Expression of the psbA Gene in the Marine Oxyphotobacteria Prochlorococcus spp’

Jose M. Garcia-Fernandez,*†,2 Wolfgang R. Hess,‡ Jean Houmard,‡ and Frédéric Partensky*

*Observatoire Océanologique de Roscoff, CNRS et Université Paris 6, Station Biologique BP 74, F-29682 Roscoff Cedex, France; †Institut für Biologie, Humboldt Universität zu Berlin, Chausseestrasse 117, D-10115 Berlin, Germany; and ‡Physiologie Microbiennne, CNRS, URA 1129, Département de Biochimie et Génétique Moléculaire, Institut Pasteur, 28 rue du Dr. Roux, F-75724 Paris Cedex 15, France

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The oxygenic photosynthetic prokaryotes Prochlorococcus marinas SS120(CCMP1375) and Prochlorococcus sp. MED4 (CCMP 1378) were previously shown to exhibit different pigmentation and ecophysiological characteristics. The former strain has a much lower divinyl-Chl a to b ratio and is adapted to lower photon flux densities than the latter. In contrast to the cyanobacteria examined so far, both strains possess only one copy of the psbA gene, encoding the D1 protein of photosystem II core. In acclimated steady-state cultures, psbA transcript levels were always higher at high irradiances in both strains. Upon a shift from low to high light, the psbA transcript levels increased in both strains but more quickly in MED4 than in SS120. They decreased during the opposite shift. Iron-starved MED4 cells overexpressed psbA at all assayed irradiances, suggesting that this species, representative of populations from naturally iron-depleted oceanic areas, may have developed a particular compensation mechanism. The similar effects of DCMU and DBMIB on the expression of psbA suggest that light regulation of psbA in Prochlorococcus may be mediated by the electron transport chain. The energy state of cells could, however, also be involved in this regulation, since cultures of both strains subjected to darkness showed psbA levels significantly lower when glucose was added.

Key Words: cyanobacteria; iron starvation; light regulation; photosystem; protein D1.

Prochlorococcus is an ubiquitous, free-living marine prokaryote of major ecological importance (1-5). Its photosynthetic apparatus shows, however, several noticeable differences with regard to that of cyanobacteria: It contains divinyl- but lacks monovinyl-Chl a; in addition, two minor proteins of photosystem I, PsaF and PsaL, are about one-third longer than usual (6). The occurrence in Prochlorococcus of a Chl a/b-binding protein as the major light-harvesting complex, while in typical cyanobacteria this function is performed by phycobilisomes, is a feature common to only two other prokaryotes, Prochlorothrix and Prochloron (7), and to chloroplasts (8). Molecular studies have, however, indicated that these so-called “prochlorophytes” are not directly related to chloroplast ancestors (7, 9-11).

A large genetic variability has recently been demonstrated among Prochlorococcus isolates, leading to their classification into at least two major clusters (12, 13). The two most studied Prochlorococcus strains, MED4 and SS120, isolated from near surface and deep (120 m) oceanic waters, respectively, are representatives of these different genetic clusters. They are also distinguishable by their respective divinyl-Chl a to b ratio, which is up to 10-fold lower in the latter strain at a given growth n-radiance (14, 15). This characteristic was shown to be due to large differences in the light-harvesting antenna complexes of these strains at both the genetic and regulational levels (7, 16). In addition, a functional operon encoding the α and β subunits of phycoerythrin has been found in SS120, but is not present in MED4 (12).
In contrast to cyanobacteria that may contain multiple copies of the \textit{psbA} gene, sometimes encoding different isoforms of \textit{D1}, the two \textit{Prochlorococcus} strains possess a single copy of this gene (11, 17), as chloroplasts do (18-21). The regulation by light intensity of \textit{psbA} expression has been well studied in \textit{Synechococcus} sp. \textit{PCC} 7942, which possesses three \textit{psbA} copies and two forms of \textit{D1}. Under low irradiance (LL), \textit{psbA} transcripts are predominant, \textit{D1:1} being the only detectable form of \textit{D1}. A shift to high irradiance (HL) provokes a rapid and transient induction of \textit{psbAII} and III and the active degradation of \textit{psbAII} transcripts, the \textit{D1:2} protein being integrated into the thylakoid membrane (20, 22, 23, 24). It has been proposed that the preferential expression of \textit{psbAII} and III at HL is due to the higher intrinsic resistance to photoinhibition (25) and higher photochemical efficiency of reaction centers containing \textit{D1:2} (22). The molecular mechanism(s) mediating light regulation of \textit{psbA} expression has not yet been clearly elucidated, but electron transport, a specific photoreceptor, the differential stability of the mRNAs resulting from the binding of effectors, endonucleolytic cleavages at specific sites into the \textit{psbA} mRNAs, and the degradation of the protein \textit{D1} having been implicated (26-32).

In the present paper, we report on the expression of the single \textit{psbA} gene in \textit{Prochlorococcus} sp. MED4 and \textit{Prochlorococcus marinus} SS120, which are adapted (in the evolutionary sense) to different growth irradiances (14, 15). The aim of this work was to study the regulation by light of their single \textit{psbA} gene and whether it might differ between the two strains. The effects of light shifts, electron transport inhibitors, and iron starvation on transcripts levels were analyzed with \textit{Prochlorococcus} cultures grown under continuous light.

**MATERIALS AND METHODS**

**Strains and growth conditions.** The two clonal \textit{Prochlorococcus} strains used in this work, MED4 (CCMP 1378) and SS120 (CCMP 1375), are available at the Culture Collection of Marine Phytoplankton (Bigelow, ME). Their origin has been described previously (2, 15). Cultures were grown at 21°C in a medium described in Ref. (12), in polycarbonate Nalgene flasks under continuous blue light and \textit{D1} (20, 22, 23, 24). Under low irradiance (LL), \textit{psbA} transcripts are predominant, \textit{D1:1} being the only detectable form of \textit{D1}. A shift to high irradiance (HL) provokes a rapid and transient induction of \textit{psbAII} and III and the active degradation of \textit{psbAII} transcripts, the \textit{D1:2} protein being integrated into the thylakoid membrane (20, 22, 23, 24). It has been proposed that the preferential expression of \textit{psbAII} and III at HL is due to the higher intrinsic resistance to photoinhibition (25) and higher photochemical efficiency of reaction centers containing \textit{D1:2} (22). The molecular mechanism(s) mediating light regulation of \textit{psbA} expression has not yet been clearly elucidated, but electron transport, a specific photoreceptor, the differential stability of the mRNAs resulting from the binding of effectors, endonucleolytic cleavages at specific sites into the \textit{psbA} mRNAs, and the degradation of the protein \textit{D1} having been implicated (26-32).

**DNA and RNA isolation.** DNA was isolated with a method modified from Ref. (33). Cells from 2-L cultures were collected by centrifugation, frozen in liquid nitrogen, and ground in a mortar. After addition of 20 mL of SET buffer (25% sucrose, 50 mM Tris-HCl, pH 7.5, 1 mM EDTA) and 5 mL 0.5 M EDTA, the extract was incubated with 50 mg of lysozyme for 1 h at 37°C; then it was incubated with 2.5 mg of proteinase K and 2.5% SDS (w/v) overnight at 45°C. The mixture was extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1) and once with chloroform/isoamyl alcohol (24:1) and precipitated with 1 volume of isopropanol. The resulting DNA was taken up by TE buffer, precipitated overnight with 0.1 volumes of 3 M sodium acetate and 2.5 volumes of ethanol, and dissolved in an appropriate volume of TE.

**For RNA isolation, cells from 1-L cultures were collected using an Avanti J25 Beckman centrifuge (total collection time, 20 min), and the final cell pellet was resuspended in 0.5 mL of 10 mM sodium acetate, pH 4.5, 200 mM sucrose and 5 mM EDTA, frozen in liquid nitrogen, and kept at -80°C until used. When taken from cultures growing under darkness, samples were maintained in the dark between centrifugations until they were frozen. One volume of 8 M guanidine-HCl, 20 mM 4-morpholineethanesulfonic acid, 20 mM EDTA, and 50 mM β-mercaptoethanol was added to samples. They were then frozen in liquid nitrogen, ground in a mortar, extracted twice with chloroform/isoamyl alcohol (25:24:1) and once with chloroform/isoamyl alcohol (24:1), and precipitated overnight with 1 volume of ethanol and 150 μL of 1 M acetic acid. The precipitated RNA was dissolved in DEPC-treated water, quantified, and precipitated to get a final concentration of 1 μg μL-1.

**Amplification of rrnA gene.** A fragment of the \textit{rrnA} gene, encoding for the 16S ribosomal RNA, was amplified by PCR using the primers PM16F (5'-ATCTGCCCCTAAGGACGGGAT-3') and PM16R (5'-CTACGATCTGAACTGACGGCAGGGT'T-3') with the following protocol: 2 min at 93°C and then 35 cycles of 1 min at 93°C, 1.5 min at 60°C, and 1.5 min at 72°C.

**RNA blotting.** RNA samples were electrophoresed in denaturing formaldehyde agarose gels and hybridized successively, after stripping with 32P-labeled \textit{psbA} and \textit{rrnA} gene probes using standard methods (34). Alternatively, the nonradioactive Gene Images system (Amersham) was used to label the probes with fluorescein, as described by the manufacturer. The \textit{psbA} probe was prepared from a \textit{PT7T318u} plasmid containing a \textit{psbA} Eco RI insert of 1.3 kb from the SS120 strain (11). The \textit{rrnA} probe was prepared directly from the PCR-amplified fragment as indicated above.

Blots were digitized using the BOP-Biocom image analysis system, and the bands were quantified using the Leefhor software (both from Biocom, Les Ulis, France). Levels of \textit{rrnA} were used as an internal standard to ensure appropriate quantification. Values at the beginning of the experiment or Time 0 were arbitrarily taken as 100%. Equivalent amounts of total rRNAs were loaded in each well.

**RESULTS**

**Effect of Light Intensity on Steady-State \textit{psbA} Levels**

\textit{Prochlorococcus} MED4 was acclimated at 8, 30, and 80 μmol photons m-2 s-1 continuous light and SS120 at the two lower irradiances only, because this strain hardly stands such high photon fluxes (15). The expression of \textit{psbA} was then quantified from RNA blots (Fig. 1). Expression levels of 16S rRNA were chosen as control for quantitation, a method that has been widely used in the literature (24, 26, 35). Quantitation of 16S rRNA levels allowed to take into account differences in the RNA extraction or transfer procedures that could occur between samples. In addition, although the content of rRNAs per cell might vary with cell cycle or growth conditions, there must be very large changes to significantly bias quantitations made by ratioing mRNA to rRNA levels.
An experiment was designed in order to examine the possible effects of iron depletion on the transcription level of the \textit{psbA} gene and how this is affected by light. \textit{Prochlorococcus} MED4 cells were acclimated to different irradiance levels as previously and then transferred three times into an iron-free medium, in order to deplete them in this metal. Iron starvation was confirmed by adding iron (up to its standard concentration in the culture medium) to a subsample of the iron-free culture; this treatment allowed a fast (within 24 h) recovery of the growth rate of the \textit{Prochlorococcus} population (not shown). Iron depletion provoked a further increase in \textit{psbA} transcript levels at all three studied irradiances compared to the levels in iron-enriched cultures (Fig. 1).

\textbf{Time Course of \textit{psbA} Transcript Levels after Light Shifts}

Dynamic changes in transcript levels were analyzed during a 24-h (or up to 38 h) time course in \textit{Prochlorococcus} cells subjected to light shifts. When MED4 cultures acclimated to 8 \(\mu\)mol photons m\(^{-2}\)s\(^{-1}\) were transferred to 15 \(\mu\)mol photons m\(^{-2}\)s\(^{-1}\), \textit{psbA} transcript levels did not change significantly (not shown). However, shifting from 8 to 55 \(\mu\)mol photons m\(^{-2}\)s\(^{-1}\) (Fig. 2A) provoked a biphasic response: a fast increase in \textit{psbA} expression (a more than 100\% increase 1 h after shifting), which gradually returned to its initial level. The SS120 strain showed a different behavior (Fig. 2A). The shift from 8 to 30 \(\mu\)mol photons m\(^{-2}\)s\(^{-1}\) produced an initial burst in \textit{psbA} transcript of lower amplitude than for MED4, but after 5 h a marked and regular increase in \textit{psbA} transcripts was detected. In the SS120 strain, \textit{psbA} transcript levels remained high even after 30 h.

The short term effect of the opposite shift (HL to LL, Fig. 2B) was to transiently decrease the \textit{psbA} transcript levels (25\%) in both strains. A strain difference was also noticed then since with MED4, the \textit{psbA} mRNA level plateaued, while, for the SS120 strain, an about 30\% higher concentration of \textit{psbA} transcripts was detected at the end of the 24-h period. Minimum values were measured after about 2 h.

\textbf{Effect of Iron Starvation on Steady-State \textit{psbA} Levels}

In microalgae such as \textit{Dunaliella tertiolecta} and \textit{Phaeodactylum tricornutum}, it is known that iron starvation provokes a loss of D1 proteins, which is rapidly compensated once iron is resupplied (36). Iron is one of the major nutrients implicated in the limitation of primary productivity in oceanic areas, such as the equatorial Pacific (37, 38). However, \textit{Prochlorococcus} proliferates in such iron-depleted environments at rates up to one division per day (39), a value close to its optimal growth rate in culture. Moreover, it has been shown that effects of iron limitation on the cellular characteristics (size and pigment content) of this organism in the field are low, compared to those on larger cells such as diatoms (40). Thus one can wonder how \textit{Prochlorococcus} can cope with iron starvation and whether the regulation of genes such as \textit{psbA} is affected, as it is in other organisms (36).
absence of glucose added to exponentially growing cultures (Fig. 3A) as well as the dynamic changes of these levels after shifting to darkness (Fig. 3B). MED4 and SS120 cultures were subjected for 72 h to continuous darkness or darkness + glucose (Fig. 3A). After acclimation to those conditions, psbA transcript levels in cultures under darkness + glucose were significantly lower (four- to fivefold) in both strains, when compared with the culture under darkness alone. Figure 3B shows the dynamic changes obtained with the SS120 strain after shifting to darkness. It induced first an increase (100% after 8 h) in psbA transcript levels, but after 24 h, they were 30% lower than in the control illuminated culture. Addition of glucose to a subsample of the culture grown under darkness produced a similar effect on psbA levels, with a higher initial increase (more than 100% after 4 h), followed by a sharper decrease after 24 h (less than 50% of the control).

In a second set of experiments, we studied the effect of two electron transport inhibitors: 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), a reducer of the plastoquinone pool, and 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), an inhibitor of the oxidation of plastoquinol (Fig. 4). For MED4, addition of either DCMU or DBMIB induced a decrease in the levels of psbA transcripts (40% compared to the control). A decrease in mRNA levels was also observed with SS120, the effect of DBMIB being more pronounced than that for MED4. The addition of the reductant dithioeryth-

![FIG. 2. Time course of psbA transcript levels in Prochlorococcus MED4 (●) and SS120 (■) cultures after the following light shifts: (A) from LL to HL (i.e., from 8 to 55 µmol photons m^{-2} s^{-1} for MED4 and from 8 to 30 µmol photons m^{-2} s^{-1} for SS120). (B) From HL to LL (i.e., from 80 to 8 µmol photons m^{-2} s^{-1} for MED4 and from 30 to 8 µmol photons m^{-2} s^{-1} for SS120).](image)

![FIG. 3. Effect of darkness and glucose on psbA transcript levels. (A) Steady-state psbA transcript levels in MED4 and SS120 cultures after 72 h subjected to darkness (shaded bars) or darkness + 12 mM glucose (dashed bars). (B) Time course of psbA transcript levels after transferring a SS120 culture from 30 µmol photons m^{-2} s^{-1} to darkness or darkness + 12 mmol glucose. ■, darkness; □, darkness + glucose.](image)

![FIG. 4. Effect of DCMU and DBMIB on psbA transcript levels, measured 24 h after the addition of the inhibitor. Three aliquots of a MED4 and SS120 culture growing at 30 µmol photons m^{-2} s^{-1} were either spiked at Time 0 with 0.06 µM DCMU (dashed bars) or 0.3 µM DBMIB (squared bars) or allowed to grow without inhibitors (control, shaded bars).](image)
ritol (DTE) did not affect the psbA transcript levels (not shown).

DISCUSSION

psbA Regulation after Light Shifts

Our results show that the psbA gene expression is regulated by light in Prochlorococcus MED4 and SS120 strains, steady-state transcript levels being higher at high irradiance. This is consistent with previous reports for different phycobilisome-containing cyanobacteria (22-24, 35, 41-43). In Synechococcus PCC 7942, psbA regulation has been shown of importance in protecting the photosynthetic machinery from the deleterious effects of photoinhibition, Dl turnover increasing with light irradiance, and the D1:2 isofrom replacing D1l (19-21). However, the presence of only one psbA copy in Prochlorococcus SS120 and MED4 (11,17), and consequently only one form of the Dl protein, makes those strains less adaptable to light shifts than cyanobacteria possessing several psbA copies and two forms of Dl (18-21).

Considering the main importance of psbA regulation to protect the photosynthetic machinery from the effects of photoinhibition in cyanobacteria (19–21), it was expected that an organism containing a single psbA copy would increase its expression under high irradiance. Although the sequence of the psbA gene from the SS120 strain is closer to that of psbAI than psbAII/III of Synechococcus (11), it is regulated more like the latter genes, i.e., the psbA mRNA level increases after shifts from LL to HL (18, 20). No similarity was found, however, between the Prochlorococcus sequences upstream of the psbA coding region and that of Synechococcus in which the light-regulatory features have been recognized (32, 44). So if the psbAI-like gene of Prochlorococcus derived from the psbAI form of an ancestral cyanobacterium, its upstream region must have changed during evolution for this gene to become regulated in a psbAII/III-like way.

The reduction of the number of psbA copies in Prochlorococcus is in agreement with the idea that this organism evolved from cyanobacteria mainly by reducing the size of its genome (11, 12), photosynthetic apparatus (12), and ultimately cell size (45). This evolution may have allowed this organism to colonize oceanic habitats where nutrients are limiting (1, 3, 4).

Despite large variations in the expression of psbA in cultures shifted from low to high irradiance (Figs. 1 and 2), steady-state levels of the Dl protein itself showed little or no variation with regards to the total pool of photosynthetic proteins in Prochlorococcus cultures grown at different irradiances (16). Increased transcription of psbA thus compensates the increase in Dl turnover that occurs under light-saturating conditions (21). It is noteworthy that for Prochlorococcus SS120 the growth rate begins to be inhibited at 37 μmol photons m⁻²s⁻¹ white light (no more growth at 140 μmol photons m⁻²s⁻¹) and for MED4 at 450 μmol photons m⁻²s⁻¹ (15), while for the marine cyanobacterium Synechococcus WH7803 high growth rates are observed up to 2000 μmol photons m⁻²s⁻¹ (46). Hence, Prochlorococcus seems to be better adapted to grow at lower irradiances than Synechococcus.

After LL to HL shifts, although the initial short-term responses for psbA transcription are comparable for both strains, the long-term effect is quite different: in MED4 a return to the initial transcript levels was found, while in SS120 they remained high. The different habitats from where these strains were isolated may explain these different behaviors. The MED4 strain originates from near the surface, i.e., an environment (the mixed layer) exhibiting much more pronounced light changes than the depth from which the SS120 was isolated, i.e., near the deep chlorophyll maximum (15). Thus the MED4 could be expected to be better acclimated to rapid changes in light than SS120, which is often long to recover or even dies after strong shifts (47).

Effect of Iron Starvation

Iron is necessary to form the heme groups of cytochrome and ferredoxin that, in turn, are required to dissipate energy from the reaction centers of the photosystems I and II. Depending on the organism, iron starvation has been shown to provoke the replacement of ferredoxin by flavodoxin (48), a reduction in the number of phycobilisomes (49), a reduction in the number of thylakoids (50), and an imbalance in the composition of PSII, with 35% reduction of the content of Dl, CP43, and CP47 (51). Iron also affects the photosynthetic efficiency of phytoplankton in the field (52, 53). The overexpression of psbA promoted by iron starvation in Prochlorococcus cultures in the exponential phase (Fig. 1) could reflect a compensating system to react against the effects of iron stress. Burnap et al. (54) also observed an overexpression of psbA transcripts in Synechococcus PCC 7942 under iron starvation. Our results reinforce the role of iron limitation as a factor with a large impact in the regulation of the photosynthetic apparatus, although in the specific case of Prochlorococcus compensation mechanisms must exist since fairly high concentrations of cells can be found in iron-depleted areas (3).

Study of the Mechanism Controlling psbA Expression

The mechanism(s) mediating the regulation of psbA expression by light in cyanobacteria is subjected to some controversy. While the necessity of a specific photoreceptor has been proposed (27), Campbell et al. (26)
showed that electron transport regulates exchange of D1 isoforms in Synechococcus PPC 7942. Our results (Fig. 4) suggest that the electron transport chain could also be involved in the regulation of the psbA expression in Prochlorococcus. Blocking of electron transport with DCMU and DBMIB induced a decrease of psbA levels with DCMU and DBMIB induced a decrease of psbA that have a similar effect on two antagonist inhibitors such as DCMU and DBMIB regulation, but likely not the plastoquinone pool, since levels in barley (55).

Addition of glucose to cultures subjected to continuous darkness provoked a decrease in psbA transcript levels (Fig. 4). This might suggest that Prochlorococcus may take up and use glucose as a source of carbon and energy. In that case, cultures growing under darkness + glucose could switch their metabolism to heterotrophy and the need for a quick D1 protein turnover would disappear, leading to the observed decrease in psbA expression. The possibility of heterotrophy in Prochlorococcus sp. would be an important metabolic feature that has to be further investigated.

REFERENCES

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