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Differential expression of antenna and core genes in *Prochlorococcus* PCC 9511 (Oxyphotobacteria) grown under a modulated light–dark cycle

Laurence Garczarek,[1] Frédéric Partensky,[1]* Horst Irlbacher,[2] Julia Holtzendorff,[2] Marcel Babin,[3] Isabelle Mary,[1] Jean Claude Thomas[4] and Wolfgang R. Hess[2]

[1]*Observatoire Océanologique de Roscoff, CNRS et Université Paris 6, Station Biologique, BP 74, F-29682 Roscoff Cedex, France.*

[2]*Humboldt-Universität zu Berlin, Institut für Biologie/ Genetik, Chausseestrasse 117, D-10115 Berlin, Germany.*

[3]*Laboratoire de Physique et Chimie Marines, Université Pierre et Marie Curie and CNRS, BP 8, 06238 Villefranche-sur-Mer Cedex, France.*

[4]*Ecole Normale Supérieure et CNRS, 46 rue d'Ulm, F-75230 Paris Cedex 05, France.*

Summary

The continuous changes in incident solar light occurring during the day oblige oxyphototrophs, such as the marine prokaryote *Prochlorococcus*, to modulate the synthesis and degradation rates of their photosynthetic components finely. How this natural phenomenon influences the diel expression of photosynthetic genes has never been studied in this ecologically important oxyphotobacterium. Here, the high light-adapted strain *Prochlorococcus* sp. PCC 9511 was grown in large-volume continuous culture under a modulated 12 h–12 h light–dark cycle mimicking the conditions found in the upper layer of equatorial oceans. The *pcbA* gene encoding the major light-harvesting complex showed strong diel variations in transcript levels with two maxima, one before the onset of illumination and the other near the end of the photoperiod. In contrast, the mRNA level of *psbA* (encoding the reaction centre II subunit D1), the monocistronic transcript of *psbD* (encoding D2) and the dicistronic transcript of *psbDC* were all tightly correlated with light irradiance, with a minimum at night and a maximum at noon. The occurrence of a second peak during the dark period for the monocistronic transcript of *psbC* (encoding one of the PS II core Chl *a* antenna proteins) suggested the involvement of post-transcriptional regulation. Differential expression of the external antenna and core genes may constitute a mechanism of regulation of the antenna size to cope with the excess photon fluxes that *Prochlorococcus* cells experience in the upper layer of oceans around midday. The 5′ ends of all transcripts were mapped, and a conserved motif, 5′-TTGATGA-3′, was identified within the putative *psbA* and *pcbA* promoters.

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Introduction

All oxyphototrophs have to deal with diel fluctuations in solar light, which profoundly influence the timing of photosynthesis and, consequently, growth processes. This timing must be precisely adapted to the ambient light level, so that photon energy can be captured and used with the highest possible efficiency, while minimizing the effects of excess excitation, which can result in severe damage to the photosynthetic apparatus. This is particularly true in the case of *Prochlorococcus*, the dominating photosynthetic organism in the intertropical areas of oceans (Chisholm *et al*., 1992). This tiny oxyphotobacterium populates the water column from surface waters exposed to extremely high irradiances down to ≈ 175 m, where it receives less than 0.1% of the incident light at the surface (for a review, see Partensky *et al*., 1999). This ability has been made possible by the occurrence in the field of populations genetically adapted to either high or low light (Moore *et al*., 1998; Urbach *et al*., 1998). Cyanobacteria and photosynthetic eukaryotes have developed different strategies to cope with strong diurnal variations in incident irradiances, including a modulation of the synthesis and degradation rates of photosynthetic components (Golden, 1994). In this context, the most extensively studied genes in cyanobacteria are those encoding the two major subunits of reaction centre II (RCII), *psbA* and *psbD*, encoding D1 and D2 respectively. Although RC proteins are highly conserved at the sequence level, three *psbA* and two *psbD* gene copies, differentially regulated by light, are present in most cyanobacteria examined to date versus only one copy of each gene per chloroplast genome in higher plants (Golden *et al*., 1986; Golden and Stearns, 1988; Bustos

and Golden, 1992). Despite the close phylogenetic relatedness of *Prochlorococcus* to marine *Synechococcus* cyanobacteria (Urbach *et al*., 1992; 1998), most *Prochlorococcus* strains have, like plants, single *psbA* and *psbD* genes (Hess *et al*., 1995; MED4 genome website at http://spider.jgi-psf.org/JGI_microbial/html/prochlorococcus_homepage.html). Differences in light-harvesting systems between these organisms are even more striking. Most cyanobacteria harvest light using phycobilisomes (PBS), which are composed of three different phycobiliprotein types, each having its specific chromophores and linkers (Gantt, 1994). In contrast, *Prochlorococcus*, as well as the other two Chl *b*-containing oxyphotobacteria known to date (*Prochloron* and *Prochlorothrix*), have developed antenna complexes that consist of either one or several (mono- or divinyl) Chl *a*/*b*-binding proteins (LaRoche *et al*., 1996; van der Staay *et al*., 1998; Garczarek *et al*., 2000). As higher plant Chl *a*/*b* proteins (CABs; Green and Durnford, 1996; Durnford *et al*., 1999), these complexes are intrinsic to the thylakoid membranes, but have six hydrophobic (i.e. putatively transmembrane) domains versus three in the CABs.

Thus, because of both its ecological significance and its photosynthetic characteristics, which are different from phycobilisome-containing cyanobacteria and green eukaryotes, *Prochlorococcus* appears to be a very interesting model for studying the regulation of photosynthetic genes by light. Several studies have shown that the growth and cell cycle of natural populations of this organism are strongly synchronized by the diel solar light cycle (e.g. Vaulot *et al*., 1995; Vaulot and Marie, 1999). In the present study, the axenic, high-light-adapted *Prochlorococcus marinus* sp. *pastoris* strain PCC 9511 (Rippka *et al*., 2000) was grown in continuous cultures and synchronized using a simulated light–dark cycle reaching a maximal irradiance of more than 900 μmol quanta m^{-2} s^{-1} (Bruyant *et al*., 2001). Such cyclostat cultures provide a particularly well-adapted means of studying light-responsive gene expression under conditions mimicking those found in the upper layer of equatorial oceans (Vaulot and Marie, 1999; Bruyant *et al*., 2001).

Results

Photosynthetic gene transcripts and gene copy numbers

The *psbA* gene produced a single monocistronic transcript of 1.35 kb in PCC 9511 (Fig. 1). This is in agreement with studies on *psbA* expression in *Prochlorococcus* MED4 (Garcia-Fernandez *et al*., 1998), a strain genetically close, if not identical, to PCC 9511 (Rippka *et al*., 2000). The *pcbA* gene, which encodes the major light-harvesting complex, yielded a monocistronic transcript of similar size (1.35 kb), providing a particularly strong signal. The transcription of

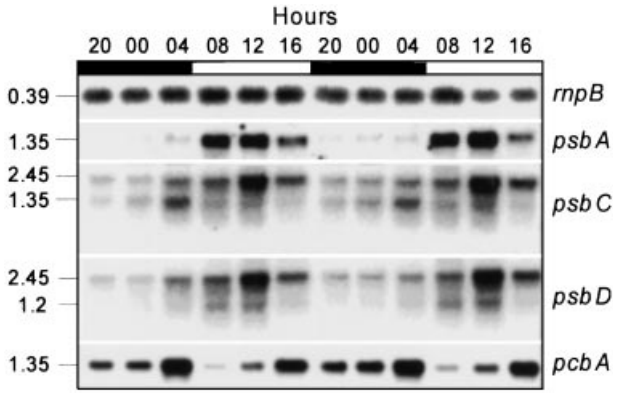

Fig. 1. Northern blot analysis of *rnpB*, *pcbA*, *psbA*, *psbC* and *psbD* mRNA levels of *Prochlorococcus* PCC 9511 grown in a simulated light–dark cycle. The transcript levels of *rnpB* were used to normalize the signals obtained with each of the individual light-regulated photosynthetic genes. Sizes of bands are in kb and were determined by comparison with ribosomal RNA bands. The light regime is presented by filled (darkness) and open (light) bars. The time scale has been shifted by 2 h with regard to the real sampling time during the workshop so that the maximum irradiance occurs at noon.

the *psbDC* operon was first studied with an RNA probe directed against the *psbC* gene. Two signals of ≈2.45 and 1.35 kb were observed (*psbC*-a and *psbC*-b respectively). Southern hybridization demonstrated that *psbC* is present in a single copy in PCC 9511 (not shown). This was confirmed further by searching the whole genome of MED4. The calculated size of the *psbDC* operon is 2451 nucleotides (nt) (Fig. 2). In MED4, genes 5′ and 3′ adjacent to this operon are located on the complementary strand. Therefore, their mRNAs cannot be part of an RNA precursor together with *psbD* and/or *psbC* (Fig. 2). Thus, we assumed that the 2.45 kb (*psbC*-a) and 1.35 kb (*psbC*-b) messages corresponded, respectively, to transcripts from the *psbDC* operon and the *psbC* locus alone. To confirm the identity of these messages, a similar experiment was performed with a *psbD*-specific probe. The rehybridization of the membrane with this probe also showed the occurrence of two transcripts, one (*psbD*-a) of identical size to *psbC*-a (2.45 kb) and the second one (*psbD*-b) slightly smaller than *psbC*-b (1.2 kb) and differently regulated by light (see below). Hence, these messages were assigned to the dicistronic *psbDC* transcript and to a monocistronic *psbD* mRNA respectively.

Effect of a light–dark cycle on growth and photosynthetic gene expression

Culturing *Prochlorococcus* PCC 9511 in a cyclostat with a modulated light regime mimicking the natural light–dark cycle (Fig. 3A) induced a strong synchronization of its cell cycle and growth (see also Bruyant *et al*., 2001; Holtzendorff *et al*., 2001). Cells entered the DNA

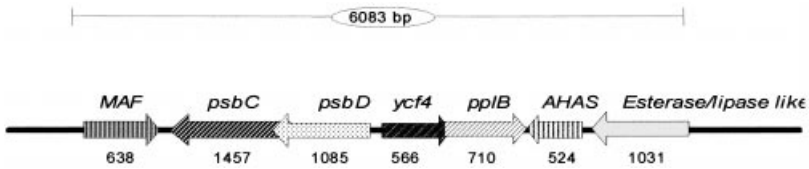

Fig. 2. Overview of a 6083 bp genomic region containing the *psbD*/*psbC* operon from MED4. Data used in this figure have been retrieved from the *Prochlorococcus* MED4 genome at http://spider.jgi-psf.org/JGI_microbial/html/prochlorococcus_homepage.html. The two genes are transcribed from the complementary strand compared with the adjacent genes and exhibit a 92 bp overlap. The lengths of the putative OFSs are indicated by numbers. *MAF*, acetyl-serotonine *N*-methyl-transferase-like gene; *pplB*, peptidyl propyl-*cis-trans*-isomerase B; *AHAS*, acetolactate synthase small subunit.

synthesis phase in the afternoon and divided during the first part of the night, as shown by sharp variations in the percentage of cells in the G1 phase of the cell cycle, i.e. non-dividing cells (Fig. 3B).

The expression of all photosynthetic genes examined in this study revealed strong diel oscillations. The transcript levels of the *psbA* and *psbD* (i.e. *psbD*-b) genes as well as of the *psbDC* operon (as seen from the independent measurements of *psbD*-a and *psbC*-a mRNA levels; see above) were strongly correlated with irradiance. The amounts of all these transcripts were very low at night and peaked in the middle of the light period, when the irradiance reached its maximum value. However, expression of *psbA* and *psbD* differed slightly. The former gene was expressed only during the light period, whereas expression of the latter gene already began in the late part of the night, i.e. before the onset of light. The behaviour of *psbC* differed from that of *psbD* as, on three of the 4 days that the study lasted, it showed an additional peak of expression before light onset. The amplitude of this peak was similar or slightly higher than the midday peak (Figs 1 and 3). As it was not possible to withdraw the large volumes needed for RNA extractions from the cyclostat more than six times per day, no sample was taken at the dark to light transition. Therefore, it is not possible to say with certainty whether the dark maximum of expression was actually located 2 h before light onset (i.e. at the time we sampled) or at the end of the dark period.

An even more surprising result was obtained for the *pcbA* gene, encoding the major antenna polypeptide of *Prochlorococcus* PCC 9511. Northern blotting revealed two peaks within one diel cycle in the expression of this gene. A first peak was observed 2 h before the dark to light transition, followed by a sharp decrease at the beginning of the light period. Then, transcript levels increased monotonously during the remaining part of the light period and gave rise to a second maximum 2 h before the light to dark transition. This pattern was highly reproducible during the 4 days that the experiment lasted (Fig. 3).

Comparative promoter mapping

To obtain information about the promoters and actual transcription initiation sites of the *psbA*, *pcbA* and *psbDC* transcripts, primer extension mapping was performed (Fig. 4). For each mRNA, two separate reactions with different sets of primers were realized in order to verify the obtained data. Furthermore, all extension experiments were repeated twice for each primer, and similar results were obtained. The sequencing ladders and the respective products of primer extension were electrophoresed alongside each other. The results for one experimental series with one primer per gene are displayed in Fig. 4A. The second primer set confirmed these data (not shown). The results clearly indicated the occurrence of single promoters for *psbA* and *pcbA* with transcription initiation sites at −63/64 and −22 respectively. For *psbD*, a strong signal was detected with both primers at −27/28. Primer psbDpe2 gave two further signals at −62/63 and −45 (not shown). However, this might result from artificial binding of primer psbDpe2 to another transcript because its last 10 nucleotides in 3′ have a perfect match at four more sites within the genome. The primer extension experiment for *psbC* provided signals arbitrarily spread over a long sequence stretch or very weak bands. We interpret this as evidence of the absence of a promoter directly upstream of *psbC*. There are no data about promoter architecture in *Prochlorococcus* yet, and we cannot rigorously exclude the possibility that some extension products might represent mRNA 5′ ends produced secondarily by degradation or maturation. However, direct comparison of sequences preceding the mapped mRNA 5′ ends shows some remarkable features: the two most highly expressed genes, *pcbA* and *psbA*, have in common the presence of a heptanucleotide 5′-TTGATGA-3′ upstream of the putative −10 element (Fig. 4B). Furthermore, 49 nt upstream of the *pcbA* transcription initiation site, there is a sequence motif 5′-TTAATAACCTTTACAA-3′ that has high similarity to a recently identified *cis* element involved in photoregulation in *Synechocystis* 6803 (Eriksson *et al*., 2000).

Discussion

When compared with phycobilisome-containing cyanobacteria, the photosynthetic apparatus of *Prochlorococcus*

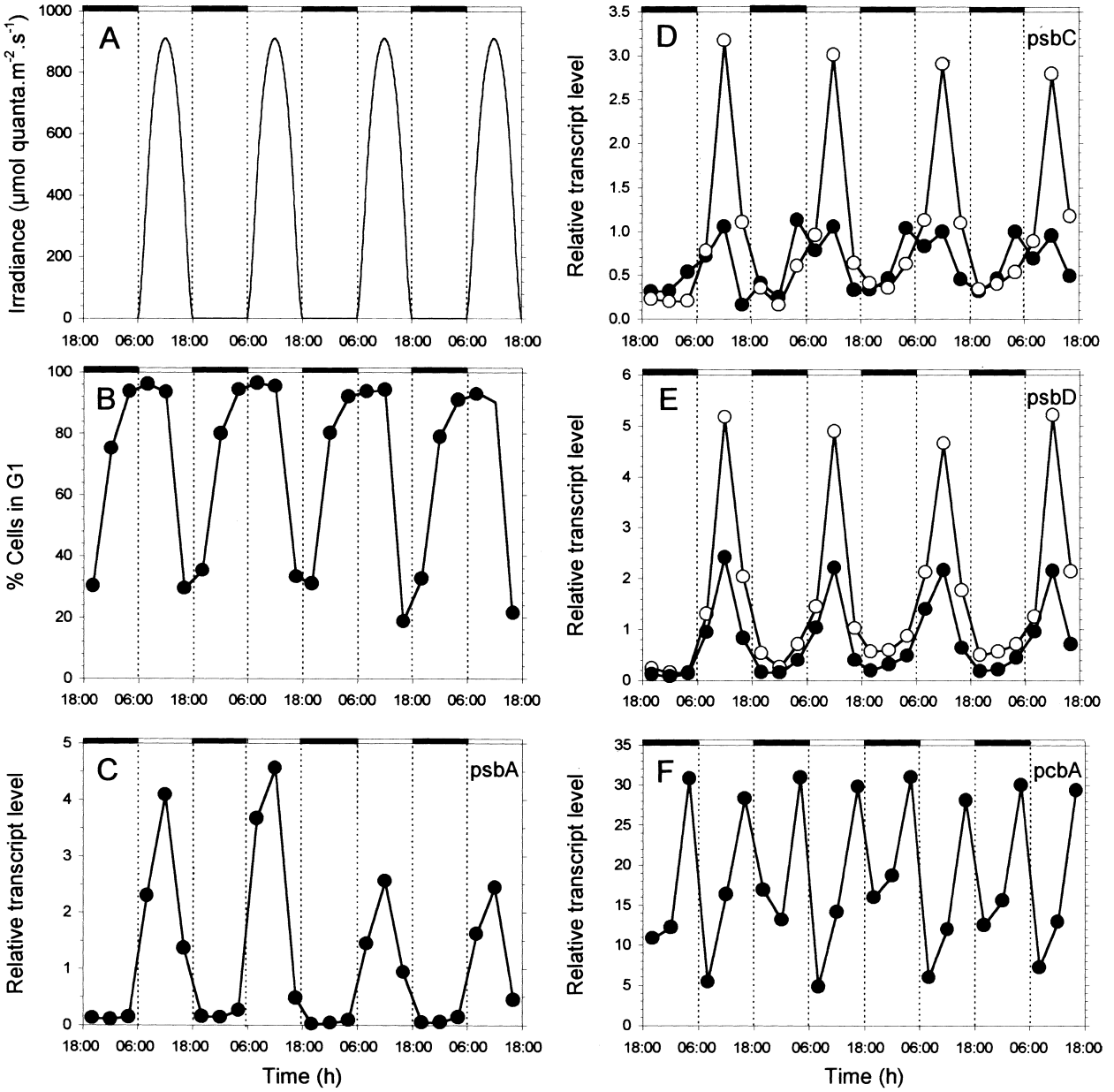

Fig. 3. Effect of a light–dark cycle on the synchronization of photosynthetic gene expression.
A. Pattern of modulated light used during the experiment.
B. Percentage of *Prochlorococcus* cells in G1 phase of the cell cycle (interphase).
C–F. Transcript pattern of *psbA*, *psbC*, *psbD* and *pcbA*. The apparently lower height of the peaks of *psbA* transcripts during the last two light dark cycles is probably an artifact resulting from unequal transfer of the RNA onto the Northern blot membrane (an independent membrane was used for the other genes).

is atypical from many viewpoints (for a review, see Partensky *et al*., 1999). Hence, it is not surprising to observe some specificities at the gene expression level as well. The first particularity concerns the expression of genes coding for the reaction centre II protein D2 (*psbD*) and PS II core antenna CP43 (*psbC*). Although *Prochlorococcus* possesses a single copy of these genes, three different transcripts have been detected: a dicistronic 2.45 kb transcript corresponding to the *psbDC* operon and two others that are individual transcripts of *psbC* and *psbD* with sizes of 1.35 and 1.2 kb respectively. By comparison, the *psbDI/C* operon of most typical cyanobacteria, such as *Synechococcus* PCC7942, gives rise to only one message (≈2.5 kbp), whereas the isolated *psbDII* gene provides a second transcript of ≈1.2 kb (Colon-Lopez and Sherman, 1998). The occurrence of an additional copy of *psbD* would provide an alternative source of this transcript when an overtranscription of D2

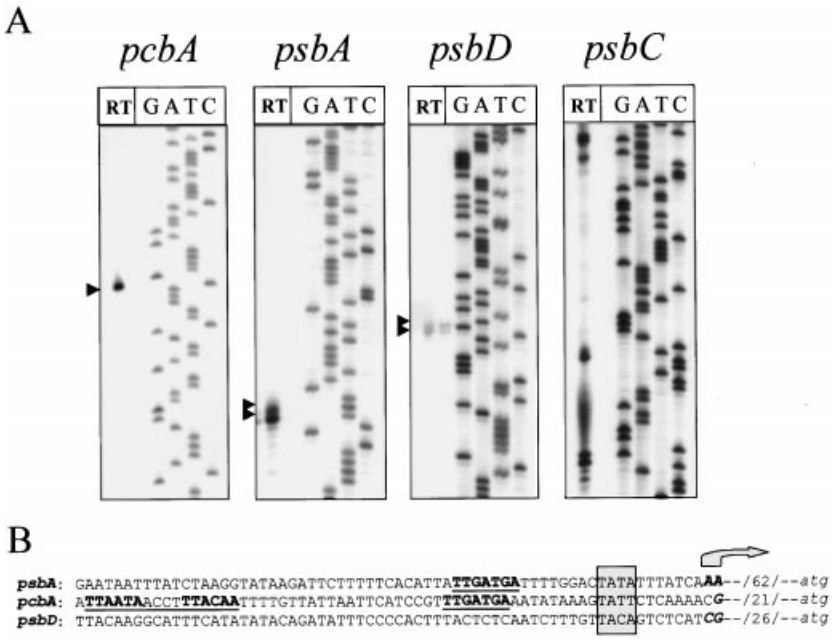

Fig. 4. Primer extension analysis of photosynthetic genes in *Prochlorococcus* PCC9511.
A. For each gene to be analysed, total RNA (15 μg for *psbA and pcbA*, and 20 μg for *psbC and psbD*) was annealed to two oligonucleotides each and extended with 200 U of Superscript II reverse transcriptase (RT). Here, the extensions with primers pcbApe1, psbam1r, psbDpe1 and psbCpe1 are shown. In the reaction for *psbD*, products of the same primer extension reaction were loaded in two adjacent lanes. Lanes G, A, T and C contain a dideoxy sequencing ladder carried out separately with the same primer. Extension products are indicated by arrowheads.
B. Alignment of putative promoter regions of *Prochlorococcus* photosynthetic genes. The mapped transcript start points are labelled by the arrow, and the respective nucleotides are italicized. The distance to the respective start codon is given in nucleotides. Putative −10 regions are boxed. A heptanucleotide motif common to the *psbA* and *pcbA* promoter is in boldface and underlined. A sequence stretch with similarity to the *Synechocystis* photoregulatory *cis* element (Eriksson *et al*., 2000) is also underlined, and the two partially repeated sequences within this element are in boldface.

with regard to CP43 is required, e.g. to compensate for the deleterious effect of high light or UV-B light (Virgin *et al*., 1988; Bustos and Golden, 1992; Jansen *et al*., 1993; Golden, 1994). The two genes within the dicistronic *psbDC* operon overlap in all cyanobacteria investigated so far (Golden *et al*., 1989), and *Prochlorococcus* does not escape this rule. This implies that the maturation of a given dicistronic precursor into monocistronic transcripts provides either a complete *psbC* mRNA or a complete *psbD* mRNA, but not both (Fig. 5). Primer extension experiments indicated that the *psbDC* operon is apparently under the control of one promoter upstream of *psbD*, but that *psbC* seemingly has no specific promoter. Thus, the differential behaviour of the monocistronic *psbD* and *psbC* transcript levels (Fig. 3) appears to result from temporal variations in the post-transcriptional maturation of *psbDC* mRNA precursors (Fig. 5). At the end of the dark period, the major part of the dicistronic transcript pool is rapidly matured into *psbC*. In contrast, during the day, about equal parts of the dicistronic pool mature into monocistronic *psbC* and *psbD*, as indicated by similar values, over the entire light period, of the ratios of *psbC-b* to *psbC*-a and of *psbD*-b to *psbD*-a transcript levels (not shown). Such a specific post-transcriptional regulation of *psbD* and *psbC* gene expression in *Prochlorococcus* PCC 9511 might counterbalance the expected lower photoacclimation capacity resulting from the absence of a second copy of *psbD* in this strain. Several other photosynthetic organisms have evolved complex regulation mechanisms of the *psbDC* operon too. In barley, for instance, 12 different mRNAs, at least four different promoters, multiple RNA processing and transcription termination events have been identified (Christopher *et al*., 1992; Christopher and Mullet, 1994). In chloroplasts of *Chlamydomonas*, several proteins are known to bind specifically to the 5′ untranslated region of *psbD*, thereby regulating the stability and translation of this mRNA (Ossenbuhl and Nickelsen, 2000). It is conceivable that analogous mechanisms act to regulate the different post-transcriptional fate of *psbC* and *psbD* in *Prochlorococcus*.

According to the strength of their hybridization signals, *psbA* and *pcbA* are probably among the most strongly transcribed protein-coding genes in *Prochlorococcus*. Thus, the finding of common promoter elements (Fig. 4B) was expected, although information on their true functional relevance will require further experiments including genetic manipulation of *Prochlorococcus*, which has not yet been mastered. The variations in *psbA* transcript abundance over time are almost perfectly superimposable on the diel irradiance curve. This suggests that *psbA* expression is strongly regulated either directly by light or by an internal clock, facilitating the synchronization of all cellular processes. Induction of *psbA* expression by light has been studied extensively in cyanobacteria (Liu *et al*., 1995; Sato *et al*., 1996; Johnson and Golden, 1999). In *Synechococcus* PCC7942, the high turnover of the D1 protein plays an important role in the

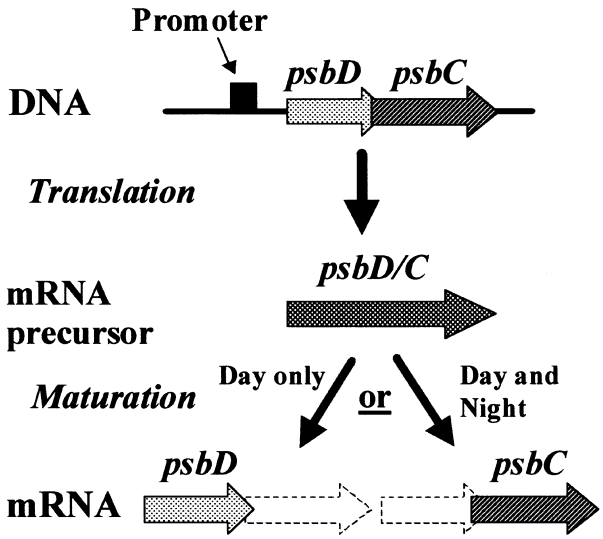

Fig. 5. Model for the expression of the *psbDC* operon in *Prochlorococcus* PCC9511.

protection of the photosynthetic apparatus against excess excitation. This photoprotective mechanism seems to imply the rapid activation by high light of the *psbAII/III* genes (coding for the form 2 of the D1 protein, D1:2) and the repression of the *psbAI* (coding for D1:1). In *Prochlorococcus* MED4, a light shift from 8 to 55 μmol quanta m^{-2} s^{-1} blue light induced a rapid increase in the mRNA level of the single *psbA* gene, followed by a gradual return to the initial level (Garcia-Fernandez *et al.*, 1998). Thus, this gene appears to be regulated like the *psbAII*/*psbAIII* genes rather than like *psbAI* from *Synechococcus* PCC 7942. Interestingly, the product of the former genes (D1:2) is 25% more photochemically efficient than D1:1 (Clarke *et al.*, 1993). Although the occurrence of a single *psbA* copy in *Prochlorococcus* could appear as a disadvantage, D1 turnover might not be as high in *Prochlorococcus* as it is in other cyanobacteria. Indeed, the occurrence of an intrinsic light-harvesting system in *Prochlorococcus* instead of the extrinsic phycobilisomes found in most cyanobacteria might result in a lower photon flux arriving at the level of RCs. The rapid turnover of RC proteins in cyanobacteria caused by excitation stress has been replaced in the intrinsic light-harvesting system of eukaryotes by more subtle mechanisms including antenna quenching (Öquist *et al.*, 1995). In much the same way, *Prochlorococcus* could have developed a less energetically costly acclimation mechanism to cope with excess excitation than is found in cyanobacteria.

The transcript accumulation of *Prochlorococcus pcbA* with two peaks within one diel cycle, a low expression when the light intensity was maximum and a high level of expression before and at the end of the light period appears to be fairly unique among photosynthetic organisms. In higher plants raised under a light–dark cycle, expression patterns are very similar for all individual *Lhc* genes: transcript levels increase after the transition from darkness to light, reach a maximum around solar noon and decrease thereafter. Only the amplitude of variation in mRNA levels varies significantly between the different *Lhc* genes (Piechulla, 1993). In the case of *Prochlorococcus*, the decrease in *pcbA* expression at the beginning of the dark period is probably linked to cell division, as indicated by cell cycle data (Fig. 3B). In fact, the expression of all other photosynthetic genes examined was at a minimum during the division processes. In contrast, the decrease in *pcbA* transcript levels during the light period could result from an inhibition of *pcbA* expression in order to regulate the antenna size and limit photoinhibitory effects. Confirmation of the putative role of modulation of antenna transcript abundance on the reduction of photodamage to the RC awaits study of the regulation of antenna complexes at the translational level.

Experimental procedures

Strain and culture conditions

The characteristics of the axenic strain *Prochlorococcus* PCC 9511 used in this study have been described recently (Rippka *et al.*, 2000). Cells were grown in PCR-S11 medium (Rippka *et al.*, 2000) at 21°C in continuous culture, as detailed elsewhere (Bruyant *et al.*, 2001). Briefly, the turbidostat culture set-up consisted of two replicate 20 l Nalgene jerry cans continuously fed with fresh medium and illuminated by a double bank of white, dimmable neon tubes providing a 12 h−12 h light–dark cycle with a progressive irradiance variation from 0 to 912 μmol quanta m^{-2} s^{-1}. Both replicate cultures were sampled for flow cytometric counting and cell cycle analyses, and these parameters showed very similar behaviour (Bruyant *et al.*, 2001). Samples of RNA used in the present study were pumped out of one of the two turbidostats. Samples (800 ml) were collected axenically at 4 h intervals for four consecutive days. Immediately after sampling, cells were harvested by centrifugation for 7 min at 4°C and 18 500 *g* with a Beckman Avanti J25 centrifuge equipped with a 6 × 500 ml JLA 10.5 rotor. Samples were then quickly resuspended in RNA buffer (10 mM sodium acetate, pH 4.5, 200 mM sucrose, 5 mM EDTA) on ice, flash frozen in liquid nitrogen and stored at −80°C until extraction, which was performed within a few days after the experiment. During dark periods, manipulation of cells was performed either in complete darkness or using a low-intensity green light, which is not absorbed by *Prochlorococcus* cells (Morel *et al.*, 1993).

RNA analysis and DNA manipulations

Total RNA was extracted as described previously (Hess *et al.*, 1995). Each RNA sample (4 μg $lane^{-1}$) was separated on a 1.2% (w/v) agarose gel and blotted onto a nylon membrane

(Gene Screen Plus; NEM Research Products). Radioactive DNA probes for *psbA* (encoding D1) and *rnpB* (encoding RNase P RNA) were prepared from gel-purified DNA fragments by random priming with a commercial kit (Ready-to-Go; Pharmacia Biotech). For *psbC*, *psbD* and *pcbA*, ^{32}P-UTP-labelled specific RNA probes were synthesized as described by Garczarek *et al*. (2000). Hybridization and membrane washing were performed as described previously (Hess *et al*., 1995) at 61.5°C for RNA probes and at 45°C for DNA probes. All hybridizations were performed on a single Northern blot, stripped and reprobed, except for the *psbA* gene, which was hybridized on a separate membrane. Detection and quantification of the transcript levels were performed using a Storm phosphorimager (Molecular Dynamics), and the signal obtained with *rnpB*, a strongly expressed housekeeping gene (Hess *et al*., 1998) whose expression is not light regulated, was used as an internal standard to normalize the relative transcript levels.

In order to generate sequencing templates for primer extension, we amplified by polymerase chain reaction (PCR) the genomic region 5′ of the respective start codon individually for *pcbA* and *psbA*. A single template was produced for *psbDC*. Each of these PCR products contained several hundred nucleotides 5′ of the respective gene or operon. After cloning into plasmid pGEMT and sequence verification, these constructs served to determine the exact mRNA 5′ ends using the ThermoSequenase kit 2.0 (Amersham). All oligonucleotide primers were selected according to data obtained from the MED4 genome website. Their sequences are as follows. For *psbA*: pcbAf, 5′-CCTGCATGA GCGACATGAGCAGCG-3′; pcbAr, 5′-GATCTGATTTGCGA TAAGACTTGGCAGC-3′; for *psbA*: psbAf, 5′-CAAGCCGCT GCTGCAAGTAGGC-3′; psbAr, 5′-CCTGTCTTTATTGGTG AGGTCGCC-3′; for *psbDC*: psbD/Cf, 5′-GCCAGAGTGTGC AATGTGTGCTCC-3′; pcbD/Cr, 5′-GGTTGAAGGATTACCT AAAGGGAGC-3′. Labeling, purification and extension of primers using Superscript II (BRL) reverse transcriptase was carried out as described previously (Vogel and Hess, 2001). For each of the genes to be investigated, two different primers were used in two independent reactions. For *pcbA*: pcbApe1, 5′-GCTACACCTGAATTACCAGCCC-3′; and pcbApe2, 5′-GG ATTACCATAGGTTTGCATTTAGG-3′; for *psbA*: psbAm2r, 5′-GTTACCCACTCACAAAACTGTGGCC-3′; and psbAm1r, 5′-C GCTGCTGCTGAATAGTTGTCATGAG-3′; for *psbD*: psbDpe1, 5′-GTCGCGCTTTAACCAATCATCAAGG-3′; and psbDpe2, 5′-GCTTCCAACGGCGATCGTCATGAG-3′; for *psbC*: psbCpe1, 5′-CGTACCAAGCGTAACCAGTTTCTTC-3′; and psbCpe2, 5′-GGCGTTTCCACGCGGAAGAACC-3′.

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