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Temporal Orchestration of Glycogen Synthase (GlgA) Gene Expression and Glycogen Accumulation in the Oceanic Picoplanktonic Cyanobacterium *Synechococcus* sp. Strain WH8103

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Glycogen is accumulated during the latter half of the diel cycle in *Synechococcus* sp. strain WH8103 following a midday maximum in *glgA* (encoding glycogen synthase) mRNA abundance. This temporal pattern is quite distinct from that of *Prochlorococcus* and may highlight divergent regulatory control of carbon/nitrogen metabolism in these closely related picocyanobacteria.

Picoplanktonic cyanobacteria (*Prochlorococcus* and *Synechococcus*) contribute substantially to primary production in the world's oceans (4, 10, 12). Like other cyanobacteria, they accumulate storage polysaccharides (glycogen) during daylight hours, which provide an important source of carbon and energy to support nocturnal respiratory activity (11). Glycogen is synthesized by the product of *glgA* (glycogen synthase) as a linear molecule of α-1,4-linked glucose subunits which is modified by a branching enzyme to produce the mature reserve polymer.

Inactivation of *glgA* not only abolishes glycogen synthesis in *Synechococcus* strain PCC7942 but also enhances the sensitivity of mutants to salt and oxidative (H_2O_2) stress (23), an intriguing phenotype that suggests additional physiological roles for glycogen in cyanobacteria of potential ecological relevance. Here, we report on the temporal regulation of *glgA* expression and glycogen metabolism in *Synechococcus* sp. strain WH8103 and show that there are marked differences in the temporal patterns of glycogen metabolism in picocyanobacteria that may reflect divergent strategies in the assimilation of carbon and nitrogen over the diel cycle.

A fragment of *Synechococcus* strain WH8103 *glgA* was amplified by PCR using the primer pair GlgA*For*/GlgA*Rev* (Table 1), cloned in pCR2.1-TOPO (Invitrogen, Paisley, United Kingdom), and sequenced bidirectionally (Source BioSciences LifeSciences, London, United Kingdom). Evolutionary analysis of the derived peptide sequence of *glgA* by the maximum likelihood method (24) placed most *Synechococcus* strains and all *Prochlorococcus* strains in a lineage distinct from other cyanobacteria of marine origin (Fig. 1). Picoplanktonic *Synechococcus* formed a monophyletic cluster encompassing subcluster 5.1 strains (5), including WH8103 in the previously designated clade III (3) and also subcluster 5.2, of which WH5701 is the type strain (5).

Prochlorococcus formed a sister group in which high-light (HL) ecotypes were found in a single well-supported cluster, whereas low-light (LL) *Prochlorococcus* ecotypes, which are probably a paraphyletic grouping (30), were less clearly resolved from *Synechococcus* subcluster 5.1 strains. The lack of phylogenetic resolution among *Prochlorococcus* LL ecotypes has been attributed to introgression due to extensive horizontal gene transfer between *Synechococcus* and these organisms (30) and, in particular, the two isolates (MIT9303 and MIT9313) with the largest genomes that cluster most closely with *Synechococcus* (Fig. 1).

To investigate the diel regulation of *glgA*, light-limited continuous cultures of *Synechococcus* sp. strain WH8103 were grown in artificial seawater (ASW) medium (29) at 60 μmol photons m^{-2} s^{-1} and at 25°C in 1-liter water-jacketed vessels (Fig. 2) under a 16-h-light–8-h-dark cycle. Illumination was provided by a "Dusk till Dawn" self-dimming lighting system fitted with T5 Aquablue Plus bulbs (D-D The Aquarium Solution Ltd., Ilford, United Kingdom) programmed to deliver simulated 30-min-long "dawn" and "dusk" periods at the beginning and end of each light cycle. Samples of the culture suspension were obtained synoptically over 1-h periods, preserved with RNAlater (Invitrogen, Paisley, United Kingdom), and subsampled for the determination of the frequency of dividing cells (FDC) (1). The remaining samples were then centrifuged at 16,000 × *g* for 20 min, and the cell pellets were fractionated for the estimation of glycogen concentrations (15, 22), protein (DC protein assay kit; Bio-Rad, Hemel Hempstead, United Kingdom), and the extraction of RNA for cDNA synthesis (28). *glgA* mRNA abundance was determined by quantitative reverse transcription-PCR (qRT-PCR) using the primer pair QGlgF/QGlgR (Table 1) and normalized between samples using the housekeeping gene *rnpB* and QRNP primer pair as described previously (28).

Cell cycle progression was synchronized to the photoperiod in *Synechococcus* sp. WH8103 (Fig. 3), with the peak in FDC appearing at subjective dusk with a similar temporal periodicity to that reported for batch cultures of this strain under a 12-h-light–12-h-dark cycle (6) and natural *Synechococcus* populations from a range of ocean provinces (e.g., see references 1, 25, and 27). Glycogen synthase expression was closely correlated with the division cycle; *glgA* mRNA abundance was at its minimum throughout the night but rose in the early part of the light phase to a midday peak coincident with the daily minimum in FDC. Following the upregulation of *glgA*, glycogen concentrations increased ~3-fold over the second half of the light phase to reach a maximum at "dusk" just prior to the *glgA* transcriptional minimum and the nocturnal decline in FDC due to cell division (Fig. 3).

Although the genes share an evolutionary origin (Fig. 1), the

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TABLE 1 Oligonucleotide primers and PCR conditions used in this study

Primer designation	Sequence (5′–3′)	Product size (bp)	Reaction conditions	Source or reference
GlgA*For*	ATGATHCCNGTNTGGATGCA	677	95°C, 2 min; (94°C, 30 s; 58°C, 30 s; 72°C, 45 s) × 25; 72°C, 10 min	This study
GlgA*Rev*	GGCTCGAANCKNAWNGGCAT	677		
QGlgF	TTCACCATCCACAACCTCAA	205	95°C, 10 min; (94°C, 15 s; 60°C, 30 s; 72°C, 60 s) × 40; increase from 55 to 95°C at 0.2°C s^{-1}	This study
QGlgR	CGAAATTGAGCAAACCATCC	205		
QRNPB F	TGAGGAGAGTGCCACAGAAA	238	95°C, 10 min; (94°C, 15 s; 60°C, 30 s; 72°C, 60 s) × 40; increase from 55 to 95°C at 0.2°C s^{-1}	28
QRNPB R	AAGAGGGTGGGTGGCTATCT	238		

control of *glgA* expression that was observed was markedly different from that of *Prochlorococcus* strain CCMP 1986 (31). In this HL ecotype, *glgA* transcription peaks in concert with *rbcLS* (encoding RubisCO) and other photosynthesis genes during the dark-to-light transition much earlier in the cell cycle (Fig. 3). While comparisons between species grown under different experimental regimes (a 14-h-light–10-h-dark cycle for CCMP 1986 versus a 16-h-light–8-h-dark cycle for *Synechococcus* sp.

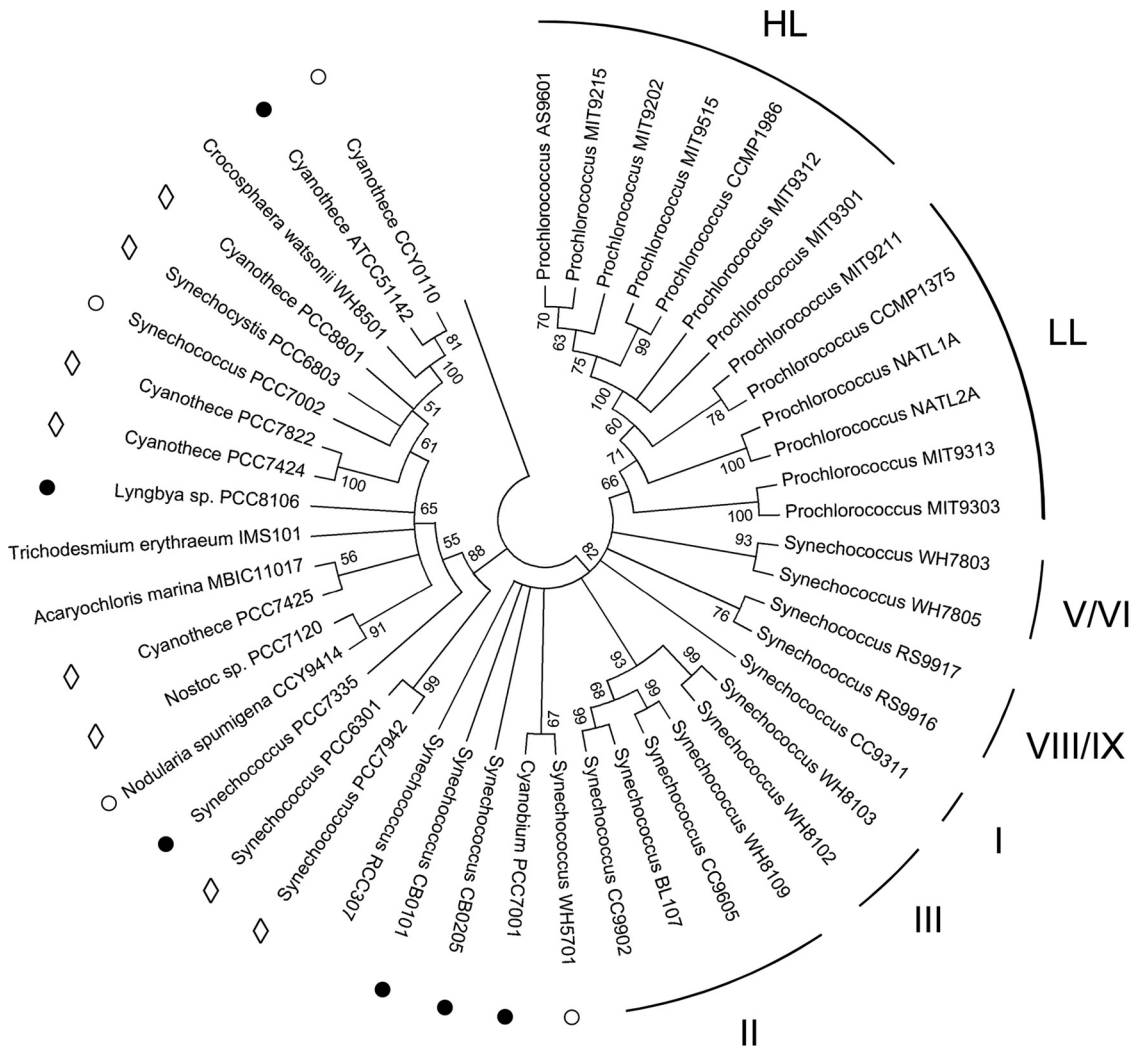

FIG 1 Consensus phylogram (500 bootstrap replicates) of cyanobacterial GlgA rooted with the orthologous peptide sequence from *Streptococcus dysgalactiae* subsp. *equisimilis* GGS124 (GenBank accession number YP_002996437). Sequences were aligned with MUSCLE using the UPGMB clustering method (2), and an evolutionary analysis based on 211 amino acid residues (gaps were removed) was conducted in MEGA5 using the maximum likelihood method based on the Dayhoff matrix model (24). All cyanobacterial taxa were isolated from open marine waters except those indicated with the following symbols: ○, coastal; ●, estuarine/intertidal; ◇, freshwater. *Prochlorococcus* ecotypes previously designated as either high-light (HL) or low-light (LL) adapted (19) are indicated. The Roman numerals correspond to the individual clades of *Synechococcus* spp. proposed by Fuller et al. (3), which are based on phylogenetic analyses of 16S rRNA gene sequences. The overall percentages of trees in which the designated branches received ≥50% support in the bootstrap test are indicated at the respective nodes.

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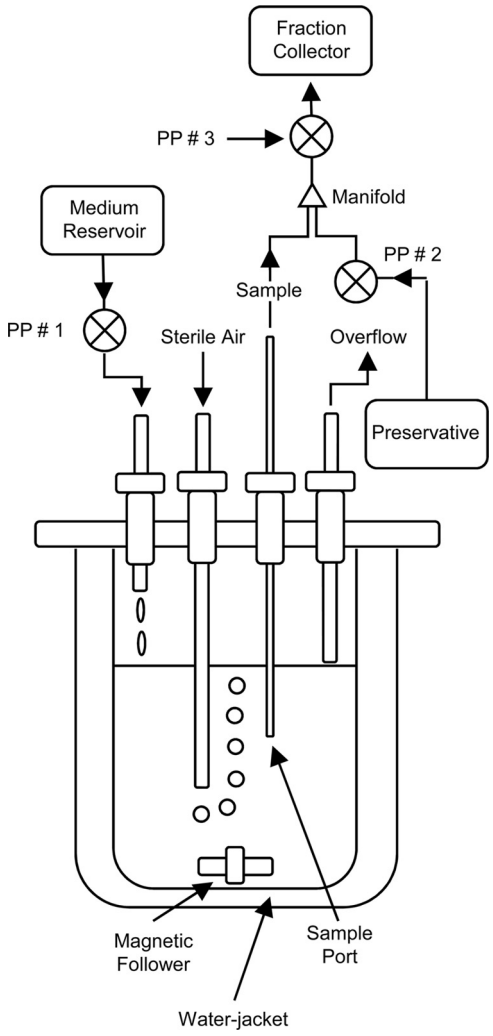

FIG 2 Diagram of the water-jacketed continuous culture apparatus used for the growth of *Synechococcus* sp. strain WH8103. Steady-state cultures were diluted with fresh medium introduced by a peristaltic pump (PP #1) at a flow rate of 14.2 ml hour^{-1} ($\mu = 0.341$ day^{-1}; g = 2.033 days). Preliminary experiments showed that the cultures were light limited; the specific growth rate was higher in more-dilute cultures during batch growth ($\mu = 0.659$ day^{-1}) and increased transiently following an increase in irradiance to 80 μmol photons m^{-2} s^{-1} until steady state was reestablished at a greater cell density. Experimental culture samples were obtained synoptically (in 1-h "bins") by withdrawing cell suspension (~5 ml hour^{-1}) through a sampling port (transit time, ~4 min inlet to outlet) into a three-way manifold in which the sample was combined with >2 volumes of the preservative RNAlater (Invitrogen, Paisley, United Kingdom) and then mixed further by passage through the rotor housing of the peristaltic pump labeled PP #3. The preserved samples were collected at 4°C in RNase-free tubes for 25 to 26 h using a programmable fraction collector.

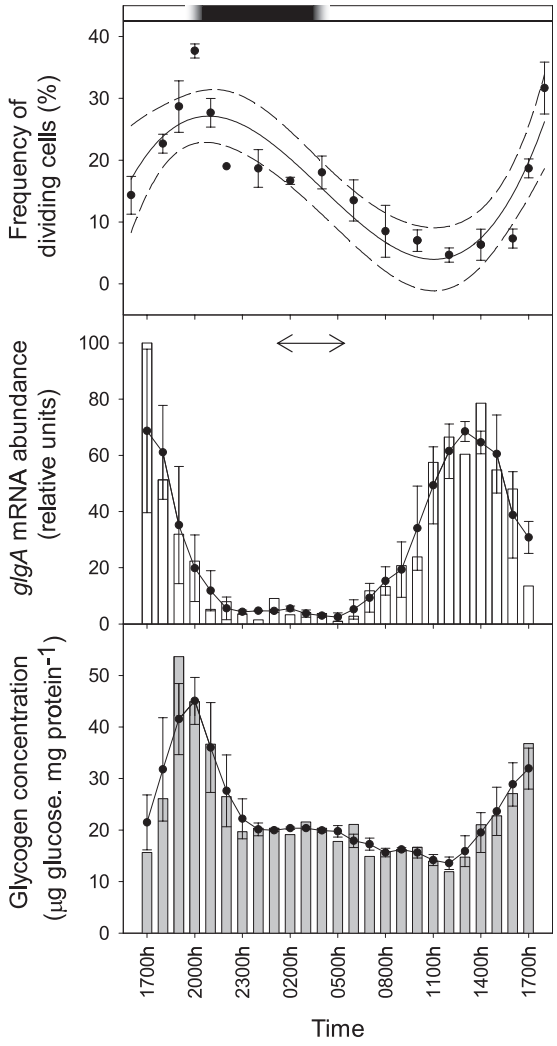

FIG 3 (Top) Diel variability (mean ± 1 standard error [SE]; $n \geq 300$ cells) in the frequency of dividing cells (FDC) (1) in *Synechococcus* sp. strain WH8103 grown in continuous cultures under a light-dark cycle. The data are fitted with a third-order linear regression curve (solid line; $r^2 = 0.766$), and the 95% confidence intervals are shown by the dashed lines. The bar at the top of the figure shows the periodicity of the light (white) and dark (black) phases over the 24-h cycle, while the 30-min-long dusk and dawn intervals are indicated by the regions of shaded gradation. (Middle) Diel variability in *glgA* mRNA abundance (bars) normalized to the housekeeping gene *rnpB* (16, 28) and expressed as a percentage of the daily maximum. The closed circles and solid line show the 3-h central moving average (±1 SE) over the diel cycle. PCR efficiency was 96.45 to 98.92% ($r^2 = 0.997$ to 0.999) and 95.11 to 102.78% ($r^2 = 0.998$) for the QGlgF/QGlgR and QRNPB F/QRNPB R primer pairs, respectively. The temporal maximum in *glgA* mRNA reported for *Prochlorococcus* strain CCMP 1986 (31) is indicated by the double-headed arrow for comparison. (Bottom) Diel variability in glycogen concentration (bars) normalized to protein content. The closed circles and solid line show the 3-h central moving average (±1 SE) over the diel cycle.

WH8103) require caution, these observations suggest some divergence of the temporal patterns of carbon (and nitrogen) metabolism between these organisms.

The diel rhythm of *rbcLS* expression is similar to *Synechococcus* (17, 26, 27), but the temporal regulation of N assimilation in *Prochlorococcus* CCMP 1986 is unusual. Ammonium assimilation genes, including *glnA* (glutamine synthetase [GS]), have late-evening expression maxima, whereas in *Synechococcus* (27), *Synechocystis* strain PCC6803, and *Synechococcus* sp. PCC7942, *glnA* is expressed maximally during mid-light phase (8, 9, 13). N assimilation is strictly light dependent in *Synechocystis* PCC6803, and GS is rapidly inactivated following transfer to darkness (18). In contrast, the 2-oxoglutarate C skeletons required for N assimilation are probably derived from dark glycogen hydrolysis in *Prochlorococcus* CCMP 1986 (31), a metabolic arrangement that mimics the temporal separation of C and N metabolism in some diazotrophic

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cyanobacteria (20, 21). The additional nighttime demand for C skeletons in *Prochlorococcus*, therefore, may underpin why *glgA* is upregulated much earlier in the cell cycle than reported here for *Synechococcus*.

If such divergent metabolic organization is typical, temporal segregation of N uptake and assimilation of potential ecological relevance may occur in those waters where these picocyanobacteria cooccur. It is not clear what might have driven the adoption of distinct carbon/nitrogen assimilation strategies in these organisms, but if glycogen accumulation also enhances oxidative stress resistance in *Prochlorococcus* (23), then diverting fixed C to glycogen throughout the daylight hours may enhance the fitness of high-light ecotypes like CCMP 1986 that are somewhat less resistant to UV radiation than *Synechococcus* (7, 14).

Nucleotide sequence accession number. The DNA sequence of *glgA* from *Synechococcus* sp. strain WH8103 has been deposited in GenBank under the accession number GU808826.

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