faster loss of the D1 protein in the mutant, which confirms an impairment of D1 turnover in slr2100.

Cyclic Nucleotide Contents and Effect of a Specific Phosphodiesterase Inhibitor—The intracellular levels of cyclic nucleotides were determined for the two strains after various periods of UV-B exposure. No significant difference was detected in the cAMP concentration in the cells kept under standard conditions (T0) as well as during the first 10 min of the UV-B treatment (Fig. 5A). In contrast, under standard conditions the steady-state level of cGMP is significantly higher in slr2100 than in the wild type strain, and it almost does not vary after the UV-B exposure (Fig. 5B). For the wild type, the cGMP concentration drops rapidly by about 40% within the first 10 min of the UV-B treatment. The UV-B stress, thus, specifically affects the intracellular cGMP concentration, and slr2100 is impaired in its ability to regulate it.

To confirm that the observed mutant phenotype could be linked to its high and not regulated level of cGMP, which may result from the lack of a phosphodiesterase activity, inhibitors were tested. Dipyridamole is known to inhibit cGMP phosphodiesterases (45). O2 evolution was monitored in wild type cells after a UV-B exposure in the presence of dipyridamole. Under these conditions cells were more sensitive than without the inhibitor (Fig. 2C). The extent of inhibition of O2 evolution observed under the UV-B stress in the presence of dipyridamole (47%) is similar to that recorded for the slr2100 mutant without inhibitor (42%). In both instances cells fully recover after white light exposure, as does the mutant.

Characterization of the slr2100 Gene

Because the slr2100 gene product can modify the response to a UV-B stress and the intracellular level of cGMP, we looked for putative regulatory sequences in front of slr2100. In Cyanobase (www.hazusa.or.jp) (46, 47), the Synechocystis PCC 6803 slr2100 sequence was annotated as an open reading frame composed of 368 amino acids, with a calculated molecular mass of 40,673 Da. No obvious ribosome binding site lies in front of the proposed ATG. The latter is nevertheless likely to be the start codon because: (i) the CheY-like phospho-acceptor domain (see below) begins immediately downstream of this ATG, and (ii) there is no other potential initiation codon preceded by a recognizable Shine-Dalgarno sequence in front of this domain. A lack of consensus ribosome

FIGURE 3. Relaxation of the variable chlorophyll fluorescence induced by a single saturating flash applied to Synechocystis PCC 6803 cells; squares, wild type strain; triangles, slr2100 mutant. A, cells before the UV-B treatment. B, after 2 h of UV-B irradiation. C, after a 1-h recovery under 50 μmol·m−2·s−1 white light. Cells at 6.5 μg of Chl·ml−1 were dark-adapted for 10 min before exposure to the saturating flash and recording of the variable fluorescence. Experiments were performed in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea, and repeated three times. The S.E. of the measurements is smaller than the size of the symbols in the figure. The curves were normalized to the same amplitude. rel.u., relative units.

FIGURE 4. D1 content of thylakoid membranes. Membranes were prepared at the given time points, and the D1 protein content was determined by immunodetection using an anti-D1 antiserum. Data are the means of three experiments. A, quantified D1 content. Circles, wild type; squares, slr2100. B, a representative immunoblot. T0 – 120, time points corresponding to the UV-B treatment; T0 – 60, time points corresponding to the recovery period under white light.
binding site has already been observed in cyanobacteria, even in front of the psbA genes, which encode the very high turnover D1 protein of photosystem II and phycobiliprotein genes (48).

Primer extension analysis was performed using a synthetic $^{32}$P end-labeled primer (nucleotides 6–26 from the slr2100 ATG) that allowed to map a stable 5’ end for the slr2100 transcripts (Fig. 6A). This potential transcriptional start site is located 22 bp upstream from the slr2100 ATG and is preceded by the sequence 5’-TTGCCC-N$_{19}$-TATACA-N$_{7}$-T-3’ (Fig. 6B). Given its sequence and the spacing between the putative −10 and −35 boxes, this promoter could be either a poor SigA-dependent or a σ class II-dependent promoter. No consensus DNA sequence known as the target for transcriptional regulators (SYCRP1 or NtcA; see for example Refs. 49 and 50) could be detected in the slr2099-2100 intergenic region. Only an imperfect match (GTAATTTTTCTCAC) to the consensus E. coli CRP binding site (TGTGA-N$_{4}$-ATCACA) can be found 280 bp downstream from the slr2100 transcriptional start site.

**Transcriptome Analysis**

Preliminary experiments were performed using macroarrays that contain PCR products for about 400 genes (~20% of the transcription units). Most of the genes chosen encode products for photosynthetic related processes and stress responses or known regulatory proteins. Representatives of the main cellular functions have also been included (see Supplemental Table 1s). Transcription profiles of slr2100 and wild type strains grown under standard conditions and after a 30-min UV-B exposure have been compared. Transcript levels were determined by measuring counts from the nylon membrane after hybridization with $^{32}$P-labeled retrotranscription products directly obtained from total RNAs without any amplification step. As could be expected, mRNA levels vary more than 4 orders of magnitude, photosynthesis-related (psa, psb, cpc, glgA, and ftrC) and ribosomal protein (rps2) genes being the most expressed. Comparing slr2100 and wild type strains adapted under standard growth conditions, changes by a factor of 2 or more

**FIGURE 5.** Evolution of the cyclic nucleotide concentrations during a UV-B stress. A. cAMP. B. cGMP. Cells were exposed to 6 μmol·m$^{-2}$·s$^{-1}$ UV-B at time 0. Data are presented as the means of two duplicates performed on two independent cultures. Solid lines and filled symbols correspond to the wild type; dashed lines and empty symbols correspond to the mutant.

**FIGURE 6.** Mapping of the stable slr2100 transcripts. A, sequencing gel with the primer extension products run along the DNA sequence performed with the oligonucleotide used to produce the extended DNA by retrotranscription. B, promoter region with relevant features (−35 and −10 boxes and translation initiation codon) in bold. The bent arrow indicates the 5’-end of the stable slr2100 mRNAs, and the dashed arrow indicates the nucleotide sequence of the oligonucleotide used for the mapping. tsp, transcriptional start site.
were observed for 30 genes. In particular, the mRNA level for *rnpB* coding for the RNA component of the RNase P (Fig. 7). This gene is classically used as an internal standard, its transcription assumed to be the less affected by the growth conditions.

As expected, because the gene was deleted, no *slr2100* transcript was found in the mutant. For 23 genes we did not detect significant differences between the wild type and *slr2100* strains grown under standard conditions (Fig. 7A). Thus, when the cells are adapted to their environment, the higher cGMP steady-state concentration present in the *slr2100* cells does not affect the transcript level for these genes. A decrease by a factor of about 2 was observed for: (i) two photosynthesis-related genes (*psbA3 cpcB*), (ii) two genes involved in PSII turnover, *phb1* (*slr1106*) and *ftsH* (*slr0228*), and (iii) a gene encoding a two-component system histidine kinase (*hik33*) proposed as a global regulator that helps coordinate cellular metabolism with growth limitations imposed by environmental conditions (51).

After 15 min of UV-B treatment, significant changes in transcript amounts were observed for 13 of the 32 genes with the wild type strain (Fig. 7B). Eight genes showed an ~2-fold or more increased mRNA level: *psbA3, psbA* (*slr0228*), *ftsH, nblA1* (small polypeptide required for phycobilisome degradation), *sigD, hliD* (a high light inducible polypeptide), and *gfb* (a glutamine synthetase inhibitor) as well as, notably, *slr2100*. Transcript levels also increased for *cya2* (guanylate cyclase), *slr2098* (*hik21*), and *slr1759* (*hik14*), which encode a two-component hybrid (sensor plus regulator) kinases. The largest decreases concern *cpcB* (phycocyanin apoprotein), *cph2* (phycocyanin-like protein), and *cpcF* (phycocyanin α-subunit phycocyanobilin lyase subunit).

**TABLE TWO**

| Gene | Function of the gene product |
|-----|-----------------------------|
| rnpB | RNA component of RNase P |
| slr1991 cya1 | Adenylate cyclase |
| stl0646 cya2 | Putative guanylate cyclase |
| stl1371 sycrp1 | cAMP receptor protein, SYCRP1 |
| slr2100 re20 | Putative phosphodiesterase, RR_HD-GYP |
| slr1164 re18 | Putative phosphodiesterase, RR_HD-GYP |
| slr1311 psbA2 | D1 protein of photosystem II |
| slr1187 psbA3 | D1 protein of photosystem II |
| slr1106 phb1 | Prohibitin 1 (PSII repair) |
| slr1768 phb2 | Prohibitin 2 (PSII repair) |
| slr0228 ftsH | Protease involved in the early stages of repair of photosystem II |
| slr0020 elpC | ATP-dependent Clp protease ATPase subunit |
| slr1157 cpcD | Apophycocyanin subunit |
| slr1151 cpcF | Phycocyanin α subunit phycocyanobilin lyase subunit |
| ssl0452 nblA1 | Polypeptide involved in phycobilisome degradation |
| slr0653 sigA | Housekeeping σ factor |
| slr0306 sigB | Group II σ factor |
| slr0212 sigD | Group II σ factor |
| slr0473 cph1 | Phychrome-like 1 (GAF_Phytochrome_His kinase) |
| slr0821 cph2 | Phychrome-like 2 (GAF_Phytochrome GGDEF_GAF_GGDEF) |
| slr1124 plpA | Required for growth under blue light ([PAS]_PAC_GAF_GAF_His kinase) |
| slr1789 hliD | High light-induced protein |
| slr2098 hik21 | Hybrid kinase ([MHTY]_PAS-PAC_His kinase (RR)_Hpt) |
| slr2099 hik40 | Hybrid kinase (RR_His kinase) |
| slr2104 hik22 | Hybrid kinase (GAF_GAF_His-Kinase_RR_Hpt) |
| slr1759 hik14 | Hybrid kinase ([PAS]_PAS-PAC2-GAF_His kinase (RR)_Hpt) |
| ssl0698 hik33 | Histidine kinase (HAMP_PAS_His kinase) |
| ssl1330 rre27 | Two-component system response regulator (RR_Treg) |
| ssl0396 rre28 | Two-component system response regulator (RR_Treg, NihB homolog) |
| slr1516 sodB | Superoxide dismutase, scavenging of O2 |
| slr1987 katG | Catalase peroxidase |
| ssl1911 gfa | Glutamine synthetase inactivating factor IF7 |
| ssl1515 gfb | Glutamine synthetase inactivating factor IF17 |

**cGMP and Adaptation to a UV-B Stress**

The transcription pattern of *slr2100* cells after the UV-B treatment clearly differs from that of the wild type for 20 of the 32 genes (Fig. 7D). Because both cAMP synthesis (achieved by *cya1*) and that of its receptor protein (SYCRP1) increase, the expression of the genes transcribed under the control of the cAMP-SYCRP1 complex likely is specifically modified in the *slr2100* mutant. The later also tends to increase protective mechanisms against damaging reactive oxygen species by producing more superoxide dismutase (SodB) and catalase peroxidase (KatG). Other important differences concern genes related to: (i) PSII repair
FIGURE 7. Histograms comparing the transcript levels determined by quantitative reverse transcription-PCR for the wild type and slr2100 strains before and after 15 min UV-B as well as the ratios for slr2100 versus wild type (wt) under standard conditions and after a 15-min UV-B exposure. The correspondence between figures and gene names is given below the figure.
DISCUSSION

Because of the presence of a HD domain and of their similarity with the cAMP-dependent phosphodiesterase RegA from *Dicyostelium discoideum*, the two open reading frames, Sll1624 and Slr2100, have been proposed as the putative phosphodiesterases needed to ensure cyclic nucleotide homeostasis in *Synechocystis* PCC 6803 cells (36). This hypothesis is supported for Slr2100 by the data obtained in this study: (i) the steady-state cGMP level under standard growth conditions is two times higher in the slr2100 null mutant than in the wild type cells, (ii) after an exposure to UV-B, the decrease in intracellular cGMP that occurs in wild type does not happen in slr2100 cells, and (iii) the increased level of slr2100 mRNAs monitored in wild type cells after the UV-B treatment correlates with the decreased concentration of cGMP observed. We have observed that the mutant cells contain more cAMP after 30 min of UV-B than the wild type (data not shown), which can be correlated with the 2-fold increase in the adenylate cyclase *cya1* mRNAs detected in the mutant compared with the wild type (Fig. 7). Cross-talks between cAMP and cGMP regulatory networks could, thus, exist.

When adapted to standard growth conditions, both the slr2100 and the wild type strains exhibit similar generation time, although the mutant contains less phycocyanin, meaning that light-harvesting is not rate-limiting. Accordingly, we detected a 2-fold lower steady-state level of *cpcD* transcripts in the mutant (Fig. 7). The expression of the five group 2 α factor genes, *sigA* (house-keeping), *sigB*, and *sigD*, was monitored. An accumulation of *sigB* transcripts has already been reported to occur under stress conditions like heat shock and high salt concentration (52), and *SigD* has been shown to contribute to the light-induced transcription of *psbA* (21), followed by translation and incorporation of new *D1* copies into the PSII reaction center complex. Although the *psbA* mRNA levels are lower in the mutant than in the wild type under standard conditions, the higher extent of UV-B induction compensates for this effect in the *slr2100* mutant under UV-B exposure. The similar and higher mRNA amounts in UV-B-stressed wild type and mutant cells show that it is not the abundance of *psbA* transcripts that limits *D1* synthesis. Therefore, the unregulated cGMP concentration observed in the mutant under the conditions of UV-B exposure should affect either the translation of *psbA* mRNA or the incorporation of newly synthesized *D1* into the PSII reaction center. Altogether, our data fit with the observation that the repair of damaged PSII differs depending on whether the inhibition was induced by UV-B or high light (17), since the difference in the repair efficiency between the *slr2100* and wild type strains only exists when cells are exposed to UV-B and not during photoinhibition by visible light.

This work points to a role for the cyclic nucleotides, more specifically cGMP, in the regulation and adaptation of the *Synechocystis* PCC 6803 photosynthetic apparatus to a UV-B stress. Even though functions such as UV-photo-protective mechanisms are common to many species, the regulatory networks by which different cyanobacteria respond to such stress seem to be achieved by different sets of proteins, or at least the domains that interact may belong to proteins that do not have the same multimodular arrangements. These differences highlight the plasticity of the ancestral cyanobacterial genome and the success of the cyanobacteria in colonizing quite different ecosystems throughout the ~3 billion years of evolution.

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