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Expression of photosynthesis genes in relation to nitrogen fixation in the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS 101

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Abstract

The daily cycle of nitrogenase expression in the marine filamentous nonheterocystous cyanobacterium *Trichodesmium* spp. is controlled by a circadian rhythm. We evaluated the rhythm of two key photosynthesis genes, *psbA* of photosystem II and *psaA* of photosystem I, in *Trichodesmium* sp. IMS 101 using the 3 criteria for an endogenous rhythm. The transcript abundance of *psbA* and *psaA* transcripts oscillated with a period of ca. 24 h under a 12 h light/12 h dark regime. At 24 °C and 28 °C the cyclic pattern of transcript abundance was maintained for at least 58 h under constant light conditions, whereas the periods were about 24 h at 24 °C, and 26–30 h at the higher temperature. The cycles of *psbA* and *psaA* gene expression were entrained using light-dark cues. Transcription of *nifHDK* was initiated prior to the light period, followed by *psbA* and finally *psaA*. There was a 90° (6 h) phase difference between the net accumulation of *nifHDK* and *psbA* transcripts, as well as between that of *psbA* and *psaA* transcripts. Results of inhibitor experiments indicated that *psbA* and *psaA* transcription was regulated differently by initiation and degradation during the light period. Short-term changes of light conditions resulted in significant effects on *psbA* transcription and nitrogenase activity, but had less of an effect on *psaA* and *nifHDK* transcription.

Introduction

Trichodesmium spp. are diazotrophic filamentous nonheterocystous marine cyanobacteria. *Trichodesmium* contributes significantly to the annual input of new nitrogen to the nutrient-limited surface waters of tropical and subtropical oceans all over the world (Carpenter, 1983; Gallon *et al.*, 1996; Capone *et al.*, 1997; Zehr *et al.*, 1998). *Trichodesmium* fixes nitrogen exclusively during the light when oxygenic photosynthesis is concurrent, without any known mechanism to either temporally or spatially separate oxygenic photosynthesis from oxygen-sensitive nitrogen fixation. How *Trichodesmium* protects nitrogenase from inactivation by oxygen is a perplexing and fascinating question.

In the past, relatively few studies have focused on photosynthesis by *Trichodesmium*. Based on parallel $^{14}CO_2$ and N_2 fixation measurements, it was suggested that the patterns of photosynthetic CO_2 fixation in both cultured and natural *Trichodesmium* populations were similar and corresponded over time to the patterns of N_2 fixation (Paerl, 1994). Microautoradiographic data from the same study also provided some evidence of a possible spatial separation between carbon and nitrogen fixation (Paerl, 1994). Results of studies using immunohistochemistry coupled with light and transmission electron microscopy

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers AF107784 (*psbA*) and AF107783 (*psaA*).

have been used to support the hypothesis that nitrogen fixation and photosynthesis are separated spatially in *Trichodesmium* (Frederiksson, 1996; Bergman *et al.*, 1997). In order to understand the link between photosynthesis and nitrogen fixation in *Trichodesmium*, molecular studies directed at the expression and regulation of photosynthesis-specific genes are necessary.

Cyanobacteria perform light-driven oxygenic photosynthesis involving the two membrane-embedded pigment-protein complexes, photosystems I and II (PS I and PS II) (Ho and Krogman, 1982). PS II is responsible for three essential reactions of photosynthesis: charge separation, quinone reduction and oxygen evolution (Vermaas and Ikeuchi, 1991). The electrons extracted by PS II are passed to PS I that passes them further to low-potential acceptors. The core of the PS II reaction center is a dimer of two structurally related proteins designated D1 and D2, both contributing ligands to the cofactors that mediate photochemistry (Anderson and Styring, 1991). D1 is encoded by the *psbA* gene that resides as a single copy in the chloroplast genome of higher plants (Zurawski *et al.*, 1982). In contrast, with one exception, the known complement of cyanobacterial *psbA* genes ranges from two to four (Golden, 1994, 1995). It has been demonstrated that each copy of the *psbA* gene family is differentially regulated by light conditions and plays different roles in photoinhibition and recovery processes in *Synechococcus* sp. strain PCC 7942 (see for reviews Golden, 1994, 1995). Furthermore, *psbAI* gene expression has been demonstrated to be under circadian rhythm control in a unicellular cyanobacterium (*Synechococus* sp. AMC149) that was transformed with a bacterial luciferase reporter gene construct (Kondo *et al.*, 1993; Golden *et al.*, 1997). Circadian rhythm expression of *psbA* genes has also been shown in the unicellular diazotrophic cyanobacterium *Cyanothece* sp. strain ATCC 51142 (Colón-López and Sherman, 1998). At the heart of the PS I reaction center is a dimer of two structurally related proteins, PsaA and PsaB, encoded by *psaA* and *psaB*, respectively (Golbeck, 1992). The *psaA* and *psB* genes have been cloned and sequenced in a few cyanobacterial species including *Synechococcus* sp. PCC 7002 (Cantrell and Bryant, 1987), *Synechocystis* sp. PCC 6803 (Smart and McIntosh, 1991), and *Anabaena variabilis* (Morden and Golden, 1989). A recent study has suggested that in the unicellular diazotrophic cyanobacterium *Cyanothece* sp. strain ATCC 51142, *psaAB* transcription is differentially regulated under different light/dark regimes and may also be controlled by a circadian rhythm (Colón-López and Sherman, 1998).

We have recently demonstrated that the circadian rhythm of nitrogenase gene expression in *Trichodesmium* sp. IMS 101 is at transcriptional and translational, as well as enzymatic activity levels (Chen *et al.*, 1998). This study deals with northern blot analyses of transcription of two key photosynthesis genes (*psbA* of PS II and *psaA* of PS I) in different light/dark regimes, as well as under different light conditions. The results have indicated that transcription of both *psbA* and *psaA* genes are also under control of the circadian rhythm. Furthermore, the study has revealed that transcription of *psbA*, *psaA* and *nifHDK* are temporally separated from each other over the diel cycle. Such phase separation between photosynthesis genes and nitrogen fixation genes, which is clearly maintained by the circadian rhythm, could be one of the underlying mechanisms that allows oxygenic photosynthesis and oxygen-sensitive nitrogen fixation to proceed simultaneously in *Trichodesmium* sp. IMS 101.

Materials and methods

Strain and growth conditions

Trichodesmium sp. IMS 101 was originally isolated from western Atlantic Ocean waters near North Carolina (Prufert-Bebout *et al.*, 1993). The cultures were grown in YBCII artificial seawater medium as previously described (Chen *et al.*, 1996). During the course of this study, stock and control cultures were maintained at 26.5 °C with a 12 h light/12 h dark cycle. The light (L) phase was from 10:00 to 22:00 and the dark (D) phase from 22:00 to 10:00, all local time. Growth irradiance was provided by cool-white fluorescent lamps at about 100 μmol photons m^{-2} s^{-1}. Biomass of the cultures was estimated by measuring chlorophyll *a* (chl *a*) (Tandeau De Marsac and Houmard, 1988). Batch cultures of *Trichodesmium* sp. IMS 101 at mid to late logarithmic growth stage were used for all experiments.

Nitrogenase activity measurement

Nitrogenase activity was assayed by the acetylene reduction technique (Capone, 1993). Ten ml aliquots were placed in 15 ml serum bottles. The vials were sealed with silicone rubber stoppers, and 0.75 ml air

in the headspace was replaced with purified acetylene at the beginning of each time point. Samples were then incubated under different experimental conditions. Ethylene production was measured by gas chromatography (Shimadzu GC-14A equipped with FID) at 1 h intervals for 2 h. Treatments and cultures were duplicated for all experiments. The ethylene production rate was normalized to chl *a*.

Isolation of chromosomal DNA

Trichodesmium sp. IMS 101 filaments from 1 liter of exponentially growing cultures were collected on 20 μm pore size Nytex membranes and resuspended in 2.5 ml of buffer (10 mM Tris pH 8.0, 1% SDS, 25 mM EDTA). The cell suspension was incubated on ice until the cells were lysed completely. The lysate was vortexed briefly with equal volume of a phenol (Tris pH 8.0 saturated)-chloroform and centrifuged for 4 min at 10 000 rpm in a micro-centrifuge. The supernatant was collected and subjected to repeated phenol-chlorofonn extraction until the interface was clear. The supernatant was then extracted once with an equal volume of chloroform. Chromosomal DNA was precipitated overnight by adding 0.1 volume of 3 M sodium acetate, 2 volumes of ethanol at −20 °C.

PCR amplification, cloning and sequencing of fragments of psbA *and* psaA *genes*

A PCR-based strategy was adopted to obtain fragments of *psbA* and *psaA* genes. Existing cyanobacterial PsbAI (D1 protein form I)/PsaA sequences were retrieved from GenBank. Degenerate primers were chosen from conserved regions (Table 1) and used to amplify *psbA/psaA* genes from genomic DNA of *Trichodesmium* sp. IMS 101 with PCR conditions as described before (Zehr and McReynolds, 1989). The sequences of degenerate primers and sizes of amplified fragments are listed in Table 1. PCR products were separated on a 1% agarose gel and the fragments of expected sizes were purified using a Qiaex II DNA kit (Qiagen) and subsequently cloned into pGEM-T vector (Promega) according to the manufacturer's instructions. The plasmids were identified as pORTER (*psbA*) and pALEALE (*psaA*). Sequencing of both *psbA* and *psaA* fragments was performed by the University of Maine DNA Sequencing Facility (Orono, ME). Resulting partial nucleotide and deduced amino acid sequences of *psbA/psaA* of *Trichodesmium* sp. IMS 101 were compared with the homologous genes from other organisms, using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) Web server (http://www.ncbi.nlm.gov).

RNA extraction

At each sampling point, 20–100 ml from each treatment were rapidly filtered onto 25 mm Magna nylon membrane (MSI, pore size 10 μm) and immediately lysed in 1 ml RNA extraction buffer (5% Triton X-100, 10% sucrose, 20 mM EDTA, 50 mM Tris, 100 mM DTT). The lysate was then extracted twice with 0.8 ml low-pH phenol (pH 4.5) equilibrated with an equal volume of chloroform, and then extracted once with 0.8 ml chloroform. Total RNA was ethanol-precipitated, washed, vacuum-dried, resuspended in RNase-free H_2O, and stored at −20 °C until analyzed.

Northern blot analysis

Northern blot analysis was performed to determine the time-course abundance of *psbA* and *psaA* transcripts of *Trichodesmium* sp. IMS 101 cultures grown under different conditions. RNA isolations and northern blot analyses were carried out following widely used protocols (Sambrook *et al.*, 1989; Ausubel *et al.*, 1990) with minor modifications. Total RNA extracted from equal amounts of biomass (ca. 1 μg chl *a* per lane) was fractionated by electrophoresis on a 1% agarose gel with 1 M formaldehyde. The intensities of ethidium bromide-stained rRNA bands were examined visually to ensure that equal amounts of total RNA were loaded for all samples. The RNA was then transferred onto a charged nylon membrane (Nytran; Schleicher & Schuell) and fixed to the membrane by baking at 80 °C for 2 h in a vacuum oven. All northern blots in this paper were initially hybridized to a *nifH* probe more than 6 months prior to this studv, and the majority of them were published in a recent paper on *nifHDK* gene expression (Chen *et al.*, 1998). Before being reprobed with either a homologous *psbA* probe (684 bp *Hin*dIII-*Pst*I fragment from pORTER) or *psaA* probe (824 bp *Hin*dIII-*Pst*I fragment from pALEALE), the blots were stripped by incubating in stripping solution (0.5× SSC, 0.5% SDS) at 95 °C for 20 min and rinsed twice with boiling distilled water. The completion of the stripping was confirmed by exposure to X-ray film (Sterling X-ray film, Bio World). The blots were prehybridized for 90 min before hybridizing overnight at 42 °C in hybridization solution containing 50% (v/v) formamide, 5× SSC

Table 1. Degenerate PCR primers used to amplify fragments of *psbA* and *psaA* genes from *Trichodesmium* sp. IMS 101, and the sizes of the fragments that were used to generate DNA probes. Y: C/T/U; N: G/C/A/T/U; R: A/G; H: A/C/T/U.

Gene	Forward primer (5′ - 3′)	Reverse primer (5′ - 3′)	Fragment size (bp)
psbA	TAYGTNGGNTGGTTYGG	CCRAARTANCCRTGNGC	684
	(corresponding positions in the D1 protein)		
	29–34	251–256	
psaA	ACNACNTGGATHTCCAA	TGNCCDATNCCCCARTT	824
	(corresponding positions in the PsaA protein)		
	45–51	319–324	

(0.75 M NaCl, 0.075 M sodium citrate), 0.1% SDS and 100 μg/ml sonicated and denatured salmon sperm DNA with homologous *psbA* or *psaA* DNA probes. The probes were labeled with α^{32}P-dCTP using the Decaprime kit (Ambion) according to the manufacturer's suggestions. The blots were then washed twice with 2× SSC for 20 min and 0.1× SSC for 30 min at 42 °C, then rinsed once with 0.2× SSC and exposed to X-ray film.

Southern analysis of psbA *genes*

Southern blotting and hybridization were done basically by following the methods described by Sambrook *et al.* (1989). Chromosomal DNA was cleaved with appropriate restriction enzymes overnight at 37 °C. The DNA was then precipitated by adding 0.1 volume of 3 M sodium acetate and 2 volumes of ethanol, and then incubated overnight at −20 °C. Precipitated DNA was then separated on a 0.7% agarose gel by electrophoresing overnight at 20 V in 1× TBE. The depurination was done by submerging the gel in 0.2 M HCl for 10 min. After the denaturation and neutralization steps, the DNA was transferred onto a nylon membrane (Schleicher & Schuell) by the capillary transfer method using 10× SSC solution for 20 h. After transfer, the DNA was fixed onto a nylon membrane by baking in a vacuum oven for 90 min at 80 °C. The membrane was probed with a homologous *psbA* probe and exposed to X-ray film following the protocols used for the northern hybridization as described above.

Densitometric analysis of northern blots

Northern autoradiograms were scanned into a Pentium processor-powered PC equipped with a HP ScanJet 4C scanner (optical resolution 600 × 600 dpi). The public domain NIH Image software (Scientific Computing Resource Center, NIH) was used to perform densitometric analysis of labeled bands. To minimize artifacts introduced during the quantitative analysis, we only compared samples on the same gel/same batch of gels and plotted them on a relative scale (percentage of maximum value). The scanned graphic images of the autoradiograms were printed on a Tektronix Phaser SDX dye-sublimation photo-realistic printer.

psbA/psaA *expression in cultures grown under different light/dark regimes*

To assess photosystem gene expression in cultures grown under different L/D regimes, *Trichodesmium* sp. IMS 101 cultures were grown for 2–6 weeks under regular culture conditions except that the incubation temperature was 28.5 °C. At the beginning of the experiments, three subcultures were established at the onset of the light period. The L/D subcultures were continuously grown under regular conditions with a 12 h L/12 h D regime; the L/L subcultures were transferred to a continuously illuminated incubator with the same light intensity used for L/D subcultures. The D/D subcultures were placed in flasks wrapped with 2 layers of black electrical tape (Scotch, 3M) and then maintained from a dark incubator. D/D samples were removed in the dark incubator and processed immediately in dim light. The incubation temperature for all subcultures was 28.5 °C. The experiment lasted for more than 60 h, during which samples were taken for RNA assays.

Temperature compensation of rhythm of psbA/psaA *gene expression*

To examine if the photosynthesis gene expression rhythms were temperature-compensated, *Trichodesmium* sp. IMS 101 cultures were incubated at 26.5 °C for 2 weeks after inoculation. The culture was separated into two subculture replicates and incubated at 24 °C, starting one week before the experiment. The temporal variation of *psbA/psaA* transcript abundance was determined, and patterns compared with those of cultures grown at 28.5 °C.

Entrainment of rhythmic psbA/psaA *gene expression to solar L/D cycle by light/dark cues*

To determine if rhythmic photosystem gene expression can be entrained to solar L/D cycles by environmental cues, we initially grew the *Trichodesmium* sp. IMS 101 culture for 3 weeks under regular conditions (12 h L/12 h D, 26.5 °C). The culture was then divided into two subcultures and placed under continuous light for at least 3 complete diel cycles (72 h). Both subcultures were given a 12 h D/12 h L/12 h D sequence as an environmental cue to reset the phase, followed by 30 h continuous illumination, during which various measurements were made to verify if the rhythmic photosystem gene expression was entrained by L/D signals. A phase difference of 90° (6 h) between the two subcultures was imposed during the entraining stage.

Turnover of psbA *and* psaA *transcripts*

In order to determine the patterns of *psbA* and *psaA* transcripts turnover during the day, rifampicin and chloramphenicol (final concentration 50 μg/ml) were added separately at different times of the day to actively growing *Trichodesmium* sp. IMS 101 cultures that were maintained under regular L/D conditions. Samples were taken at short intervals for RNA analysis. Densitometric measurements of relevant bands from the initial sample points, normally within the first hour after the addition of inhibitor, were used to estimate the half-life of transcripts. Data of transcript abundances were subjected to exponential regression analysis to calculate half-lives.

Impact of short-term changes of light conditions on psbA/psaA *transcription and nitrogen fixation*

To examine the impact of short-term changes of light conditions on photosynthesis gene expression, *Trichodesmium* sp. IMS 101 cultures actively growing under regular conditions were split into three subcultures immediately before the experiment, which started at 12:00 (2 h into light period). Subculture L remained under regular light conditions (light intensity 100 μE m^{-2} s^{-1}), while subculture H was transferred to higher light intensity (300 μE m^{-2} s^{-1}), and subculture D was placed in darkness. Each subculture was further divided into two with one of them incubated with the transcription inhibitor rifampicin (50 μg/ml). Samples were taken at short intervals for RNA analysis. Densitometric measurements of relevant bands from the initial sample points, normally within the first hour after the addition of inhibitor, were used to estimate the half-life of transcripts. Samples from different subcultures were also taken every two hours for nitrogen fixation rate measurements.

Results

Characterization of psbA/psaA *genes in* Trichodesmium *sp. IMS 101*

Fragments of *psbA* and *psaA* genes of *Trichodesmium* sp. IMS 101 were obtained by PCR. The degenerate primers used in the PCR were designed on the basis of conserved regions within the protein coding regions, and the resulting fragments were internal gene sequences including part of a open reading frame of each gene (Table 1). The nucleotide sequence of the *Trichodesmium* sp. IMS 101 684 bp *psbA* partial coding region is very similar to *psbA* sequences of *Cyanothece* ATCC 51142, *psbAII/psbAIII* of *Synechocystis* sp. PCC 6803, *psbAI* of *Synechocystis* sp. PCC 6714, *psbAI/II/IV* of *Anabaena* sp. PCC 7120, and *psbAI* of *Synechococcus vulcanus*, with identities ranging from 75% to 85%. The deduced partial amino acid sequence (Dl protein) of *Trichodesmium* sp. IMS 101 *psbA* fragment is also very similar to the D1 proteins of all cyanobacteria studied with identities ranging from 90% to 97%. The similarity of *Trichodesmium psaA* nucleotide (in the range of 70% identical) and the deduced amino acid sequences (ca. 80% identical) (PsaA) to sequences from other cyanobacterial species are significantly lower than is the case for *psbA*/D1.

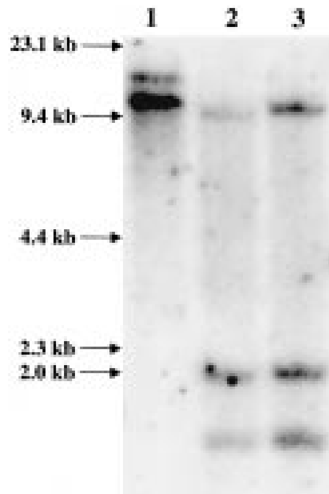

Figure 1. Southem blot analysis of *psbA* genes in *Trichodesmium* sp. IMS 101 genomic DNA hybridized with a 684 bp internal fragment of homologous *psbA* probe. Genomic DNA was digested by *Eco*RI (lane 1), by *Eco*RI and *Eco*RV (lane 2), and by *Eco*RV (lane 3). Fragment sizes were determined by comparison to *Hin*dIII-digested λDNA, and indicated on the left side.

Southern blot analysis of psbA *genes in* Trichodesmium *sp. IMS 101 genomic DNA*

The presence of multiple copies of *psbA* genes has been demonstrated in all cyanobacteria whose photosystem II genes have been investigated (Golden, 1994). In order to characterize *psbA* genes of *Trichodesmium* sp. IMS 101, we conducted Southern blot analysis. When restricted by *Eco*RI, two bands with an estimated size of roughly 12 and 16 kb hybridized to the *psbA* specific probe (Figure 1). Genomic DNA from *Trichodesmium* sp. IMS 101 restricted by *Eco*RV produced three bands at about 10.5, 2.1 and 1.6 kb, all of which hybridized to the *psaA* probe. Three bands hybridizing to the *psbA* probe (ca. 10, 2.11 and 1.6 kb) were detected when *Trichodesmium* genomic DNA was digested by a combination of *Eco*RI and *Eco*RV.

psbA/psaA *gene expression in cultures grown under different light/dark regimes*

The results of time course measurements of *psbA* and *psaA* transcript abundance in *Trichodesmium* sp. IMS 101 cultures grown under different L/D regimes are summarized in Figures 2 and 3.

The results of northern analysis indicated that in L/D cultures, *psbA* transcripts appeared near the onset of the light period, increased gradually before reaching the maximum level 2–5 h before the end of the light period, decreased rapidly upon onset of the dark period, and were virtually undetectable 4 h into the dark period (Figures 2A and 3A). In L/L cultures, while the pattern of cyclic abundance of *psbA* transcripts resembled that of L/D cultures, there appeared to be a daily shift in phase of the peak, suggesting a free-running period close to 30 h, much longer than 24 h in the L/D cultures. The period of net accumulation of the transcripts increased from 7–10 h in the L/D culture to l2–15 h (Figures 2A, 3A, 3B). In addition, though the overall levels of the transcript dropped about 40% after the first diel cycle, the transcripts continued to be present during the subjective dark phases in L/L cultures (Figures 2B, 3B). Although completely shielded from light since the prior dark period, the pattern of *psbA* transcript abundance in D/D cultures was similar to that of the regular L/D culture in the first diel cycle with much lower overall transcript levels; however, no transcripts were detected during the rest of the experiment (Figures 2A, 3C).

The results of northem analysis of *psaA* transcripts indicate that in L/D cultures, although present continuously, *psaA* transcript levels started to increase 4 h after the onset of the light period, reached its peak 1–4 h after the commencement of the dark period, and rapidly decreased before reaching the minimum in the beginning of the light period (Figure 2A, 3A). A similar cyclic pattern was observed for the *psaA* transcripts in L/L cultures; however, the overall abundance of transcripts dropped dramatically after the first diel cycle, to less than 20% of that of day 1, on days 2 and 3 (Figures 2B, 3B). In addition, the daily shift in the phase of the peak was less consistent than that of *psbA* transcripts, especially on day 3 (Figures 2B, 3B). In D/D cultures, *psaA* transcripts continued to decrease before becoming undetectable on days 2 and 3 (Figures 2B, 3C).

Temperature compensation of rhythm of psbA/psaA *gene expression*

Natural populations of *Trichodesmium* inhabit waters that range in temperature from ca. 20 °C to ca. 30 °C. The rhythmic patterns of *psbA* and *psaA* transcripts displayed in *Trichodesmium* sp. IMS 101 cultures grown at 28.5 °C were examined to determine if they were maintained when the cultures were grown at different temperatures within its physiological limit. The time-course analysis of abundance of *psbA* and *psaA* transcripts from the cultures grown at 24 °C is summarized in Figure 4.

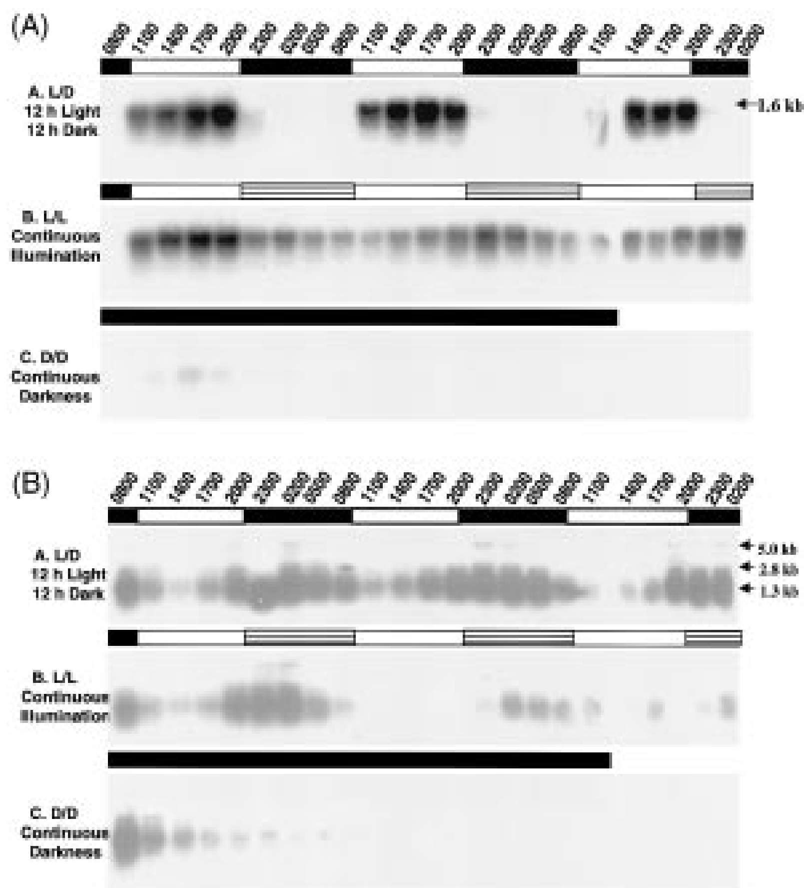

Figure 2. Circadian rhythm of *psbA* (A) and *psaA* (B) transcript abundance in *Trichodesmium* sp. IMS 101 cultures grown under different L/D regimes at 28.5 °C. A. A 1.6 kb *psbA* band hybridized to the homologous probe. B. Three bands hybridized to the homologous probe, which corresponded to 1.3, 2.8, and 5.0 kb transcripts.

Results of northem blots revealed a rhythmic pattern of *psbA* transcript abundance which mimicked that in L/L cultures grown at a higher temperature (28.5 °C) (Figure 4A). The length of the period was maintained at 24 h, and the overall level of the transcript did not change significantly on days 2 and 3 compared with that of the first day (Figure 4B). Northern blots of *psaA* transcripts displayed a pattern that resembled that of the *psaA* transcripts in the L/D culture grown at 28.5 °C (Figure 4). Unlike that of *psaA* in the L/L culture grown at 28.5 °C, the cyclic pattern of *psaA* transcript abundance was well maintained in the L/L culture grown at 24 °C, with a period of 24 h. Furthermore, the precipitous decrease of overall *psaA* transcript levels on days 2 and 3 in the L/L culture at 28.5 °C did not occur in the L/L culture at 24 °C (Figures 2B, 3B, 4).

Entrainment of rhythmic psbA/psaA *gene expression to solar L/D cycle by light/dark cues*

Experiments were carried out to examine whether light/dark signals can reset the phase of the *Trichodesmium* circadian rhythm, i.e., if the rhythmic photosynthesis gene expression can be entrained by environmental cues such as light/dark signals. The results are summarized in Figure 5.

Rhythmic abundance of *psbA* and *psaA* transcripts with a period of about 24 h was observed in two subcultures after they were entrained with separate light/dark cues that were out of phase by 6 h (Figure 5). Characteristics of the diel transcript abundance pattern of both genes in the two subcultures were well maintained, and the 6 h phase difference for transcripts of both genes was generally maintained between the two subcultures (Figure 5).

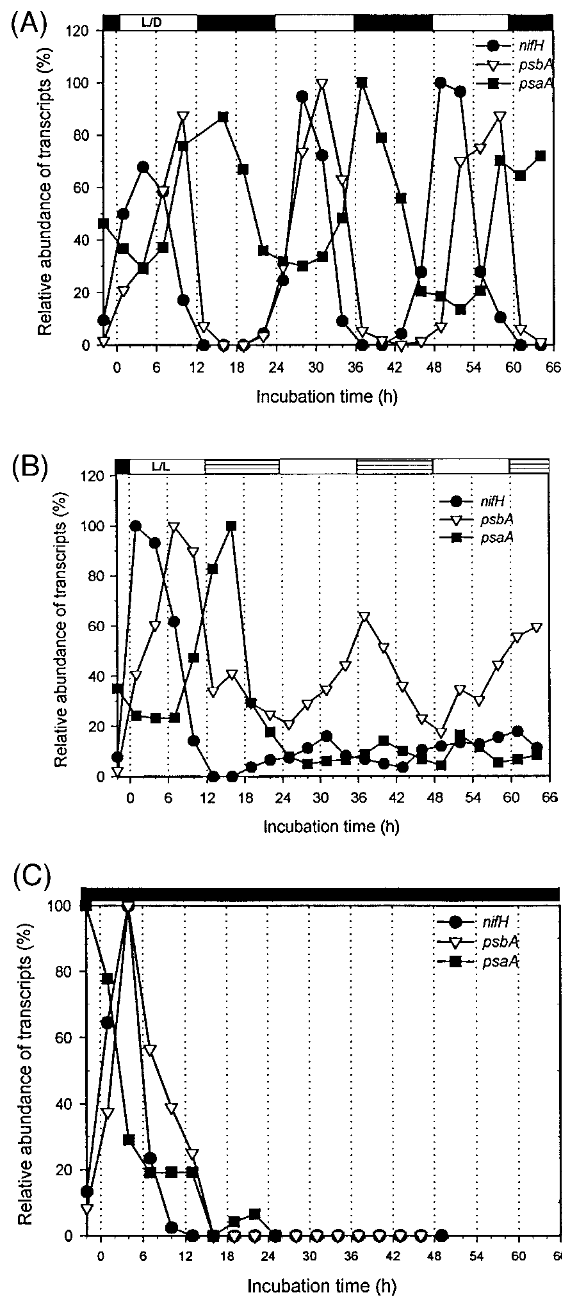

Figure 3. Densitometric analysis of the northern results of *psbA* and *psaA* transcript abundance from Figure 2. A. 12 L/12 h D. B. Constant illunination. C. Constant darkness. Previous results of densitometric analysis of *nifH* transcript abundance (Chen *et al.*, 1998) were replotted for comparison purposes.

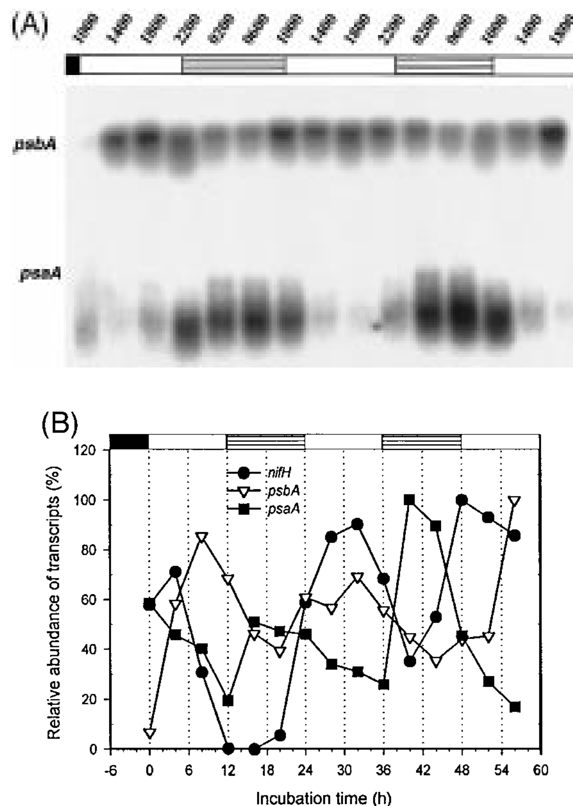

Figure 4. A. Circadian rhythm of *psbA* and *psaA* transcript abundance in *Trichodesmium* sp. IMS 101 cultures grown under constant illumination at 24 °C. Bold boxes indicate subjective dark phase. B. Densitometric analysis of the northern blot results of *psbA* and *psaA* transcript abundance from A. Previous results of densitometric analysis of *nifH* transcript abundance (Chen *et al.*, 1998) were replotted for comparison.

Table 2. Half-lives of *psbA* and *psaA* transcripts in the presence of rifampicin (50 μg/ml) during the light period (10:00–22:00) in the *Trichodesmium* sp. IMS 101 culture. All estimates of half-lives of *psaA* transcript were based on the abundance of the 5.0 kb transcript. R^2 values for all half-life calculations are 0.8 or better. See Figure 6 for corresponding northern analysis of *psbA/psaA* transcripts.

Time when inhibitor added	Half-life (min)	
	psbA	*psaA*
10:00	11	N/A
16:00	41	49
18:00	23	28

Turnover of psbA/psaA *transcripts during the light period*

In order to characterize the regulation of photosystem gene expression at both transcriptional and transla-

Table 3. Half-lives of *psbA* and *psaA* transcripts in the presence of rifampicin upon transfer to different light conditions in *Trichodesmium* sp. IMS 101 culture. Rifampicin (50 μg/ml) was added at 12:00 local time when subcultures were placed under different light conditions. All estimates of half-lives of *psaA* transcript were based on the abundance of 5.0 kb size transcript. R^2 values for all half-life calculations are 0.8 or better. See Figure 7 for corresponding northern analysis of *psbA/psaA* transcripts.

Light conditions	Half-life (min)	
	psbA	*psaA*
L (100 μE m^{-2} s^{-1}, control)	14	20
H (300 μE m^{-2} s^{-1})	18	12
D (darkness)	32	12

tional levels during the diel cycle, the half-lives of *psbA* and *psaA* transcripts were estimated. Results of Northern analysis and estimated half-lives are summarized in Figure 6 and Table 2, respectively. For *psbA* transcripts, the half-life increased substantially from 11 min in the beginning of the light period to 41 min at the mid-light period before decreasing to 23 min in the late light period (Figure 6A, Table 2). The half-life of *psaA* transcripts appeared to decrease from 49 min to 28 min as the light period proceeded (Figure 6, Table 2). At each time point, while *psbA* transcripts disappeared rapidly in cultures treated with rifampicin, they increased dramatically in chloramphenicol-treated cultures (Figure 6). In contrast, *psaA* transcripts decreased in cultures treated with either inhibitor (Figure 6).

Impact of short-term changes of light conditions on psbA *and* psaA *transcription*

The impact of short-term changes of light conditions on photosynthesis gene expression in *Trichodesmium* sp. IMS 101 was examined by analysing changes of *psaA* and *psbA* transcript abundance in response to changing light intensity. Compared with that of the control culture maintained under 100 μE m^{-2} s^{-1}, *psbA* transcripts increased several-fold in the culture transferred to 300 μE m^{-2} s^{-1}, while decreasing slightly in the culture placed in the dark (Figure 7A). In contrast, *psaA* transcript abundance appeared to decrease in the culture shifted to the higher light intensity and increased in the culture shifted to the dark, though changes were rather moderate in both cases (Figure 7B). Results of the rifampicin experiment indicated that the apparent half-life of the *psbA* transcript more than doubled when the culture was transferred from light to dark, compared to little change when the culture was shifted to higher light intensity (Figure 7A, Table 3). On the other hand, an apparent decrease of half-life occurred for the *psaA* transcripts in the cultures shifted to high light and to dark (Figure 7B, Table 3). Compared with the control subculture, the subculture transferred to higher light increased nitrogenase activity by 30%, 3 and 5 h after the change of light conditions. The subculture transferred to darkness lost all nitrogenase activity during the same period of time (Figure 8). Regardless of how light conditions were changed, there was little difference in abundance of *nifHDK* transcripts among the three different subcultures (data not shown).

Discussion

Nitrogen fixation in *Trichodesmium* sp. IMS 101 has been shown to be under the control of a circadian rhythm (Chen *et al.*, 1998). The cyclic patterns of *psbA* and *psaA* genes were examined to confirm that a circadian rhythm controls photosynthesis gene expression at the transcriptional level.

The periods of oscillation of *psbA* and *psaA* gene transcription were within the range of circadian rhythms reported in other organisms (Johnson and Hastings, 1986; Golden *et al.*, 1997). All rhythms were entrained to the daily L/D cycle with a period of about 24 h following a dark/light/dark cue. The imposed phase difference between the two subcultures in the entrainment experiment was maintained for both *psbA* and *psaA* transcription. The results clearly indicated that both *psbA* and *psaA* gene expression rhythms of *Trichodesmium* sp. IMS 101 satisfies all three criteria for a circadian rhythm, i.e., persistence under constant conditions, temperature compensation of the period, and entrainment to the daily L/D cycle by environmental cues (Schweiger *et al.*, 1986; Wilkins, 1992).

Incubation temperature affected rhythmic photosynthesis gene expression in a different way than it affected nitrogenase gene expression (Chen *et al.*, 1998). The amplitude and periodicity of *psbA* and *psaA* gene expression varied as a function of temperature under continuous light conditions. The results clearly indicated that while expression of both genes is controlled by a circadian clock, they are regulated differently by the L/D regime and temperature.

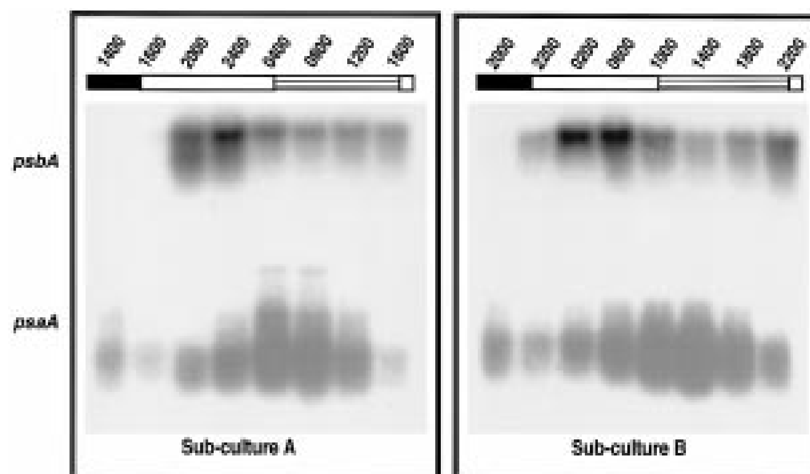

Figure 5. Entrainment of *psbA* and *psaA* transcription rhythm by 12 h dark/12 h light/12 h dark pulses in *Trichodesmium* sp. IMS 101. A 6 h phase difference was imposed during the entrainment. Subculture A: subjective light phase 16:00–04:00, subjective dark phase 04:00–16:00. Subculture B: subjective light phase 22:00–10:00, subjective dark phase 10:00–22:00. Bold boxes indicate subjective dark phase.

Genetic and transcriptional analysis of the *psbA* genes has been conducted in several cyanobacterial species. In all studied cyanobacteria species, *psbA* genes are present as a small gene family with 2–4 copies per genome, often encoding two different forms of the D1 protein (Golden, 1994, 1995). The multiple bands from the restriction digestion of the genomic DNA of *Trichodesmium* sp. IMS 101 indicate that there is probably more than one copy of the *psbA* gene. The *psbA* transcript is much larger than the *psbA* transcripts in most cyanobacteria, but is similar in size to the second form of the *psbAII* transcript in *Synechococcus* sp. PCC 7942 (Bustos *et al.*, 1990). In *Synechococcus* sp. PCC 7942, the *psbAII* open reading frame is also present on a 1.6 kb transcript that initiates 419 bp upstream of the 1.2 kb transcript and carries an additional 342 bp open reading frame of unknown function. It should be pointed out that the *psbA* probe used in this study was derived from an internal fragment of the *Trichodesmium* sp. IMS 101 *psbA* gene, which is most likely to be highly conserved among all *psbA* genes. Therefore, the *psbA* probe should hybridize to all *psbA* genes in the Southern blot or to the *psbA* pool of all transcripts in the northern blots. The *psbA* gene family, as well as their transcription, has yet to be characterized in *Trichodesmium* sp. IMS 101.

Genetic and transcription studies of cyanobacterial PS I genes (*psaA* and *psaB*) have been very limited. In all cyanobacteria studies thus far, the genes are arranged in tandem with *psaA* upstream of *psaB* (Cantrell and Bryant, 1987; Smart, *et al.*, 1991; Nyhus *et al.*, 1992). In the northern blot analysis of *Synechocystis* sp. PCC 6803, both *psaA* and *psaB* probes hybridized to a 5.0 kb transcript as well as a smear of smaller bands, indicating that they are likely co-transcribed. In addition, the *psaA* probe also hybridized to a 2.4 kb transcript, while the *psaB* probe hybridized to a 2.0 kb transcript (Smart *et al.*, 1991). The northern blot analysis of *psaAB* transcription in *Cyanothece* sp. ATCC 51142 revealed a single band, and a smear below the band which hybridized to the *psaAB* probe, and the size of the band increased from 5.2 kb in the culture grown in L/D to 5.9 kb in the culture grown under constant light (Colón-López and Sherman, 1998). The results of northern blots of *psaA* transcription in *Trichodesmium* sp. IMS 101 revealed 3 bands that hybridized to a 824 bp homologous internal *psaA* probe. The 5.0 kb band is likely to be co-transcribed *psaAB* transcripts as is the case in other cyanobacteria, while the 2.8 kb band may be the *psaA* transcript as in *Synechocystis* sp. PCC 6803 (Smart *et al.*, 1991). In this study, the 5.0 kb size band was used to quantify the *psaA* transcript level, which largely agreed with results based on the 2.8 kb band (data not shown).

Transcription of *psbA* in *Trichodesmium* is initiated in the beginning of the light period, peaks near the end of the light period and decreases in the dark period. In contrast, *psbA* transcription normally reaches maximum levels 2–4 h before the midpoint of the light period in the cyanobacteria *Cyanothece* sp. ATCC 51142 (Colón-López and Sherman, 1998),

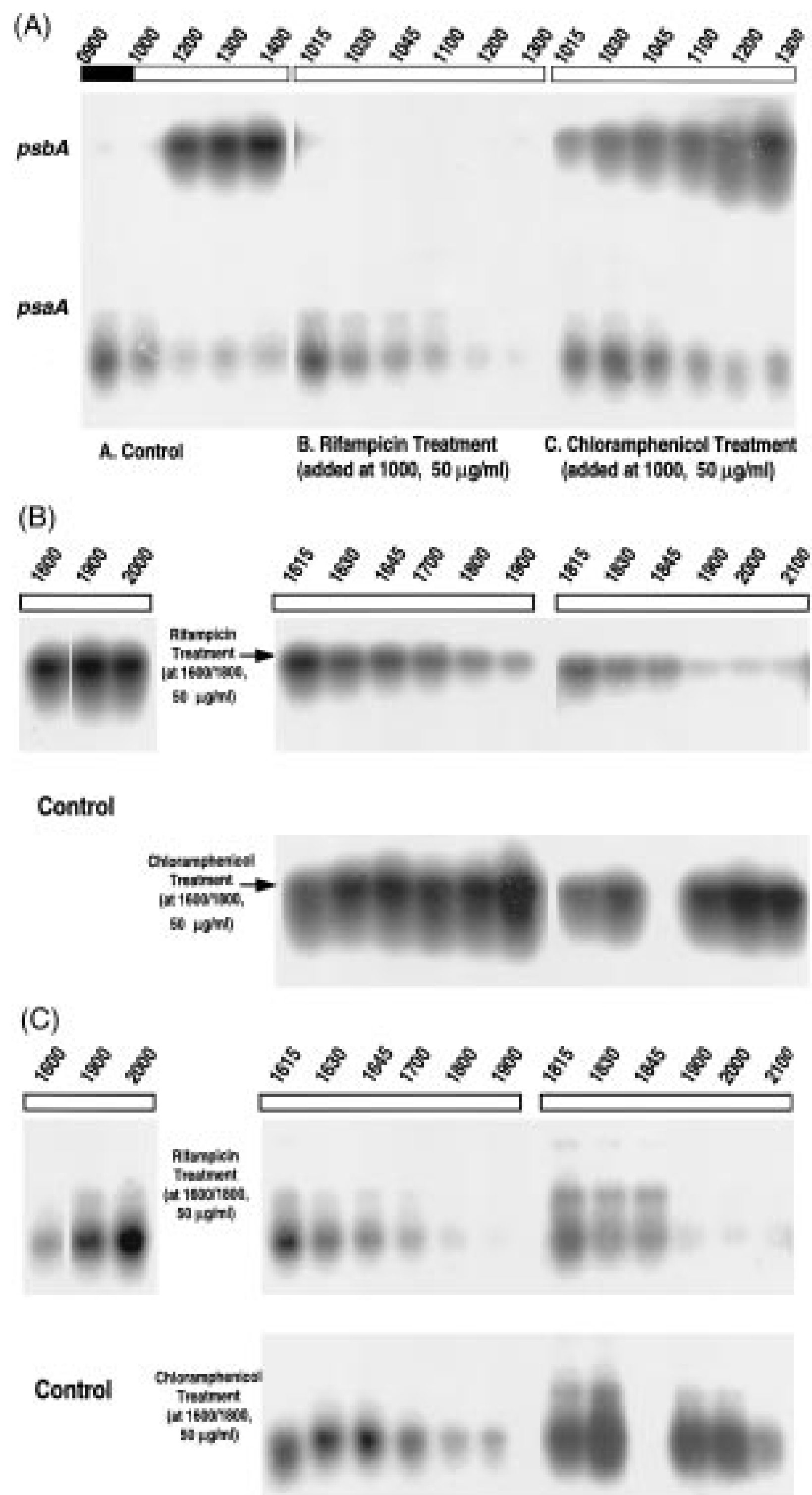

Figure 6. Northern blot assay of *psbA/psaA* transcript turnover in *Trichodesmium* sp. IMS 101 during the light period (10:00–22:00). A. Both *psbA* and *psaA* treatments started at 10:00, the onset of light period. B. *psbA* treatments started at 16:00 and 18:00. C. *psaA* treatments started at 16:00 and 18:00.

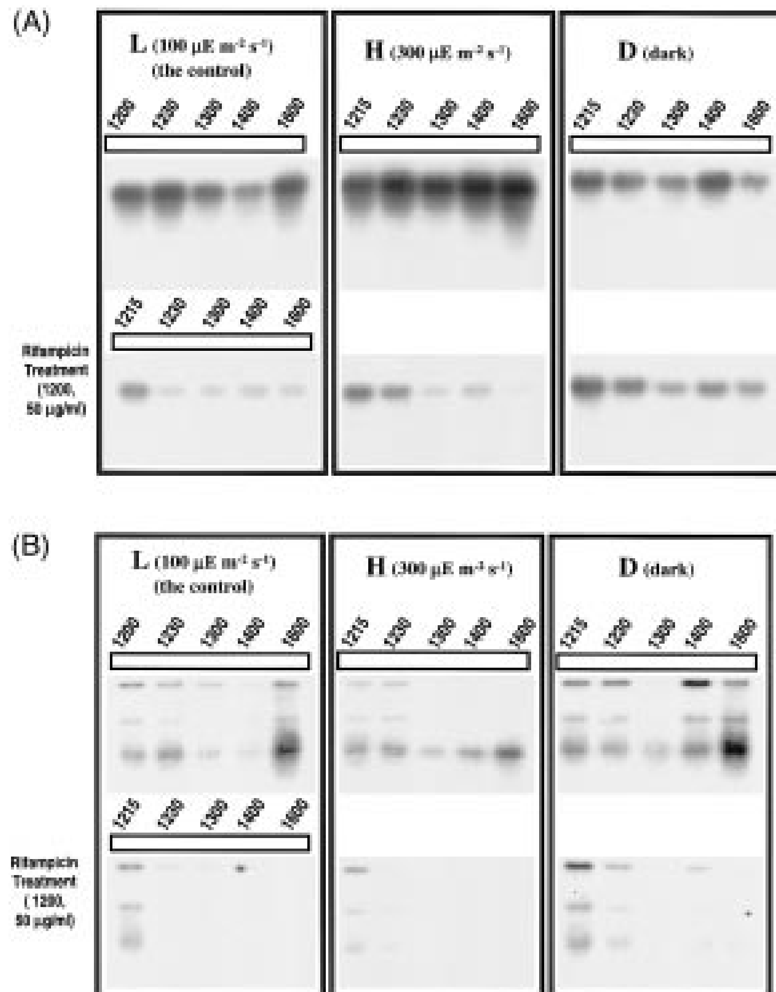

Figure 7. Northern blot assay of time course abundance as well as transcript turnover of *psbA* (A) and *psaA* (B) in *Trichodesmium* sp. IMS cultures subjected to short-term changes of light conditions. Rifampicin (50 μg/ml) was added immediately prior to 12:00, when the changes of light conditions were imposed.

Synechococcus sp. PCC 7942 (Kondo *et al.*, 1993), and in the eukaryotic algae *Chlamydomonas reinhardtii* (Huang *et al.*, 1996) and *Heterosigma carterae* (Doran and Cattolico, 1997). The transcription of *psaA* in *Trichodesmium* sp. IMS 101 started a few hours after the beginning of the light period, reached a maximum early in the dark period, and then decreased over the rest of the dark period. However, *psaA* transcription reached the peak 4 h before a dark period in *Cyanothece* sp. ATCC 51142 (Colón-López and Sherman, 1998), or even earlier in *Chlamydomonas reinhardtii* (Huang *et al.*, 1996). The differences of diel transcription of both *psbA* and *psaA* transcription between *Trichodesmium* and other organisms are very intriguing with respect to the nitrogen fixation gene expression. Under the regular L/D regime, *nifHDK* transcription in *Trichodesmium* sp. IMS 101 is initiated 4–6 h before the onset of the light period, reached the maximum level 1–4 h into the light period and rapidly decreased thereafter (Chen *et al.*, 1998). Therefore, a roughly 90° (6 h) phase difference occurred between *nifHDK* and *psbA* transcription, as well as between *psbA* and *psaA* transcription (Figure 3A). The phase differences among transcription of *nifHDK*, *psbA* and *psaA* were generally maintained in *Trichodesmium* cultures grown under constant light conditions at different temperatures. In the diazotrophic unicellular *Cyanothece* sp. ATCC 51142, the phase differences between photosynthesis genes *psbA*, *psaA* and the nitrogen fixation genes *nifHDK* were suggested to be

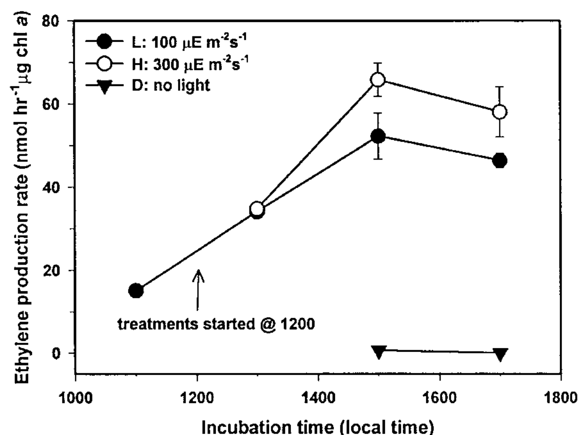

Figure 8. Nitrogenase activity in *Trichodesmium* sp. IMS 101 cultures shifted to different light conditions. Treatment started at 12:00 local time. L culture: under 100 μE m^{-2} s^{-1} as the control; H culture: shifted from 100 to 300 μE m^{-2} s^{-1}; D culture: shifted from 100 μE m^{-2} s^{-1} to darkness. Duplicate samples were taken every 2 h.

the molecular mechanisms that underlie the temporal separation of oxygenic photosynthesis and oxygen-sensitive nitrogen fixation (Colón-López *et al.*, 1997; Colón-López and Sherman, 1998). The same studies also suggested that circadian rhythms make the separation of the two processes possible by maintaining the phase differences between photosynthesis and nitrogen fixation gene expression. The phase differences between the expression of the two groups of genes in *Trichodesmium* are not 'complete' as is the case in *Cyanothece* sp. ATCC 51142 (Colón-López *et al.*, 1997; Colón-López and Sherman, 1998). While it is known that photosynthesis and nitrogen fixation proceed simultaneously in *Trichodesmium* cells during the light period (Carpenter and Price, 1976; Capone *et al.*, 1990; Paerl, 1994), it is still possible that the phase differences between the two types of genes at the transcriptional level may provide *Trichodesmium* with partial temporal separation between the two incompatible processes and therefore minimize the conflict between them. A previous field study of natural populations of *Trichodesmium* indicated that transcription of *nifH* was repressed by oxygen in the afternoon but not in the morning (Zehr *et al.*, 1993). Future studies measuring diel evolution of photosynthetic oxygen as well as photosynthesis gene expression at posttranscriptional levels are needed to fully understand the implication of the partial temporal separation of transcription between nitrogen fixation and photosynthesis genes.

When grown in constant light at 28.5 °C, the overall nitrogen fixation rate of the *Trichodesmium* culture decreased precipitously over time (Chen *et al.*, 1998). During the same time, while *psbA* transcription only decreased by 40% with the rhythm well maintained, both *nifH* and *psaA* transcription decreased by 80% or more with increasingly variable diel patterns. On days 2 and 3, the overall nitrogen fixation rate of the *Trichodesmium* culture grown at 24 °C under constant light conditions was about 40% of that in the first subjective light phase (Chen *et al.*, 1998). During the same period of time, the overall abundance of all three transcripts (*psbA*, *psaA* and *nifM*) did not change substantially, and their rhythms of abundance were well maintained, resulting in the well maintained phase differences among each other. The implication of these results are mainly two-fold. First, expression of photosynthesis genes, especially *psaA*, may somehow be related to nitrogen fixation in *Trichodesmium.* Second, the rigid phase differences between the transcription of each gene may be important for *Trichodesmium* to maintain normal nitrogen fixation activity. The rigid phase differences between *nifHDK*, *psbA* and *psaA* were maintained in the L/D culture as its circadian clock was reset each day. They were also maintained in the L/L culture grown at 24 °C whose period length was about 24 h, but not in the L/L culture grown at 28.5 °C where the period was at least 26 h and had longer and more variable rhythms for all three gene transcripts. This indicates that the circadian rhythm which temporally separates the two incompatible processes of photosynthesis and nitrogen fixation in some cyanobacteria (Mitsui *et al.*, 1986; Stal and Krumbein, 1987; Golden *et al.*, 1997), may also be a pivotal mechanism allowing the two processes to proceed simultaneously in *Trichodesmium.*

The estimated half-lives of *psbA* transcripts varied dramatically during the light period, whereas the estimated half-lives of *psaA* transcripts are much longer. While the stability of *psbA* and *psaA* transcripts may contribute to the diel regulation of expression of both genes, it is yet to be demonstrated that a circadian clock controls the stability of these two gene transcripts over the diel cycle, as has been described for *tufA* transcripts in *Chlamydomonas* (Huang *et al.*, 1996). At all three time points during the light period, *psbA* transcripts in cultures treated with the translational inhibitor chloramphenicol increased to levels higher than those of the control cultures, while during the same time, degradation was also enhanced as evidenced by the much longer smears below the normal

psbA band (Figure 6A, B). In contrast, *psaA* transcripts in chloramphenicol-treated cultures decreased steadily over the same time period (Figure 6A, C). The results suggested the possibility that a specific protein factor may be involved in *psbA* transcript degradation, but not in the degradation of *psaA* or *nifHDK* transcripts (Chen *et al.*, 1998). Involvement of a specific protein factor in degradation of *psbAI* transcripts has been observed in *Synechococcus* sp. PCC 7942 cultures shifted from low to high light intensity, but unlike the protein factor in *Trichodesmium*, *de novo* synthesis of this protein was blocked by rifampicin or chloramphenicol (Kulkarni *et al.*, 1992). The *psaA* transcripts of *Trichodesmium* sp. IMS 101 treated with chloramphenicol degraded faster than those of the cultures treated with the RNA synthesis inhibitor rifampicin, especially for the larger transcripts (5.0 and 2.8 kb) (Figure 6A, C), indicating that protein synthesis, or more specifically, peptide elongation, could be important for the stability of *psaA* transcripts, as may be the case for *psbA* and *nifHDK* transcripts (Chen *et al.*, 1998). It should be pointed out that while the concentrations of inhibitors used in this study were comparable to those mainly used in *Trichodesmium* spp. studies (Zehr *et al.*, 1993; Huang *et al.*, 1996), they were only 10–25% of those used in other cyanobacterial photosynthesis gene expression studies (Lönneborg *et al.*, 1988; Mohamed and Janson, 1991; Tyystjärvi *et al.*, 1996). Therefore, the half-life estimation in this study should be considered as conservative. Nonetheless, the interpretation of the turnover experiment might be complicated by the fact that the inhibitors could have indirect effects on degradative processes.

The short-term change of light conditions in the *Trichodesmium* culture during the early light period had different impacts on *psbA* and *psaA* transcription. The results indicate that the increase in *psbA* transcript levels in the culture placed under higher light intensity was mainly due to increased transcription rather than decreased degradation. An increase of total *psbA* transcripts in response to a shift to higher light intensity has been reported in other cyanobacterial species (Lönneborg *et al.*, 1988; Mohamed and Jansson, 1989; Kulkarni *et al.*, 1992; Constant *et al.*, 1997). The inverse correlation between *psbA* transcription and *psbA* transcript stability observed in the *Trichodesmium* culture shifted to the dark has also been reported in *Synechocystis* sp. PCC 6803 (Mohamed and Jansson, 1991). In this case, however, the *psbA* half-life of the *Synechocystis* culture placed in the dark was much longer (about 7 h), and was suggested to be the result of shut-down of photosynthetic electron transport.

The abundance of *psaA* transcripts decreased slightly in the culture shifted to the higher light intensity while increasing in the culture shifted to the dark. The reduction in apparent half-life of transcripts in both cultures suggested that while the decrease of *psaA* transcripts in the culture shifted to high light may be due to accelerated degradation, the increase in *psaA* transcripts in the culture shifted to the dark may be the result of enhanced transcription of the *psaA* gene (Table 3). Interestingly, in the *Trichodesmium* culture grown under the regular L/D regime, the abundance of *psaA* transcripts started to increase late into the light period and reached its maximum level early into the dark period (Figure 2B). In *Cyanothece* sp. ATCC 51142, while *psaA* transcript abundance peaked late in the light period, the PS I reaction center proteins increased during the dark phase (Colón-López and Sherman, 1998). In addition, the capacity of electron transport is higher in the dark phase than in the light phase in L/D-grown *Cyanothece* sp. ATCC 51142 culture. It has been proposed that cellular bioenergetic needs may result in the enhanced expression of PS I genes in dark (Colón-López and Sherman, 1998). Specifically, the cell emphasizes noncyclic electron flow in the light phase (demands PS II gene expression) but shifts to cyclic electron flow in the dark (demands PS I gene expression) (Colón-López and Sherman, 1998). It is unclear how PS I functions in the dark period. More studies are also needed to characterize the light-dependent regulation of *psbA* and *psaA* gene expression and their impact on bioenergetics and nitrogen fixation in *Trichodesmium* cells.

Nitrogen fixation rates were affected significantly by the short-term change of light conditions. The patterns of *psaA* transcripts differed from those of the *psbA* transcripts and nitrogenase activity (decreased in the higher light intensity but increased in the dark). These results indicate the possibility that nitrogen fixation in *Trichodesmium* is linked to PS II more directly and closely than to PS I. The results also suggest that while previous light conditions determine the diel pattern of *nifHDK* transcription and therefore the long-term nitrogen fixation potential, the *in situ* light conditions could affect the short-term nitrogen fixation rates at the translational or post-translational levels by directly affecting the expression of photosynthesis genes such as *psbA*. With adjustable buoyancy provided by gas vesicles, natural populations of *Trichodesmium* routinely migrate in the water

column (Walsby, 1992). Future studies of photosynthesis gene expression in continuously changing light conditions and their impact on nitrogen fixation in *Trichodesmium* will enhance our understanding of nitrogen fixation in vertically migrating populations of *Trichodesmium* in the ocean environment.

In summary, we have demonstrated that cyclic *psbA* and *psaA* gene expression persists under constant conditions, that the rhythms can be entrained to daily L/D cycles by light/dark cues, and that the rhythms are temperature-compensated between 24 °C and 28.5 °C. We conclude that, as for *nifHDK*, *psbA* and *psaA* gene expression in *Trichodesmium* sp. IMS 101 is controlled by a circadian rhythm. The transcriptional phase differences among *psbA*, *psaA* and *nifHDK*, maintained by the circadian clock, could be important for simultaneous photosynthesis and nitrogen fixation in *Trichodesmium*. Inhibitor experiments and light experiments indicate that the expression of *psbA* and *psaA* genes is regulated differently.

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References

Anderson, B. and Styring, S. 1991. Photosystem II: molecular organization, function and acclimation. Curr. Top. Bioenerg. 16: 1–81.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (Eds.), 1990. Current Protocols in Molecular Biology, Greene Publishing/Wiley-Interscience, New York.

Bergman, B.B., Gallon, J.R., Rai, A.N. and Stal, L.J. 1997. N_2 fixation by non-heterocystous cyanobacteria. FEMS Microbiol. Rev. 19: 139–185.

Bustos, S.A., Schaefer, M.R. and Golden, S.S. 1990. Different and rapid responses of four cyanobacterial *psbA* transcripts to changes in light intensity. J. Bact. 172: 1998–2004.

Cantrell, A. and Bryant, D.A. 1987. Molecular cloning and nucleotide sequence of the *psaA* and *psaB* genes of the cyanobacterium *Synechococcus* sp. PCC 7002. Plant Mol. Biol. 9: 453–468.

Capone, D.G. 1993. Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure. In: P.F. Kemp, B.F. Sherr, E.B. Sherr and J.J. Cole (Eds.), Current Methods in Aquatic Microbiology, Lewis Publishers, New York, pp. 621–631.

Capone, D.G., O'Neil, J.M., Zehr, J. and Carpenter, E.J. 1990. Basis for diel variation in nitrogenase activity in the marine planktonic cyanobacterium *Trichodesmium thiebautii*. Apll. Environ. Microbiol. 56: 3532–3536.

Capone, D.G., Zehr, J.P., Paerl, H.W., Bergman, B. and Carpenter, E. 1997. *Trichodesmium*, a globally significant marine cyanobacterium. Science 276: 1221–1229.

Carpenter, E.J. 1983. Physiology and ecology of marine *Oscillatoria* (*Trichodesmium*). Mar. Biol. Lett. 4: 69–85.

Carpenter, E.J. and Price, C.C. 1976. Marine *Oscillatoria* (*Trichodesmium*): explanation for aerobic nitrogen fixation without heterocysts. Science 19: 1278–1280.

Chen, Y.-B., Zehr, J.P. and Mellon, M.T. 1996. Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS 101 in defined media: evidence for a circadian rhythm. J. Phycol. 32: 916–923.

Chen, Y.-B., Dominic, B., Mellon, M.T. and Zehr, J.P. 1998. Circadian rhythm of nitrogenase gene expression in the filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS 101. J. Bact. 180: 3598–3605.

Colón-López, M.S. and Sherman, L.A. 1998. Transcriptional and translational regulation of photosystem I and II genes in light-dark- and continuous-light-grown cultures of the unicellular cyanobacterium *Cyanothece* sp. strain ATCC 51142. J. Bacteriol. 180: 519–526.

Colón-López, M.S., Sherman, D.M. and Sherman, L.A. 1997. Transcriptional and translational regulation of nitrogenase in light-dark- and continuous-light-grown cultures of the unicellular cyanobacterium *Cyanothece* sp. strain ATCC 51142. J. Bacteriol. 179: 4319–5327.

Constant, S., Perewoska, I., Alfonso, M. and Kirilovsky, D. 1997. Expression of the *psbA* gene during photoinhibition and recovery in *Synechocystis* PCC 6714: inhibition and damage of transcriptional and translational machinery prevent the restoration of photosystem II activity. Plant Mol. Biol. 34: 1–13.

Doran, E. and Cattolico, R.A. 1997. Photoregulation of chloroplast gene transcription in the comophytic alga *Heterosigma carterae*. Plant Physiol. 115: 773–781.

Frederiksson, C. 1996. Nitrogenase localisation reveals cell differentiation in filamentous, non-heterocystous cyanobacteria. Stockholm University.

Gallon, J.R., Jones, D.A. and Page, T.S. 1996. *Trichodesmium*, the paradoxical diazotroph. Algol. Stud. 83: 215–243.

Golbeck, J.H. 1992. Structure and function of photosystem I. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43: 293–324.

Golden, S.S. 1994. Light-responsive gene expression and the biochemistry of the photosystem II reaction center. In: D.A. Bryant (Ed.), The Molecular Biology of Cyanobacteria, Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 693–714.

Golden, S.S. 1995. Light-responsive gene expression in cyanobacteria. J. Bacteriol. 177: 1651–1654.

Golden, S.S., Ishiura, M., Johnson, C.H. and Kondo, T. 1997. Cyanobacterial circadian rhythms. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48: 327–354.

Ho, K.K. and Krogman, D.W. 1982. Photosynthesis. In: N.G. Carr and B.A. Whitton (Eds.), The biology of Cyanobacteria, Blackwell Scientific Publications, Oxford, pp. 191–214.

Huang, S., Kawazoe, R. and Herrin, D.L. 1996. Transcription of *tufA* and other chloroplast-encoded genes is controlled by a circadian clock in *Chlamydomonas*. Proc. Natl. Acad. Sci. USA 93: 993–1000.

Johnson, C.H. and Hastings, J.W. 1986. The elusive mechanism of the circadian clock. Am. Scient. 74: 29–36.

Kondo, T., Strayer, C.A., Kulkarni, R.D., Taylor, W.I.M., Golden, S.S. and Johnson, H. 1993. Circadian rhythms in prokaryotes: luciferase as a reporter of circadian gene expression in cyanobacteria. Proc. Natl. Acad. Sci. USA 90: 5672–5676.

Kulkarni, R.D., Schaefer, M.R. and Golden, S.S. 1992. Transcriptional and posttranscriptional components of *psbA* response to high light intensity in *Synechococcus* sp. strain PCC 7942. J. Bact. 174: 3775–3781.

Lönneborg, A., Kalla, S., Samuelsson, G., Gustafsson, P. and Öquist, G. 1988. Light-regulated expression of the *psbA* transcript in the cyanobacterium *Anacystis nidulans*. FEBS Lett. 240: 110–114.

Mitsui, A., Kumazawa, S., Takahashi, A., Ikemoto, H., Cao, S. and Arai, T. 1986. Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically. Nature 323: 720–722.

Mohamed, A. and Jansson, C. 1989. Influence of light on accumulation of photosynthesis-specific transcripts in the cyanobacterium *Synechocystis* 6803. Plant Mol. Biol. 13: 693–70.

Mohamed, A. and Jansson, C. 1991. Photosynthesis electron transport controls degradation but not reduction of *psbA* transcripts in the cyanobacterium *Synechocystis* 6803. Plant Mol. Biol. 16: 891–897.

Morden, C.W. and Golden, S.S. 1989. *psbA* genes indicate common ancestry of prochlorophytes and chloroplasts. Nature 337: 382–385.

Nyhus, K.J., Ikeuchi, M., Inoue, Y., Whitmarsh, J. and Pakrasi, H.B. 1992. Purification and characterization of photosystem I complex from the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413. J. Biol. Chem. 267: 12489–12495.

Ohki, K. and Fujita, Y. 1992. Light-dependent maintenance of active nitrogenase in the non-heterocystous cyanophyte *Trichodesmium* sp. NIBB 1067. In: N. Murata (Ed.), Research in Photosynthesis, Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 103–106.

Paerl, H.W. 1994. Spatial segregation of CO_2 fixation in *Trichodesmium* spp.: linkage to N_2 fixation potential. J. Phycol. 30: 790–799.

Prufert-Bebout, L., Paerl, H.W. and Lassen, C. 1993. Growth, nitrogen fixation, and spectral attenuation in cultivated *Trichodesmium* species. Appl. Environ. Microbiol. 59: 1367–1375.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (Eds.), 1989. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schweiger, H.G., Hartwig, R. and Schweiger, M. 1986. Cellular aspects of circadian rhythms. J. Cell. Sci. Suppl. 4: 181–200.

Smart, L.B. and Mclntosh, L. 1991. Expression of photosynthesis genes in the cyanobacterium *Synechocystis* sp. PCC 6803: *psaA-psaB* and *psbA* transcripts accumulate in dark-grown cells. Plant Mol. Biol. 17: 959–971.

Stal, L.J. and Krumbein, W.E. 1987. Temporal separation of nitrogen fixation and photosynthesis in the filamentous, non-heterocystous cyanobacterium *Oscillatoria* sp. Arch. Microbiol. 149: 76–80.

Tandeau De Marsac, N. and Houmard, J. 1988. Complementary chromatic adaptation: physiological conditions and action spectra. In: L. Packer and A.N. Glazer (Eds.), Cyanobacteria, Academic Press, San Diego/New York/Tokyo, pp. 318–328.

Tyystjärvi, T., Mulo, P., Mäenpää, P. and Aro, E.-M. 1996. D1 polypeptide degradation may regulate *psbA* gene expression at transcriptional and translational levels in *Synechocystis* sp. PCC 6803. Photosynth. Res. 47: 111–120.

Vermaas, W.J.F. and Ikeuchi, M. 1991. Photosystem II. In: L. Bogorad and I.K. Vasil (Eds.), The Photosynthetic Apparatus: Molecular Biology and Operation, Academic Press, San Diego, CA, pp. 25–111.

Walsby, A.E. (1992. The gas vesicles and buoyancy of *Trichodesmium*. In: E.J. Carpenter, D. Capone and J.G. Rueter (Eds.), Marine Pelagic Cyanobacteria: *Trichodesmium* and Other Diazotrophs, Kluwer, Dordrecht, Netherlands, pp. 141–161.

Wilkins, N.B. 1992. Circadian rhythms: their origin and control. New Phytol. 121: 347–375.

Zehr, J.P. and McReynolds, L.A. 1989. Use of degenerate oligonucleotides for amplification of the *nifH* gene from the marine cyanobacterium *Trichodesmium* spp. Appl. Environ. Microbiol. 55: 2522–2526.

Zehr, J.P., Wyman, M., Miller, V., Duguay, L. and Capone, D.G. 1993. Modification of the Fe protein of nitrogenase in natural populations of *Trichodesmium thiebautii*. Appl. Environ. Microbiol. 59: 669–676.

Zehr, J.P., Dominic, B., Chen, Y.-B., Mellon, M.T. and Meeks, J.C. 1998. Nitrogen fixation in the marine cyanobacteria *Trichodesmium*: a challenging model for ecology and molecular biology. In: G.A. Peschek, W. Loffelhardt and G. Schmetterer (Eds.), Phototrophic Prokaryotes, Plenum Press, New York, pp. 485–500.

Zurawski, G., Bohnert, H.J., Whitefeld, P.R. and Bottomley, W. 1982. Nucleotide sequence of the gene for the Mr 32,000 thylakoid membrane protein from *Spinacea oleracea* and *Nicotiana debneyi* predicts a totally conserved primary translation product of Mr 38,950. Proc. Natl. Acad. Sci. USA 79: 7699–7703.