

Cell Cycle Regulation by Light in *Prochlorococcus* Strains

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The effect of light on the synchronization of cell cycling was investigated in several strains of the oceanic photosynthetic prokaryote *Prochlorococcus* using flow cytometry. When exposed to a light-dark (L-D) cycle with an irradiance of 25 μmol of quanta $\cdot \text{m}^{-2} \text{s}^{-1}$, the low-light-adapted strain SS 120 appeared to be better synchronized than the high-light-adapted strain PCC 9511. Submitting L-D-entrained populations to shifts (advances or delays) in the timing of the "light on" signal translated to corresponding shifts in the initiation of the S phase, suggesting that this signal is a key parameter for the synchronization of population cell cycles. Cultures that were shifted from an L-D cycle to continuous irradiance showed persistent diel oscillations of flow-cytometric signals (light scatter and chlorophyll fluorescence) but with significantly reduced amplitudes and a phase shift. Complete darkness arrested most of the cells in the G₁ phase of the cell cycle, indicating that light is required to trigger the initiation of DNA replication and cell division. However, some cells also arrested in the S phase, suggesting that cell cycle controls in *Prochlorococcus* spp. are not as strict as in marine *Synechococcus* spp. Shifting *Prochlorococcus* cells from low to high irradiance translated quasi-instantaneously into an increase of cells in both the S and G₂ phases of the cell cycle and then into faster growth, whereas the inverse shift induced rapid slowing of the population growth rate. These data suggest a close coupling between irradiance levels and cell cycling in *Prochlorococcus* spp.

The marine oxyphotobacteria of the genus *Prochlorococcus* are the smallest phytoplanktonic organisms known to date (7, 26). They are widely distributed both latitudinally (over a 40°S to 45°N band) and vertically (over the whole euphotic zone), with maximum concentrations exceeding 2×10^5 cells ml^{-1} . Since the discovery of this genus and its description by Chisholm and coauthors (7), numerous field studies have been performed to describe the structure of picoplanktonic communities, and these studies have revealed the significant role of *Prochlorococcus* spp. in biomass and production of oligotrophic areas such as those of the central North Pacific Ocean or the northern Atlantic (for a review, see reference 25).

This genus presents some remarkable characteristics in terms of light acclimation (18, 20, 24). Two ecotypes, distinguishable both by their divinyl-chlorophyll (-Chl) *b* to -Chl *a* ratio and their genetic signatures, can be discriminated in the field and have been brought into the lab and maintained in culture (19). These two ecotypes correspond to populations adapted to either dim or bright light, e.g., to irradiance levels encountered in the field at greater depths or in surface waters, respectively. The molecular bases of the physiological differences between low- and high-light-adapted strains are beginning to be unveiled (e.g., see reference 9). Among the strains described to date, *Prochlorococcus* SS 120 and MED 4 are good examples of these two respective ecotypes (or possibly subspecies [28]) and have been the subject of several comparative studies in culture (18, 24).

Although the distribution of *Prochlorococcus* spp. depends on a variety of physical and chemical factors, such as temperature or stability of the water column, and on biological con-

trols, such as grazing and probably viral lysis (see reference 26 and references therein), light is clearly one of the most important factors (19, 38). Besides the vertical-light gradient, the alternation of day and night has a major effect on the short-term population dynamics of *Prochlorococcus* populations. Recent studies have shown that cell division of these populations is strongly synchronized by the natural light-dark (L-D) cycle, both in culture (29) and in the field (29, 36). In most cases, the main DNA replication burst (indicated by the timing of the peak of S cells) occurs in late afternoon or at the L-D transition, and division is completed during the early part of the night. Whether these diel variations may simply be explained by a direct control of light over a defined phase of the cell cycle (30) or by a circadian clock (31) is not clear. The latter hypothesis has been clearly demonstrated for *Synechococcus* spp. (31), and its genetic basis has been uncovered (10).

In the present study, we examined the synchronization processes in *Prochlorococcus* spp. with a special emphasis on the factors that could be involved in setting up synchrony (onset of light and irradiance level). For this purpose, we investigated the effect on cell cycle and optical properties of (i) an L-D cycle consisting of 12 h of light and 12 h of dark (12 h-12 h L-D cycle) with a fixed irradiance during the photoperiod, (ii) continuous darkness or constant light following L-D entrainment, and (iii) changes in the timing of the "light on" signal for populations entrained by an L-D cycle. We have also looked at the dynamic response of the *Prochlorococcus* cell cycle after shifting cells from low light (LL) to high light (HL) and conversely. All analyses were performed using flow cytometry.

MATERIALS AND METHODS

Cultures. The LL-adapted type strain *Prochlorococcus marinus* SS 120 (also called CCMP [Center for Culture of Marine Phytoplankton, Bigelow, Maine] 1375 or RCC [Roscoff Culture Collection, Roscoff, France] 156) and the two HL-adapted strains PCC (Pasteur Culture Collection, Paris, France) 9511 (RCC 168) and MED 4 (also called CCMP 1378 or RCC 153) were used in this study.

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The latter two strains have very similar genetic and pigment signatures (28). PCC 9511 offers the advantage of being bacteria free, which under certain conditions such as HL facilitates cell cycle analyses. Thus, strain PCC 9511 was used preferentially to MED 4 (the strain we chose initially) in two experiments (see below).

Growth conditions. Batch *Prochlorococcus* cultures were grown in the seawater-based medium PCR-S11 (26, 28). Cultures were maintained in 500-ml polyethylene Nalgene flasks (Bioblock, Illkirch, France) under blue light provided by daylight fluorescent TLD 18W/827, Philips bulbs wrapped with "moonlight blue" filters (Lee Filters, Panavision, France). Light intensity was measured inside a culture flask filled with filtered seawater, using an LI-1000 quantum sensor (Li-Cor Ltd., Lincoln, Nebr.) equipped with a 4 π spherical sensor. Cultures were placed in a temperature-controlled room, and the average temperature was 20 \pm 2°C for all experiments.

Experimental conditions. The first experiment, done on both strains PCC 9511 and SS 120, consisted of testing the effect of a 12 h-12 h L-D cycle with a constant irradiance of 25 μmol of quanta \cdot m $^{-2}$ s $^{-1}$ during the light period. A second set of experiments, performed with MED 4, consisted of changing the timing of the light-on signal (i.e., by advancing or delaying light bulb switching by 4 h) of cultures previously acclimated to an L-D cycle. The third set of experiments consisted of shifting L-D-entrained cells to either darkness (experiment done with strain MED 4) or constant light (experiment done with strain PCC 9511). For the latter experiment, two different levels of continuous irradiance were tested. One culture was kept under 25 μmol of quanta \cdot m $^{-2}$ s $^{-1}$, whereas a duplicate culture was wrapped with a neutral filter (Lee), reducing the light irradiance to 10 μmol of quanta \cdot m $^{-2}$ s $^{-1}$. The last experiments (done with MED 4) consisted of shifting cultures preacclimated to grow under continuous LL (8 μmol of quanta \cdot m $^{-2}$ s $^{-1}$) or HL (57 μmol of quanta \cdot m $^{-2}$ s $^{-1}$) to the reverse condition. Duplicate cultures for each condition were left at the initial irradiance (LL and HL controls). Data on photosynthesis and photosynthetic pigments from this last set of experiments have been reported elsewhere (4). For all experiments, populations were acclimated for at least 2 weeks, during which cultures were periodically transferred into fresh medium in order to maintain them at an exponential growth rate.

Culture sampling. For all experiments except those involving light shifts, cultures were sampled during the exponential-growth phase either once or twice per hour. Sampling was performed automatically using a remotely controlled peristaltic pump (Masterflex; Bioblock) and a custom-designed fraction collector, modified from the description by Jacquet and coauthors (11). Samples were kept until analysis or fixation in a large Plexiglas tank filled with circulating water at 4°C coming from a Minichiller system (Bioblock). Storage at 4°C for up to 10 h was previously shown to result in minimal effects on the parameters measured by flow cytometry, including cell cycle (11). During the light shift experiments, cultures were sampled by hand over approximately 4 days at short time intervals for the first 12 h and then at longer intervals for the rest of the experiment (see below).

Sample processing. Samples were divided into two aliquots, one for the analysis of flow-cytometric cell parameters (i.e., cell number, light scatter, and Chl fluorescence) and another one for cell cycle analysis. The first aliquot was analyzed fresh (i.e., with no preservatives), after dilution with filtered (0.2- μm pore size) seawater to avoid coincidence problems due to excessive count rates. Efforts were made to reduce the interval between sampling and analysis for samples collected at night (this delay never exceeded 8 h). The second aliquot was fixed for 15 min with glutaraldehyde (0.25% final concentration), frozen in liquid nitrogen, and stored at -80°C for later cell cycle analysis. Prior to analysis, this aliquot was thawed and incubated at 37°C for 1 h in the presence of a 0.1-g liter $^{-1}$ mixture of RNase A and B (R-4875 and R-5750, 1:1 [wt/wt]; Sigma, Saint-Quentin Fallavier, France). After dilution using 0.2- μm -pore-size filtered seawater, samples were immediately stained with SYBR Green I (1/10,000 final concentration) for at least 10 min (16). For light shift experiments, samples for DNA analysis were fixed with paraformaldehyde (0.5% final concentration), frozen in liquid nitrogen, and stored at -80°C until further analysis. Samples were thawed at room temperature and stained with 1 μg of Hoechst 33342 (Sigma) ml $^{-1}$ for 30 min.

Flow-cytometric analysis. Most samples were analyzed with a FACSort flow cytometer (Becton Dickinson, San Jose, Calif.) with 488-nm excitation. We recorded the right-angle light scatter (RALS), which is related to the cell size and refractive index of the cells (21), and two fluorescence signals referred to as "green" (530 \pm 15 nm) and "red" (>630 nm) that are related to the DNA and Chl contents of the cells, respectively. For analyses of fresh samples, 0.2- μm -pore-size-filtered seawater was used as the sheath fluid. Cell parameters were normalized to 0.95- μm fluorescent beads (Polysciences, Warrington, Pa.). Acquisition was performed at a high rate (90 to 100 μl min $^{-1}$) for fresh samples and

TABLE 1. Growth of two *Prochlorococcus* strains as measured during fixed L-D cycle.

Growth parameters ^a	Values for strain	
	PCC 9511	SS 120
T_S (h)	4	5.3
T_{G_2} (h)	4	4.7
Δt (h)	4	5
μ_{CC} (day $^{-1}$)	0.48	0.57
T_g (h)	35	29
S_R (h)	0.23	0.34

^a T_S , estimated duration of S phase; T_{G_2} , estimated duration of G_2 phase; Δt , time between peaks of cells in S and G_2 phases; S_R , estimated rate of synchronization from the ratio $(T_S + T_{G_2})/(T_{G_1} + T_S + T_{G_2})$.

at a medium rate (25 to 30 μl min $^{-1}$) for cell cycle analysis, since lower speed allows a better discrimination between the different cell cycle phases. For the light shift experiments, all measurements were made with an EPICS V flow cytometer (Coulter) equipped with a 5-W Argon laser (Coherent). Laser emission was set at 488 nm for analyses on unstained samples and at 353 to 357 nm for Hoechst-stained cells. Optical setups have been detailed previously (35).

Data analysis. Data were collected as listmode files and then analyzed on a computer using the custom-designed freeware CYTOWIN (34; available through <http://www.sb-roscoff.fr/Phyto/cyto.html#cytowin>). Cell cycle analyses were performed using MultiCYCLE (P. S. Rabinovitch, Phoenix Flow Systems, San Diego, Calif.).

Specific growth rate. Division rate (μ_{CC}) was estimated from cell cycle data using the equation of Carpenter & Chang (6):

$$\mu_{S+G_2} = \frac{\sum_{i=1}^n \ln[1 + f_S(t_i) + f_{G_2}(t_i)]}{n + (T_S + T_{G_2})} \times 24 \quad (1)$$

where μ_{S+G_2} is an estimate of the division rate μ_{CC} (day $^{-1}$), n is the number of samples collected at fixed intervals during one subjective day, $T_S + T_{G_2}$ (hours) is the sum of the duration of the S and G_2 phases, computed as twice the delay (Δt [Table 1]) between the peaks of cells in these phases [$2(T_{G_2\text{max}} - T_{S\text{max}})$], and $f_S(t_i)$ and $f_{G_2}(t_i)$ are the fractions of cells in S and G_2 phases at time t_i . Average generation time (hours [Table 1]) was calculated as follows:

$$T_g = 24 \times (\ln 2 / \mu_{CC}) \quad (2)$$

RESULTS

Effect of the L-D cycle. Figure 1 shows the variations of flow-cytometric parameters RALS and Chl fluorescence (A and B) and of the percentage of cells in the S (C) and G_2 (D) phases of the cell cycle for both of the *Prochlorococcus* ecotypes (PCC 9511 and SS 120) grown under a 12 h-12 h L-D cycle. This culture condition led to clear diel patterns for all cellular parameters measured. Maximum relative values for RALS and Chl fluorescence were recorded around the L-D transition, whereas the minimum values occurred at the onset of light. The mean RALS and Chl fluorescence of SS 120 cells were significantly higher than those of PCC 9511 cells (Table 2), as reported previously for SS 120 and MED 4 (24), and the range of variation for these parameters was slightly larger in SS 120 than in PCC 9511 (Table 2). The RALS of SS 120 began to increase 2 to 3 h after the light was switched on, whereas there was no delay recorded for PCC 9511. No photoquenching was recorded at this irradiance for either strain. The cell cycle, characterized by a discrete DNA synthesis S phase surrounded by two well-defined G_1 and G_2 phases, was well phased to the L-D cycle, but data revealed some differences between the two strains. First, the percentages of cells in both S and G_2 phases

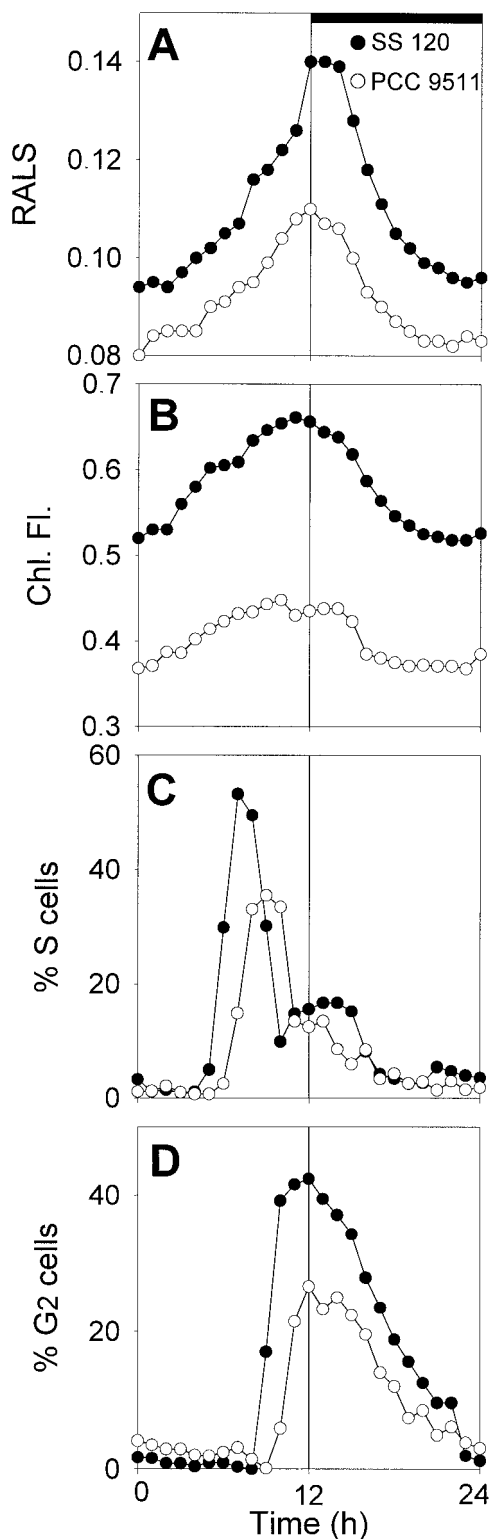


FIG. 1. Effect of a 12 h-12 h L-D cycle on *Prochlorococcus* PCC 9511 and SS 120 (A) RALS, (B) chlorophyll fluorescence, and the percentage of cells in (C) S and (D) G_2 phases of the cell cycle. Dark bars, night period.

TABLE 2. Effect of 12 h-12 h L-D diel cycle on *Prochlorococcus* strains

<i>Prochlorococcus</i> strain	Flow-cytometric parameter			
	RALS		Red Chl fluorescence	
	Mean	% Variation ^a	Mean	% Variation ^a
PCC 9511	0.09	33	0.40	20
SS 120	0.11	42	0.58	25

^a Variations were estimated from the ratio (maximum value – minimum value)/mean, expressed in percent.

were clearly higher in strain SS 120 than in strain PCC 9511. Second, there was a 1- to 2-h delay in the start of the S and G_2 phases for PCC 9511. There was also a slightly higher proportion of cells in the G_2 phase, outside the active DNA replication and cell division period, for PCC 9511 than for SS 120 (Fig. 1D). For both strains, a second peak of cells in S phase was recorded a few hours after the major one, although this peak was more marked for SS 120 cells. In contrast, no minor peak of cells in G_2 was observed. Phase duration and growth rate were slightly higher for strain SS 120 than for strain PCC 9511 (Table 1).

Effect of continuous darkness. *Prochlorococcus* MED 4 cells, acclimated to a 12 h-12 h L-D cycle, were transferred to complete darkness, and cell parameters were monitored for the next 48 h (Fig. 2). During the first 12 h of darkness (corresponding to the normal dark period), cells of the experimental culture behaved similarly to those of the control (left under an L-D cycle). Following this period, the behavior of the two cultures diverged. In the dark culture, cells were not able to restart growth, and diel patterns disappeared. The RALS remained constant for almost 6 h, then decreased for 7.5 h before stabilizing at a lower value than the minimum observed in the control culture (Fig. 2A). Chl fluorescence in the dark treatment was constant for 6 h and then decreased steadily (Fig. 2B). A few cells entered the S phase during the first day of darkness, apparently forming three successive cohorts (Fig. 2C). However, these cells did not seem to complete DNA synthesis and move towards division since no significant increase in G_2 cells was observed afterwards (Fig. 2D). These cells probably died, as confirmed by the decrease in cell number (data not shown), which roughly paralleled the decrease in RALS.

Effect of constant light. To analyze the effect of constant light, two replicate cultures of PCC 9511 were first acclimated under a 12 h-12 h L-D cycle. Although both replicates grew exponentially at similar rates and had similar densities the day before sampling began (data not shown), flow-cytometric data showed that they were not strictly identical at $t = 0$ and during the first 24 h of sampling, although the two cultures were maintained under a similar L-D cycle. Differences included a slight shift in the response patterns of RALS and Chl fluorescence (Fig. 3). At $t = 24$ h, both cultures were transferred to constant light, one at $25 \mu\text{mol}$ of quanta $\cdot \text{m}^{-2} \text{s}^{-1}$ and the other at $10 \mu\text{mol}$ of quanta $\cdot \text{m}^{-2} \text{s}^{-1}$. After 36 h, i.e., when darkness should have occurred, RALS (Fig. 3A) and Chl fluorescence (Fig. 3B) of the culture placed at $25 \mu\text{mol}$ of quanta $\cdot \text{m}^{-2} \text{s}^{-1}$ began to decrease, as observed at the beginning of the previous dark

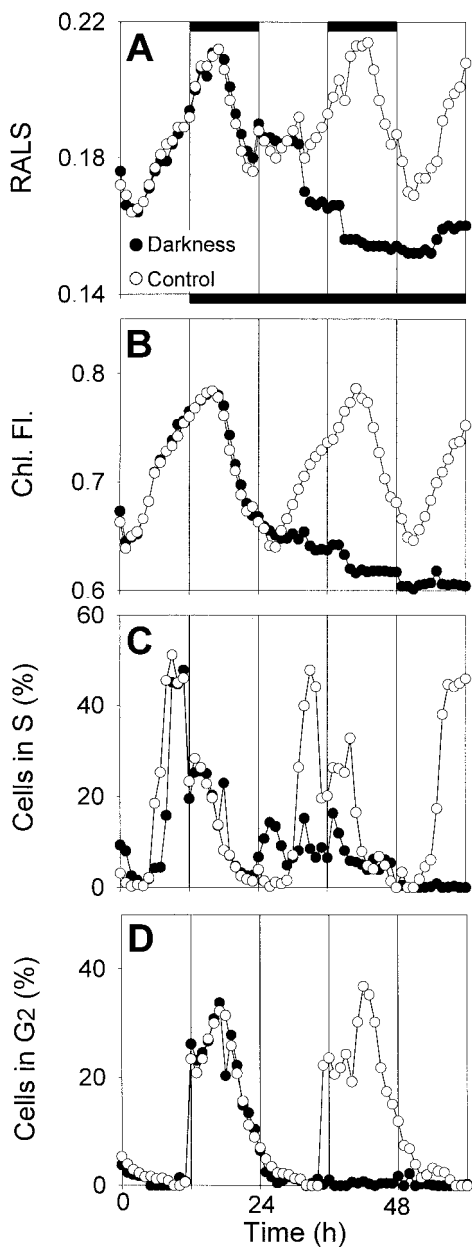


FIG. 2. Effect of complete darkness following L-D entrainment on *Prochlorococcus* MED 4 (A) RALS, (B) chlorophyll fluorescence, and the percentage of cells in (C) S and (D) G₂ phases of the cell cycle. Dark symbols represent the population placed in the dark compared to the control (white symbols) maintained in 12 h-12 h L-D cycle.

period, but the amplitude of the decrease was much smaller. RALS and fluorescence patterns then showed several oscillations, but they were out of phase with respect to the patterns observed during the first 36 h of the experiment. Patterns of variation in the percentages of cells in the active phases of the cell cycle (S and G₂) in the time interval 36 to 42 h compared well with those previously observed during the first 12 to 18 h. Then, during the period from 42 to 96 h, two oscillations were observed, albeit reduced ones. The peaks of S (Fig. 3C) and G₂ (Fig. 3D) cells occurred about 4 h earlier compared to the pattern observed at the begin-

ning of the experiment. At 10 μmol of quanta $\cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the evolution was approximately the same, but the decreases of fluorescence and, to a lesser extent, of RALS after 36 h were more limited. At 10 μmol of quanta $\cdot \text{m}^{-2} \cdot \text{s}^{-1}$, fewer cells entered the S phase during the photoperiod following the light shift (around $t = 36$ h), and there was no clear second peak of S cells at $t = 78$ h.

Effect of shifting the light-on signal. To determine the role of the light-on signal on all cycling, an experiment was conducted

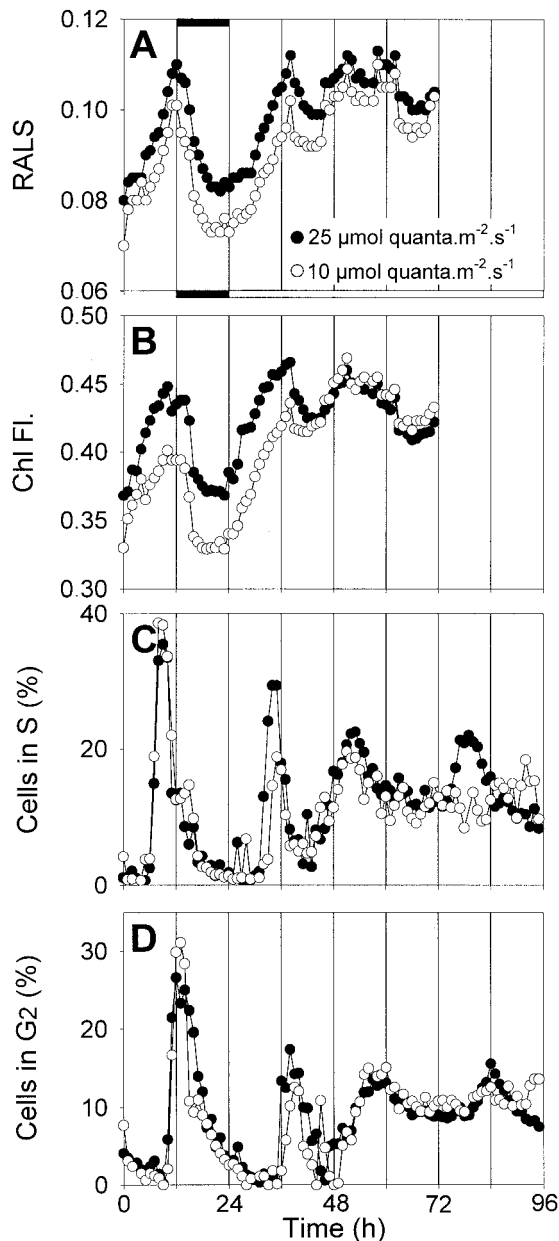


FIG. 3. Effect of constant light following L-D entrainment on *Prochlorococcus* PCC 9511 (A) RALS, (B) chlorophyll fluorescence, and the percentage of cells in (C) S and (D) G₂ phases of the cell cycle. Dark and white symbols represent the cultures grown under free running conditions at 25 and 10 μmol quanta $\cdot \text{m}^{-2} \cdot \text{s}^{-1}$, respectively. No data were available for fresh samples for the last day of experiment due to flow-cytometric problems.

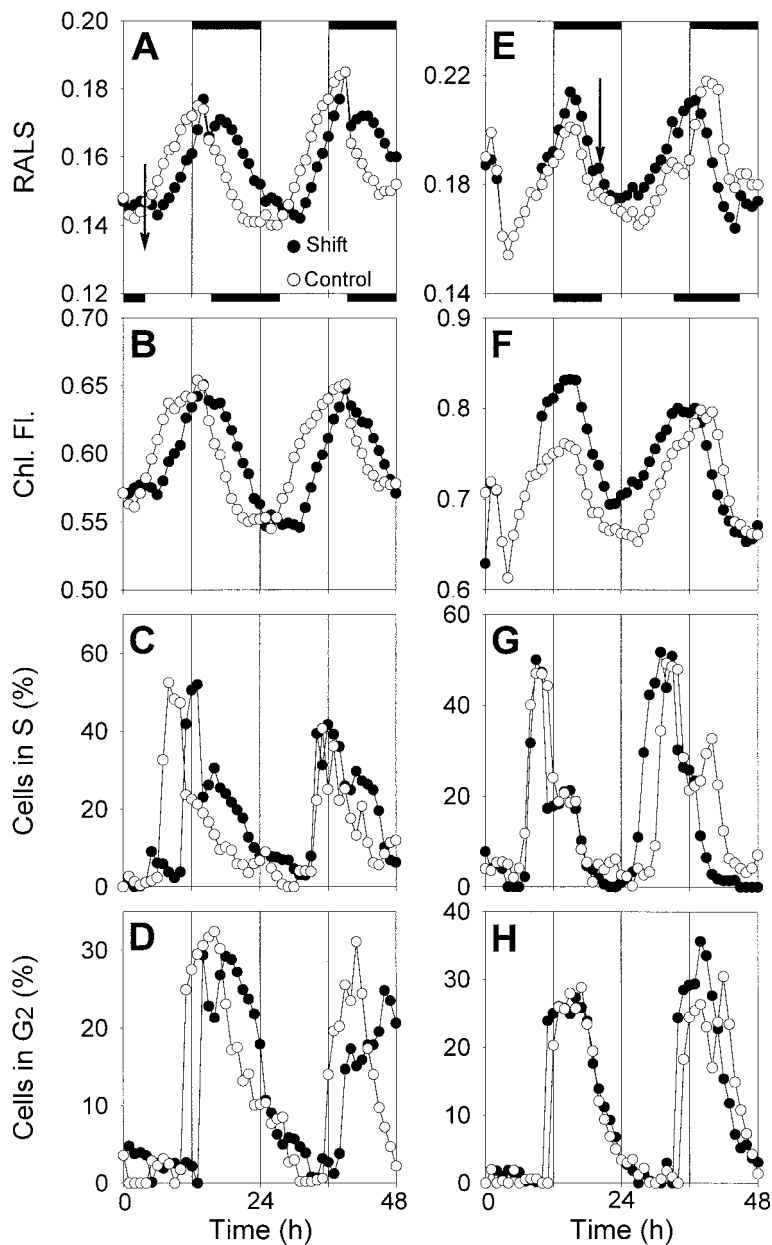


FIG. 4. Effect of a 4 h delay (+) and a 4 h advance (-) of light onset on *Prochlorococcus* MED 4 (A, E) RALS, (B, F) chlorophyll fluorescence, and the percentage of cells in (C, G) S and (D, H) G_2 phases of the cell cycle. Black arrows symbolize the moment of the shift. Dark and white symbols represent data for the shift and the control, respectively.

using replicate *Prochlorococcus* MED 4 cultures entrained by a 12 h-12 h L-D cycle. For one of these cultures, the time at which light was switched on was set 4 h later, and for another culture it was set 4 h earlier than the usual time (Fig. 4). When the light-on signal was delayed by 4 h, RALS began to increase slowly; then, after 3 h, it began to drop (Fig. 4A). A new increase began 2 h after the new light-on signal, in a parallel manner to that recorded for the control (Fig. 4A). The maximum value of RALS was recorded at the end of the new light period, as occurs in normal conditions (Fig. 4A). The Chl fluorescence pattern was very similar to that of RALS (Fig. 4B). The maximum percentages of cells in the S and G_2 growth phases were recorded 5 h and 4 h later,

respectively, than in the control (Fig. 4C and D). There was no significant change in the proportion of cells in S and G_2 phases for both conditions. When light was switched on 4 h earlier than the normal time, RALS rapidly stopped decreasing and then began to increase, reaching a maximum 2 h earlier than that of the control (Fig. 4E). Chl fluorescence began to increase 5 h before that of the control and peaked 4 h earlier compared to the control (Fig. 4F). The peak of the S phase seemed to occur only 2 h earlier than that of the control (Fig. 4G), whereas the G_2 phase was most populated 4 h earlier (Fig. 4H). As for the delay, the advanced light shift induced no significant change in the proportion of cells in both phases.

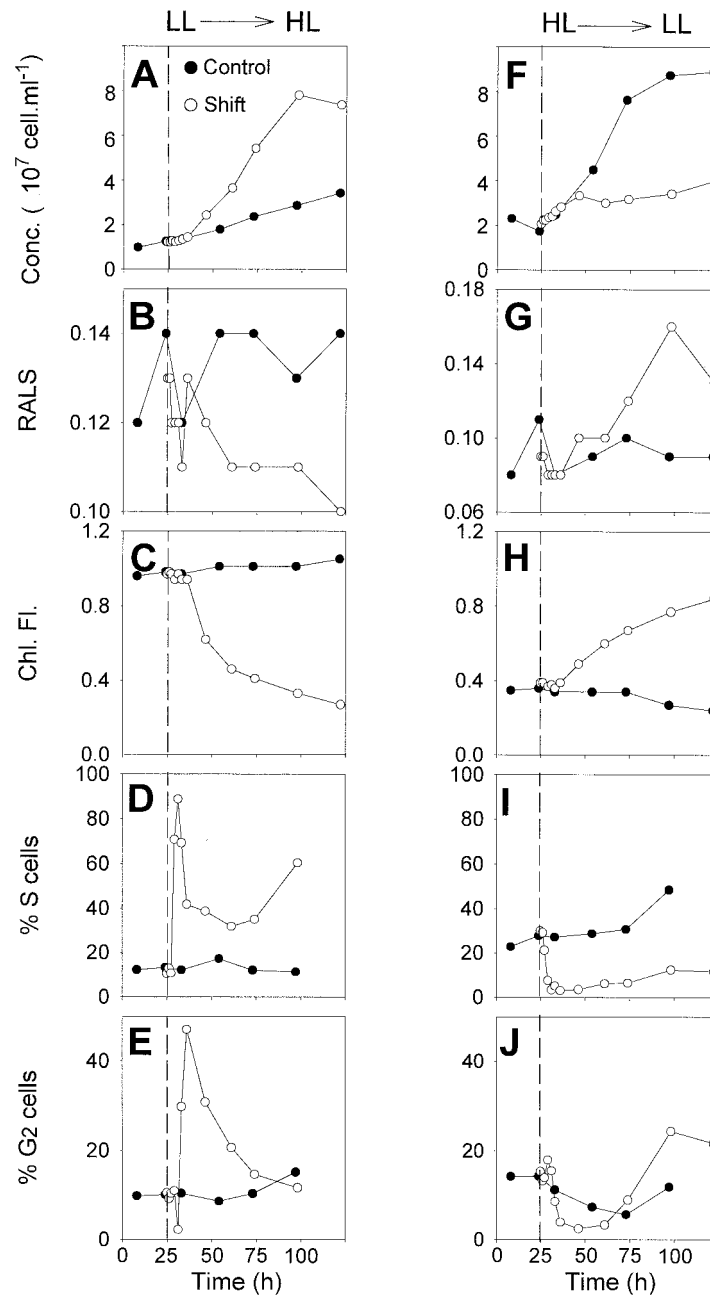


FIG. 5. Photoacclimation experiments performed on *Prochlorococcus* MED 4. Cells were shifted from 8 to 57 $\mu\text{mol quanta} \cdot \text{m}^{-2} \text{s}^{-1}$ (i.e., from LL to HL) and inversely (from HL to LL). Changes are reported for (A, F) cell concentration, (B, G) RALS, (C, H) chlorophyll fluorescence, and DNA distribution in (D, I) S and (E, J) G_2 phases of the cell cycle. Dark and white symbols represent the control and the shifts in irradiance level, respectively.

Effect of light level shift. The photoacclimation dynamics of *Prochlorococcus* growth were investigated in strain MED 4 by monitoring variations in cellular parameters (including cell numbers) and cell cycle. Cultures were grown in replicate at two constant light intensities (8 and 57 μmol of quanta $\cdot \text{m}^{-2} \text{s}^{-1}$) and transferred at time $t = 0$ from one level to the other (Fig. 5). We refer to the transfer of cells from LL to HL as the “shift up” and to the transfer of cells from HL to LL as the “shift down.” Although cell concentrations at $t = 0$ were comparable in all cultures, cells grown at HL grew much faster than

those left at LL, such that the abundance in the HL control was much higher than in the LL control at the end of the experiment (Fig. 5A and F). Shifting cultures either from LL to HL or vice versa caused significant changes in all cellular parameters. During the shift up, the increase in cell concentration paralleled that of the LL control for about 12 h. Then cells increased their division rate until reaching that of the HL control (Fig. 5A). During the shift down, the variation of cell concentration followed closely that of the HL control for 12 h, and then the division rate began to decrease to match the rate

observed in the LL control (Fig. 5F). Patterns of RALS and Chl fluorescence remained fairly constant in the HL and LL controls. The relative values of these two parameters were systematically higher at LL than HL (Fig. 5B, C, G and H), as expected for fully acclimated cells (18). During the shift-up experiment, RALS and Chl fluorescence progressively decreased throughout the experiment and finally approached the values of the HL control (Fig. 5B and C). The converse phenomenon occurred during the shift-down experiment (Fig. 5G and H). However, at the end of the experiment, the Chl fluorescence of cells shifted to LL was clearly below that of the LL control, whereas the RALSs of these cultures were similar. The shift up induced a very fast and dramatic response in the cell cycle, with a very high proportion of cells moving through the S and G₂ phases just after the light shift (Fig. 5D and E). During the shift down experiment, the opposite trend was observed with steadily fewer cells entering the S and G₂ phases at the end of the experiment, as cells became acclimated to their new growth conditions (Fig. 5I and J).

DISCUSSION

One of the most prevalent phenomena in natural systems is the never-ending alternation of light and darkness over a 24-h period. As a direct consequence, living organisms from prokaryotes (13) to human beings (8) display clear daily periodicities in their activity. Members of the genus *Prochlorococcus* are no exception to this rule. Both field (29, 36) and cultured *Prochlorococcus* populations entrained by L-D cycles show marked diel patterns in abundance, and cellular parameters and cell division are tightly synchronized with the daily light cycle (29; S. Jacquet, F. Partensky, J.-F. Lennon, and D. Vaulot, submitted for publication).

In the present study, we found that RALS and Chl fluorescence displayed very similar patterns whatever the conditions tested. When *Prochlorococcus* spp. were entrained by the L-D cycle, there was an overall increase of both parameters during the light period and a decrease during the night. Continuous darkness provoked a clear decrease in relative values of scatter and fluorescence, whereas the converse pattern was recorded under constant light. These parallel patterns of cell fluorescence and light scatter suggest that pigment synthesis and cell growth are tightly coupled. A similar observation was done for field *Prochlorococcus* populations at depths (37). In contrast, in surface waters, Chl synthesis and fluorescence quenching have opposite effects and translate into a marked decrease of the Chl fluorescence in the middle of the day, which is not paralleled by the light scatter pattern. Such a midday decrease in Chl fluorescence did not occur in our experiments, likely due to the nonsaturating irradiance levels used. We also noticed a good correlation between RALS variation and growth rate (see Table 1 and Fig. 5). Such coupling has already been reported for field populations (37) and might reflect the fact that cells must reach a critical size before division can proceed. This hypothesis was recently supported in the marine cyanobacterium *Synechococcus* WH 8101 (3), a close relative of *Prochlorococcus* spp.

Although culturing *Prochlorococcus* cells under an L-D cycle systematically provoked a strong oscillation of light scatter values, it must be noted that, contrary to what is observed in

the field (37), relative RALS values here never doubled. Moreover, the percentage of cells in the active phases of the cell cycle (S and G₂) never equaled zero. This indicates that the synchronization of the *Prochlorococcus* population was only partial in our culture conditions. The better synchrony generally observed in nature (e.g., 29, 36, 37) is probably mainly due to the temporal modulation of natural sunlight. Preliminary growth experiments in which we compared the effect of an L-D cycle with a constant light of 25 μmol of quanta $\cdot \text{m}^{-2} \text{s}^{-1}$ during the photoperiod to a stepwise L-D cycle with a maximum irradiance around noon of 43 μmol of quanta $\cdot \text{m}^{-2} \text{s}^{-1}$ (cells received a similar daily light dose in both experiments) were inconclusive (S. Jacquet, unpublished data). However, growing *Prochlorococcus* PCC 9511 cells under a bell-shaped L-D cycle and a very high maximum irradiance of about 1,000 μmol of quanta $\cdot \text{m}^{-2} \text{s}^{-1}$ resulted in almost perfect cell cycle synchronization (5).

The *Prochlorococcus* growth rate appears to be tightly controlled by irradiance levels. This can clearly be inferred from the light shift experiment. When MED 4 cells were shifted from LL to HL (Fig. 5), there was a rapid and dramatic increase in the proportion of cells in S and G₂ phases, which soon translated into an increased division rate. The reverse effect was observed when cells were transferred from HL to LL. Similarly, when cells were shifted from an L-D cycle to constant light conditions, fewer cells entered the S phase when they were placed at 10 rather than 25 μmol of quanta $\cdot \text{m}^{-2} \text{s}^{-1}$, a phenomenon which was accompanied by an apparently quicker loss of cell cycle synchronization in the LL condition. An increase in the percentages of cells in the S and G₂ phases of the cell cycle in response to higher irradiances was previously observed in surface waters of the Mediterranean Sea for *Synechococcus* cells (12). It was also observed along the vertical light gradient for populations of *Prochlorococcus* spp. in the equatorial Pacific from the bottom of the euphotic zone up to about 35 m (36). However, there was a reversal of this trend higher in the water column, due to the combined negative effects of excess light on *Prochlorococcus* growth as well as to a delayed entry of cells in the S phase, likely due to UV light.

Depletion or complete starvation of essential resources such as nutrients or light has been shown to induce cell cycle arrest at specific blocking points in the cell cycle, most commonly at the end of the G₁ phase (30, 32). When cells have passed this point, the environmental factor is no longer required for transit through the later phases of the cell cycle, and cells are committed to divide. The effect of suboptimal irradiances for growth is different depending on whether the population is synchronized or not. For an asynchronous population, a limiting light level induces an expansion in the G₁ duration, rather than a true blockage of cells in G₁ (22). We show here that in the case of *Prochlorococcus* cells, slow progression through G₁ can be rapidly reversed when cells are transferred to higher light. Conversely, this progression can be quickly slowed down when cells are transferred to lower light (Fig. 5). In contrast, when populations are synchronized, as is the case for *Prochlorococcus* spp. either in the field or submitted to an artificial L-D cycle, the observed decrease in growth rate at LL implies that only a part of the population (proportional to the irradiance level) can proceed thru S and G₂ phases and then divide at precise times during the L-D cycle. The rest of the popula-

tion is committed to wait, at a blocking point in G_1 , until the following day.

When *Prochlorococcus* cells are placed into complete darkness, most cells arrest at the blocking point in phase G_1 . However, it is noteworthy that some of them do not stop in phase G_1 but in phase S, as observed previously in phosphorus-starved *Prochlorococcus* cells (23). The increase of cells in the S phase was apparently not artefactual, since there was no significant increase in the coefficient of variation of the G_1 peak that could result in an overestimation of the proportion of cells in the S phase (1). By comparison, cells of the marine *Synechococcus* strain WH 8101 complete DNA synthesis in the dark and do not arrest in S phase (1). In fact, the latter strain seems to have blocking points both in the G_1 and G_2 phases, like diatoms (32). For *Prochlorococcus*, cells that start the S phase in the dark apparently cannot complete DNA replication or enter G_2 . The exact reasons behind such an effect remain unexplained. However, these data along with those of Parpais et al. (23) suggest that cell cycle control in *Prochlorococcus* spp. is not as strict as in *Synechococcus* spp. or other eukaryotic phytoplankters, which are totally prevented from entering the critical phase of genome replication in cases of energy deprivation.

In the present study, we have also examined which signals may be responsible for the synchronization. We found that a shift in the time of the dark-to-light transition was accompanied by a parallel shift in the timing of entry of cells into S phase. When cells were subjected to complete darkness following the L-D cycle, the cell cycle was disrupted; i.e., cells stopped cycling as if they were waiting for the stimulus of entrainment (the light-on signal). These results strongly suggest that the onset of light is a critical signal for triggering cell cycling in *Prochlorococcus* spp. This conclusion is in agreement with a previous study suggesting that S-phase initiation is linked to a light-triggered timer in *Prochlorococcus* spp. (29). These results also confirm previous assumptions about the importance of this signal in synchronizing natural picoplanktonic populations (12, 37). The sensor of the light stimulus is not yet known in *Prochlorococcus* spp. In other photosynthetic bacteria, it has recently been shown to be a phytochrome (39).

Circadian clocks, i.e., endogenous oscillators responsible for the daily patterns recorded in the activity of cells in the absence of entrainable stimuli, have been extensively studied in unicellular algae (e.g., see reference 15). Since the discovery of biological timers in unicellular marine cyanobacteria (17), cell cycle clocks in these organisms have been intensively investigated (e.g., see reference 13 for a review), and cell division in *Synechococcus* spp. has been shown to obey clock-controlled circadian regulation (10, 14, 31). In the genus *Prochlorococcus*, *rbcl* gene expression has been suggested to be endogenously controlled (27). Shalapyonok et al. (29) also reported that the timing of cell cycle events in *Prochlorococcus* spp. may be controlled by a circadian clock. In the present study, *Prochlorococcus* cells displayed some features that may point to the existence of a clock (oscillations after shift from L-D to continuous light and resetting of division timing by light shift). However, these features can also be easily explained by a direct effect of light on the cell cycle (35). *Prochlorococcus* spp., because they can be synchronized so tightly, would be an excellent model to further address the question of the interaction between light and cellular processes and the eventual role of a central clock.

A refined study of genes implicated in diel rhythms in the *Prochlorococcus* genus is now needed in order to better understand the coupling between light, cellular processes, and possibly a biological clock. It would also be worthwhile to determine how the regulation of these genes differs from that of marine *Synechococcus* spp., which often co-occur with *Prochlorococcus* spp. in the field, but whose diel patterns of cellular parameters as well as cell cycle regulation mechanisms are clearly different (2; S. Jacquet et al., submitted).

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REFERENCES

- Armbrust, E. V., J. D. Bowen, R. J. Olson, and S. W. Chisholm. 1989. Effect of light on the cell cycle of a marine *Synechococcus* strain. *Appl. Environ. Microbiol.* **55**:425-433.
- Binder, B. J., and S. W. Chisholm. 1995. Cell cycle regulation in marine *Synechococcus* sp. strains. *Appl. Environ. Microbiol.* **61**:708-717.
- Binder, B. 2000. Cell cycle regulation and the timing of chromosome replication in a marine *Synechococcus* (cyanobacteria) during light- and nitrogen-limited growth. *J. Phycol.* **36**:120-126.
- Bricaud, A., K. Allali, A. Morel, D. Marie, M. J. W. Veldhuis, F. Partensky, and D. Vaultot. 1999. Divinyl chlorophyll *a* specific absorption coefficients and absorption efficiency factors for *Prochlorococcus* sp.: kinetics of photoacclimation. *Mar. Ecol. Prog. Ser.* **188**:21-32.
- Bruyant, F., M. Babin, A. Sciandra, D. Marie, B. Genty, H. Claustre, J. Blanchot, A. Bricaud, R. Rippka, S. Boulben, and F. Partensky. *J. Appl. Physiol.*, in press.
- Carpenter, E. J., and J. Chang. 1988. Species-specific phytoplankton growth rates via diel DNA synthesis cycles. I. Concept of the method. *Mar. Ecol. Prog. Ser.* **43**:105-111.
- Chisholm, S. W., R. J. Olson, E. R. Zettler, R. Goericke, and J. B. Waterbury. 1988. A novel free living prochlorophyte abundant in the oceanic euphotic zone. *Nature* **334**:340-343.
- Czeiler, C. A., et al. 1999. Stability, precision, and near 24-hour period of the human circadian pacemaker. *Science* **284**:2177-2181.
- Garczarek, L., W. R. Hess, J. Holtzendorff, G. W. M. van der Staay, and F. Partensky. 2000. Multiplication of antenna genes as a major adaptation to low light in a prokaryote. *Proc. Natl. Acad. Sci. USA* **97**:4098-4101.
- Ishiura, M., S. Kutsuna, S. Aoki, H. Iwasaki, C. R. Andersson, A. Tanabe, S. S. Golden, C. H. Johnson, and T. Kondo. 1998. Expression of a gene cluster *kaiABC* as a circadian feedback process in cyanobacteria. *Science* **281**:1519-1523.
- Jacquet, S., J.-F. Lennon, and D. Vaultot. 1998. Application of a compact automatic sea water sampler to high frequency picoplankton studies. *Aquat. Microbiol. Ecol.* **14**:309-314.
- Jacquet, S., J.-F. Lennon, D. Marie, and D. Vaultot. 1998. Picoplankton population dynamics in coastal waters of the N. W. Mediterranean Sea. *Limnol. Oceanogr.* **43**:1916-1931.
- Johnson, C. H., S. S. Golden, M. Ishiura, and T. Kondo. 1996. Circadian clocks in prokaryotes. *Mol. Microbiol.* **21**:5-11.
- Kondo, T., C. A. Strayer, R. D. Kulkarni, W. Taylor, M. Ishiura, S. S. Golden, and C. A. Johnson. 1993. Circadian rhythms in prokaryotes: luciferase as a reporter circadian gene expression in cyanobacteria. *Proc. Natl. Acad. Sci. USA* **90**:5672-5676.
- Lloyd, D. 1998. Circadian and ultradian clock-controlled rhythms in unicellular microorganisms. *Adv. Microb. Physiol.* **39**:291-338.
- Marie, D., F. Partensky, S. Jacquet, and D. Vaultot. 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid dye SYBR-Green I. *Appl. Environ. Microbiol.* **63**:186-193.
- Mitsui, A., S. Kumazakawa, A. Takahashi, H. Ikemoto, S. Cao, and T. Arai. 1986. Strategy by which unicellular cyanobacteria grow photoautotrophically. *Nature* **323**:720-722.
- Moore, L. R., R. Goericke, and S. W. Chisholm. 1995. Comparative physi-

- ology of *Synechococcus* and *Prochlorococcus*: influence of light and temperature on growth, pigments, fluorescence and absorptive properties. *Mar. Ecol. Prog. Ser.* **116**:259–275.
19. Moore, L. R., G. Rocap, and S. W. Chisholm. 1998. Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. *Nature* **393**:464–467.
 20. Moore, L. R., and S. W. Chisholm. 1999. Photophysiology of the marine cyanobacterium *Prochlorococcus*: ecotypic differences among cultured isolates. *Limnol. Oceanogr.* **44**:628–638.
 21. Morel, A. 1991. Optics of marine particles and marine optics. In S. Demers (ed.), *Particle analysis in oceanography*. NATO ASI Ser. **27**:141–188.
 22. Olson, R. J., and S. W. Chisholm. 1986. Effects of light and nutrient limitation on the cell cycle of the dinoflagellate *Amphidinium carteri*. *J. Plank. Res.* **8**:785–793.
 23. Parpais, J., D. Marie, F. Partensky, P. Morin, and D. Vault. 1996. Effect of phosphorus starvation on the cell cycle of the photosynthetic prokaryote *Prochlorococcus*. *Mar. Ecol. Prog. Ser.* **132**:265–274.
 24. Partensky, F., N. Hoepffner, W. K. W. Li, O. Ulloa, and D. Vault. 1993. Photoacclimation of *Prochlorococcus* sp. (Prochlorophyta) strains isolated from the North Atlantic and the Mediterranean Sea. *Plant Physiol.* **101**:285–296.
 25. Partensky, F., J. Blanchot, and D. Vault. 1999. Differential distribution and ecology of *Prochlorococcus* and *Synechococcus* in oceanic waters: a review, p. 431–449. In L. Charpy and A. W. D. Larkum (ed.), *Marine cyanobacteria and related organisms*. Oceanographic Museum, Monaco.
 26. Partensky, F., W. R. Hess, and D. Vault. 1999. *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Mol. Microb. Ecol. Rev.* **63**:106–127.
 27. Pichard, S. L., L. Campbell, J. B. Kang, F. R. Tabita, and J. H. Paul. 1996. Regulation of ribulose biphosphate carboxylase gene expression in natural phytoplankton communities. I. Diel rhythms. *Mar. Ecol. Prog. Ser.* **139**:257–265.
 28. Rippka, R., T. Coursin, W. R. Hess, C. Lichtlé, D. J. Scanlan, K. A. Palinska, I. Iteman, F. Partensky, N. Tandeau de Marsac, J. Houmard, and M. Herdman. 2000. *Prochlorococcus marinus* Chisholm et al. 1992, subsp. nov. *pastoris*, strain PCC 9511, the first axenic chlorophyll a2/b2-containing cyanobacterium (Oxyphotobacteria). *Int. J. Syst. Evol. Microbiol.* **50**:1833–1847.
 29. Shalapyonok, A., R. J. Olson, and L. S. Shalapyonok. 1998. Ultradian growth in the marine planktonic photosynthetic prokaryote *Prochlorococcus*. *Appl. Environ. Microbiol.* **64**:1066–1069.
 30. Spudich, J. L., and R. Sager. 1980. Regulation of the *Chlamydomonas* cell cycle by light and dark. *J. Cell Biol.* **85**:136–145.
 31. Sweeney, B. M., and M. B. Borgese. 1989. A circadian rhythm in cell division in a prokaryote, the cyanobacterium *Synechococcus* WH7803. *J. Phycol.* **25**:183–186.
 32. Vault, D., R. J. Olson, and S. W. Chisholm. 1986. Light and dark control of the cell cycle in two marine phytoplankton species. *Exp. Cell Res.* **167**:38–52.
 33. Vault, D., and S. W. Chisholm. 1987. A simple model of the growth of phytoplankton populations in light/dark cycles. *J. Plank. Res.* **9**:345–366.
 34. Vault, D. 1989. CytoPC: processing software for flow cytometric data. *Signal Noise* **2**:8.
 35. Vault, D., and F. Partensky. 1992. Cell cycle distributions of prochlorophytes in the North Western Mediterranean Sea. *Deep-Sea Res.* **39**:727–742.
 36. Vault, D., D. Marie, R. J. Olson, and S. W. Chisholm. 1995. Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the Equatorial Pacific Ocean. *Science* **268**:1480–1482.
 37. Vault, D., and D. Marie. 1999. Diel variability of photosynthetic picoplankton in the equatorial Pacific. *J. Geophys. Res.* **104**:3297–3310.
 38. West, N. J., and D. J. Scanlan. 1999. Niche-partitioning of *Prochlorococcus* populations in a stratified water column in the eastern north Atlantic Ocean. *Appl. Environ. Microbiol.* **65**:2585–2591.
 39. Yeh, K.-C., S.-H. Wu, J. T. Murphy, and J. C. Lagarias. 1997. A cyanobacterial phytochrome two-component light sensory system. *Science* **277**:1505–1508.