RESEARCH LETTER

Temporal separation of cell division and diazotrophy in the marine diazotrophic cyanobacterium *Trichodesmium erythraeum* IMS101

Gustaf Sandh, Rehab El-Shehawy, Beatriz Díez & Birgitta Bergman

Department of Botany, Stockholm University, Stockholm, Sweden

Correspondence: Gustaf Sandh, Department of Botany, Stockholm University, S-106 91 Stockholm, Sweden. Tel.: +46 8 163 918; fax: +46 8 165 525; e-mail: sandh@botan.su.se

Present address: Beatriz D´ez, Departament de Biologia Marina i Oceanograa, Institut de Ciències del Mar (CMIMA, CSIC), E-08003 Barcelona, Spain.

Received 24 March 2009; accepted 26 March 2009.
First published online 27 April 2009.

DOI:10.1111/j.1574-6968.2009.01608.x

Editor: Hermann Bothe

Keywords
Trichodesmium; cyanobacteria; cell division; nitrogen fixation; diazocyte differentiation.

Abstract

Examination of the diurnal patterns of basic cellular processes in the marine nonheterocystous diazotrophic cyanobacterium *Trichodesmium* revealed that the division of cells occurred throughout the diurnal cycle, but that it oscillated and peaked at an early stage in the dark period. Transcription of the early cell division gene *ftsZ* and the occurrence of the FtsZ protein showed a similar diurnal rhythmicity that preceded the division of cells. DNA replication (*dnaA* gene transcription) occurred before the transcription of *ftsZ* and *hetR*, the latter encoding the key heterocyst differentiation protein. Transcription of *ftsZ* and *hetR* in turn preceded the development of the nitrogen-fixing diazocytes and *nifH* transcription, and were at the minimum when diazotrophy was at the maximum. The *nif*H gene transcription showed a negative correlation to the circadian clock gene *kaiC*. Together, the data show a temporal separation between cell division and diazotrophy on a diurnal basis.

Introduction

The marine nonheterocystous diazotrophic cyanobacterium *Trichodesmium* is a globally important organism, as it sequesters large quantities of carbon dioxide (via photosynthesis) and atmospheric nitrogen gas (via nitrogen fixation) into the biogeochemical cycles of warm'er oligotrophic oceans (Karl *et al*., 2002). To accomplish this, *Trichodesmium* shows a unique physiological and developmental adaptation to accommodate the nitrogen fixation process while at the same time performing oxygenic photosynthesis. The genus *Trichodesmium* fixes nitrogen in the light period (Saino & Hattori, 1978), although this is the supposed exclusive norm for heterocystous cyanobacteria. Both natural and cultured *Trichodesmium* spp. (including the genus *Katagnymene*; Lundgren *et al*., 2005) synthesize the nitrogen-fixing enzyme nitrogenase in a specific cell type, the diazocyte, a strategy to protect the oxygen-sensitive nitrogenase enzyme complex (see Fredriksson & Bergman, 1997; Berman-Frank *et al*., 2001). The frequency of diazocyte cells varies on a diurnal basis (Fredriksson & Bergman, 1995; Lin *et al*., 1998), in a pattern that positively correlates with the diazotrophic activity in *Trichodesmium*.

Cell division generally precedes cell differentiation and a connection between the processes was recently proposed for a heterocystous cyanobacterium (Sakr *et al*., 2006a). As for *Trichodesmium*, many cyanobacteria, under natural conditions, time their physiological activities to distinct periods during light/dark cycles. The nondiazotrophic unicellular genera *Prochlorococcus* and *Synechococcus* often show a highly synchronized cell division that takes place either late in the light period or at the beginning of the dark period (Jacquet *et al*., 2001; Asato, 2003). A contrasting pattern is seen in the marine unicellular diazotrophic cyanobacterium *Cyanothece* ATCC 51142, where both the cell division and the transcription of the cell division gene *ftsZ* peak early in the light period (Stöckel *et al*., 2008; Toepel *et al*., 2008). To our knowledge, the only filamentous cyanobacterium studied so far is the heterocystous *Anabaena flos-aquae*, which showed a nonsynchronized cell division that was confined to the light period (Lee & Rhee, 1999).

Diurnal patterns in cell division and related molecular regulatory mechanisms are unknown in filamentous, non-heterocystous cyanobacteria. This is also the case for any coupling between cell division and development of diazocytes in the cyanobacterium *Trichodesmium*. As cell division and diazocyte development in *Trichodesmium* may relate to its profound global ecological success in the oligotrophic oceans (Karl *et al.*, 2002) and to its unique behavior as a diazotroph, these processes were examined at the structural and molecular level over the diurnal light/dark cycle.

Materials and methods

Material and growth conditions

Cultures of *Trichodesmium erythraeum* IMS101 were grown in YBCII media (Chen *et al.*, 1996) in 2-L polycarbonate culture flasks (Nalgene) with constant aeration. The cultures were maintained at 25–27 °C with a 12-h/12-h light/dark cycle, with a constant irradiance of 70–80 μmol photons m^{-2} s^{-1}. Growth was monitored by measuring chlorophyll *a* concentrations (Meeks & Castenholz, 1971). A minimum of three biological replicas were used in each subsequent analysis.

Diurnal sampling

Trichodesmium samples were initially collected every 2 h (light period) and 4 h (dark period) over the 24-h interval, while every 2 h in later experiments (light and dark). The samples were placed in sterile 50-mL Falcon tubes. From these, 15 and 30 mL were used for RNA and protein extraction, respectively, and 1 mL for light microscopy (LM) analyses. The cell samples were filtered onto 5 μm (for RNA and protein extractions) or 8 μm (for LM analyses) Nuclepore polycarbonate filters (Whatman). The RNA samples were quickly immersed in RLT buffer (RNeasy kit, Qiagen) and frozen in liquid nitrogen and kept at − 80 °C until further processing. The protein samples were filtered and frozen in liquid nitrogen and stored at − 80 °C.

LM

To increase the contrast when identifying and quantifying cell division in *Trichodesmium* filaments, samples were collected at each sample point (as above) and directly fixed/stained in a 5% (v/v) acidic iodine–potassium iodine solution according to Lugol (Carl Roth). Cell division patterns were examined using bright-field LM (Olympus BX60 microscope, Olympus). Quantifications of the frequency of dividing cells were based on 500–1000 cells per sample. The percentage of diazocytes in the cell population was also counted at each time point ($\geq$1000 cells per sample).

Transmission electron microscopy

Filaments were harvested from an actively growing aerated *Trichodesmium* culture 4 h into the dark period (during a 12-h/12-h light/dark cycle) and were immediately fixed for 2 h in 2.5% (w/v) glutaraldehyde dissolved in YBCII medium. Subsequent dehydration and embedding steps were performed according to Lundgren *et al.* (2001). Ultrathin sections were obtained using an ultramicrotome (2088 Ultrotome V, LKB Bromma, Sweden) and the sections were placed on Cu grids and poststained with lead citrate and uranyl acetate. The sections were viewed in a Zeiss EM 906 transmission electron microscope at 80 kV.

Fluorescence *in situ* immunolocalization

Filaments from *Trichodesmium* were collected by filtration onto an 8-μm Nuclepore membrane filter (Whatman) at mid-day and the filters were submerged in ice-cold absolute ethanol. The cells were stored at − 20 °C until further processing. Samples were next transferred onto glass slides, air dried and incubated in 200-μL drops of 0.5% dimethyl sulfoxide for 15 min. Following 3 × 2 min washes in phosphate-buffered saline (PBS), the samples were incubated for 1 h with a polyclonal rabbit anti-NifH *Rhodospirillum rubrum* antibody, diluted 1 : 100 in PBS. The samples were then washed 3 × 2 min in PBS and the secondary goat anti-rabbit antibody conjugated to a fluorescent marker (Alexa 350, Molecular Probes) diluted 1 : 200 was added. The incubation lasted for 45 min at room temperature. After 3 × 2 min washes in PBS, an antifade reagent (Prolong Gold, Molecular Probes) was added. The blue fluorescent-labeled cells were identified using a Zeiss Axiovert M-200 microscope (Zeiss) equipped with filter set 49 (Zeiss, G 365, FT 395, BP 445/50).

Immunogold localization

Trichodesmium filaments were harvested at mid-day and were fixed in 3% (w/v) freshly made paraformaldehyde for 2 h. The cells were then dehydrated and embedded in LR-White (TAAB Laboratories Equipment), and ultrathin sections were placed on Ni grids according to the protocol described in Lundgren *et al.* (2001). The grids with sections were blocked in PBS containing 10% (w/v) bovine serum albumin for 1 h, followed by incubation in the primary antibody, a polyclonal rabbit anti-NifH *R. rubrum* antibody, diluted 1 : 100 in PBS, at 4 °C overnight. The samples were then washed 3 × 10 min in PBS. The secondary antibody, a polyclonal goat anti-rabbit antibody conjugated to 5 nm colloidal gold (GE Healthcare), was diluted 1 : 20 in PBS. Drops of this solution were placed to cover the sections on the grids and the incubation lasted for 1 h at room temperature. After subsequent washing of the sections with PBS

(2×10 min) and dH_2O (2×10 min), the samples were air dried and viewed as above.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting

Cell-free protein extracts of *Trichodesmium* were prepared by adding 200 μL sample buffer [2% (w/v) SDS, 2.5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, 25 mM Tris-HCl, pH 6.8, and 0.1 tablet mL^{-1} protease inhibitor cocktail; Complete Mini, Roche] to the filtered filaments, followed by boiling for 5 min. The lysate was cleared from cell debris by centrifugation for 10 min at 15 800 ***g*** at 4 °C, and the supernatant was collected. Equal loading was achieved by determining the protein concentrations using a protein assay kit (RC DC, Bio-Rad) before loading. SDS-PAGE was performed using 10% (v/v) (29 : 1) polyacrylamide gels. The proteins were transferred from the gels and immunolabeled according to Klint *et al.* (2007) using a polyclonal rabbit anti-FtsZ *Anabaena* PCC 7120 diluted 1 : 500 in PBS-T and a secondary goat anti-rabbit antibody conjugated

Table 1. Gene-specific primers used for real-time RT-PCR, presented in the 5′ → 3′ mode

Genes	Forward primers	Reverse primers
ftsZ	GACGACGCCGAATTACTCAG	TGCAATTATGAAAGCCTCCTG
dnaA	GGAGGGAGCTTTAACAAGGG	GCCTCTGGTGAAGCCTCTAA
16S rRNA	GCAACTTCGAACGGACTCTTC	AGTGTGGCTGCTCATCCTCT
kaiC	GGGGTACAACCCACATGAAG	AACCCCCACCACACATTTTA
*nifH**	CGGTGGCATTAAGTGTGTTG	ACCTAAACGGACACCACCAG
*hetR**	TGAACCCAAACGGGTTAAAG	GCTTCACTTAGAGGCATCCG

*Designed by El-Shehawy *et al*. (2003).

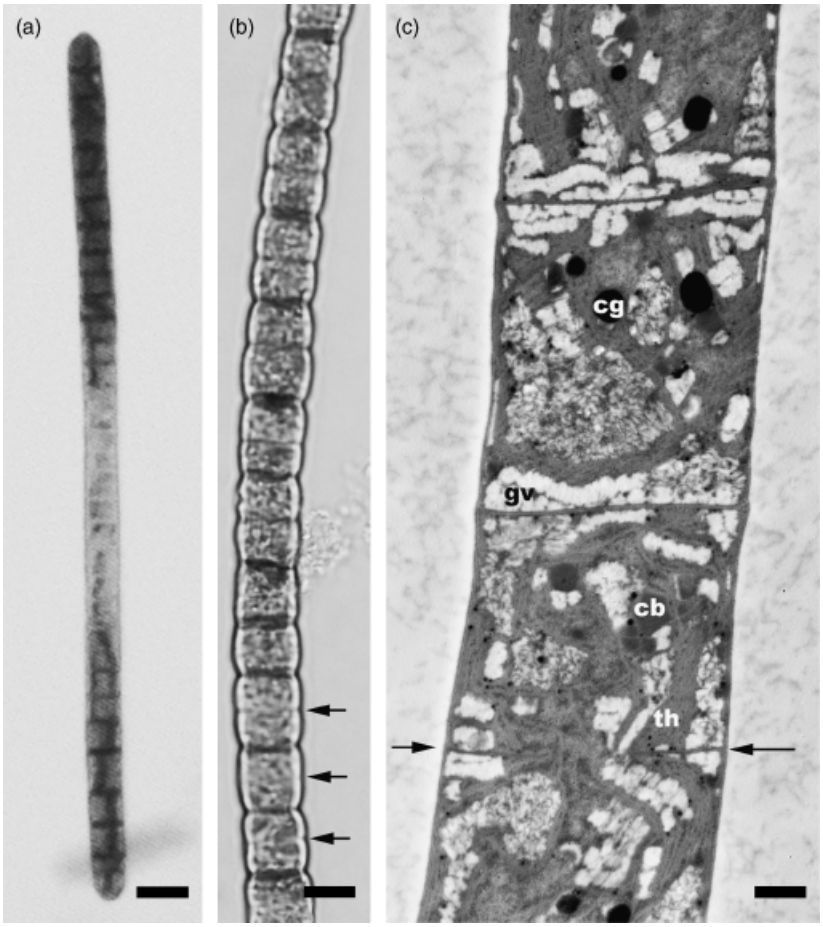

Fig. 1. Characteristics of cell division in *Trichodesmium erythraeum* IMS101. (a) Light micrograph of a filament stained with 5% Lugol's solution. Note the prominent, lighter stained, zone of diazocytes in the center of the filament. Scale bar = 10 μm. (b) Group of dividing cells (arrows) in a filament stained with Lugol's solution. Scale bar = 5 μm. (c) Transmission electron microscopy micrograph of a longitudinally sectioned filament depicting a dividing cell with developing septa (lower part). Also seen are electron-dense cyanophycin granules (cg), medium electron-dense polyhedral carboxysomes (cb), thylakoid membranes (th) and electron-transparent gas vacuoles (gv). Scale bar = 1 μm.

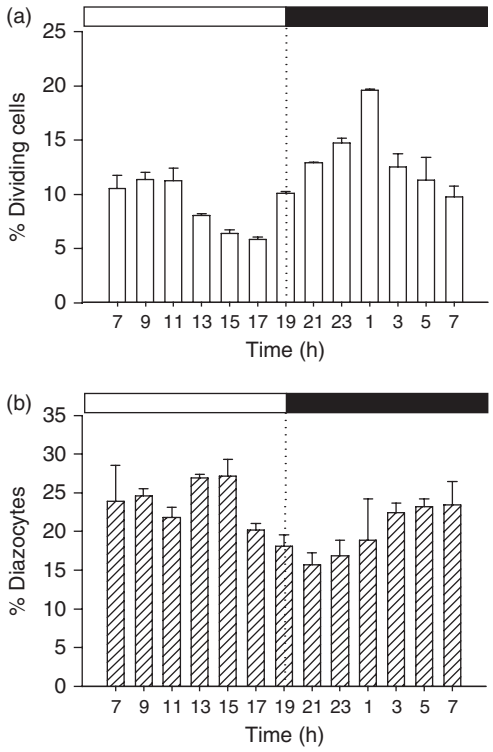

Fig. 2. Diurnal variations in cell division and diazocyte abundance in filaments of *Trichodesmium erythraeum* IMS101. (a) Frequency of dividing cells within filaments. (b) Diurnal variations in diazocyte frequency given as a proportion of the total cell number. The cells were fixed/stained with 5% Lugol's solution and examined using LM.

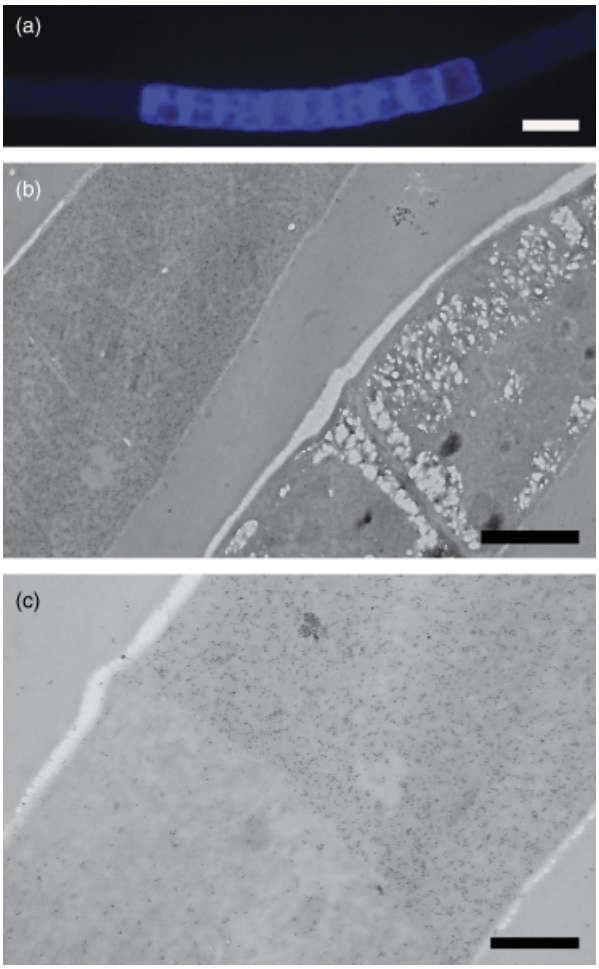

Fig. 3. Immunolocalization of nitrogenase (NifH) in filaments of *Trichodesmium erythraeum* IMS101. (a) Fluorescence *in situ* immunolocalization of NifH in an intact filament of *Trichodesmium*. Scale bar = 10 μm. (b) Longitudinally sectioned *Trichodesmium* filaments illustrating the subcellular ultrastructure of 5-nm immunogold-labeled diazocytes (left) and vegetative cells lacking immunogold label (right). Scale bar = 2 μm. (c) A diazocyte zone border with an immunogold-labeled diazocyte (right) and vegetative cells lacking an immunogold label but resembling the typical diazocyte subcellular ultrastructure. Scale bar = 1 μm.

to horse radish peroxidase (Dako), diluted 1 : 5000 in PBS-T. Detection of the secondary antibody was achieved using a chemiluminescent reagent (ECL Plus, GE Healthcare) according to the manufacturer's instruction and the staining was visualized using a ChemiDoc XRS system (Bio-Rad).

RNA extraction

RNA was isolated from the *Trichodesmium* cells using a commercial extraction kit (RNeasy Mini, Qiagen). After purification, the RNA samples were treated with an additional step of DNAse ($0.17\,U\,\mu L^{-1}$) in $1\times$ DNAse buffer (200 mM Tris-HCl, pH 7.5, 10 mM EDTA and 75 mM $MgCl_2$) at 37 °C for 30 min, followed by 68 °C for 10 min. The samples were then ethanol precipitated and spun down for 10 min at 15 800 ***g*** before being resuspended in 30 μL RNAse-free dH_2O, and stored at −80 °C. The concentration of RNA was measured spectrophotometrically, and real-time reverse transcriptase (RT)-PCR analyses were performed with RNA as a template to test for and avoid possible DNA contamination.

DNA extraction

Genomic DNA was isolated from 200 mL of actively growing *Trichodesmium* culture. After filtration onto a 5-μm Nuclepore polycarbonate filter (Whatman), DNA was extracted using a commercial kit (DNeasy Tissue Kit, Qiagen).

Real-time RT-PCR

Real-time RT-PCR was carried out in a two-step reaction. A cDNA pool was generated from each RNA sample using the iScript™cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's protocol. The real-time-PCR reaction was

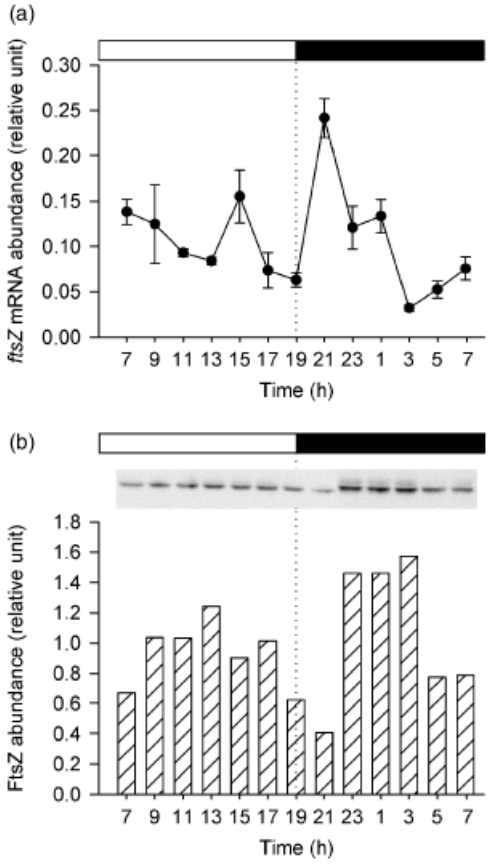

Fig. 4. Diurnal expression profiles of the early cell division gene *ftsZ* in *Trichodesmium erythraeum* IMS101. (a) The relative transcription levels of *ftsZ* over a diurnal cycle normalized to 16S rRNA gene transcription levels. A representative graph is shown, coinciding with data from at least three additional experiments. (b) Immunoblot analysis of the diurnal occurrence of FtsZ, using a polyclonal anti-*Anabaena* 7120 FtsZ antibody. One band with an M_r of c. 50 000 is found throughout the diurnal cycle. The relative abundance of the FtsZ protein is given as bars in the figure below (pixel intensities; IMAGEJ program), with the average FtsZ protein level over the 24-h period set to 1.

then carried out on an iCycler iQ (Bio-Rad) using iQ^{TM}SYBR® Green Supermix (Bio-Rad). The gene-specific PCR primers designed for the second step are presented in Table 1; primers for *hetR* and *nifH* were taken from El-Shehawy *et al.* (2003). The reaction mixture was prepared according to the manufacturer's protocol and the reaction was performed as follows: 95 °C for 5 min, 40 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. Fluorescence levels were monitored at 72 °C and the relative quantification of cDNA was performed using serial dilutions of a known concentration of *Trichodesmium* genomic DNA and normalized to the abundance of 16S rRNA gene. Melt curve analysis and sequencing of the products were used to confirm the specificity of the PCR reactions and the identity of the PCR products (data not shown).

Results

Filaments of *T. erythraeum* IMS101 (hereafter referred to as *Trichodesmium*), grown under controlled (12-h/12-h light/dark cycles) nitrogen-fixing (combined nitrogen-free medium) conditions, showed a doubling time of *c.* 2.4 days. Dividing cells were identified by the following criteria: (1) longer cells (5–10 μm); (2) development of division septa (Fig. 1, arrows); and (3) Lugol staining, being particularly pronounced at the cell walls connecting two vegetative cells (Fig. 1a). Division never took place synchronously in the whole filament, but was restricted to small groups of cells that spread along the filaments.

Cell division in *Trichodesmium* occurred throughout the diurnal cycle, although at varying relative quantitative levels (Fig. 2a). The proportion of dividing cells, out of the total number of cells, varied from 5% to 20%. A period of high cell division frequency was apparent a few hours into the dark period. This was always preceded by a considerable decline in cell division during the later half of the light period (Fig. 2a).

As found earlier, groups of lighter cells (more transparent) were apparent in the *Trichodesmium* filaments when subject to Lugol staining (Fig. 1a; Bryceson & Fay, 1981; El-Shehawy *et al.*, 2003). An almost identical distribution of nitrogenase-containing cells was also detected using *in situ* immunolocalization (Fig. 3a), and similar to the situation in natural populations (Fredriksson & Bergman, 1997), the nitrogenase-containing cells showed distinct subcellular rearrangements, including reduced amounts of gas vacuoles (Fig. 3b and c). The lighter cells after Lugol staining match both the pattern and the subcellular appearance of nitrogenase-containing cells along filaments of *Trichodesmium*, confirming a weaker labeling of diazocytes by Lugol staining (Figs 1a and 3a–c).

The lighter-stained diazocytes constituted on average 22% of the total cell number (Fig. 2b), and the frequency ranged from 15% to 27% on a diurnal basis (Fig. 2b), being the highest in the light and the lowest in the dark period. The transcription of *nifH*, encoding one of the proteins in the nitrogenase complex, was the lowest when the frequency of diazocytes was at minimum (Fig. 5a).

Diurnal transcription levels of *ftsZ* (Fig. 4a) and the occurrence of the corresponding cell division protein, FtsZ (Fig. 4b), were next examined in *Trichodesmium* using real-time RT-PCR and immunoblotting, respectively. The *ftsZ* gene was transcribed throughout the diurnal light/dark cycle and showed a small increase late in the light period and a pronounced peak during the first half of the dark period (Fig. 4a). A polyclonal anti-*Anabaena* PCC 7120 FtsZ

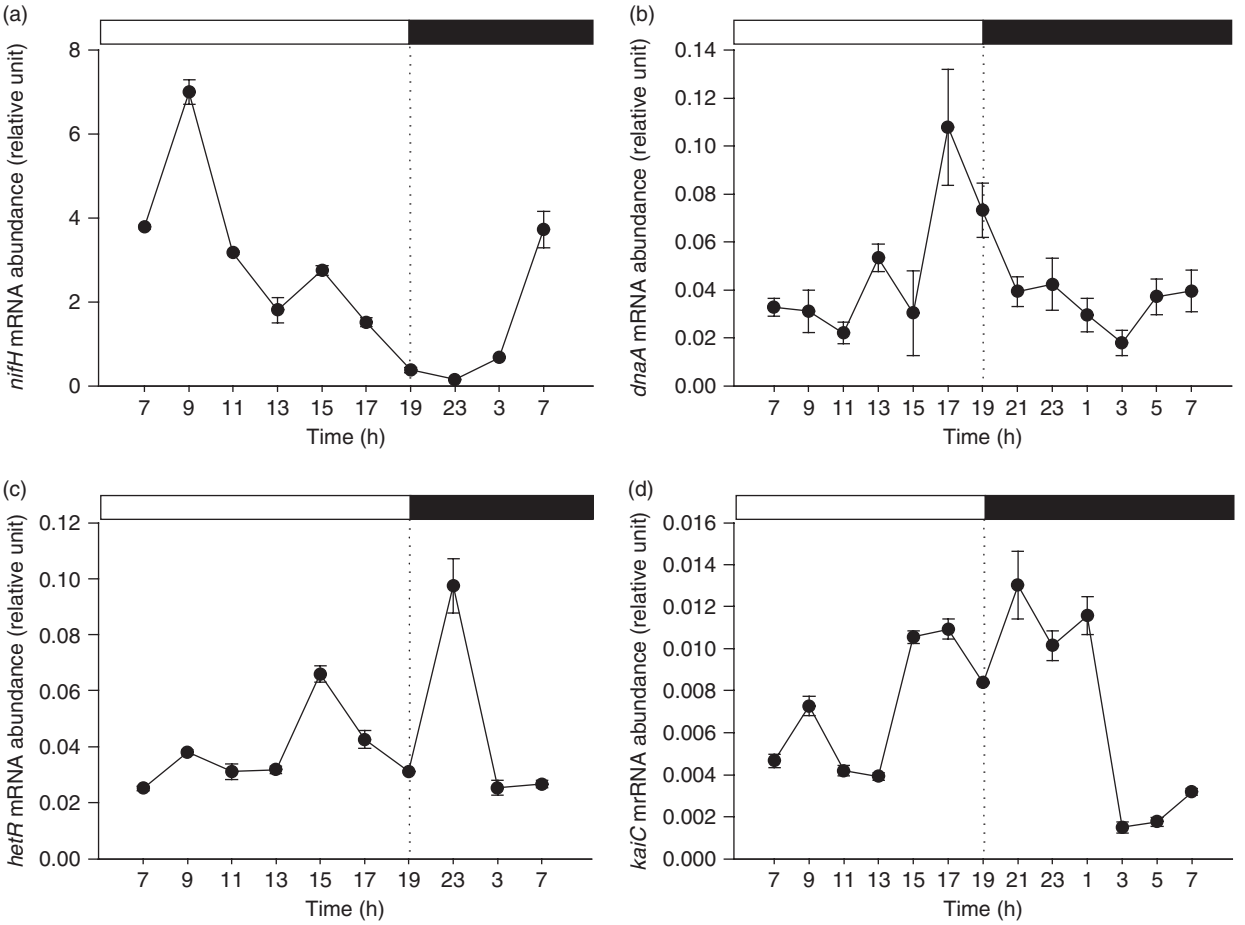

Fig. 5. Diurnal transcription profiles in *Trichodesmium erythraeum* IMS101 of genes involved in nitrogen fixation, DNA replication, cell differentiation and the circadian clock. The relative transcription levels of (a) *nifH*, (b) *dnaA*, (c) *hetR* and (d) *kaiC* are normalized to *16S rRNA* transcription levels. Representative graphs are shown, coinciding with data from at least three additional experiments.

antibody (Klint *et al.*, 2007) revealed fourfold fluctuations in the FtsZ protein levels over the diurnal cycle, with high FtsZ levels being apparent towards the middle of the dark period (Fig. 4b). The synthesis of the FtsZ protein followed the *ftsZ* transcription pattern with an *c.* 2-h delay and correlated positively with the cell division patterns and the appearance of diazocytes (Fig. 2b).

Diurnal transcription patterns of the DNA replication gene *dnaA* were also examined in the same cultures of *Trichodesmium*. As seen in (Fig. 5b) *dnaA* transcription correlated positively with *ftsZ* transcription, and was at the maximum *c.* 2 h before *ftsZ* in replicate experiments. The transcription of *hetR* (Fig. 5c), encoding the master gene for heterocyst differentiation and potentially involved in diazocyte development as suggested earlier (El-Shehawy *et al.*, 2003), peaked together with the transcription of *ftsZ* in *Trichodesmium*. The larger peak also coincided with the development of diazocytes (Fig. 2b).

As the basic components of a cyanobacterial circadian clock (Dong & Golden, 2008) are present in the genome of *Trichodesmium* (http://www.jgi.doe.gov), the transcription of *kaiC*, encoding the master clock protein KaiC, was followed. The transcript levels of the *kaiC* gene were highest in the middle of the dark period, followed by an approximately sixfold decline in expression towards the light period (Fig. 5d). The expression of the *kaiC* gene showed a pattern reciprocal to the transcription of *nifH* (Fig. 5a).

Discussion

As shown previously, both photosynthesis and nitrogen fixation are confined to the light/day period in *Trichodesmium*, while here we show that cell division, including *ftsZ* transcription, FtsZ synthesis and septum formation, is primarily confined to the dark period. It is also clear that patches of cells along the filaments of *Trichodesmium* divide

nonsynchronously, although a small proportion divides throughout the diurnal period. However, most importantly, a distinct time period of enhanced cell divisions was discovered in the dark, and cell division consistently appeared just after DNA replication. Likewise, cell division has also been shown to be coupled to DNA replication in the unicellular cyanobacterium *Microcystis* (Yoshida *et al.*, 2005). Moreover, the nonsynchronized cell division pattern found in the *Trichodesmium* filaments, with small groups of cells dividing at a given time point, may be due to different positions in the cell division cycle of the individual cells, perhaps in combination with nutrient gradients caused by the semi-regular distribution of groups of nitrogen-fixing diazocytes along the filaments.

Compared with cell division, a reciprocal pattern was seen in diazocyte abundance and *nifH* transcription, the latter coinciding with earlier data (Chen *et al.*, 1998; Lin *et al.*, 1998; El-Shehawy *et al.*, 2003). Hence, key physiological processes, such as photosynthesis and nitrogen fixation, take place in the light (our data, Berman-Frank *et al.*, 2001), probably as a consequence of their high energy requirements, while cell division in *Trichodesmium* is confined to the dark hours. A similar but inverted strategy with respect to diazotrophy and cell division is apparent in the unicellular diazotrophic cyanobacteria *Cyanothece* ATCC 51142 (Stöckel *et al.*, 2008; Toepel *et al.*, 2008) and *Gloeothece* PCC 6909 She^{-} (Peschek *et al.*, 1991). In these unicellular diazotrophs, cell division is confined to the light and nitrogen fixation to the dark period.

Cell division is known to be required for the initiation of heterocysts differentiation in *Anabaena* PCC 7120 (Sakr *et al.*, 2006a, b). The timing of cell division in *Trichodesmium* shows a tight sequential coupling to the emergence of diazocytes and *ftsZ* coexpresses with the heterocyst differentiation regulator *hetR*, previously implicated in diazocyte differentiation (El-Shehawy *et al.*, 2003). It therefore appears that cell division may be required for diazocyte development in *Trichodesmium*. As opposed to heterocysts, diazocytes retain their division capacity (Janson *et al.*, 1994; Fredriksson & Bergman, 1997). Therefore, changes in diazocyte abundance (Fig. 2b; Fredriksson & Bergman, 1995; Lin *et al.*, 1998) may originate from either dividing diazocytes and/or from dividing and differentiating vegetative cells.

Many essential metabolic processes are known to be regulated by the circadian clock, composed in cyanobacteria of the core proteins KaiA, KaiB and KaiC (Johnson *et al.*, 2008). The presence of a clustered *kai*ABC operon and several of the input and output regulators of the circadian clock (http://www.jgi.doe.gov), together with the oscillating pattern of *kaiC* presented here, supports the operation of a circadian clock in *Trichodesmium* and strengthens data obtained previously (Chen *et al.*, 1998). Although *kaiC* was expressed at low levels, increased levels of *kaiC* coincided in time with a higher expression of *ftsZ*, *dnaA* and *hetR*, while there seems to be a negative correlation between *kaiC* and *nifH* expression. Further studies will, however, be needed before linking the circadian clock to the regulation of basic cellular processes, for example cell division, DNA replication and cell differentiation, in *Trichodesmium*.

In summary, our data show for the first time that cell division in the globally successful cyanobacterium *Trichodesmium* takes place in the dark period on a diurnal basis, and that there exists a clear diurnal separation between cell division and cell development, on the one hand, and nitrogen fixation and photosynthesis, on the other. This separation may represent an important means to optimize the energy-demanding daytime diazotrophic potential of this marine primary producer.

Acknowledgements

Financial support from the Swedish Research Council (to B.B.) and from K. Wallenberg and A. Wallenberg's Foundation is gratefully acknowledged, as are research stipends from Hierta-Retzius Foundation and Stockholm Marine Research Center (to G.S.). S. Lindwall is acknowledged for excellent experimental assistance.

References

Asato Y (2003) Toward an understanding of cell growth and the cell division cycle of unicellular photoautotrophic cyanobacteria. *Cell Mol Life Sci* **60**: 663–687.

Berman-Frank I, Lundgren P, Chen YB, Küpper H, Kolber Z, Bergman B & Falkowski P (2001) Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium *Trichodesmium*. *Science* **294**: 1534–1537.

Bryceson I & Fay P (1981) Nitrogen fixation in *Oscillatoria* (*Trichodesmium*) *erythraea* in relation to bundle formation and trichome differentiation. *Mar Biol* **61**: 159–166.

Chen YB, Zehr JP & Mellon M (1996) Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS101 in defined media: evidence for a circadian rhythm. *J Phycol* **32**: 916–923.

Chen YB, Dominic B, Mellon MT & Zehr JP (1998) Circadian rhythm of nitrogenase gene expression in the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. strain IMS101. *J Bacteriol* **180**: 3598–3605.

Dong G & Golden SS (2008) How a cyanobacterium tells time. *Curr Opin Microbiol* **11**: 541–546.

El-Shehawy R, Lugomela C, Ernst A & Bergman B (2003) Diurnal expression of *hetR* and diazocyte development in the filamentous non-heterocystous cyanobacterium *Trichodesmium erythraeum*. *Microbiology* **149**: 1139–1146.

Fredriksson C & Bergman B (1995) Nitrogenase varies diurnally in a subset of cells within colonies of the non-heterocystous

cyanobacteria *Trichodesmium* spp. *Microbiology* **141**: 2471–2478.

Fredriksson C & Bergman B (1997) Ultrastructural characterization of cells specialized for nitrogen fixation in a non-heterocystous cyanobacterium, *Trichodesmium* spp. *Protoplasma* **197**: 76–85.

Jacquet S, Partensky F, Lennon JF & Valout D (2001) Diel patterns of growth and division in marine picoplankton in culture. *J Phycol* **37**: 357–369.

Janson S, Carpenter EJ & Bergman B (1994) Compartmentalisation of nitrogenase in a non-heterocystous cyanobacterium: *Trichodesmium* contortum. *FEMS Microbiol Lett* **118**: 9–14.

Johnson CH, Mori T & Xu Y (2008) A cyanobacterial circadian clockwork. *Curr Biol* **18**: R816–R825.

Karl D, Michaels A, Bergman B, Capone D, Carpenter E, Letelier R, Lipschultz F, Paerl H, Sigman D & Stal L (2002) Dinitrogen fixation in the world's oceans. *Biogeochemistry* **57/58**: 47–98.

Klint J, Rasmussen U & Bergman B (2007) FtsZ may have dual roles in the filamentous cyanobacterium *Nostoc/Anabaena* sp. strain PCC 7120. *J Plant Physiol* **164**: 11–18.

Lee DY & Rhee GY (1999) Circadian rhythm in growth and death of *Anabaena flos-aquae* (Cyanobacteria). *J Phycol* **35**: 694–699.

Lin S, Henze S, Lundgren P, Bergman B & Carpenter EJ (1998) Whole-cell immunolocalization of nitrogenase in marine diazotrophic cyanobacteria, *Trichodesmium* spp. *Appl Environ Microb* **64**: 3052–3058.

Lundgren P, Söderbäck E, Carpenter EJ & Bergman B (2001) *Katagnymene*: characterization of a novel marine diazotroph. *J Phycol* **37**: 1052–1062.

Lundgren P, Janson S, Singer A, Jonasson S & Bergman B (2005) Unveiling of novel radiations within the *Trichodesmium* cluster by *hetR* gene sequence analysis. *Appl Environ Microb* **71**: 190–196.

Meeks JC & Castenholz RW (1971) Growth and photosynthesis in an extreme thermophile *Synechococcus lividus* (Cyanophyta). *Arch Mikrobiol* **78**: 25–41.

Peschek GA, Villgrater K & Wastyn M (1991) 'Respiratory protection' of the nitrogenase in dinitrogen-fixing cyanobacteria. *Plant Soil* **137**: 17–24.

Saino T & Hattori A (1978) Diel variation in nitrogen fixation by the marine blue-green alga, *Trichodesmium thiebautii*. *Deep-Sea Res* **25**: 1257–1263.

Sakr S, Jeanjean R, Zhang CC & Arcondeguy T (2006a) Inhibition of cell division suppresses heterocyst development in *Anabaena* sp. strain PCC 7120. *J Bacteriol* **188**: 1396–1404.

Sakr S, Thyssen M, Denis M & Zhang CC (2006b) Relationship among several key cell cycle events in the developmental cyanobacterium *Anabaena* sp. strain PCC 7120. *J Bacteriol* **188**: 5958–5965.

Stöckel J, Welsh EA, Liberton M, Kunnvakkam R, Aurora R & Pakrasi HB (2008) Global transcriptomic analysis of *Cyanothece* 51142 reveals robust diurnal oscillation of central metabolic processes. *P Natl Acad Sci USA* **105**: 6156–6161.

Toepel J, Welsh E, Summerfield TC, Pakrasi HB & Sherman LA (2008) Differential transcriptional analysis of the cyanobacterium *Cyanothece* sp. strain ATCC 51142 during light–dark and continuous-light growth. *J Bacteriol* **190**: 3904–3913.

Yoshida T, Maki M, Okamoto H & Hiroishi S (2005) Coordination of DNA replication and cell division in cyanobacteria *Microcystis aeruginosa*. *FEMS Microbiol Lett* **251**: 149–154.