Biochemical and Biophysical Research Communications **255,** 47–53 (1999)
Article ID bbrc.1998.9996, available online at http://www.idealibrary.com on IDEAL®

Light-Dependent and Rhythmic *psbA* Transcripts in Homologous/Heterologous Cyanobacterial Cells

Ganesh Kumar Agrawal, Munehiko Asayama, and Makoto Shirai[1]
Laboratory of Molecular Microbiology, School of Agriculture, Ibaraki University, Ami-machi, Inashiki-gun, Ibaraki 300-0332, Japan

Received December 10, 1998

The *psbA2* gene exhibits light-dependent and rhythmic expression in a unicellular cyanobacterium, *Microcystis aeruginosa (Synechocystis)* K-81. To further understand the *psbA2* expression, biological analyses were performed in homologous and heterologous cyanobacterial cells. The results of the experiments using the K-81 cells revealed that (i) the light-dependent expression appeared on transcriptional and/or post-transcriptional level(s) under light/dark cycles, (ii) circadian-rhythmic transcripts were also observed under the control of an endogenous clock. To assess whether light-dependent and rhythmic *psbA2* expression occurs in heterologous cyanobacterium, *Synechococcus* sp. strain PCC 7942, the K-81 *psbA2* 5′-upstream region of which the promoter and its around sequences share with those of PCC 7942 *psbAII*, was fused to the bacterial lacZ reporter gene, introduced into the genome of PCC 7942 and the *psbA2* transcripts were directly investigated by primer extension. The K-81 *psbA2* specific transcripts were also light-dependent and rhythmic in PCC 7942, strongly demonstrating that a common regulatory mechanism exists *per se* for the *psbA2* expression in both strains. Furthermore, *psbA2* expression in the recombinant PCC 7942 strain, AG400 in which the region from −404 to +111 of *psbA2* is fused to *lacZ*, exhibited clear rhythmicity, while very little or no rhythmicity was observed in AG429 (−38 to +14, the only promoter region), suggesting that the region(s) around the promoter was essentially required for clear rhythmic expression.

The *Microcystis aeruginosa* K-81 (hereafter called K-81), a unicellular and colony-forming cyanobacterium capable of driving oxygenic photosynthesis, was previously isolated (1) and characterized (2–4). In cyanobacteria, the D1 protein of the Photosystem II is encoded by a multigene family, termed *psbA* genes (5, 6). These genes differentially express under altered environmental conditions (7–9). Moreover, most of these photosynthetic genes are presumed to be regulated by a biological clock for efficient photosynthesis to occur for development of the organism under changeable environmental cues. These biological clocks, which exist in most eukaryotic organisms (10, 11) and also in some prokaryotes (12), are known to have approximately 24 hours periodicity but not exactly 24 hours under constant conditions. Recently, the *psbA2* gene, which also encodes D1 protein homolog of Photosystem II, was clones from K-81 and its transcription exhibited light-responsiveness and rhythmicity under light-dark (L/D) cycle conditions (13). In addition, structural similarities of the *psbA* promoters among cyanobacteria and a novel DNA curvature, *CIT* (*c*hangeable bending-center sites of an *i*ntrinsic curvature under *t*emperature conditions), were also explored at the 5′-upstream region (−141 to −184) of K-81 *psbA2* (14, 15). To further extend our knowledge on light-dependent and rhythmic expression of K-81 *psbA2*, its transcripts were characterized in homologous and heterologous cyanobacteria cells. In this study, we report evidence that light-dependent and possibly circadian-rhythmic expression are controlled on transcriptional and/or post-transcriptional level in both types of cyanobacteria, implying that a common regulatory mechanism exists for *psbA* expression.

MATERIALS AND METHODS

Cyanobacterial strains, media, and culture. The *M. aeruginosa* K-81 and recombinant *Synechococcus* sp. strain PCC 7942 (AG400 and AG429) were grown in CB (4) and BG11 liquid medium (16) containing spectinomycin sulfate (40 μg/ml), respectively, at 30°C under continuous light illumination (1,700 lux) until the mid-log phase (OD_{750} = 0.4 to 0.5) followed by either L/D (12 h-dark/12 h-light of 1,700 lux), light (1,700 lux) or high light (8,500 lux) illumination.

Constructions of a reporter plasmid and a recombinant Synechococcus strain. The pHNL7-up plasmid DNA (15), carrying the 5′-upstream promoter region of the K-81 *psbA2* gene, was digested with

[1] Corresponding author. Fax: (+81)298-88-8653. E-mail: shirai@agr.ibaraki.ac.jp.

0006-291X/99 $30.00

*Sma*I and *Bgl*II and the resultant fragment (−404/+111) was inserted into the *Sma*I-*Bgl*II site of pAM990 (17), which is a promoter-probe vector with a *lacZ* reporter gene, to creat pAG400. A fragment (−38/+14) was amplified by PCR with primers [GKA7, 5′-ttg-*ccccggg*CCCTTTACATAACTAT-3′; GKA34, 5′-cgga*agatct*TCTTTG-TTTGACATGA-3′: upper case letters correspond to sequences of *psbA2* and its 5′-upstream regions and lower case letters in the GKA primers are tag sequences containing *Bgl*II or *Sma*I site (italics)] and pHNL7-up. This amplified fragment (−38/+14) was inserted into the *Sma*I-*Bgl*II site of pAM990 to yield pAG429. The sequence of the insert at the junction site to *lacZ* on pAG400 or 429 for frame analysis was verified by DNA sequencing (18). To construct recombinant PCC 7942 strains, PCC 7942 was naturally transformed by a double cross-over reaction with donor pAG400 and 429 DNAs (19, 20) and spectinomycin-resistant cells, called AG400 and 429, were selected on BG11 plates containing spectinomycin sulfate. Genomic DNAs were isolated from AG400 or 429 and recombination was confirmed by Southern analysis at 55°C using a *Bam*HI-*Eco*RV fragment, carrying the *psbA2* 5′-upstream region (−404/+111) and the part of *lacZ* derived from pAG400, as a probe.

RNA isolation and primer extension analysis. The total RNA, isolated from cell cultures (50 ml) and the primer extension analyses were carried out as described previously (13). The 5′-ends of a D2II synthetic oligonucleotide (5′-TGTTGTAGAGTTGTA-3′ according a position from +72 to +58 of the *psbA2*structural gene) were labeled with [γ-^{32}P]ATP (5,000 Ci/mmol) and used as a primer. Since AG429 recombinant strain contains the only −38/+14 regulatory region of *psbA2*, the primer extension analysis was performed with a *lacZ* internal primer, called *lacZ*-RI (5′-AGTTGGGTAACGCCAGGGT-TTTCCCA-3′). After reaction of the extension, samples were denatured at 95°C for 5 min, quickly chilled on ice for 2 min and then 1 μl in case of K-81 and 3 μl in recombinant PCC 7942 strains (out of 7 μl) were loaded on a 6% polyacrylamide gel containing 8 M urea. Electrophoresis was performed with 1/2X TBE buffer at 2,500 constant voltage for 50 min followed by X-ray exposure at −80°C for 24 to 48 hours. The signal intensity of the band corresponding to the *psbA2* transcripts on the original gels was measured by Ultraviolet BIO-PROFIL (Vilber Lourmat, Cedex, France), and the relative abundances were plotted.

RESULTS

The light-dependent psbA2 expression on transcriptional and/or post-transcriptional level(s) in K-81. Previous Northern analysis revealed that the K-81 *psbA2* transcript was light-dependent (13). To further understand light-dependent *psbA2* expression under a sequencial condition of the L/D cycles in K-81, the *psbA2* transcripts were investigated by primer extension analysis. The K-81 cells grown under a continuous light condition until the mid-log phase were subjected to the L/D regime. The cells were collected in the second stage of the cycle (Fig. 1A). The *psbA2* transcripts in total RNA isolated from the cells significantly increased under the light cycle, reaching its transcript maxima at 6 hours followed by gradual decrease in the dark cycle. The 5′-end of *psbA2* transcripts was also observed at the same position according to the transcription start point (+1). These results are compatible with those previously reported (12, 13). Furthermore, to characterize the expression, antibiotics for transcriptional or translational inhibitors were added to the cell cultures and their effects against to the light-dependent *psbA2* expression were assessed (Fig. 1B,C).

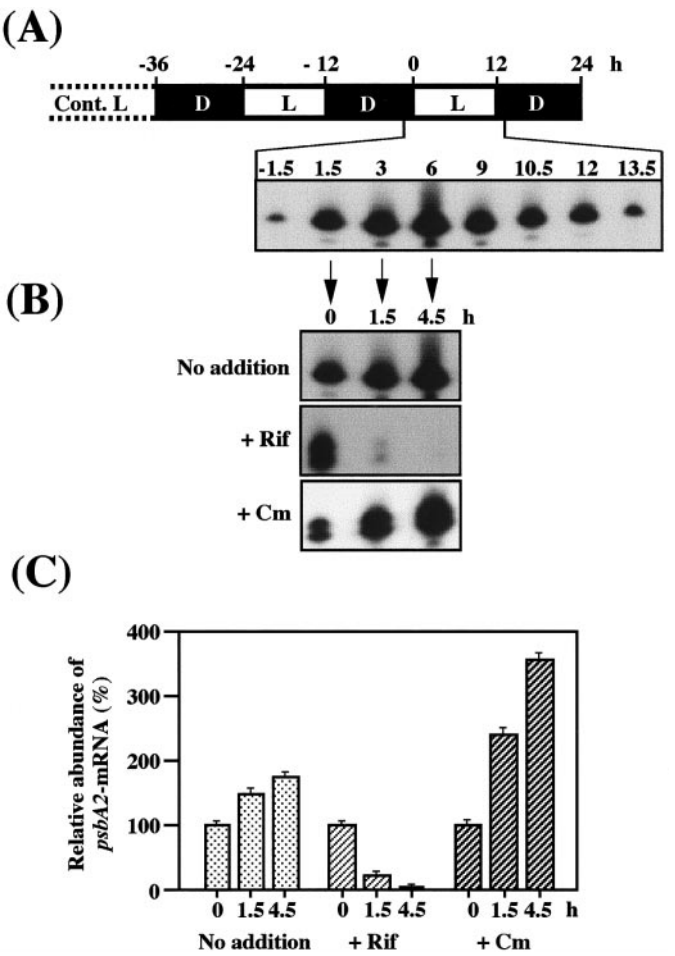

FIG. 1. The light-dependent *psbA2* transcripts in the K-81 cells. (A) L/D (12 h/ 12 h) cycles and the transcript under light conditions. The *M. aeruginosa* K-81 cells were grown under a continuous light (Cont. L) condition until the mid-log growth phase at 30°C, then entrained into the L/D cycles. Total RNA was isolated from the cells and subjected to primer extension analysis. The signal bands corresponding to the transcription start point (+1) of *psbA2* on an X-ray film are shown. Sequential harvesting time is shown at the top of each lane. (B) Effect of antibiotics on the light-dependent expression. Rifampicin (Rif, 200 μg/ml) or chloramphenicol (Cm, 250 μg/ml) was added into the cell culture at 1.5 hours in the second light period, considering it as 0 hour followed by further sampling at 1.5 and 4.5 hours. (C) Relative abundance of *psbA2*-mRNA according to the signal intensities in panel (B) is given. The error rate is less than 15%.

When rifampicin (Rif) was added to the K-81 cells at 1.5 hours in the second light cycle (Fig. 1A) and incubation continued for 4.5 hours (Fig. 1B), the synthesized transcripts within 1.5 hours consequently resulted in a decrease. These signal intensities of the transcript were also measured and are represented in Fig. 1C. If the *psbA2* mRNA is stable, a constant amount of the transcripts must appear after the addition of rifampicin because this bacteriocidal antibiotic inhibits RNA synthesis by binding to and inhibiting the β-subunit of RNA polymerase. However, the quantity of the transcript was actually not constant but

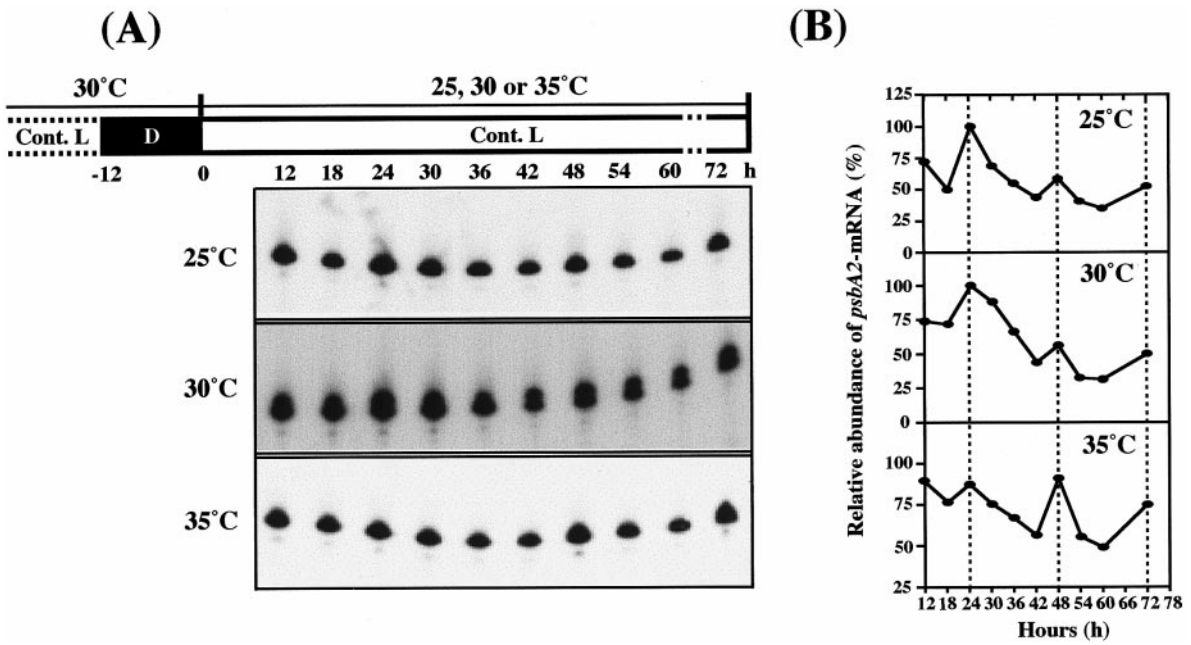

FIG. 2. Circadian rhythmic *psbA2* transcript in K-81. (A) Temperature compensation assay. The cells grown under a continuous light condition until the mid-log growth phase at 30°C were subjected to 12 hours of Dark (D) pulse followed by continuous light condition at 25, 30, and 35°C, respectively, as permissive growth conditions. Cell harvesting was performed every 6 hours, total RNA was isolated from the cells and subjected to primer extension. Signals are presented the same as in Fig. 1. (B) The relative abundance of the *psbA2*-mRNA. The signal intensities in panel (A) are shown.

degradable, indicating that its mRNA is not stable in the light condition. Whereas, light-induced *psbA2* transcripts were still observed under the conditions with chloramphenicol (Cm), as well as no addition of the antibiotics (Fig. 1B). Interestingly, the ratio of light-induced transcripts is higher (the value is 3.5 at 4.5 hours) with chloramphenicol than that (1.8 at 4.5 hours) with no antibiotic, suggesting that the addition of chloramphenicol causes up shifting of the *psbA2* light-inducible expression. Since chloramphenicol inhibits protein synthesis by interacting with the 50S ribosomal subunit and inhibiting the peptidyltransferase reaction, there is a possibility that synthesis of some negative protein which acts on the *psbA2* light-inducible expression was hindered by the addition of chloramphenicol and the *psbA2* mRNA synthesis and/or stability was eventually increased. Furthermore, we essentially observed the same results for the light-inducible expression with the addition of erythromycin which inhibits protein synthesis, as well as that of chloramphenicol (data not shown). These results indicate that the light-dependent *psbA2* expression in the K-81 cells is regulated on transcriptional and/or post-transcriptional level(s).

Circadian-rhythmic expression in K-81. Three criteria of (i) persistence under constant conditions, (ii) phase resetting by light and dark signals and (iii) temperature compensation of the period (10, 12), are required to satisfy the evidence for a circadian clock. Although the *psbA2* expression exhibited rhythmicity under constant darkness (13), it remains unclear whether the K-81 cells possess the circadian clock for *psbA2*. To clear this point, we performed temperature compensation assay (Fig. 2). The K-81 cells grown at 25, 30, or 35°C, respectively, under continuous light condition followed by 12 hours of dark adaptation were collected, the *psbA2* transcripts in the cells were directly analyzed by primer extension (Fig. 2A) and the signal intensities were measured (Fig. 2B). Peaks of *psbA2* expression were observed periodically at approximate 24 hour intervals for each temperature (Fig. 2B, vertical broken lines), indicating that rhythmicity was also exhibited under the constant light condition referring to the first criteria. This rhythmicity was shifted by a phase resetting of a dark pulse (not shown), according the second criteria. In addition, the calculated Q_{10} value for frequency (1/period) of the rhythm is 1.0 (Fig. 2B), referring the third criteria. This value is compatible with those of previous reports for the circadian clock and is far from that of most biological processes, such as growth or respiration, for which the Q_{10} values are usually between 2 and 3. This evidence satisfies the three criteria and shows that the *psbA2* expression possesses circadian rhythm.

The light-dependent psbA2 transcripts in a heterologous cyanobacterium, PCC 7942. The nucleotide sequence of the region upstream of K-81 *psbA* involving a promoter exhibits similarities among cyanobacterial

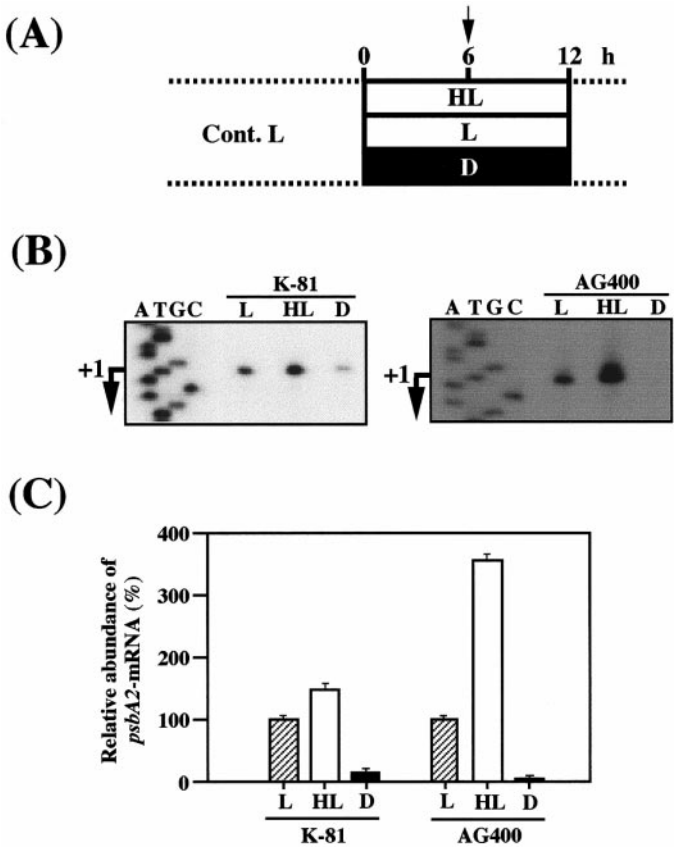

FIG. 3. The light-dependent *psbA2* transcripts in homologous and heterologous cells. (A) The K-81 and AG400 cells, grown in the CB and BG11 media until mid-log growth phase, respectively, at 30°C under continuous light (L; 1,700 lux), were subjected to high light (HL; 8,500 lux), dark (D; complete darkness), and light (L; 1,700 lux). The cells were collected after 6 hours (shown by arrow). (B) 5′-end mapping of the *psbA2* transcript. Total RNA isolated from the cells (A) was subjected to primer extension. The transcription start point (+1) is indicated by arrows. The sequence ladders (A, T, G, and C) were also created by using the same primer, D2II and pHNL7-up as a template DNA. (C) The relative abundance of the *psbA2*-mRNA is shown in the panel (B). The error rate is less than 13%.

psbA genes (14). Especially, the sequence of K-81 *psbA2* well shares to that of PCC 7942 *psbAII* which exhibits the high light inducible transcription. If there is a common regulatory system for the *psbA* expression in cyanobacteria, the K-81 *psbA2* light-dependent (and high light inducible) transcript can be also observed in a heterologous cyanobacterium, PCC 7942 of which the transformation system has been established (19, 20). To examine this possibility, we constructed pAG400 carrying the *psbA2* 5′-upstream region (−404/+111), introduced into the PCC 7942 genome as a monocopy, then the K-81 *psbA2* specific transcript was analyzed by primer extension in the recombinant cell, AG400, under various growth conditions (Fig. 3). When the AG400 cells were cultivated for 6 hours-light (L), -high light (HL), and -dark (D) conditions (Fig. 3A) following a continuous light (Cont. L), high light inducible (the ratio of values in high light and light conditions, HL/L) and light dependent (the ratio of values in light and dark conditions, L/D) transcripts were observed (Fig. 3B) in the heterologous cells. This pattern is also essentially the same as observed in the original K-81 cells as a control. A signal corresponding to the transcriptional start point (+1) was also detected at the same position in both K-81 and AG400 cells. These results showed that the K-81 *psbA2* light-responsive transcription also occurs in the heterologous PCC 7942 cells, suggesting that the common regulatory system for the *psbA* expression exists in both cells. From the results displayed in Fig. 3C, the ratio of values referring to high light induction (the ratio is 3.5 in HL/L) and light-dependent (20 in L/D) in PCC 7942 was higher than those (1.5 and 5.0, respectively) in K-81, indicating that the K-81 *psbA2* light-responsiveness under both HL/L and L/D was enhanced in the PCC 7942 cells. Furthermore, we investigated the K-81 light-dependent expression under the sequencial conditions of L/D cycles in both cells (Fig. 4). In the K-81 cells, the *psbA2* transcript detected by primer extension increased under the light cycle, reaching a maxima at 6 hours as observed in Fig. 1A, but decreased under darkness. The same result was observed in the AG400 cells, but the strength of the signal intensities was clearer than that in K-81, as shown in Fig. 3. Since the K-81 *psbA2* light-dependent transcripts were detected in the heterologous cyanobacterium, the affect of antibiotics on expression was investigated (data not shown). Similar patterns of signals, except for the slower decay of *psbA* mRNA in the presence of rifampicin, were also observed using AG400, as exhibited in K-81 (Fig. 1), strongly confirming that a common regulatory system for light-dependent *psbA2* expression exists on transcriptional and/or post-transcriptional level(s) in both cells. Since the AG400 construct carries the region from −404 to +111 of K-81 *psbA2*, only this

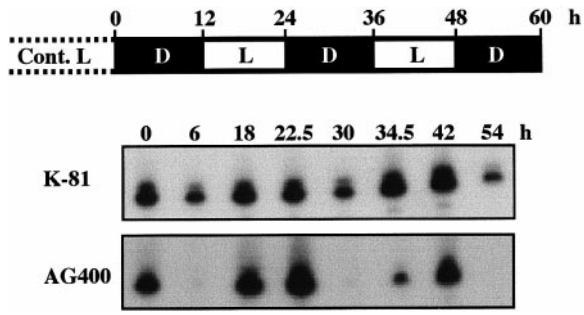

FIG. 4. The light-dependent *psbA2* transcripts in K-81 and AG400 under L/D cycles. The K-81 and AG400 cells grown under a continuous light condition (Cont. L; 1,700 lux) until the mid-log growth phase at 30°C were subjected to the L/D cycles. The total RNA isolated from the cells were subjected to primer extension and the signals according to the *psbA2* transcript are shown the same as in Fig. 1.

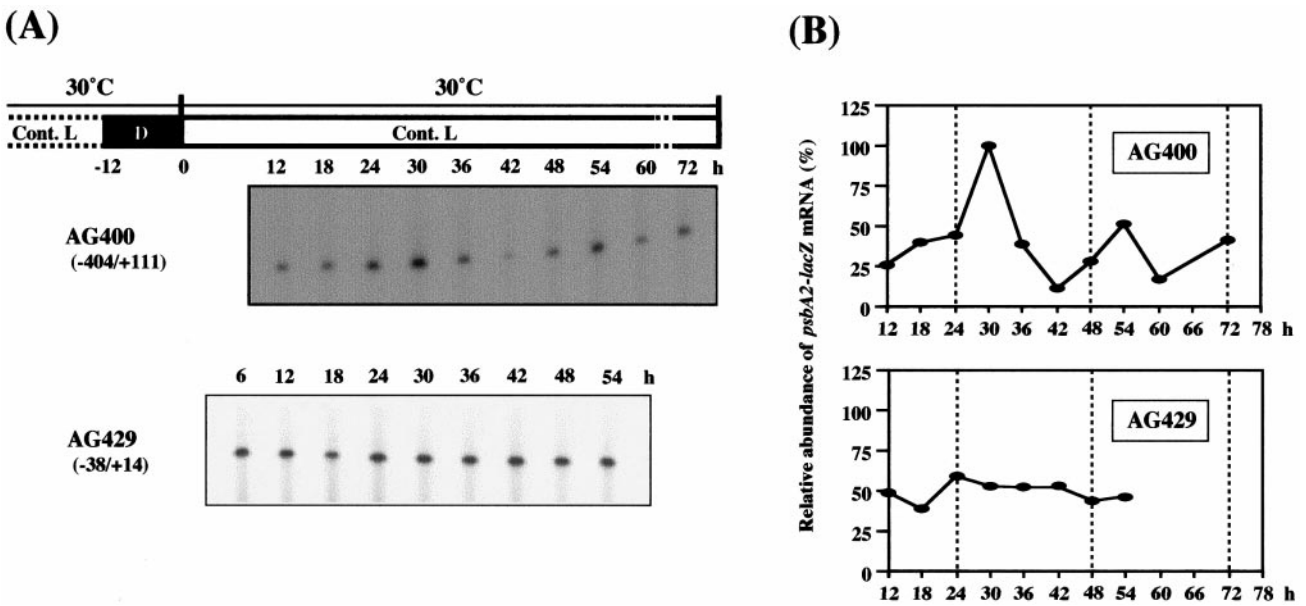

FIG. 5. The rhythmic *psbA2* expression in the AG400 cells. (A) A sampling condition. The cells were cultivated under a continuous light condition until the mid-log phase at 30°C, then given 12 hours of Dark (D) pulse followed by continuous light condition at 30°C. (B) The relative abundance of *psbA2-lacZ* mRNA according to the signal intensities in panel (A) is presented.

cis-region may be sufficient for light-dependent expression in the heterologous cell.

Rhythmic expression in PCC 7942. Although the *psbA2* transcription exhibits circadian rhythms in K-81, whether the K-81 *psbA2* 5′-upstream also maintains the rhythmic fluctuation in PCC 7942 remains unclear. To ascertain this, the *psbA2* transcript in AG400 cells was investigated by primer extension under the same continuous light condition at 30°C as shown in Fig. 2 (Fig. 5). The results show a rhythmic pattern of periodicity close to 24 hours, implying that a possible circadian clock for the K-81 *psbA2* expression exists in PCC 7942. This result is not contradictous to evidence that gene expression of endogenous genes in PCC 7942 are dependent on the circadian clock (21). Since the rhythmic *psbA2* transcripts were observed in AG400 (−404/+111), we investigated whether the rhythmicity is observed in AG429 (−38/+14) which carries a minimal *cis*-element with the promoter for transcription. The result indicated that very little or no rhythmicity in AG429 was observed on the original gel, suggesting that the region(s) around the promoter was required for clear rhythmicity.

DISCUSSION

This is comparative studies that the *psbA* light-dependent and rhythmic expression have been performed in heterologous cells of a cyanobacterium in which the *psbA* gene expresses the same as in the original K-81 cells. Although unicellular cyanobacteria, *Microcystis (Synechocystis)* strains are taxonomically far from the *Synechococcus*-group (22), the *psbA* light-dependent and rhythmic expression can be observed in both cells. Findings in this work suggest a possible common regulatory system for *psbA* expression at transcriptional and/or post-transcriptional level(s) exists in phylogenetically distant cyanobacteria. Specific recognition of the K-81 *psbA2* promoter by RNA polymerases containing principal sigma factors has been reported (14). This *psbA2* expression was light-dependent in K-81 whereas it was constitutive in *E. coli* under the light/dark cycles. Therefore, such a unique regulatory system for the light-dependence and rhythmicity could exist only in phototrophic organisms. Potential *trans*-acting factors and ribonucleases have been known to associate and modify light-responsive gene expression on mRNA synthesis and its decay, respectively, in cyanobacteria and plants (23–28). It is also implied that the *psbA2* mRNA degradated under the light cycle (Fig. 1). Which *psbA* transcript is more stable under the light or dark condition? An analysis of the mRNA stability under dark conditions with antibiotics in the L/D regime sheds light on the question. Cyanobacteria are photoautotrophic organisms capable of oxygen-producing photosynthesis similar to that in eukaryotic algae and plants, involving light-dependent and circadian-rhythmic gene expression. Evidence from DNA sequence and phylogenetic analyses indicate that chloroplasts are close relatives of cyanobacteria and are derived from an endosymbiotic event (29, 30). Therefore, it would be interesting to compare common regulatory system between plant and cyanobacteria.

We can also discuss minor but noteworthy differences in the K-81 *psbA2* light-dependent (involving the mRNA stability) and rhythmic gene expression between K-81 and recombinant PCC 7942 cells as follows: (i) strength of signal intensities referring to the *psbA2* transcript in PCC 7942 were clearer than that in K-81 (Figs. 3 and 4), (ii) the mRNA stability with addition of rifampicin might be lower in K-81 (Fig. 1), and (iii) a time lag of the peaks in rhythms (Figs. 2 and 5). At first, the *M. aeruginosa* K-81 strain can grow under the L/D cycle condition in the CB (or BG11) medium for one month at least, whereas the *Synechococcus* sp. strain PCC 7942 can grow only for several days. We consequently set up the condition in which each strain was subjected to preincubation under continuous light condition, then entrained into the L/D cycle for several days in CB medium for K-81 or BG11 medium for PCC 7942 (Figs. 1, 3 and 4). Previously our experiment revealed that almost all of the K-81 *psbA2* transcript rapidly diminished until 1.5 hours in dark conditions in the L/D cycle from the inoculation of the cells (13), however, the mRNA was still detected in this study (at 13.5 hours in Fig. 1). The continuous L/D cycles from the inoculation are required for effective *psbA* light-dependent expression in K-81, whereas one cycle of L/D regime may be sufficient for them in the recombinant PCC 7942 cell, AG400 (Fig. 4). Whether this difference arises from the media (CB or BG11) or the cells (K-81 or recombinant PCC 7942), the K-81 cells can grow in the BG11 medium and the *psbA2* transcripts should be observed the same as in Fig. 4. The decay-rate of *psbA2* mRNA was more rapid in K-81 than in PCC 7942 under the situation with rifampicin in the light cycle (Fig. 1), and this might be caused by the differences in sensibilities against the antibiotics or in activities of *trans*-acting factors (*e.g.* ribonucleases) against the mRNA in both cells. Approximately 6 hours of phase delay was observed in the recombinant PCC 7942 cells, AG400, which carries the *psbA2* (−404 to +111)-*lacZ* fragment (Fig. 5). The cloned region from *psbA2* might affect the rhythm in heterologous cells and this rhythm may be rectified to the same pattern without the time lag in K-81 by several L/D pulses prior the continuous light conditions. However, these phenomena are not substantial obstacles for analysis of light-dependent and rhythmic *psbA* expression in heterologous cells.

Gene expression is generally controlled at the transcriptional, post-transcriptional, translational and post-translational levels. It is also known that the *psbA* expression is regulated at each level in phototrophic organisms (8, 23, 31–34). Our results revealed the K-81 *psbA2* light-dependent and circadian rhythmic transcripts. Furthermore, its expression might be controlled at least at the transcription and/or post-transcription. Especially on the transcriptional and post-transcriptional regulation, it is known in plants that RNA polymerases containing sigma factors can control transcription and the synthesized transcripts were edited by ribonucleases (25, 28, 35–37). Although the participation of these in cyanobacteria have not been solved sufficiently at present, an RNA polymerase with the principal sigma factor can specifically recognize the *psbA* gene (14, 38, 39) and an alternative type of sigma factor can modify circadian expression (40). Since the results found in this study also show the robust rhythm of the *psbA2* expression in AG400 (−404/+111) whereas very low or no amplitude in AG429 (−38/+14), effective *cis*-elements around the promoter region can attend to the regulation for clear rhythmicity (Fig. 5). Characterization of *cis*-elements and *trans*-acting factors for light-dependent and rhythmic *psbA* expression and functional analyses in the homologous/heterologous cyanobacteria are required and are being currently performed in our laboratory.

ACKNOWLEDGMENT

This work was supported by a grant for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports, and Culture of Japan.

REFERENCES

1. Shirai, M., Matumaru, K., Ohtake, A., Takamura, Y., Aida, T., and Nakano, M. (1989) *Appl. Environ. Microbiol.* **55,** 2569–2571.
2. Shirai, M., Ohtake, A., Sano, T., Matsumoto, S., Sakamoto, T., Sato, A., Aida, T., Harada, K., Shimada, T., Suzuki, M., and Nakano, M. (1991) *Appl. Environ. Microbiol.* **57,** 1241–1245.
3. Ohtake, A., Shirai, M., Aida, T., Mori, N., Harada, K., Matsuura, K., Suzuki, M., and Nakano, M. (1989) *Appl. Environ. Microbiol.* **55,** 3202–3207.
4. Sakamoto, T., Shirai, M., Asayama, M., Aida, T., Sato, A., Tanaka, K., Takahashi, H., and Nakano, M. (1993) *Int. J. Syst. Bacteriol.* **43,** 844–847.
5. Golden, S. S., Brusslan, J., and Haselkorn, R. (1986) *EMBO J.* **5,** 2789–2798.
6. Osiewacz, H. D., and McIntosh, L. (1987) *Nucleic Acids Res.* **15,** 10585.
7. Vrba, J. M., Curtis, S. E. (1989) *Plant Mol. Biol.* **14,** 81–92.
8. Mohamed, A., Eriksson, J., Osiewacz, H. D., and Jansson, C. (1993) *Mol. Gen. Genet.* **238,** 161–168.
9. Golden, S. S. (1995) *J. Bacteriol.* **177,** 1651–1654.
10. Sweeney, B. M. (1987) Rhythmic Phenomena of Plants, Academic Press, San Diego.
11. Sweeney, B. M., and Borgese, M. B. (1989) *J. Phycol.* **25,** 183–186.
12. Johnson, C. H., Golden, S. S., Ishiura, M., and Kondo, T. (1996) *Mol. Microbiol.* **21,** 5–11.
13. Sato, M., Shibato, J., Aida, T., Asayama, M., and Shirai, M. (1996) *J. Gen. Appl. Microbiol.* **42,** 381–391.
14. Shibato, J., Asayama, M., and Shirai, M. (1998) *Biochim. Biophys. Acta* **1442,** 296–303.
15. Agrawal, G. K., Asayama, M., and Shirai, M. (1997) *FEMS Microbiol. Lett.* **147,** 139–145.
16. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., and Stanier, R. Y. (1979) *J. Gen. Microbiol.* **111,** 1–61.
17. Li, R., and Golden, S. S. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11678–11682.

18. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
19. Golden, S. S., and Sherman, L. A. (1984) *J. Bacteriol.* **158,** 36–42.
20. Bustos, S. A., and Golden, S. S. (1992) *Mol. Gel. Genet.* **232,** 221–230.
21. Liu, Y., Tsinoremas, N. F., Johnson, C. H., Lebedeva, N. V., Golden, S. S., Ishiura, M., and Kondo, T. (1995) *Genes Dev.* **9,** 1469–1478.
22. Wilmotte, A. (1995) *in* Molecular Evolution and Taxonomy of the Cyanobacteria (Bryant, D. A., Eds.), pp. 1–25. Kluwer Academic Publishers Press, Dordrecht.
23. Isono, K., Niwa, Y., Satoh, K., and Kobayashi, H. (1997) *Plant Physiol.* **114,** 623–630.
24. Kulkarni, R. D., Schaefer, M. R., and Golden, S. S. (1992) *J. Bacteriol.* **174,** 3775–3781.
25. Staub, J. M., and Maliga, P. (1994) *EMBO J.* **6,** 547–553.
26. Yohn, C. B., Cohen, A., Rosch, C., Kuchka, M. R., and Mayfield, S. (1998) *J. Cell. Biol.* **142,** 435–442.
27. Kulkarni, R. D., and Golden, S. S. (1997) *Mol. Microbiol.* **24,** 1131–1142.
28. Memon, A. R., Meng, B., and Mullet, J. E. (1996) *Plant Mol. Biol.* **30,** 1195–1205.
29. Ohyama, K., *et al.* (1986) *Nature* **322,** 572–574.
30. Kaneko, T., *et al.* (1996) *DNA Res.* **3,** 109–136.
31. Alexander, C., Faber, N., and Klaff, P. (1998) *Nucleic Acids Res.* **26,** 2265–2272.
32. Kim, J., Klein, P. G., and Mullet, J. E. (1994) *J. Biol. Chem.* **269,** 17918–17923.
33. Elich, T. D., Edelman, M., and Mattoo, A. K. (1993) *EMBO J.* **12,** 4857–4862.
34. Danon, A., and Mayfield, S. P. (1994) *Science* **266,** 1717–1719.
35. Tanaka, K., Tozawa, Y., Mochizuki, N., Shinozaki, K., Nagatani, A., Wakasa, K., and Takahashi, H. (1997) *FEBS Lett.* **413,** 309–313.
36. Tozawa, Y., Tanaka, K., Takahashi, H., and Wakasa, K. (1998) *Nucleic Acids Res.* **26,** 415–419.
37. Tiller, K., and Link, G. (1993) *EMBO J.* **12,** 1745–1753.
38. Asayama, M., Tanaka, K., Takahashi, H., Sato, A., Aida, T., and Shirai, M. (1996) *Gene* **181,** 213–217.
39. Asayama, M., Suzuki, H., Sato, A., Aida, T., Tanaka, K., Takahashi, H., and Shirai, M. (1996) *J. Biochem.* **120,** 752–758.
40. Tsinoremas, N. F., Ishiura, M., Kondo, T., Andersson, C. R., Tanaka, K., Takahashi, H., Johnson, C. H., and Golden, S. S. (1996) *EMBO J.* **15,** 2488–2495.