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# Redox Control of *psbA* Gene Expression in the Cyanobacterium *Synechocystis* PCC 6803. Involvement of the Cytochrome $b_6/f$ Complex

**Miguel Alfonso[1,2], Irène Perewoska, and Diana Kirilovsky***

Unité Mixte de Recherche 8543, Centre National de la Recherche Scientifique, "Photorégulation et Dynamique des Membranes Végétales," Ecole Normale Supérieure, 46 rue d'Ulm, 75230 Paris cedex 05, France

**We investigated the role of the redox state of the photosynthetic and respiratory electron transport chains on the regulation of *psbA* expression in *Synechocystis* PCC 6803. Different means to modify the redox state of the electron carriers were used: (a) dark to oxidize the whole electron transport chain; (b) a shift from dark to light to induce its reduction; (c) the chemical interruption of the electron flow at different points to change the redox state of specific electron carriers; and (d) the presence of glucose to maintain a high reducing power in darkness. We show that changes in the redox state of the intersystem electron transport chain induce modifications of *psbA* transcript production and *psbA* mRNA stability. Reduction of the intersystem electron carriers activates *psbA* transcription and destabilizes the mRNA, while their oxidation induces a decrease in transcription and a stabilization of the transcript. Furthermore, our data suggest that the redox state of one of the electron carriers between the plastoquinone pool and photosystem I influences not only the expression of the *psbA* gene, but also that of other two photosynthetic genes, *psaE* and *cpcBA*. As a working hypothesis, we propose that the occupancy of the $Q_0$ site in the cytochrome $b_6/f$ complex may be involved in this regulation.**

Light is a major regulator of the expression and synthesis of many genes and proteins related to photosynthesis (for reviews, see Rochaix, 1992; Tandeau de Marsac and Houmard, 1993; Mayfield et al., 1995). Tight regulation of these genes allows photosynthetic organisms to adapt to changes in light quality and intensity. The *psbA* gene that encodes the D1 protein is one of these light-regulated genes. The D1 protein, together with the D2 subunit, provides the protein backbone in which several cofactors such as chlorophyll (Chl) or quinones are attached, forming the photosystem II (PSII) reaction center. This membrane protein complex catalyzes the light-driven reduction of plastoquinone (PQ) with electrons derived from water, producing oxygen as a side product. In addition, D1 is continuously damaged and replaced during illumination (Mattoo et al., 1984; Ohad et al., 1984). The well-known rapid and light-intensity-dependent turnover of D1 enables PSII function by counteracting photodamage (for reviews, see Prasil et al., 1992; Aro et al., 1993). Indeed, for a large range of light intensities, the rate of damage is balanced by its rate of repair and an optimal efficiency of photosynthesis is maintained. The regulation of D1 synthesis by light is therefore essential for the survival of photosynthetic organisms.

In higher plants and algae, the expression of the chloroplastic *psbA* gene is mainly regulated at post-transcriptional steps, i.e. mRNA stability and translation (for review, see Mullet, 1988; Rochaix, 1992; Mayfield et al., 1995). In contrast, in cyanobacteria, light essentially modulates the transcription of the *psbA* gene, which is generally present as a family of genes (for review, see Golden, 1995). Most data come from work performed with *Synechococcus* PCC 7942 and to a lesser extent with *Synechocystis* PCC 6803 and PCC 6714. In these strains there are three *psbA* genes: *psbA*I, *psbA*II, and *psbA*III (Golden, 1995). The regulation of the expression of the *psbA* gene within *Synechococcus* PCC 7942 and *Synechocystis* PCC 6803 seems to be quite different. In *Synechocystis* PCC 6803 and 6714, the divergent *psbA*I copy is not expressed at all, while the *psb*AII and *psb*AIII copies are almost identical and encode only one form of D1 (Mohamed and Jansson, 1989; Bouyoub et al., 1993). Nevertheless, these two copies are differentially expressed: about 95% of *psbA* transcripts originate from *psbA*II, while only 5% originate from *psbA*III in cells grown at low- or high-light conditions (Bouyoub et al., 1993; Mohamed et al., 1993). Shifting cells to higher irradiances increases the steady-state levels of both *psbA*II and *psbA*III transcripts (Mohamed et al., 1993). This rise is due to an increase in transcription activity (Constant et al., 1997). In *Synechococcus* PCC 7942, the three genes encode two distinct forms of D1 (Golden et al., 1986). When grown at low irradiance, more than 80% of *psbA* transcripts corresponded to the divergent *psb*AI copy encoding the D1:1 form. When cells were shifted to high light, there was an increase of *psb*AII and *psb*AIII transcription, both encoding the D1:2 form, with a parallel decrease of *psbAI* transcript levels (Bustos et al., 1990).

Light can be sensed by specific photoreceptors such as the blue-light receptor or the phytochrome and/or via redox sensors of the photosynthetic electron transport chain (reviewed by Allen, 1993; Bowler and Chua, 1994). Different models have been proposed. Work carried out in

[1] M.A. was the recipient of a postdoctoral fellowship from the Departamento de Educación, Universidades e Investigación, Eusko Jaularitza-Gobierno Vasco.

[2] Present address: Departamento de Nutricion Vegetal, E.E. "Aula Dei," Consejo Superior de Investigaciones Científicas, Apdo 202, 50080 Zaragoza, Spain.

* Corresponding author; e-mail kirilov@wotan.ens.fr; fax 33–1–44323935.

the green alga *Chlamydomonas reinhardtii* suggests that light regulates *psbA* mRNA translation via a redox mechanism. The binding of putative translational factors to the *psbA* mRNA may depend on the redox state of thioredoxin and on the ADP levels of the cell (Danon and Mayfield, 1991, 1994a, 1994b). In chloroplasts of higher plants, transcription of *psbA* and *psaAB* genes seems to be regulated by the redox state of the PQ pool (Pfannschmidt et al., 1999).

Our understanding of the mechanism by which light regulates *psbA* expression in cyanobacteria is still insufficient. Tsinoremas et al. (1994) reported that in the cyanobacterium *Synechococcus* PCC 7942, expression of different copies of the *psbA* gene is regulated via a blue light photoreceptor. Another group has reported, in the same strain, a modulation of the expression of the different *psbA* copies by lowering the culture growth temperature without changing light quality or intensity (Campbell et al., 1995). These investigators attributed the D1 exchange to changes in the rate of photosynthetic electron transport. Finally, other studies have suggested that D1 degradation products may regulate *psbA* gene expression at both the transcriptional and translational levels in *Synechocystis* (Tyystjärvi et al., 1996). Transcription of the *psbA* gene induced by transfer form low to high light seems to be under the control of PSII activity (Alfonso et al., 1999) in *Synechocystis*. Nothing is known about light regulation of *psbA* expression in other cyanobacterial strains. Due to these multiple and controversial propositions, the regulation of *psbA* expression needed further investigation.

The aim of our work was to study the role of photosynthetic and respiratory activity on the regulation of *psbA* expression in *Synechocystis* PCC 6803. Cyanobacteria perform aerobic respiration and oxygenic photosynthesis in the same membrane. The PQ pool, the cytochrome (Cyt) $b_6/f$ complex (Cyt $b_6/f$), and plastocyanin (or Cyt c553) are common components of both respiratory and photosynthetic electron transport chains (Hirano et al., 1980; Aoki and Katoh, 1983; Peschek, 1987). The redox state of both photosynthetic and respiratory electron transport chains was varied by changing light and nutrient regimes and by the addition of different inhibitors and electron acceptors. Since light seems to regulate not only *psbA* mRNA production but also mRNA stability (Bustos et al., 1990; Mohamed and Jansson, 1991), both the levels of the *psbA* transcript and the stability of *psbA* mRNA were measured under these different conditions. To differentiate between specific and general mechanisms involved in the regulation of *psbA* expression, we also studied the redox regulation of two other photosynthetic genes: *psaE*, encoding a small subunit of PSI, and *cpcBA*, encoding the $\beta$- and $\alpha$-subunits of the phycocyanin. In contrast to D1, these proteins are stable under illumination and their turnover is independent of light intensity. Our results demonstrate the existence of a redox regulation not only of *psbA* expression but also of *psaE* and *cpcBA* expression. The redox state of one of the electron carriers between the PQ pool and the PSI seems to be involved. This regulation is likely specific, since several housekeeping genes, such as *rpnB* and *trpA*, do not respond to dark/light transitions or to changes in the redox state of the photosynthetic electron transport chain.

## MATERIALS AND METHODS

### Strain Culture Conditions and Chl Measurements

Wild-type *Synechocystis* PCC 6803 cells were grown in a mineral medium as described in Herdman et al. (1973) with twice the concentration of nitrate. Cells were grown in a rotary shaker (120 rpm) at 32°C under a 5% (v/v) $CO_2$-enriched atmosphere and continuous illumination from fluorescent white lamps giving an intensity (photosynthetic photon flux density) of about 90 $\mu$E $m^{-2}$ $s^{-1}$. The Chl concentration was spectrophotometrically determined in methanol as previously described (Constant et al., 1997). When necessary, cells were grown in a 0.1% (w/v) Glc-containing medium for at least five generations before use.

### Light Treatments

*Synechocystis* PCC 6803 cells were first harvested by centrifugation and resuspended in fresh sterile culture medium containing 50 mM 2-*N*-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (pH 6.8) at a final concentration of 30 $\mu$g Chl $mL^{-1}$ (approximately $3 \times 10^8$ cells/mL) for dark experiments or 15 $\mu$g Chl $mL^{-1}$ (approximately $1.5 \times 10^8$ cells/mL) for dark to light experiments. For dark experiments, the cells were preincubated under low light (90 $\mu$E $m^{-2}$ $s^{-1}$, 32°C) for 15 min and then transferred to darkness. For dark to light experiments, cells dark-adapted for 1 h were transferred to low white light intensities in the absence or presence of inhibitors. When indicated, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (20 $\mu$M), 2,5-dibromo-3-methyl-6-isopropyl-benzoquinone (DBMIB) (20 $\mu$M), or methyl viologen (MV) (300 $\mu$M or 2 mM) was added. DBMIB was added 10 min before the transfer of cells from dark to light; DCMU and MV were added 30 min before. Rifampicin was used as an inhibitor of transcription to determine the stability of transcripts. Rifampicin was added in excess at a final concentration of 300 $\mu$g $mL^{-1}$ to avoid problems of antibiotic degradation. Samples were collected at different times (as indicated in "Results") during the different light and inhibitory treatments. For RNA isolation, cells were immediately pelleted and frozen in liquid nitrogen. All samples were stored at −80°C until used.

### RNA Methods

Total RNA was isolated from 10 mL of *Synechocystis* PCC 6803 cells using hot phenol and LiCl as precipitating agents (Constant et al., 1997). Once isolated, total RNA concentration was spectrophotometrically determined by absorption at 260 nm and samples were stored in aliquots (10 $\mu$g) at −80°C until gel experiments. For northern-blot experiments RNA samples were denatured for 3 min at 70°C and separated by electrophoresis on a 1.2% (w/v) agarose gel containing formaldehyde as a denaturing agent. The gel was transferred onto a charged nylon membrane (Hybond-$N^+$, Amersham-Pharmacia Biotech, Buckinghamshire, UK) by capillary blotting and fixed to the membrane by 5 min UV and 2 h at 80°C. All solutions were treated with 0.1% (w/v) diethyl pyrocarbonate (Sigma-Aldrich, St. Louis) as

previously described. Blots were hybridized with different radioactive probes at 42°C for *psbA*, *psaE*, *rnpB*, and *trpA* and at 40°C for *cpcBA* and *16S* rRNA. Northern blots were exposed to x-ray film (Eastman Kodak, Rochester, NY) to obtain autoradiograms.

### Hybridization Probes

#### *psbA Probe*

A 0.7-kb *Kpn*I-*Kpn*I fragment containing the *psbA*II gene region of *Synechocystis* PCC 6714, encoding the 3′ half of the gene that contains the sequence of the $Q_B$ niche, was used as a homologous probe (Ajlani et al., 1989). This probe recognized the two expressed *psbA* copies, *psbA*II and *psbA*III. Due to the similarity between the two copies (including the upstream untranslated region), it was impossible to construct specific probes for each copy.

#### *psaE Probe*

A 0.35-kb *Ava*I-*Eco*24I fragment, derived from the plasmid pBSPsaE (gift from B. Lagoutte [Rousseau, 1992]) and containing the whole *psa*E sequence from *Synechocystis* PCC 6803, was used as a probe.

#### *cpcBA Probe*

The *cpcBA* probe was prepared from a 1.3-kb *Pvu*II fragment isolated from the plasmid pPM62 (gift from V. Capuano and N. Tandeau de Marsac). This fragment contains the coding sequences of the $\beta$- and $\alpha$-subunits of phycocyanin 2 from the cyanobacterium *Calothrix* PCC 7601 (Capuano et al., 1988).

#### *rnpB Probe*

Coding sequence of the *rnpB* gene of *Anacystis nidulans* (*Synechococcus* PCC 6301) (gift from A. Vioque, Sevilla, Spain) (Vioque, 1992).

#### *trpA Probe*

A 792-bp fragment containing the complete coding sequence of the *trpA* gene encoding the trypthophan synthase subunit A involved in aromatic aminoacid synthesis was amplified with oligos trpA1 (5′-ATGAACGCTGTTGCCGCTTG-3′) and trpA2 (5′-ACTGATGGCCGTTTTCAGTTC-3′). These oligos were deducted from the *Synechocystis* PCC 6803 genome map available at Cyanobase.

#### *rRNA Probes*

To verify the equal loading of the gels, the membranes were always probed with a 1.8-kb *Pst*I-*Eco*RI fragment containing the 16S rRNA gene of the brown algae *Pylaiella littoralis* (gift from C. Passaquet). The probes were radiolabeled by the random priming method using the multiprime DNA labeling system (Amersham-Pharmacia Biotech).

### Autoradiogram Scanning

The autoradiograms were analyzed using a combination of a scanner (studio *Ii*si, AGFA, Berlin) and a Macintosh Power 7100/80 computer using the public software domain NIH Image program (developed at U.S. National Institutes of Health and available from the Internet by anonymous FTP from http://www.zippy.nimh.nih.gov or on floppy disc from the National Technical Information Service, Springfield, VA, part no. PB95–500195GEI).

### Fluorescence Measurements to Determine the Redox State of the PQ Pool

To determine the redox state of the PQ pool in darkness, cells (30 $\mu$g Chl $mL^{-1}$) were incubated for 1, 5, 15, 60, and 90 min in the dark in the absence of any inhibitor. Then, the cells were diluted at 2 $\mu$g Chl $mL^{-1}$ and fluorescence induction curves were monitored. DBMIB (10 $\mu$M) was added to the cell suspension just before the measurement. Under our conditions, DBMIB completely inhibited oxygen evolution (data not shown). At each time, maximum PSII fluorescence in the dark-adapted state ($F_m$) was determined in the presence of DCMU (20 $\mu$M) and DBMIB (10 $\mu$M). Under these conditions the cells were always in a low fluorescence state. The area between the induction curve in the presence of DBMIB alone and that in the presence of DCMU plus DBMIB were calculated for each time. Chl fluorescence induction during the first second of illumination was monitored with a home-built fluorimeter. The time of the opening of the shutter was 2 ms. Excitation light was provided by a tungsten lamp equipped with 4–96 filters (Corning, Corning, NY). Fluorescence was detected in the red region through a 2–64 filter (Corning) and a 90 filter (Kodak Wratten, Rochester, NY). The recording was made with a multichannel analyzer connected to a personal computer.

## RESULTS

### Behavior of the *psbA* Transcript after Transfer of Cells from Light to Dark

The effect of transfer cells from light to dark on *psbA* mRNA levels and on transcript stability was studied. *Synechocystis* PCC 6803 cells were incubated for 15 min under illumination and then transferred to dark conditions. Total RNA extracted from cells incubated for different times under dark conditions was subjected to northern-blot analysis. Using a 0.7-kb *Kpn*I *psbA*II gene fragment containing the 3′-half coding sequence of D1 as a probe, a 1.2-kb mRNA originating from the two homologous copies: *psbA*II (95% of total *psbA* transcript) and *psbA*III (5% of total *psbA* transcript) was detected (Mohamed et al., 1993). The level of *psbA* mRNA slowly decreased. Even after 7 h of dark incubation, a high *psbA* mRNA level was observed (Fig. 1A).

The half-life of the *psbA* mRNA was determined using rifampicin, an inhibitor of transcription. Under illumination, the half-life of the transcript was 15 min (Fig. 1B).

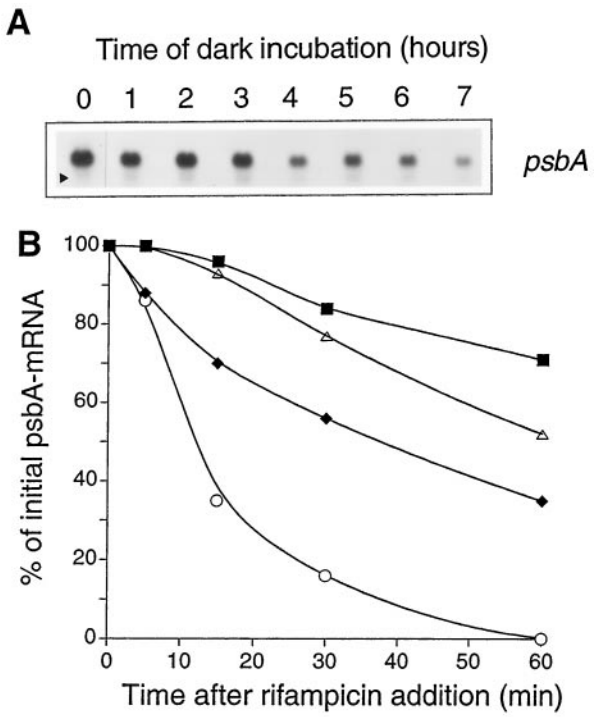


**Figure 1.** Effect of darkness on the *psbA* mRNA level and stability. A, *psbA* transcript level under dark conditions. Low-light-adapted cells (30 $\mu$g Chl mL$^{-1}$) were transferred to dark and samples for RNA isolation were taken at the indicated times. The blots were probed with the *psbA* probe. B, Rate of degradation of the *psbA* transcript under dark conditions. Rifampicin was added at the start of dark incubation (♦), and after 30 min (△) or 120 min of dark incubation (■). Degradation of the *psbA* transcript under low-light conditions (○) is also shown. Data were obtained by densitometry of the autoradiograms from two independent experiments. The position of the 0.9-kb truncated *psbA* transcript is indicated by the arrow.

Dark incubation resulted in a progressive stabilization of the mRNA: the half-life of the *psbA* transcript increased from 30 to 35 min (rifampicin added at time 0) to more than 2 h (rifampicin added at time 120 min) (Fig. 1B). In parallel, a new *psbA* transcript of 0.9 kb appeared (Fig. 1A, see also Fig. 3). This transcript has been previously observed in dark-adapted cells (Mohamed and Jansson, 1989, 1991) and in photoinhibited cells (Constant et al., 1997). It has been already demonstrated that this 0.9-kb transcript is a truncated *psbA* transcript that lacks the 5′ end (Mohamed et al., 1993; Constant et al., 1997).

### Redox State of the Electron Transport Chain under Dark Conditions

Under illumination, the PQ pool is mainly reduced by electrons coming from PSII but also by an NAD(P)H-quinone oxidoreductase via cyclic electron transport and respiration (Mi et al., 1992). In the dark, the PQ pool is reduced only by electrons coming from NAD(P)H by the NAD(P)H-quinone oxidoreductase. Thus, in darkness, the redox state of the PQ pool and the Cyt $b_6/f$ depends on the reducing power of the cells (Mi et al., 1994). To check the possible relationship between the changes observed on the expression of *psbA* after cell transfer to darkness and the redox state of the electron transport chain, the redox state of the PQ pool during dark incubation was measured by fluorescence induction kinetics. In the presence of DBMIB, the area over the induction curve is proportional to the number of equivalents of PQ that can be reduced by light (Vernotte et al., 1990). Cells were incubated in darkness for different times, and then fluorescence induction curves were recorded in the presence of DBMIB alone or DCMU plus DBMIB to determine the $F_m$ level. The incubation of *Synechocystis* cells in darkness induced an increase in the complementary area above the induction curve in the presence of DBMIB (Fig. 2), indicating that during dark incubation the PQ pool and therefore Cyt $b_6/f$ became progressively more oxidized than under illumination.

### Behavior of the *psbA* Transcript after Transfer of Cells from Dark to Light

Cells were placed in the dark for 1 h, conditions under which the *psbA* mRNA was shown to be stable. Cells were then transferred to light or kept in darkness. During the 1st h of light incubation, a large increase (400%) in *psbA* transcript levels was observed (Fig. 3), while at the same time the *psbA* transcript became less stable; in darkness, the half-life of the transcript was 90 min, while under illumination it was 12 min (Fig. 3). Upon illumination, the oxidized electron carriers become more reduced via the PSII. To determine whether the accumulation of the *psbA* mRNA is related to the reduction of the electron transport chain, the cells were transferred to light in the presence of DCMU

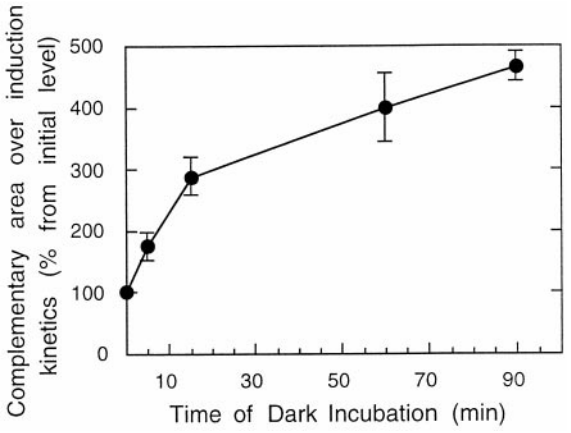


**Figure 2.** Kinetics of PQ reoxidation during dark incubation expressed as increase of the complementary area over the induction fluorescence curve in the presence of DBMIB. Low-light-adapted cells were transferred to darkness in the absence of inhibitors. Samples were taken after 1, 5, 15, 60, and 90 min of dark incubation. At each time, the cells were diluted to 2 $\mu$g Chl mL$^{-1}$ and fluorescence induction curves were monitored upon addition of DBMIB alone or DBMIB plus DCMU to determine the $F_m$ level. The area between the induction curve in the presence of DBMIB alone and that in the presence of DBMIB plus DCMU was calculated for each time. Data from three independent experiments were averaged and normalized to the initial area obtained at time 0 of dark incubation; $n = 3 \pm$ SE.

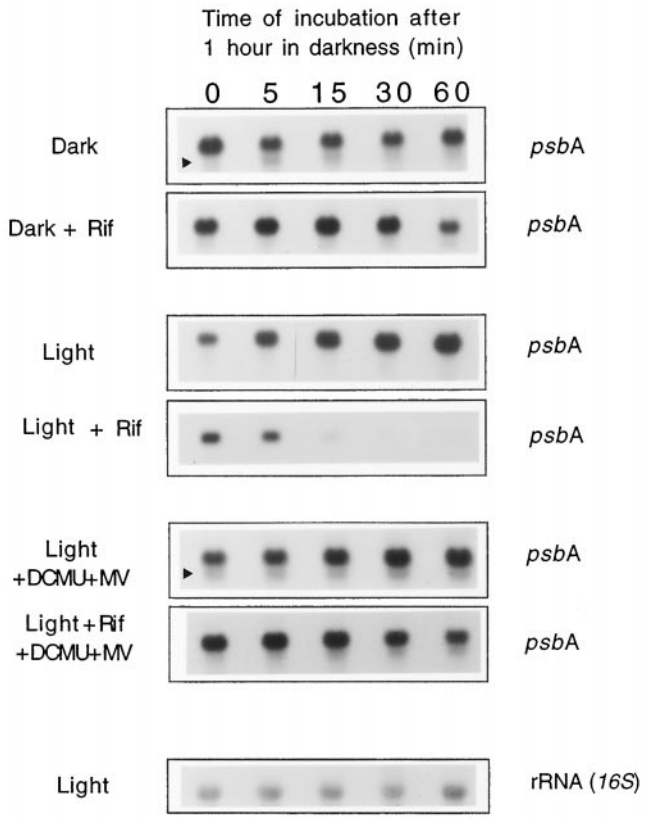


**Figure 3.** Northern-blot analysis of the *psbA* transcript levels after the transfer of dark-adapted cells to low-light conditions in the absence and presence of photosynthetic electron transport inhibitors. Cells (15 $\mu$g Chl mL$^{-1}$) were preincubated for 1 h in darkness. The cells were then left in darkness or illuminated in the absence or presence of rifampicin, in the absence of photosynthetic inhibitors, or in the presence of DCMU (20 $\mu$M) plus MV (300 $\mu$M). Total RNA isolated from cells left in darkness or illuminated for 0, 5, 15, 30, and 60 min was separated on agarose-formaldehyde gels (10 $\mu$g per lane), transferred onto a nylon membrane, and hybridized with the *psbA* probe. Then 16S rRNA was always used as loading control. The membrane used in the light experiment without inhibitors, dehybridized, and probed with a *Sma*I-*Pst*I fragment containing the 16S rRNA gene is shown in the figure. The position of the 0.9-kb truncated *psbA* transcript is indicated by the arrow.

plus MV. These chemicals inhibit linear and cyclic photosynthetic electron transfer. Under these conditions, a large inhibition of *psbA* transcript accumulation was observed: the level of *psbA* mRNA increased only 40%. On the other hand, the transcript remained very stable upon illumination: its half-life was similar to that observed in darkness (Fig. 3).

### Effect of Reducing the PQ Pool and Cyt $b_6/f$ in Darkness

The relationship between the redox state of the cells and *psbA* expression was further studied using Glc-grown cells. In this type of cells, a high concentration of NADPH is maintained for a long time under dark conditions by Glc metabolization. As a consequence, in darkness the PQ pool and Cyt $b_6/f$ are more reduced than in cells that have been grown in the absence of Glc (Mi et al., 1994). We determined whether the availability of reducing power can mimic in darkness the light effect on *psbA* expression. Figure 4 compares the light and dark stability of the *psbA* mRNA in cells that were grown in a Glc-containing medium. Rifampicin was added after 1 h of dark or light incubation. The half-life of the *psbA* mRNA that was calculated from autoradiograms of three independent experiments was 18 min in light and 20 min in darkness. In the absence of rifampicin, the *psbA* mRNA levels remained constant (Fig. 4).

### Is a Specific Electron Carrier Involved in the Redox Control of *psbA* Expression?

The results presented above suggested that the redox state of one or several carriers involved in electron transport may play an important role in the regulation of the expression of the *psbA* gene. To elucidate which electron carrier is involved (PQ pool, Cyt $b_6/f$, or electron acceptors of PSI), we studied the effect of different chemicals (DCMU, DBMIB, and MV) on *psbA* mRNA accumulation (Fig. 5B) and *psbA* stability (Fig. 5C) after transfer of dark-adapted cells (for 1 h) to light.

The site of action of the different electron transport inhibitors is described in Figure 5A. Under illumination in the presence of DCMU, an inhibitor of the electron transport between the primary ($Q_A$) and secondary ($Q_B$) electron acceptor quinones of the PSII, the PQ pool and Cyt $b_6/f$ cannot be further reduced by PSII. However, cyclic electron transport and respiration are still able to partially reduce both carriers. In the presence of MV, a synthetic PSI electron acceptor, the PQ pool, and the Cyt $b_6/f$ can be reduced via PSII and respiration. MV reduction prevents the reduc-

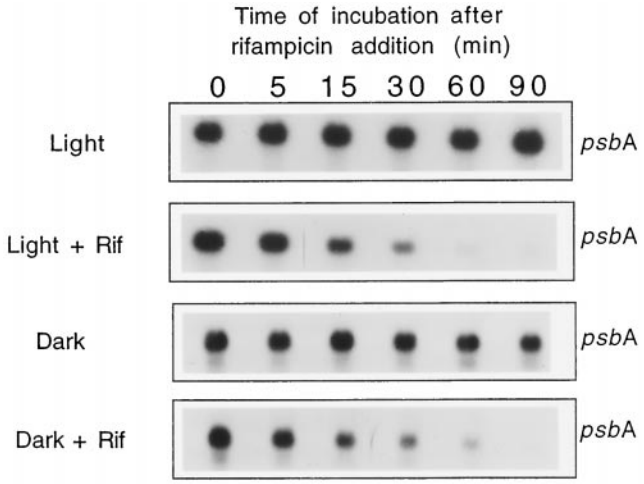


**Figure 4.** Effect of Glc on *psbA* transcript levels and *psbA* mRNA stability. *Synechocystis* PCC 6803 cells were adapted to grow in the presence of Glc for at least five generations. Cells (30 $\mu$g Chl mL$^{-1}$) were resuspended in a Glc-containing medium, incubated for 1 h under light or dark conditions, and then maintained in darkness or light in the absence or presence of rifampicin. RNA was isolated at the indicated times. Time 0 coincided with 1 h of dark or light incubation. Blots were hybridized with the *psbA* probe.

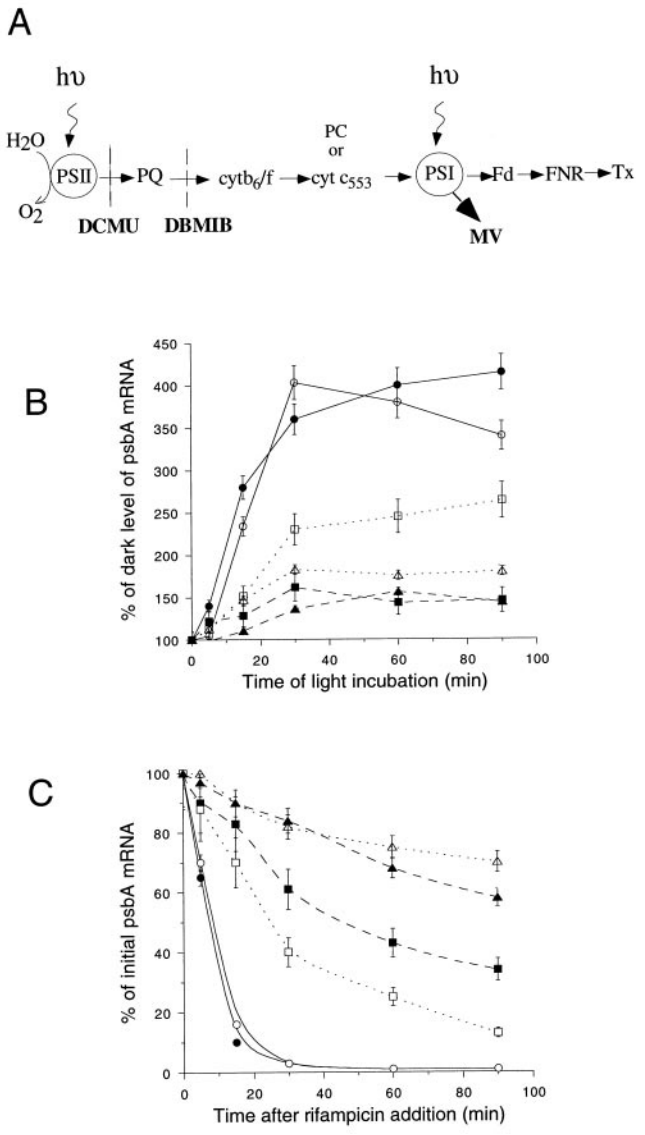


**Figure 5.** Relative levels of *psbA* transcript and *psbA* mRNA stability after cell transfer from dark to light in the absence and presence of photosynthetic electron transport inhibitors. A, Schematic representation of the action sites of the different chemicals used in this study. PC, Plastocyanin; Fd, ferredoxin; FNR, ferredoxin-NADH-reductase; Tx, thioredoxin. B, Quantification of steady-state *psbA* transcript levels following cell transfer from dark to light in the absence or presence of photosynthetic electron transport inhibitors. For experimental conditions, see "Materials and Methods." C, Rate of disappearance of the *psbA* transcript in the presence of rifampicin after the shift from dark to light. Rifampicin was added at time 0, which coincided with the time of the shift to light. ●, No inhibitors; ○, with 2 mM MV; □, DCMU (20 μM) alone; ■, DBMIB (20 μM) alone; △, DCMU (20 μM) plus MV (300 μM); ▲, DBMIB (15 μM) plus MV (300 μM). The average of three separate experiments is shown. Data were obtained by densitometry of autoradiograms.

tion of the natural electron acceptors of PSI and inhibits cyclic electron transport. Therefore, treatment of cells with DCMU plus MV produces a large oxidation of the photosynthetic electron transport chain, with the exception of $Q_A$. DBMIB largely inhibits linear and cyclic electron transports as well as respiration via the Cyt $b_6/f$ by its binding to the $Q_0$ site of the Cyt $b_6/f$; it also prevents PQ reoxidation. In its presence, while the PQ pool is mainly reduced, the other electron carriers (including Cyt $b_6/f$) are mostly oxidized. The combination of DBMIB and MV increases the level of oxidation of the electron carriers, with the exception of $Q_A$ and the PQ pool, which remain reduced.

Measurements of oxygen-evolving activity showed that DCMU (20 μM) and DBMIB (20 μM) completely inhibited oxygen evolution when added under illumination. DBMIB is relatively unstable and is easily converted to other compounds. When added 10 min before cell transfer to light, its presence still inhibited 90% of oxygen evolution after illumination: without additions, 150 μmol $O_2$ $mg^{-1}$ Chl $h^{-1}$; in the presence of DBMIB, 18 μmol $O_2$ $mg^{-1}$ Chl $h^{-1}$. However, if added 1 h before cell shift to light, only 30% to 40% of oxygen evolution was inhibited upon illumination (data not shown). When MV was added under illumination, the time needed for maximal oxygen consumption by the Mehler reaction (250 ± 30 μM $O_2$ $mg^{-1}$ Chl $h^{-1}$) was dependent on the MV concentration: 2 min for 2 mM of MV and 5 min for 300 μM of MV. Our measurements indicated that a large quantity of electrons arriving at the PSI go to MV and not to $NADP^+$.

Figure 5B shows that MV (added 30 min before illumination) had no effect on the accumulation of the *psbA* mRNA during the first 30 min of light incubation. The relative decrease of mRNAs (Figs. 5 and 8) observed at 60 and 90 min of cell incubation in the presence of 2 mM MV could be related to general oxidative damage. A decrease of variable fluorescence observed after 30 min of cell incubation under illumination and in the presence of 2 mM MV may also suggest oxidative damage (data not shown). DCMU partially inhibited *psbA* mRNA accumulation, while DBMIB (added 10 min before illumination) or the combinations DCMU plus MV or DBMIB plus MV largely suppressed the increase on the level of the *psbA* mRNA (Fig. 5B). The chemicals also influenced the stability of the *psbA* transcript. In the presence of MV alone, the stable dark *psbA* transcript became less stable upon illumination, as was the case in the absence of inhibitors. The transcript remained more stable upon illumination in the presence of DCMU or DBMIB (half-life: 30 and 45 min, respectively) or DCMU plus MV or DBMIB plus MV (half life: more than 2 h) (Fig. 5C).

In darkness, DBMIB, which binds to the $Q_0$ site of the Cyt $b_6/f$ complex, prevents Cyt $b_6/f$ reduction by plastoquinol via the respiratory electron transport chain. In Glc-adapted cells, where respiration via the Cyt $b_6/f$ is maintained for a long time under dark conditions, the *psbA* mRNA was rapidly degraded even in darkness (Figs. 4 and 6). We tested whether addition of DBMIB to dark Glc-adapted cells had any effect on *psbA* mRNA stability. Figure 6 shows that the presence of DBMIB increased the stability of the *psbA* transcript in dark Glc-adapted cells. The results presented in this section indicate that the reduction of the PQ pool itself or that of the electron acceptors of PSI are not the critical factors controlling the stability and accumulation of *psbA* transcripts.

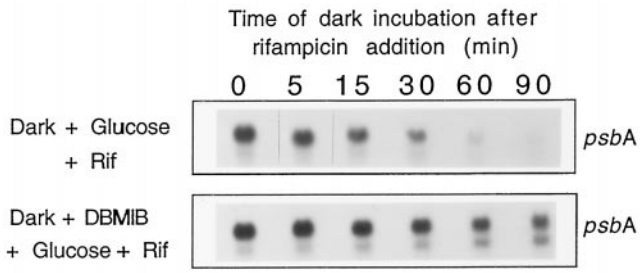


**Figure 6.** Effect of DBMIB on *psbA* mRNA stability. Glc-grown *Synechocystis* PCC 6803 cells (30 $\mu$g Chl mL$^{-1}$) were resuspended in a Glc-containing medium and incubated for 1 h under dark conditions in the presence or absence of DBMIB (20 $\mu$M). Rifampicin was then added and the cells were maintained in darkness. RNA was isolated at the indicated times. Time 0 coincided with 1 h of dark incubation. Blots were hybridized with the *psbA* probe.

### Response of *psaE* and *cpcBA* Transcripts to Dark and Light Transitions and to Electron Transport Inhibitors

We also studied the redox regulation of other photosynthetic genes: *psaE*, encoding a small subunit of PSI, and *cpcBA*, encoding the $\beta$- and $\alpha$-subunits of the phycocyanin. Light-dark and dark-light transitions also affected the levels of the *psaE* and *cpcBA* transcripts. Cell shift from light to dark induced a rapid decrease in the levels of both transcripts. This decrease was faster than that of the *psbA* mRNA: they were no more detectable after 2 h of dark incubation (Fig. 7). A half-life of 12 min was found for both transcripts under light conditions, while it was about 20 min in darkness (data not shown). The effect of dark was largely avoided by the presence of Glc (Fig. 7). The light level of the transcripts was maintained for a long time in darkness.

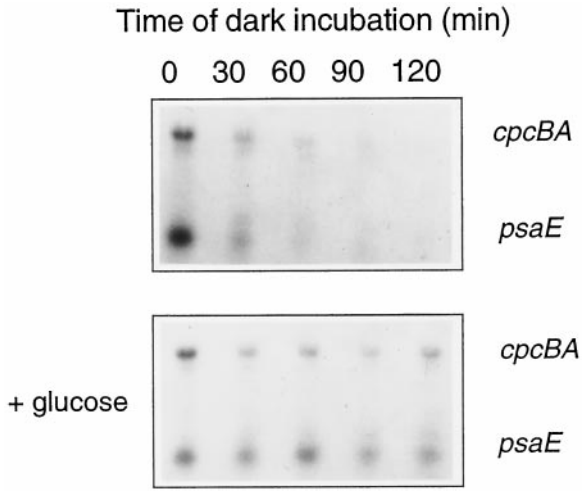


**Figure 7.** Effect of darkness on *psaE* and *cpcBA* transcript levels. Cells were grown in the absence or presence of Glc. Low-light-adapted cells (30 $\mu$g Chl mL$^{-1}$) were transferred to dark in the absence or presence of Glc and samples for RNA isolation were taken at the indicated times. Blots were hybridized with the *cpcBA* probe and the *psaE* probe. The sizes of *psaE* and *cpcBA* transcripts were determined to be 0.35 and 1.6 kb, respectively.

The *psaE* and *cpcBA* transcript levels increased when dark-adapted cells were transferred to light (Fig. 8). Therefore, we investigated possible redox control of this accumulation. Treatment of cells with DBMIB or DCMU completely inhibited *psaE* and *cpcBA* transcript accumulation during the dark to light shift. In contrast, the presence of MV had no effect (Fig. 8).

### Response of *trpA* and *rnpB* Transcripts to Dark and Light Transitions and to Electron Transport Inhibitors

Figure 9 shows the behavior of two housekeeping genes in darkness and in light: the *rnpB* gene, encoding the constitutive RNA component of RNAse P, and the *trpA* gene, encoding the Trp synthase enzyme subunit A. The level of neither mRNA decreased during the 1st h of dark incubation. Moreover, after 8 h of dark incubation a significant

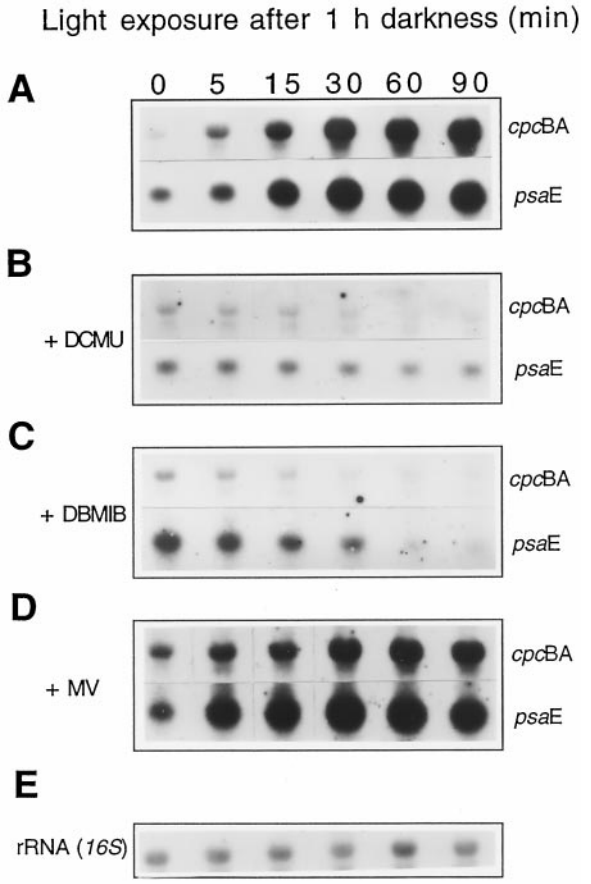


**Figure 8.** Effect of photosynthetic electron transport inhibitors on *psaE* and *cpcBA* transcript accumulation following the shift of cells from dark to light. Northern-blot analysis of *psaE* and *cpcBA* transcript levels after the transfer of dark-adapted cells to light conditions. Cells were preincubated for 1 h in darkness and then illuminated in the absence (A) or presence of the photosynthetic inhibitors DCMU (20 $\mu$M) (B), DBMIB (20 $\mu$M) (C), or MV (2 mM) (D). Samples for RNA isolation were taken at the indicated times. Blots were hybridized with the *psaE* and the *cpcBA* probes. 16S rRNA was always used as loading control. The membrane used in experiment A dehybridized and probed with a *Sma*I-*Pst*I fragment containing the 16S rRNA gene is shown in E.

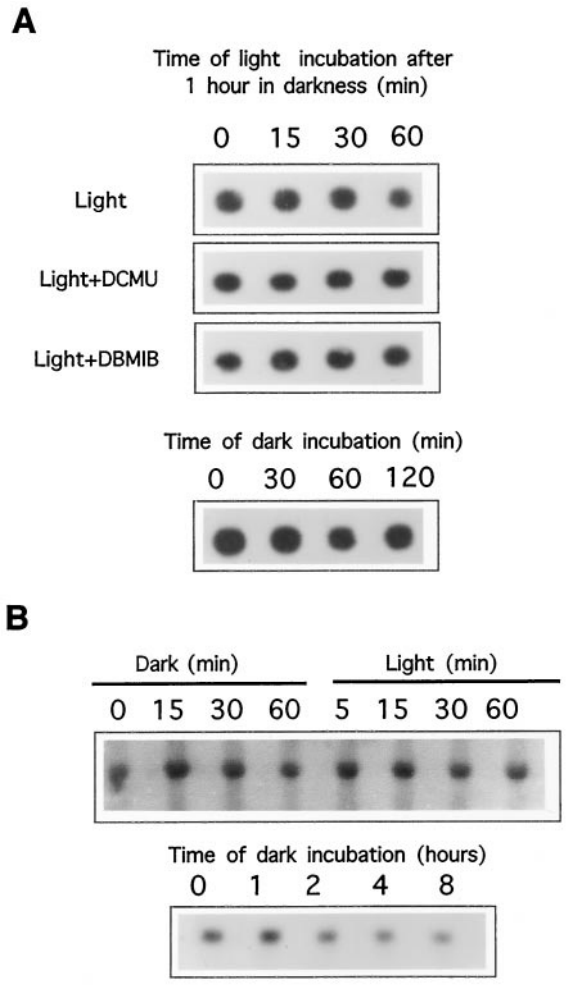


**Figure 9.** Behavior of *rnpB* and *trpA* mRNAS under light and dark conditions. A, Effect of DCMU and DBMIB northern-blot analysis of *rnpB* transcript level after the transfer of dark-adapted cells to light conditions. Cells were preincubated for 1 h in darkness, and then illuminated in the absence or presence of DCMU (20 $\mu$M) or DBMIB (20 $\mu$M). The levels of the *rnpB* transcript during the dark incubation are also shown. B, *trpA* transcript levels under dark conditions and after the transfer of dark-adapted cells to light conditions.

level of *trpA* mRNA was still observed (Fig. 9B). In contrast to what happened with *psbA*, *psaE*, and *cpcBA* mRNAs, the level of the *rnpB* and *trpA* mRNAs did not increase upon transfer of dark-adapted cells to light. Moreover, the mRNA levels did not decrease in the presence of DBMIB or DCMU (Fig. 9A and data not shown). These results suggested that the changes observed in *psbA*, *psaE*, and *cpcBA* gene expression with light were specific and were not a consequence of a general response of the cell.

## DISCUSSION

Our results demonstrated the existence of a redox regulation of *psbA* expression. In *Synechocystis* PCC 6803, photosynthetic electron transport has an important role not only in the stability of *psbA* mRNA, as previously suggested (Mohamed and Jansson, 1991), but also in the regulation of *psbA* transcript accumulation.

### Action of Light via Photosynthetic Electron Transport on the Stability of *psbA* mRNA

The *psbA* transcript is more stable in the dark than in the light. The stability of the *psbA* mRNA seems to be controlled, directly or indirectly, by the redox state of the intersystem electron carriers of the electron transport chain more than by light itself (Mohamed and Jansson, 1989; this article). The *psbA* transcript remained very stable upon illumination, when the reduction of the PQ pool, Cyt $b_6/f$, and PSI electron acceptors was hindered. Under dark conditions, the presence of Glc, which increased the reducing power of the cell, induced the destabilization of the transcript. The effect of the redox state of the electron transport chain on the stability of the *psbA* mRNA seems to be specific. The rates of *psaE* and *cpcBA* mRNA degradation were only slightly slowed down under dark conditions. The stability of the *rbcL-S* and *psbD* mRNAs is not larger in darkness than in light (Mohamed and Jansson, 1989). These results suggest that synthesis of new mRNA was not required for rapid degradation of transcripts in *Synechocystis* PCC 6803 cells, and that a decrease of general RNAse activity was not the only cause of the large stability of the *psbA* mRNA in darkness. The fact that in darkness, the decrease of the steady-state levels of the *psaE*, *cpcBA* (this article), *psbD*, and *rbcL-S* (Mohamed and Jansson, 1989) mRNAs was faster than that of the *psbA* mRNA could suggest that the *psbA* transcript was more stable than the other mRNAs, even when general transcription was not inhibited.

The redox control of the stability of the *psbA* mRNA could be explained by the following hypothesises: (a) the binding of the factors influencing the stabilization of the mRNA depends directly on the redox state of the photosynthetic electron transport chain. (b) The active degradation of the mRNA depends on D1 translation that progressively decreases under dark conditions (S. Constant and D. Kirilovsky, unpublished data). Under conditions where *psbA* mRNA translation is inhibited, such as light plus lincomycin (Constant et al., 1997) or after prolonged light stress (Constant et al., 1997), the *psbA* transcript stability increases. These data suggest that in *Synechocystis*, like in higher plants (Klaff and Gruissem, 1991), the formation of translation complexes could be a mechanism to initiate and/or facilitate the turnover of *psbA* mRNA.

### Expression of *psbA* Is Regulated by the Redox State of the Electron Transport Chain

We have already demonstrated that the accumulation of *psbA* transcripts following low to high light transitions is due to an increase in the *psbA* mRNA production, while the stability of the transcript is independent of the light inten-

sity (Constant et al., 1997). The redox state of PSII seems to be involved in this regulation (Alfonso et al., 1999). In the present study, we show that dark-light and light-dark transitions affect the expression of the *psbA* gene. Shift of dark-adapted cells to light induces a large increase in the level of the *psbA* transcript, which is accompanied by a rapid destabilization of the mRNA, while transfer of light-adapted cells to darkness induces a slow decrease of the *psbA* mRNA level as a result of a decrease in transcription and a progressive stabilization of the mRNA.

In *Synechocystis* PCC 6803 cells the photosynthetic electron transport chain, including the PQ pool, becomes more oxidized during dark incubation due to a decrease of the NADPH plus $H^+$ concentration (Vernotte et al., 1990; Mi et al., 1994; this work). In contrast, the ATP content decreases slowly in darkness (only 10% after 6 h of starvation; Vernotte et al., 1990). Thus, the decrease of the reducing power, and not that of the ATP level, seems to be responsible for the decrease in *psbA* expression. Moreover, in darkness in the presence of Glc, the *psbA* gene seems to be transcribed. The steady-state levels of the *psbA* mRNA remained constant for a long time in darkness, although the half-life of the transcript was only 20 min. In addition, He and Vermaas (1998) showed that in the absence of Glc, no significant amount of *psbA* mRNA was detectable in 48-h dark-adapted *Synechocystis* cells, while a high level of this transcript was observed when Glc was present in the growth medium.

Our results clearly demonstrate that the light induction of *psbA* accumulation can be modified by artificially changing the redox state of electron carriers of the electron transport chain. When dark-adapted cells are transferred to light, the entire electron transport chain becomes more reduced as a result of photosynthesis. This reduction can be avoided by inhibiting linear and cyclic electron transfer. Under these conditions, *psbA* mRNA accumulation is largely inhibited.

The redox state of the electron transport chain is also involved in the regulation of the expression of other genes in *Synechocystis* cells. In this article, we show that the expression of *psaE* and *cpcBA* genes is modulated by the redox state of one of the photosynthetic electron carriers between the PQ pool and PSI. The expression of genes such as *glnA* and *glnB*, encoding enzymes involved in nitrogen metabolism, seems to be similarly redox controlled in *Synechocystis* PCC 6803 (Reyes and Florencio, 1995; Garcia-Dominguez and Florencio, 1997). Other non-photosynthetic genes, such as the *secA* gene (Mazouni et al., 1998) or the *dnA*-like gene (Richter et al., 1998), also seem to be regulated by the redox state of the cells. However, there are other genes that are not dependent on the redox state of the cell or on the presence of light. We show that the *rnpB* and *trpA* genes do not respond to light. The ORF sll0615 also does not increase under light conditions (Mazouni et al., 1998). Moreover, in *Synechococcus* 7002, the transcription of the *lrtA* gene is inhibited by light (Tan et al., 1994). The specific dark expression of other proteins was reported in *Synechococcus* 6301 and *Anacystis nidulans* (Singer and Doolittle, 1975; Suranyi et al., 1987). The occurrence of genes that do not respond to light or that are negatively regulated by light supports the idea that light and redox controls are specific for certain types of genes and not part of a general metabolic control system.

In the green alga *Chlamydomonas reinhardtii*, light regulation of *psbA* translation seems to be under redox control through a thioredoxin-dependent system (Danon and Mayfield, 1994b). Recently, Pfannschmidt et al. (1999) proposed that in mature chloroplasts, the redox state of the PQ pool controls the rate of transcription of genes encoding reaction-center apoproteins of PSI and PSII. Our results indicate that in *Synechocystis* cells, the involvement of the redox state of PSI electron acceptors in the regulation of *psbA* expression is unlikely. Transcript accumulation induced by cell shifting from dark to light was not inhibited in the presence of MV alone, which prevents the reduction of the natural electron acceptors of the PSI. In addition, this inhibitor did not induce any change in mRNA stability. In contrast, a large effect was observed when the electron carriers between PSII and PSI were oxidized. The largest inhibition of the accumulation of *psbA* transcripts (like that of *psaE* and *cpcBA* transcripts) was observed not only in the presence of DCMU plus MV but also in the presence of DBMIB plus MV. Since DCMU and DBMIB have opposite effects on the redox state of the PQ pool, we conclude that *psbA* expression is not directly correlated with the redox state of the PQ pool.

It has been recently demonstrated that occupancy of the $Q_0$ site in the Cyt $b_6/f$ by a plastoquinol molecule is the signal for the activation of a light-dependent kinase of light-harvesting complex II involved in the balance of excitation energy between the two photosystems (Vener et al., 1995, 1997; Zito et al., 1999). DBMIB, which prevents the PQ reoxidation by its binding to the $Q_0$ site of the Cyt $b_6/f$, largely inhibits the accumulation of *psbA* mRNA upon cell illumination or in darkness in the presence of Glc. Thus, as a working hypothesis, we propose that a similar mechanism could be involved in the regulation of the expression of the *psbA* gene and other photosynthetic genes, such as *psaE* and *cpcBA*: the absence of a plastoquinol molecule in the $Q_0$ site of the Cyt $b_6/f$ complex might induce the repression of transcription, while a turnover of plastoquinol at the $Q_0$ site might be required to allow transcription.

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