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Effect of phosphorus starvation on the cell cycle of the photosynthetic prokaryote *Prochlorococcus* spp.

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ABSTRACT: The influence of phosphorus (P) starvation on the cell cycle of *Prochlorococcus* was studied for several strains from the Mediterranean Sea, the Atlantic and Pacific Oceans. P starvation could only be induced at high N/P ratio (≥150). In P-starved stationary phase, the fraction of cells in G_1 dropped markedly, while cells accumulated not only in G_2, but also in the DNA synthesis phase S, indicating that cells were not arrested at a precise cell cycle location but in all cell cycle phases. The percentage of P-starved cells in each phase varied slightly between strains. For the Mediterranean Sea strain CCMP 1378, cell cycle arrest in G_1 and G_2 could be reversed by addition of phosphates, while cells arrested in S could not resume their cycle and died. Such irreversible arrest in S had never been reported previously in phytoplankton. This suggests that cell cycle controls are not very tight in *Prochlorococcus*, since cells are not prevented from entering the critical S phase in the absence of phosphorus.

KEY WORDS: *Prochlorococcus* · Phosphate starvation · Cell cycle · Flow cytometry · Growth rate

INTRODUCTION

The cell cycle consists of a succession of steps leading from the birth of a cell to its subsequent division into 2 daughter cells (Murray & Hunt 1993). In eukaryotes, the cell cycle is composed of 4 phases called G_1, S, G_2 and M; S corresponding to DNA synthesis and M to mitosis. The study of the marine phytoplankton cell cycle has recently received increased attention in marine ecology for 2 reasons. First, the cell cycle is the basis of a very elegant and accurate method of estimating the cell division rate of natural populations (Carpenter & Chang 1988, Vaulot et al. 1995). Second, the cell cycle behavior of natural populations can reveal very useful information on the nutritional status of the cells (e.g. Vaulot & Partensky 1992). We will be concerned with the latter aspect in the present paper.

In order to be able to correctly interpret cell cycle patterns observed in the field, the effect of limiting factors such as light and nutrients must be examined in laboratory cultures. In general, the deprivation of a key nutrient arrests eukaryotic cells in the G_1 phase: for example in yeast, starved cells cease progressing in their cell cycle when they reach the 'Start' point, a critical point beyond which they are committed to replicate DNA (S phase) and to divide (Murray & Hunt 1993). In eukaryotic phytoplankton the same rule more or less applies: for example the absence of light or nitrogen induces cells to arrest in G_1 (see review in Vaulot 1995).

In contrast to eukaryotes, for which DNA synthesis occurs during a discrete phase of the cell cycle (the S phase), the DNA replication of rapidly growing prokaryotes is continuous. Moreover, DNA replication can be uncoupled from cell division, such that individual cells may have more than 2 copies of the genome, resulting in DNA distributions with multiple peaks (e.g. Binder & Chisholm 1995). However, the cell cycle of slowly growing photosynthetic prokaryotes resembles that of eukaryotes most of the time, with a well-defined S phase and no cells with more than 2 copies of the genome (e.g. Armbrust et al. 1989, Vaulot & Partensky 1992). The effect of limiting factors on cell cycling of oceanic photosynthetic prokaryotes has been little studied (see Armbrust et al. 1989, Binder & Chisholm 1995). However, these organisms, and in particular *Prochlorococcus* (Chisholm et al. 1988, 1992), are impor-

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tant contributors to the chlorophyll biomass and primary production in oligotrophic areas of temperate and subtropical oceans (Olson et al. 1990, Li et al. 1992, Campbell & Vaulot 1993, Veldhuis & Kraay 1993) as well as in some high nutrient-low productivity waters such as the Equatorial Pacific (Vaulot et al. 1995).

In this paper we investigate the response of the *Prochlorococcus* cell cycle to P-limitation in culture. This study is motivated by the fact that *Prochlorococcus* is present and sometimes abundant in the Mediterranean Sea (Vaulot et al. 1990, Vaulot & Partensky 1992, Li et al. 1993, Bustillos-Guzman 1995), an area where phosphorus, rather than nitrogen, is hypothesized to be a key limiting factor (Berland et al. 1980, Krom et al. 1991, Thingstad & Rassoulzadegan 1995).

MATERIAL AND METHODS

Strains used and culture conditions. *Prochlorococcus* strains from different geographic areas were used in this study (Table 1). Cultures were grown routinely at 19 ± 1°C in 1 l polycarbonate Nalgene flasks (Nalgene Brand Products, Rochester, NY, USA) under cool-white fluorescent bulbs wrapped with moonlight blue LEE filter (Panavision) and providing 7 µmol photon m^{-2} s^{-1} of continuous blue light. The culture medium consisted of 0.2 µm Millipore-filtered, nutrient-poor sea water enriched with 150 µM nitrogen (final concentration) provided as 50 µM urea and 50 µM NH_4Cl, 10 µM Na_2 glycerophosphate (Gly-P, $C_3H_7Na_2O_6P$) and trace metals as in K/10 (–Cu) medium (Chisholm et al. 1992). This 'standard' medium was modified in several ways for this study.

In all experiments, orthophosphate (Na_2HPO_4 hereafter shortened to PO_4^{3-}) was used instead of Gly-P, because only the former could be precisely measured by spectrophotometry (see below).

In a first experiment, growth of *Prochlorococcus* CCMP 1378 was compared between media enriched with either Gly-P or PO_4^{3-} to check whether cells could grow in both media in a similar way.

A second experiment aimed at determining the PO_4^{3-} concentration at which cells became limited. *Prochlorococcus* strain CCMP 1378 was grown in duplicate in a range of initial PO_4^{3-} concentrations (provided as Na_2HPO_4) with a fixed nitrogen (N) concentration. 0.3 µM PO_4^{3-} (N/P = 500), 1 µM PO_4^{3-} (N/P = 150), 3 µM PO_4^{3-} (N/P = 50) or 10 µM PO_4^{3-} (N/P = 15, the standard culture conditions). The inoculum was grown in medium with an initial concentration of 1 µM PO_4^{3-}. The volume of the inoculum was adjusted to minimize the PO_4^{3-} addition resulting from the inoculum below 1%. Growth and cell cycle were surveyed daily. When the stationary phase was reached, PO_4^{3-} was added to a final concentration of 10 µM in one of the replicates to monitor recovery from P limitation.

In a third experiment, the growth and cell cycle of 5 *Prochlorococcus* strains (NATL1, NATL2, TATL1, TATL2 and PAC1) were monitored in media with PO_4^{3-} concentrations of 10 µM (standard culture conditions) and 0.3 µM (P limitation).

Measurements of PO_4^{3-} concentrations. Two replicate 50 ml samples were filtered on Whatman GF/F filters, and stored at –20°C for subsequent analysis. PO_4^{3-} concentrations were measured using the method of Murphy & Riley (1962) with a Shimadzu UV 120 spectrophotometer with 10 cm cell length.

Flow cytometric measurements. For cell counting and cell cycle analyses, 900 µl samples were fixed with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentrations) for 20 min in the dark, frozen in liquid nitrogen and stored at –80°C until analysis (modified from Vaulot et al. 1989).

For cell cycle analyses of strain CCMP 1378, samples were diluted 10 to 100 times in 250 µl of a 30 mM sodium citrate buffer in the presence of 1 g l^{-1} of a mixture of RNAse A and B (Sigma R-4875 and R-5750, 1:1, w:w) and 30 nM of the nucleic acid stain YOYO-1 (Molecular Probes, Eugene, OR, USA). For measurements on the other strains performed later, Picogreen (Molecular Probes) was used instead of YOYO-1, because it allows easier differentiation of *Prochlorococcus* cells from heterotrophic bacteria. Picogreen was added at a final concentration of $^1/_{1000}$ of the commercial stock solution (molarity not communicated by manufacturer) in a buffer consisting of 30 mM sodium

Table 1. *Prochlorococcus*. Origin of strains used in this study

Strain	Clonal/unialgal	Origin	Latitude, longitude	Isolation depth	Isolated by
CCMP 1378	Clonal	Mediterranean Sea	43° 12' N, 6° 52' W	5 m	D. Vaulot & F. Partensky
NATL1	Unialgal	North Atlantic	37° 39 'N, 40° 01' W	30 m	F. Partensky
NATL2	Unialgal	North Atlantic	38° 59' N, 49° 33' W	10 m	F. Partensky
TATL1	Unialgal	Tropical Atlantic	21° 02' N, 31° 08' W	20 m	F. Partensky
TATL2	Unialgal	Tropical Atlantic	20° 25 'N, 31° 08' W	30 m	F. Partensky
PAC1	Unialgal	Tropical Pacific	22° 45' N, 158° W	100 m	L. Campbell

citrate buffer, 10 mM Tris pH 7.4 and 1 mM EDTA. These samples were incubated in presence of RNAse as described above.

For strain CCMP 1378, analyses were performed using an EPICS 541 (Coulter, Hialeah, FL, USA) flow cytometer equipped with a Biosense flow cell and a confocal lens to increase sensitivity (Olson et al. 1990, Vaulot et al. 1990). Cell counting was done using a Micro Sample Delivery System delivering a precise volume of sample at a fixed rate. Samples were illuminated with 1300 mW of blue light (488 nm). Chlorophyll red fluorescence was collected through a 670 nm long pass filter. For cell cycle analysis, YOYO-1-DNA green fluorescence was collected through a 530 nm short pass filter. From 20000 to 50000 cells were analyzed per sample.

For the other strains, cell cycle analyses were performed using a FACSort™ flow cytometer (Becton Dickinson, San Jose, CA, USA). A laser set at 488 nm was used for excitation. Chlorophyll red fluorescence was collected through a 650 long pass filter and green fluorescence of Picogreen-DNA through a 525 band pass filter.

Fluorescent standard beads (0.95 µm diameter, Polyscience, Warrington, PA, USA) were added in each sample to monitor instrument accuracy and normalize fluorescence signals.

Data were acquired in list mode and transferred to a PC-compatible computer for subsequent analyses using CYTOPC (Vaulot 1989) and WinMDI (Joe Trotter). Cell cycle histograms were analyzed using MULTICYCLE (Phoenix Flow Systems, San Diego, CA). Cell cycle phase durations during exponential phase were computed following Slater et al. (1977).

RESULTS

Source of phosphorus

The usual medium for growing *Prochlorococcus* contains an organic source of phosphorus, Gly-P (Chisholm et al. 1992). However, Gly-P cannot be monitored easily, in particular with spectrophotometry. Therefore, in this study it seemed appropriate to use orthophosphate (PO_4^{3-}) instead. To verify that the latter form of phosphorus is assimilated by *Prochlorococcus*, we monitored growth of strain CCMP 1378 in media supplemented with 10 µM of either Gly-P or PO_4^{3-} (Fig. 1). After 2 transfers, both growth rate and final yield appeared identical (Fig. 1). The only difference was that cells seemed to maintain themselves for a longer duration in stationary phase in medium with Gly-P. While little or no PO_4^{3-} should have been detected during growth in the latter medium, concentrations of PO_4^{3-} measured by spectrophotometry were in fact unexpectedly identical in both media (data not shown). This suggested that Gly-P was rapidly remineralized in culture. In order to determine the kinetics of this process, the CCMP 1378 strain was inoculated in duplicate into the 2 media (Fig. 2A). Since *Prochlorococcus* strains are not axenic, a replicate experiment was performed in the dark (Fig. 2B) to assess the respective importance of heterotrophic versus autotrophic processes in Gly-P transformation. Gly-P was completely transformed into PO_4^{3-} within 48 h. No difference was observed between light and dark condi-

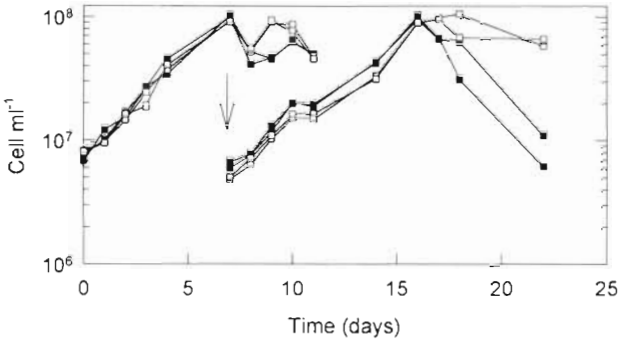

Fig. 1. *Prochlorococcus*. Replicate growth curves of strain CCMP 1378 during 2 successive cultures in media initially containing 10 µM Gly-P (□) or 10 µM PO_4^{3-} (■). Arrow marks the time at which the culture was diluted

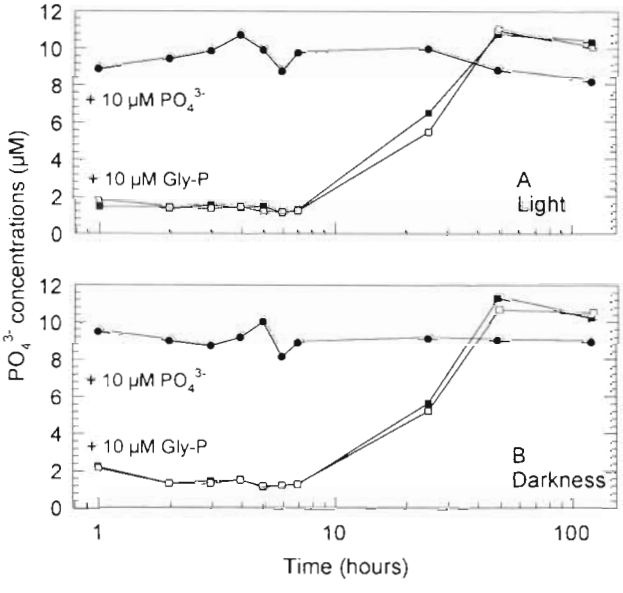

Fig. 2. *Prochlorococcus*. Kinetics of Gly-P transformation into PO_4^{3-} in a culture of strain CCMP 1378 inoculated in a medium with 10 µM of Gly-P. Each point represents the average of 2 PO_4^{3-} measurements. (□, ■) Replicate cultures with Gly-P; (●) control with PO_4^{3-} added initially. (A) Light; (B) darkness

tions (Fig. 2). In contrast a control initially spiked with PO_4^{3-} exhibited no change during this time period (Fig 2). For this reason, an inorganic source of phosphorus (Na_2HPO_4) was used in all following experiments.

Effect of P starvation on *Prochlorococcus* strain CCMP 1378

In order to determine initial PO_4^{3-} concentrations resulting in P starvation, the growth of *Prochlorococcus* strain CCMP 1378 was monitored in 4 different media containing equal amounts of nitrogen and decreasing amounts of PO_4^{3-} resulting in N/P ratios of 15, 50, 150, and 500 (Fig. 3). After stationary phase was reached, PO_4^{3-} was added to one replicate at a final concentration of 10 µM to check whether it induced growth recovery. Initial PO_4^{3-} concentrations did not affect the growth rate (μ_{avg}: 0.48 ± 0.04 d^{-1}; Table 2) but did affect final cell yield (Fig. 3). Cultures with initial PO_4^{3-} concentrations of 10 µM and 3 µM reached a final cell yield of ca 10^8 cell ml^{-1} while those with initial concentrations of 1 and 0.3 µM had a markedly lower final yield. PO_4^{3-} concentrations measured in stationary phase were similar for cultures initially containing 3, 1 and 0.3 µM PO_4^{3-} and ranged around the detection limit (20 to 30 nM; Table 2). In contrast, PO_4^{3-} was still present in stationary phase for the culture with an initial PO_4^{3-} concentration of 10 µM. The 10 and 3 µM cultures did not resume growth when PO_4^{3-} was added during stationary phase, in contrast to the cultures with 1 and 0.3 µM PO_4^{3-}. For the latter, the final yield after PO_4^{3-} addition approached that of the standard 10 µM culture. In cultures not supplemented with PO_4^{3-}, cell concentrations rapidly declined (Fig. 3).

The cell cycle distributions obtained for exponentially growing *Prochlorococcus* in P-replete conditions

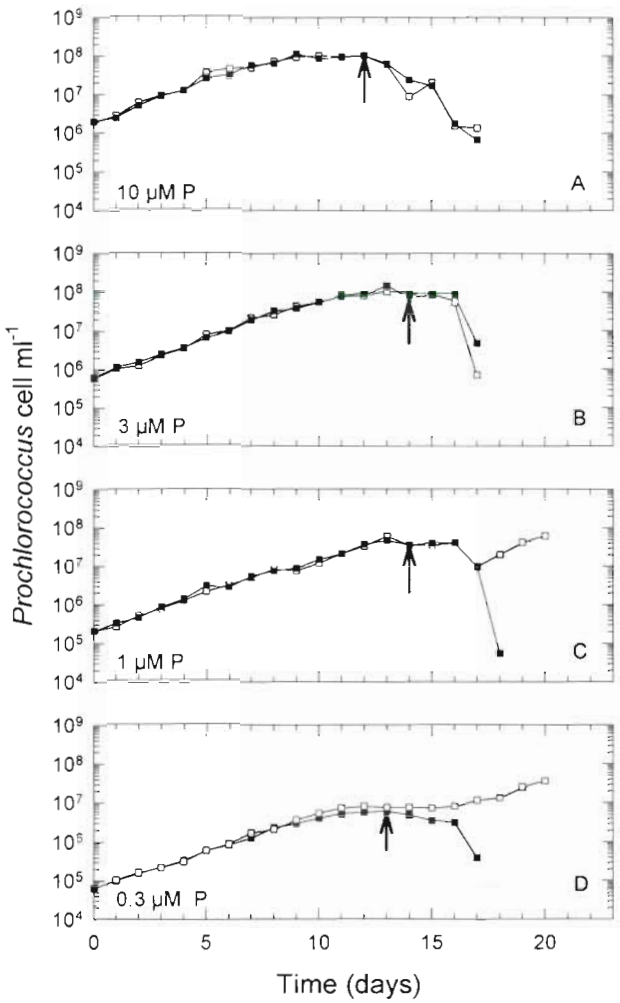

Fig. 3. *Prochlorococcus*. Growth curves of strain CCMP 1378 in different initial concentrations of PO_4^{3-}. (A) 10 µM; (B) 3 µM; (C) 1 µM; (D) 0.3 µM. (■) Control cultures; (□) cultures to which PO_4^{3-} was added at the beginning of the stationary phase (arrows)

Table 2. *Prochlorococcus*. Growth of strain CCMP 1378 in media with different initial PO_4^{3-} concentrations. Each row corresponds to a replicate (see Fig. 3). μ: growth rate; t_d: generation time; t_{G1}, t_S and t_{G2}: durations of the G_1, S and G_2 phases of the cell cycle, respectively. nd: not determined

N/P initial	PO_4 concentration (µM) Initial	Stationary phase	Cell concentration (cell ml^{-1}) Initial	Stationary phase	μ (d^{-1})	t_d (d)	t_{G1} (d)	t_S (d)	t_{G2} (d)
15	10	4.500	2.0×10^6	1.1×10^8	0.48	1.43	1.16	0.23	0.04
15	10	4.600	2.0×10^6	1.1×10^8	0.46				
50	3	0.035	5.8×10^5	9.2×10^7	0.49	1.41	1.12	0.25	0.05
50	3	0.017	6.2×10^5	1.5×10^8	0.52				
150	1	0.023	2.1×10^5	4.8×10^7	0.46	1.50	1.14	0.28	0.08
150	1	0.035	2.1×10^5	4.2×10^7	0.45				
500	0.3	0.035	6.3×10^4	6.0×10^6	0.48	1.43	1.07	0.29	0.08
500	0.3	nd	6.6×10^4	8.0×10^6	0.47				

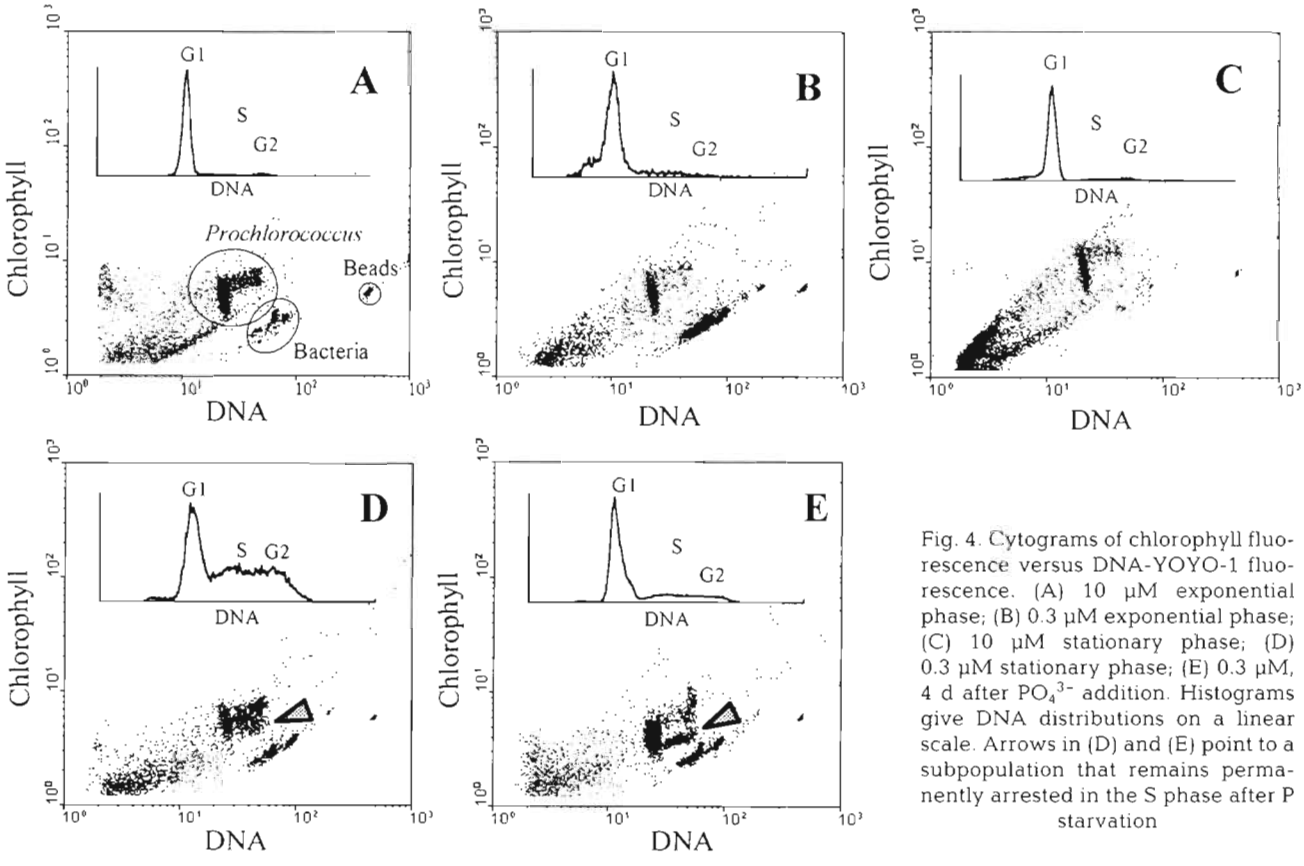

Fig. 4. Cytograms of chlorophyll fluorescence versus DNA-YOYO-1 fluorescence. (A) 10 µM exponential phase; (B) 0.3 µM exponential phase; (C) 10 µM stationary phase; (D) 0.3 µM stationary phase; (E) 0.3 µM, 4 d after PO_4^{3-} addition. Histograms give DNA distributions on a linear scale. Arrows in (D) and (E) point to a subpopulation that remains permanently arrested in the S phase after P starvation

(Fig. 4A) displayed 2 peaks corresponding to the G_1 and G_2 phases (Vaulot & Partensky 1992) and a well-defined S phase. Initial phosphate conditions only slightly affected cell cycle phase distributions as long as cells were in exponential phase (Fig. 4B). Cells were mostly in the G_1 phase of the cell cycle. The average durations of G_1, S and G_2 were equal to 1.14, 0.26 and 0.06 d respectively (Table 2). The only visible effect of low initial PO_4^{3-} concentrations (1 and 0.3 µM) was a slight increase of the duration of S and G_2 at the expense of G_1. In stationary phase, most cells of P-replete cultures (initial N/P = 15 and 50) were in G_1 (Fig. 5). In both cases, P addition during stationary phase had no effect on the cell cycle distribution. In P-starved cultures (initial N/P = 150 and 500), the relative percentage of cells in G_1 (G_1%) decreased and conversely G_2% increased to 20–40 % (Figs. 5 & 6). Moreover, when *Prochlorococcus* began to be P-starved, a subpopulation of cells accumulated in the S phase (Fig. 4D arrowhead). These cells gradually lost their red chlorophyll fluorescence (Fig. 4E). This loss of chlorophyll was not reversed when P was resupplied. In contrast, cells arrested in G_1 and G_2 did not loose fluorescence (Fig. 4E). When P was resupplied, the fraction of cells in G_2 decreased as the result of cell division and cells finally accumulated in G_1 as in the P-replete case (Figs. 5 & 6).

Comparison of the effect of P starvation on other *Prochlorococcus* strains

In order to compare the effect of P starvation on *Prochlorococcus* originating from different geographic areas, 5 other strains from the Atlantic and the Pacific Oceans (Table 1) were grown with either 10 or 0.3 µM PO_4^{3-} (Fig. 7). For all those strains, as for CCMP 1378, initial PO_4^{3-} concentration did not affect growth rates (Table 3). However, these strains grew ca 2–2.5 times more slowly than CCMP 1378 (Table 2), either because they had a higher growth irradiance optimum or because they were incompletely acclimated to light conditions. In terms of cell cycle phase durations, G2 was much longer than in CCMP 1378 and initial PO_4^{3-} conditions had a more marked effect. For PAC1, TATL1 and TATL2, S was longer in low PO_4^{3-} medium while it was not affected for the 2 other strains. For all strains G_2 was shorter under P-limited condition.

Final cell yields were much lower with 0.3 µM than with 10 µM initial PO_4^{3-} concentration (Table 3) as observed for CCMP 1378. Similarly, for all the strains but NATL2, S and G_2% increased for P-starved stationary cultures (Fig. 7). The most dramatic changes, similar to those previously observed for CCMP 1378, were found for TATL2 (Fig 7H), the other strains

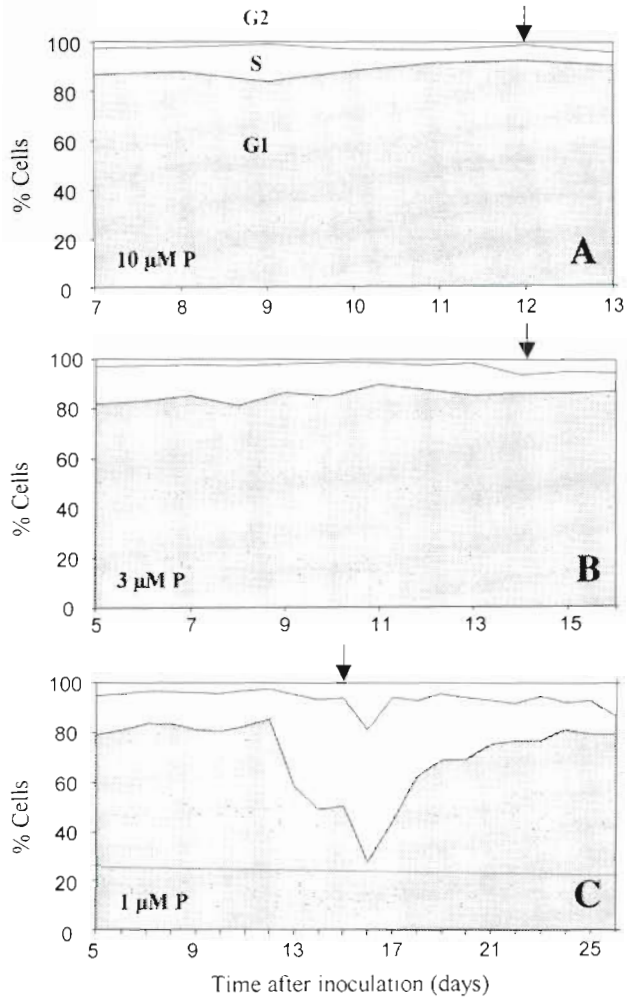

Fig. 5. *Prochlorococcus*. Percentage of cells in the G_1, S and G_2 phases during growth of strain CCMP 1378 in different initial phosphate concentrations. (A) 10 µM PO_4^{3-}; (B) 3 µM PO_4^{3-}; (C) 1 µM PO_4^{3-}. PO_4^{3-} was added during stationary phase to a final concentration of 10 µM (arrows; see Fig. 3)

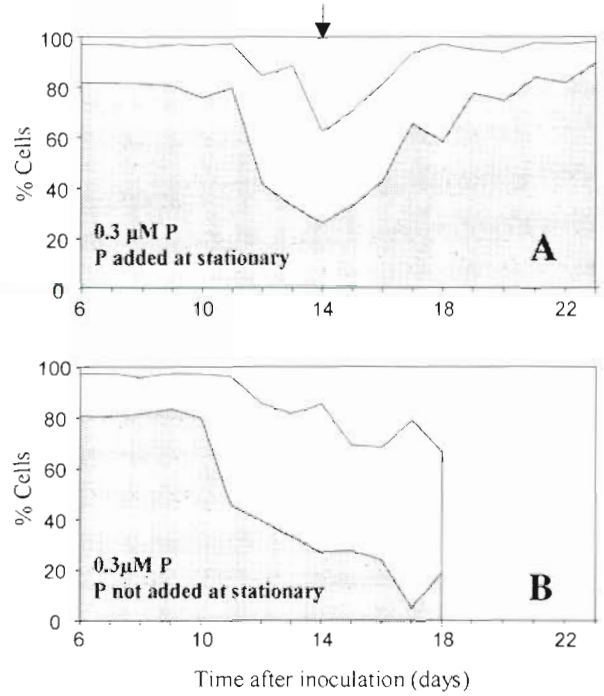

Fig. 6. *Prochlorococcus*. Percentage of cells in the G_1, S and G_2 phases during growth of strain CCMP 1378 with an initial concentration of 0.3 µM PO_4^{3-}. (A) PO_4^{3-} added in stationary phase to a final concentration of 10 µM (arrow). (B) No PO_4^{3-} added in stationary phase

showing a much more progressive increase of G_1 and S% as cells became P-starved. For NATL2, only S, not G_2%, increased at the stationary phase (Fig. 7d). Under P-replete conditions, TATL2 again showed the closest behavior to CCMP1378 with almost no changes in the percentage of cells in the different cell cycle phases during the stationary phase. For the other strains G_2% had a tendency to increase at the end of the exponential growth.

DISCUSSION

Gly-P is the phosphorus source classically used in the medium for growing *Prochlorococcus* (Chisholm et al. 1992) since it is derived from the K medium (Keller

Table 3. *Prochlorococcus*. Final cell concentrations and growth rates (μ), generation times (t_d) and durations of the G_1, S and G_2 phases of the cell cycle (t_{G1}, t_S, t_{G2} respectively) during the exponential phase for different strains in the standard medium (N/P = 15, PO_4^{3-} initial = 10 µM) and in P-limited medium (N/P = 500, PO_4^{3-} initial = 0.3 µM)

Strain	Final cell conc. (cell ml^{-1})		μ (d^{-1})		t_d (d)		t_{G1} (d)		t_S (d)		t_{G2} (d)	
	N/P = 15	N/P = 500	N/P = 15	N/P = 500	N/P = 15	N/P = 500	N/P = 15	N/P = 500	N/P = 15	N/P = 500	N/P = 15	N/P = 500
NATL1	1.1×10^8	2.9×10^6	0.21	0.27	3.29	2.61	1.94	1.67	0.45	0.47	0.90	0.47
NATL2	1.8×10^8	1.5×10^6	0.21	0.22	3.32	3.15	1.80	2.01	0.96	0.89	0.56	0.26
TATL1	8.1×10^7	1.5×10^6	0.20	0.20	3.54	3.41	2.08	2.00	0.42	0.80	1.04	0.61
TATL2	1.5×10^8	8.8×10^5	0.19	0.20	3.69	3.47	2.79	2.10	0.48	1.03	0.42	0.34
PAC1	7.2×10^7	4.0×10^6	0.25	0.24	2.80	2.89	1.40	2.06	0.37	0.54	1.02	0.29

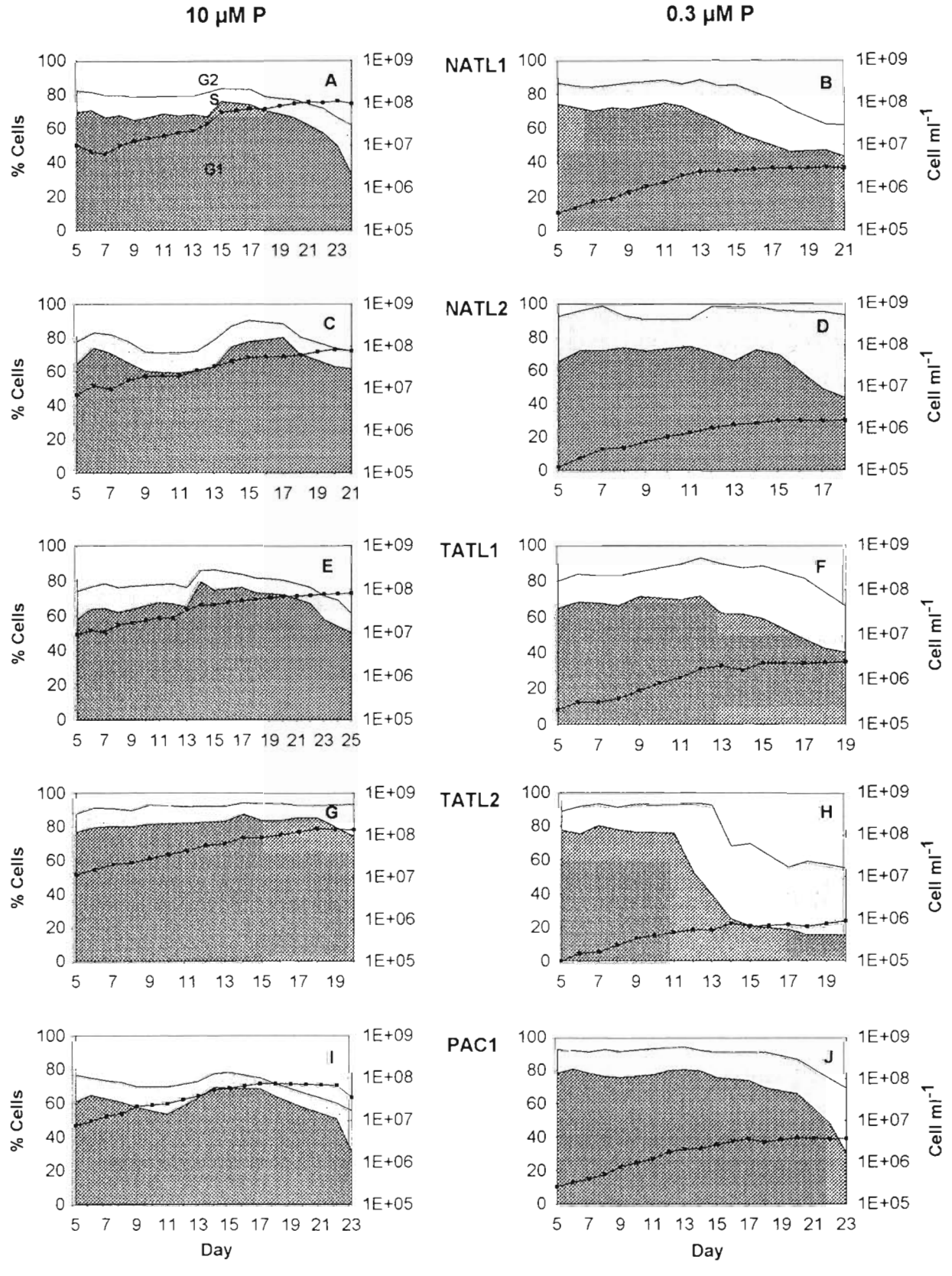

Fig. 7. *Prochlorococcus*. Cell concentration and percentage of cells in the different cell cycle phases in media with initial PO_4^{3-} concentrations of 10 µM (left) and 0.3 µM (right) for strains NATL1 (A, B), NATL2 (C, D), TATL1 (E, F), TATL2 (G, H) and PAC1(I, J)

et al. 1987) initially designed for neritic photosynthetic eukaryotes and containing this form of phosphorus. However, we demonstrated that, at least for non-axenic *Prochlorococcus* strains (no axenic strain is available yet), inorganic PO_4^{3-} is perfectly adequate for *Prochlorococcus* growth. Moreover, Gly-P appears to be rapidly degraded in *Prochlorococcus* cultures. Since the process is similar under light and dark conditions, it is probably mediated by heterotrophic contaminating bacteria, releasing phosphatase activity (Cembella et al. 1983a).

Change in the initial PO_4^{3-} concentration and in particular a decrease down to 0.3 µM had virtually no effect on the initial population growth rate for any of the tested strains. Cells could divide for at least 3 generations in such low P medium. Since final P concentration in stationary phase was ca 0.03 µM, it is likely that P-limitation for *Prochlorococcus* only occurs at low concentrations (10 to 100 nM). Indeed, for strain CCMP 1378, only a very high initial N/P ratio (≥ 150) led to unambiguous P starvation in stationary phase as demonstrated by the triple evidence of lower final cell yield with respect to P-replete conditions (N/P= 15), of near detection limit PO_4^{3-} concentration in stationary phase and of resumption of growth after P addition. For all strains, N/P ratios of 15 and 500 also corresponded to P-replete and P-starved conditions respectively. At the intermediate N/P ratio of 50, the final cell yield of strain CCMP 1378 was slightly reduced with respect to P-replete condition, PO_4^{3-} concentration was very low, but growth did not restart after P addition, suggesting a double limitation by P and another factor (probably N). N/P ratios at which one nutrient limitation replaces the other have also been found to be different from the Redfield ratio for eukaryotic microalgae (in the range 7 to 53) and have been suggested to correspond to the ratio of minimum N and P cell quotas (Cembella et al. 1983b). Raven (1994) predicted that the N/P ratio should be higher than the Redfield ratio for small cyanobacteria cells. The present data for *Prochlorococcus* seem to confirm this prediction.

In all strains except NATL2, P starvation induced accumulation of cells into the S and G_2 phases. Accumulation at the end of the cell cycle in response to an environmental stress is quite unusual. For example, nitrogen starvation induces, in all phytoplankton species studied to date, cells to accumulate in the G_1 phase (e.g. Olson et al. 1986, Vaulot et al. 1987). This is also apparently true for *Prochlorococcus* field populations (Vaulot & Partensky 1992) as well as cultured strains (this study).

Cell arrest in G_2 in response to limiting environmental factors has rarely been reported (Murray & Hunt 1993). One exception is diatoms that may arrest in G_2 in response to silica starvation (Vaulot et al. 1987, Brzezinski et al. 1990), since silica is required for new frustule formation prior to cell separation. Similarly, light deprivation can block diatom cells in G_2 (Vaulot et al. 1986), but again this appears to be related to light-dependent silica uptake prior to frustule deposition. Closer to *Prochlorococcus*, darkness induces the cyanobacterium *Synechococcus* to arrest in G_2 (Armbrust et al. 1989, Binder & Chisholm 1990).

Several hypotheses could explain an arrest in G_2 in the present case. Either the energetic stock (ATP) that relies heavily on P (Cembella et al. 1983b) is too low to allow cell division, or there is a lack of P for the synthesis of major cellular components required for division, such as the phospholipids needed to make up novel cell membranes. Although our data set does not allow us to distinguish between these hypotheses, it appears clearly that cells blocked in this phase can resume cycling once PO_4^{3-} is provided again.

Are P-starved *Prochlorococcus* cells arrested in G_1 and G_2 blocked in a single location (block point), as observed for light arrested cells of the coccolithophorid *Pleurochrysis carterae* (Vaulot et al. 1986), or are they uniformly distributed over each phase? In the former case, one would expect PO_4^{3-} addition to induce synchronous release from G_1 and G_2, rapidly resulting in a major wave of cells going from G_1 to S and from G_2 to G_1, followed by damped oscillations (see for example Vaulot & Chisholm 1987). Although we did not sample with sufficient temporal resolution after PO_4^{3-} addition to see waves of cells leaving G_1 and G_2, the slow decrease in the fraction of cells in S and G_2 after P addition suggests uniform arrest rather than sharp blocking points. This is also suggested by the occurrence of cells arrested throughout the whole S phase (Fig. 4E).

The most remarkable cell cycle behavior we observed in all strains is clearly the arrest of cells in the S phase. This is a most unusual feature not only for phytoplankton, but for both eukaryotes and prokaryotes in general. In eukaryotes, the S phase is the most critical one. In particular gene expression is partly inactivated as DNA undergoes replication. All conditions must be right for S phase completion before cells can begin DNA synthesis. This phase is under the control of multiple check points and feedback mechanisms (Murray & Hunt 1993). Even in prokaryotes, which have much looser cell cycle controls (for example DNA replication and cell division can be somewhat uncoupled; Helmstetter & Cooper 1968), cells starved for light (autotrophs) or organic carbon (heterotrophs) usually terminate DNA synthesis before arrest (Binder & Chisholm 1990, Lebaron & Joux 1994). In the case of P starvation of *Prochlorococcus*, it looks as if no mechanism ensures that cells entering the S phase hold enough internal P stores to successfully complete DNA

replication, a process that requires a lot of P. The very critical nature of the S phase is further illustrated by the irreversible nature of the arrest. In all strains, cells arrested in S progressively lost chlorophyll fluorescence and, at least for CCMP 1378, P addition did not induce those cells to cycle again.

Although the different strains we examined have been isolated from widely different locations both in terms of geography and of depth (Table 1) and differ both phenotypically (F.P. unpubl. data) and genotypically (Scanlan et al. 1996), they displayed a relatively uniform response to P starvation. This is surprising in view of the wide variety of cell cycle behaviors observed in strains of the genetically closely related cyanobacterium *Synechococcus* (Binder & Chisholm 1995). The only marked differences between strains concerned the duration of the G_2 phase during exponential growth and the fact that P-starved cells of strain NATL2 did not accumulate in G_2. These differences between the different strains could be constitutive as has been shown for *Synechococcus* (Binder & Chisholm 1995). However CCMP 1378 is genetically further apart from TATL2 than from NATL2 (Scanlan et al. 1996), although it has a cell cycle behavior much closer to that of the former strain. Alternatively, it could also be explained by the history of cells. While strain CCMP 1378 was acclimated for numerous generations before the P-limitation experiment was conducted, the other strains had less time to acclimate to their light environment, which could explain their lower generation time.

From an ecological point of view, this study suggests that the examination of the cell cycle status of natural populations could be used quite easily to determine whether they are limited by P or another factor. Observing large cell populations in the S phase would suggest that P is limiting, in the same way that arrest in G_1 and growth restart after N supply suggested N limitation of *Prochlorococcus* cells in the Mediterranean Sea (Vaulot & Partensky 1992).

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