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Change in Photosynthetic Capacity over the Cell Cycle in Light/Dark-Synchronized *Amphidinium carteri* Is Due Solely to the Photocycle[1]

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ABSTRACT

Cell cycle dependent photosynthesis in the marine dinoflagellate *Amphidinium carteri* was studied under constant illumination and light/dark (L/D) photocycles to distinguish intrinsic cell cycle control from environmental influences. Cells were grown in constant light and on a 14:10 L:D cycle at light intensities that would yield a population growth rate of 1 doubling per day. In the former case division was asynchronous, and cells were separated according to cell cycle stage using centrifugal elutriation. Cells grown on the L:D cycle were synchronized, with division restricted to the dark period. Cell cycle stage distributions were quantified by flow cytometry. Various cell age groups from the two populations were compared as to their photosynthetic response (photosynthetic rate *versus* irradiance) to determine whether or not the response was modulated primarily by cell cycle constraints or the periodic L/D cycle. Cell cycle variation in photosynthetic capacity was found to be determined solely by the L/D cycle; it was not present in cells grown in constant light.

In populations synchronized by environmental cues, it is difficult to distinguish cell cycle dependent physiology from responses to the environment. Periodicity in the environment can entrain independently both the cell cycles and the circadian clocks of the individuals in a population (2, 29). Since both of these mechanisms can give rise to coordinated population behavior, it is not clear which is the important factor. Further complicating the problem is the fact that cells have direct reactions to their surroundings. For example, a plant cell transferred into the light may respond to its changed energy status regardless of its cell cycle or clock position. The well known periodic change in photosynthetic capacity in cells grown in photocycle is an example of such an ambiguous situation; while photosynthetic rhythms have been described in detail (6, 13–15), the relative contributions of the clock, the photocycle, and the cell cycle in controlling such rhythms have not been well established. In particular, the contribution of the cell cycle *per se* (*i.e.* in the absence of previous entraining stimuli) has never been evaluated.

In all cells, specific physiological events such as DNA synthesis, mitosis, and cytokinesis define progression through the cell cycle. Cell cycle dependent physiology has been invoked to help explain phasing of cell division in periodic environments and to explain diel changes in cell behavior. For example, phasing of cell division to periodic L/D[3] exposure in some phytoplankton can be modeled as resulting from a light dependent portion in the cell cycle (28, 30). This light dependent segment causes cells to arrest in specific cell cycle stages in the dark and to lengthen the duration of those stages at light levels that are subsaturating for growth (23, 30).

Cell cycle dependent physiology has also been used to explain the temporal separation of photosynthesis and nitrogen fixation in the nonheterocystous cyanobacterium, *Synechococcus* spp. (20). Similarly, L/D synchronized *Platymonas striata* exhibits periodicity in many cellular activities including the synthesis of RNA, carbohydrate, protein, and photosynthetic pigments (26). Finally, Howell *et al.* (8) observed a 3- to 12-fold fluctuation in the synthesis rate of 20 proteins with cell cycle progression in *Chlamydomomas reinhardtii* maintained on a periodic light regime. As these authors point out, the results of all these experiments are ambiguous since environmental change is coincident with the cell cycle progression.

The difficulty in separating environmental effects from cell cycle dependent events has been formally addressed by John *et al.* (11). According to their categorization, 'primary' events in the cell cycle, such as a light requiring phase of the cycle and the synthesis of DNA, are those that occur under all environmental conditions and are inseparable from the cell cycle. 'Secondary' events are directly involved in cell cycle progression, but may be necessary only under some circumstances. For example, although tubulin is necessary for completion of the cycle, the cell may only synthesize it if its internal stores are insufficient. The final, 'tertiary,' processes are coincident with, but not controlled by, the cell cycle. These are, in fact, responses to the changing environment that occur simultaneously with the synchronized population's cycling. John *et al.* hypothesize that the majority of changes in physiological parameters (*i.e.* photosynthesis, nutrient uptake, etc.) are of this type.

This dichotomy between cell cycle *coincident* events in environmentally entrained populations and true cell cycle *dependent* activities has been well established in nonphotosynthetic systems. In yeast and animal cell cultures it has long been recognized that synchronization of populations by nu-

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[3] Abbreviations: L/D, light/dark; FSW, filtered seawater.

trient starvation or inhibitors might lead to artifactual changes with the progress of the cell cycle (4, 19). For example, Maltese and Sheridan (16) have shown conclusively that a previously observed peak in sterol biosynthesis in late G_1 LM cells is an artifact of the synchronization process. Cells stopped developing at a specific point in their cell cycle in response to a synchronizing agent, but at the same time they were changing their pattern of sterol synthesis in direct response to the same agent. The ambiguity arose because the arrest within the cell cycle and response to the environmental change occurred simultaneously. It is plausible that similar phenomenon occur during the synchronization of algal cell division by environmental cues such as periodic L/D.

Further complicating the situation is the action of the circadian clock within the cells, particularly in photosynthetic cells. The cell cycle can be coupled to this endogenous clock, which in turn is entrained by the diel photocycle when population growth rates are less than one doubling per day (2). This entrainment is maintained upon the withdrawal of the cue, and cell division synchrony can persist in constant conditions for many generations. In a periodic environment, the clocks of the entire population are reset, and thus synchronized, daily. In a constant environment, on the other hand, each cell's clock will continue to keep time according to its free running period, resulting in the ultimate decay of population synchrony. Thus, the circadian clock can serve as another interface between the environment and the cell cycle. Its mechanisms are elusive, as is its degree of coupling to the cell cycle under different environmental conditions.

In this paper, we have used centrifugal elutriation to segregate a constantly illuminated, asynchronous population of *Amphidinium carteri* into various cell cycle stage enriched fractions. By analysis of these fractions and comparison with samples from cultures synchronized by periodic L/D exposure, we investigate whether the well known diel variation in photosynthesis is cell cycle dependent or regulated by the light history of the cells. Although this study cannot shed light on the role of an endogenous clock in photosynthetic periodicity, we were able to establish that the cell cycle *per se* has very little influence on photosynthetic performance relative to that of the diel L/D cycle.

MATERIALS AND METHODS

Culture Conditions

Cultures of the dinoflagellate *Amphidinium carteri* were maintained in f/2 culture media (23) at 20°C. Population growth and cell volume were monitored using a Coulter Electronics (Hialehia, FL) model Z_m electronic particle counter with an accompanying Channelizer model 256. Cells were maintained in nutrient replete growth by dilution with fresh f/2 media when cell concentrations approached 3×10^4 cells mL^{-1}.

Asynchronous cultures were kept in constant illumination of approximately 150 $\mu E\ m^{-2}\ s^{-1}$ while synchronized cultures received 10 h of 260 $\mu E\ m^{-2}\ s^{-1}$ illumination followed by 14 h of dark each day. Under both illumination regimes the population-growth rate was one doubling per day. Both cultures were maintained and monitored for at least 20 generations prior to experimentation to assure that cells had fully acclimated to their environment.

Centrifugal Elutriation

Elutriation was performed using a JE-6B elutriating rotor with the standard Beckman chamber in the J2-21 refrigerated centrifuge equipped with a stroboscopic rotor speed monitor (Beckman Instruments Inc., Palo Alto, CA). Fluid flow was provided by a peristaltic pump (Cole-Palmer Masterflex model No. 900-197 with the 7014 pump head). Cells were loaded at a concentration of 2×10^5 cells/mL and elutriated with f/2 media. The rotor and the cell cultures were maintained at room temperature during elutriation, to avoid changes in fluid temperature.

Beckman Instruments (1) provides a nomogram of rotor speed and fluid flow rate to aid in the establishment of correct conditions for the elutriation of different sized cells. This nomogram was developed for typical animal cells which are less dense than most algae, thus we had to establish elutriation conditions by trail and error. Elutriation of *A. carteri* was successful at a rotor speed of 1335 rpm and with media flow ranging from 11 to 28 mL min^{-1}.

In an effort to make fine separations among the small, common cells and to maximize the number of cells per fraction with the larger, rarer cells, the change in the media flow rate varied from small (2 mL min^{-1}) steps initially to larger (6 mL min^{-1}) steps in later fractions. At each flow rate, two 50 mL fractions were collected and stored in dim light (25 $\mu E\ m^{-2}\ s^{-1}$) at room temperature (20°C) for approximately 30 min prior to analysis. *A. carteri* cells displayed approximately 1 h of reduced motility following elutriation. Rates of photosynthesis in elutriated samples, however, were quite similar to the rates observed in the exponentially growing population.

Following elutriation, each 50 mL fraction was counted with a Coulter electronic particle counter (model Z_m) and cell volume distributions were also collected.

Cell Cycle Stage Distributions

Following sampling, approximately 5×10^5 cells were prepared for flow cytometric analysis as described in Olson *et al.* (24). Cells were collected by centrifugation at 2000*g* for 5 min, resuspended in 0.5 mL of the remaining media, and injected through a 24 gauge hypodermic needle into 10 mL of ice-cold methanol. Samples were stored at 4°C at least overnight to assure complete extraction of Chl.

In preparation for staining, cells were pelleted out of methanol by centrifugation at 2000*g* for 5 min, resuspended in 1 mL of FSW and recentrifuged at 14,000*g* for 1 min. Cells were again resuspended in 1 mL FSW and stained using the DNA specific fluorescent dye Hoechst 33342 at a final concentration of 5 $\mu g\ mL^{-1}$ (24). Cells were exposed to the stain for at least 30 min prior to flow cytometric analysis.

Analysis was performed on a microscope based flow cytometer (24) using ultraviolet (300-400 nm) excitation from a mercury arc lamp. DNA-Hoechst fluorescence was measured as emitted light passing a 430 to 470 nm bandpass filter. Data was collected on 5×10^4 cells and 256 channel histograms

were stored on discs using an IBM Instruments 9000 computer. DNA histograms were subsequently analyzed to determine the proportion of each G_1, S, and G_2 + M using a algorithm developed by Vaulot *et al.* (30). Following elutriation, no fraction contained greater than 5% S phase cells. This was likely due to volume overlap between the cell cycle stages preventing the isolation of fractions enriched with S phase cells. Our attention was thus concentrated on the G_1 and G_2 + M cell cycle stages.

Chl Concentration

Ten mL of sample was filtered onto Schleicher and Schuell 24 mm No. 34 glass filters using a vacuum of <1 psi and extracted in 90% acetone. Chl concentration was determined fluorometrically as described by Parsons *et al.* (25).

Photosynthesis *versus* Irradiance Curves

A light gradient of eight intensities ranging from 24 to 821 μE m^{-2} s^{-1} was made using neutral density screening and eight 30 W cool-white fluorescent bulbs. The temperature was held at 25°C at all sample positions throughout the experiment.

Photosynthesis was measured using a modification of the $^{14}CO_2$ uptake technique of Parsons *et al.* (25). Ten mL of diluted culture at 10^4 cells mL^{-1} were exposed to 1 μCi of $NaH^{14}CO_2$ (ICN Radionuclides, Irvine, CA) in 10^{-2} M total inorganic carbon. Dark fixation was monitored by incubation in aluminum foil-wrapped tubes. After incubation, cells were filtered at <1 psi onto Schleicher and Schuell No. 34 glass filters, which were then fumed over concentrated HCl to volatilize unreduced $^{14}CO_2$. After 24 h equilibration with 5 mL of Beckman Ready Safe scintillation cocktail, the radioactivity in the samples was determined. The rate of $^{14}CO_2$ fixation in the dark was subtracted from each sample. Photosynthesis *versus* irradiance curves were fit iteratively by a least squares procedure to a linearization of the relationship:

$$P(I) = P_m \tanh(\alpha I/P_m) \quad (1)$$

where $P(I)$ is the photosynthetic rate at a given irradiance, I is the irradiance, α is the slope of the relationship at low irradiance, and P_m is the light saturated rate of photosynthesis (10). While this relationship provides a good description of the data, the nonlinear nature of the hyperbolic tangent function makes it difficult to assign confidence intervals to P_m and α, the parameters of interest in our analysis. To avoid this problem, two simple linear functions were fit to the hyperbolic data such that values and confidence intervals were obtained for α and P_m. The parameters resulting from the two functions are α' and P_m'. The first line had a slope α', and a y-intercept of zero, fit to the $P(I)$ values at the lowest light levels. The P_m' parameter was fit to the data from saturating light levels by a line with zero slope and a y-intercept of P_m'. Since the iterative fit to the hyperbolic tangent relationship uses all the data in assigning values to α and P_m it was used as a check on the simple linear descriptions of α' and P_m'. In all cases the parameters obtained by the two different methods (α and α', P_m and P_m') were very similar. Thus, they were considered equivalent and will be referred to as α and P_m in the remaining discussion.

RESULTS

Separation of Cell Cycle Stages by Elutriation

Centrifugal elutriation is a gentle way of separating cells on the basis of their fluid drag and their density by balancing the forces of fluid flow and centrifugal acceleration (1). It is particularly useful for separating young cells from old cells for analysis of cell cycle dependent physiology. The details of the elutriation proceedure used in this work can be found in Gerath (3), which is available upon request from the second author.

Elutriation of the asynchronous *Amphidinium carteri* population grown in constant light resulted in good separation of the cells by volume. The average volume of the elutriated fractions increased steadily from 360 μm^3 to 600 μm^3 (Fig. 1A) with most fractions containing at least 10% of the total population of cells loaded (data not shown) The portion of the elutriation profile in which average cell volume increased between fractions (between flow rates of 11 and 23 mL min^{-1}) contained 80% of the loaded cells; 83% of the cells were recovered in all.

Analysis of the DNA histograms from flow cytometric analysis of these populations revealed a steady decrease in the percentage of G_1 cells from 94 to 40% with increasing average volume (Fig. 1A). Concurrent with this decrease in the proportion of G_1 cells was an increase in the percentage of cells in G_2 + M from 5 to 55%. These are substantial changes from the asynchronous pre-elutriation population which had a distribution of 71% G_1, 7% S, and 22% G_2 + M. The proportions of G_1 and G_2 + M were directly related to cell volume (Fig. 1B), but under no circumstance could we obtain complete separation of the G_1 cell cycle stage from the G_2 + M stage. This is consistent with results from studies using other cell types (4, 12) and probably results from the fact that the largest G_1 cells are the same volume as many of the G_2 + M cells (12).

In the constantly illuminated population, there was a direct relationship between the proportion of G_2 + M and the Chl per cell (Fig. 1C). In contrast to the observed doubling in cell volume with the same transition (Fig. 1B), extrapolation of the Chl relationship from 0 to 100% G_2 + M does not yield a doubling of Chl per cell. Since cellular Chl increases more slowly than cellular volume, a decrease in cellular Chl concentration with cell aging is implied. The explanation of this is not clear but may be due to selective loss of the youngest and/or oldest cells during elutriation, to a potential increased rate of Chl synthesis early or late in the cell cycle, or to both factors acting together.

Cell Division Patterns in L/D Synchronized Cultures

In populations grown in a 10:14 L/D cycle, cell volume increased steadily during the 10 h light period while the number of cells remained constant (Fig. 2A). With the onset of darkness the cells abruptly stopped growing and even decreased slightly in volume. Cell division began after approximately 4 h of darkness and continued until the number of cells had doubled lO h later. As expected, the average cell volume declined as cell division proceeded.

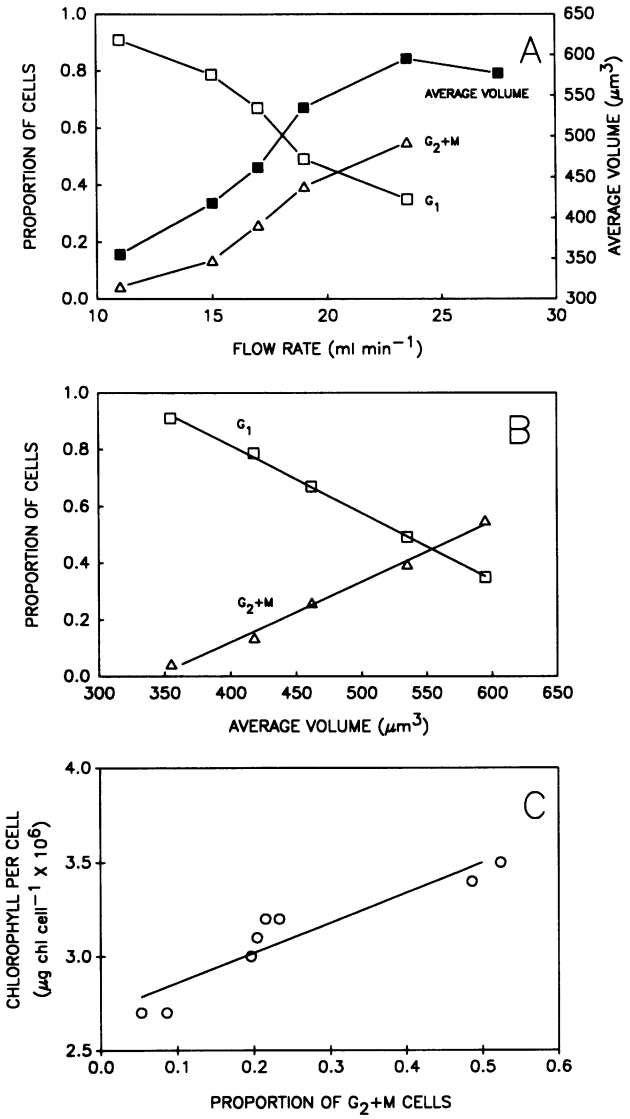

Figure 1. Separation of *A. carteri* cells in different cell cycle stages using centrifugal elutriation. The population was grown in constant light before the elutriation was performed. A, The average cell volume and cell cycle stage distributions as determined by flow cytometry in an elutriated fraction are plotted against the fluid flow rate yielding the fraction. The distribution of cell cycle stages before elutriation was $G_1/S/G_2 + M = 0.71/0.07/0.22$. B, Variation of cell cycle stage proportion with average cell volume. C, Average Chl per cell as a function of the proportion of $G_2 + M$ cells.

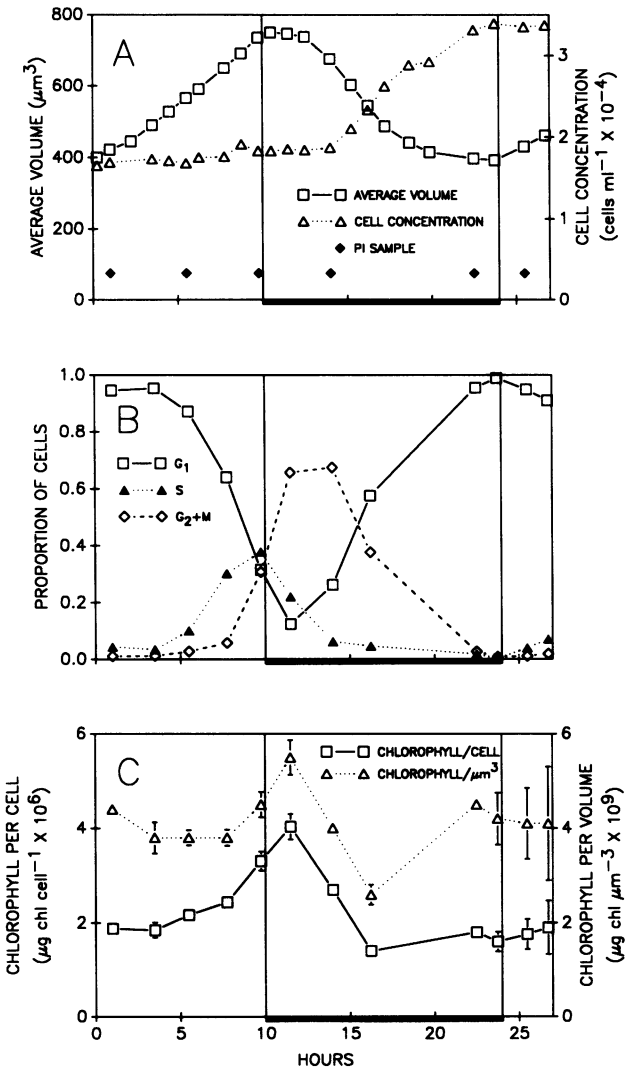

Figure 2. Characteristics of L/D synchronized *A. carteri*. A, Average cell volume and cell concentration in culture synchronized on L/D 10/14. Cell concentration and volume are the averages of four replicate cultures. Diamonds indicate the times when samples were taken to measure photosynthetic performance. B, Proportions of cell cycle stages. C, Chl content per cell and cell volume. Error bars represent the 95% confidence interval.

Flow cytometric analysis of DNA per cell confirmed that the cells were synchronized in their cell cycle stages (Fig. 2B). In the early portion of the light period the cells were nearly all in G_1. The cells progressed through their cell cycles as a cohort in a manner consistent with the cell number data (Fig 2A). Accompanying this progression through the cell cycle were obvious changes in the rate of cell growth, division, and Chl content. The increase in cell volume was restricted to the light period, while the cells are in G_1 and S (Fig. 2A). The population exhibited an increase in Chl per cell through the light period and into the early dark period (Fig. 2C). Although it appears that the amount of chlorophyll per cell doubled and then was reduced by half by cell division, the decline in Chl per cell actually precedes the onset of cell division. Furthermore, the volume-normalized Chl dropped sharply and then increased, apparently reflecting a loss and resynthesis during the mid-dark period.

Contrasting these results with those from the elutriated population we find distinctly different patterns of Chl per cell and Chl per volume were evident between the two light regimes. Despite the steady increase in Chl per cell observed with cell cycle progression under constant illumination, under periodic conditions the cells lost much of their Chl with the entry into $G_2 + M$. This loss was also reflected in a sharp decline in the Chl per volume through the mid-dark period. The Chl loss probably results from cessation of synthesis,

since the observed loss rate is consistent with Chl turnover times of approximately 1 h (5). Chl per volume began to increase before the dark to light transition, suggesting the operation of a cellular clock (2).

Photosynthetic Responses Over the Cell Cycle in Constant Light

Examination of the photosynthetic response curves of the elutriated fractions from the constantly illuminated cultures revealed little change in photosynthetic performance over the cell cycle (Fig. 3). Since α and P_m are often well correlated (10) and this is borne out in our data (3) we limit our analysis to changes in P_m. The photosynthesis-irradiance relationship for the large (55% G_2 + M) and medium sized (77% G_1 and 19% G_2 + M) cells were virtually identical when photosynthesis was normalized to number of cells (Fig. 3A). P_m for the smaller (94% G_1) cells was slightly reduced. This difference was no longer evident when the photosynthetic rate was expressed in terms of Chl (Fig. 3B). Thus, the P_m parameter normalized by cell number or Chl concentration changed by less than 20% despite a 10-fold change in the proportion of G_2 + M cells in the population (Fig. 3C).

Photosynthetic Response in L/D Entrained Populations

Significant changes in the light saturated rate of photosynthesis were evident when a L/D cycle was superimposed on the cell cycle (Fig. 4A). Light saturated photosynthesis per cell was relatively constant through the entire light period except for an apparent increased sensitivity to high light at 5.5 h into the light period (Fig. 4, A and B). This reduction in photosynthetic rate was seen consistently in replicate experiments and was absent from either of the surrounding time points. Cellular photosynthetic capacity was greatly reduced in the dark period: 4.2 h after the onset of darkness the photosynthetic rate had declined by 75%. The rate doubled through the next 8.5 h of darkness and doubled again in 3 h with the beginning of illumination. The initial slope (α) underwent similar changes over time (data not shown).

When the photosynthetic rate is normalized to the amount of Chl (Fig. 4C) it is clear that efficiency of photosynthesis declined as Chl was synthesized through the day. This decline proceeded at a constant rate and lasted into the dark period (Fig. 4D). Although a sharp change in the rate of carbon fixation was observed at the dark-light transition, a recovery in the rate was again evident before this transition. Again, the a parameter changed in parallel to P_m (data not shown).

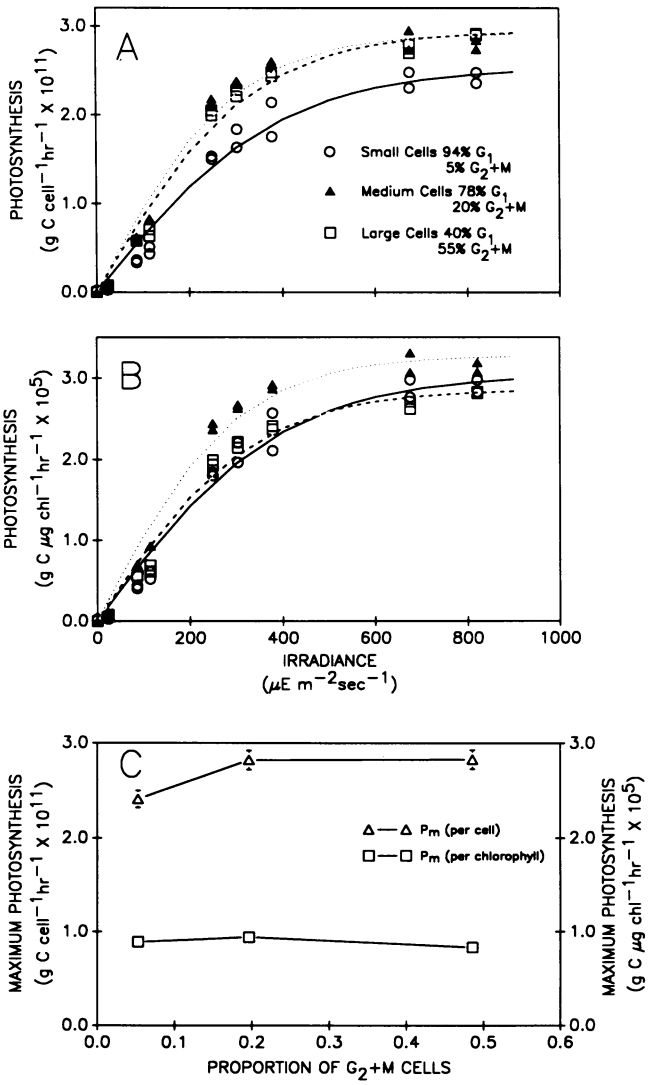

Figure 3. Photosynthetic rate as a function of irradiance in cells grown in constant light, and fractionated into different cell cycle stages through elutiation. A, Photosynthetic rate normalized to cell number; B, photosynthetic rate normalized to cellular Chl; C, light saturated photosynthetic rate (P_m) from (A) and (B) *versus* proportion of G_2 + M in the population. Error bars are the 95% confidence interval.

DISCUSSION

Amphidinium carteri cells synchronized by a periodic photocycle exhibit dramatic variation in photosynthetic capacity and Chl, coincident with progression through the cell cycle. Since such changes are not apparent as cells progress through their cell cycle in a constant light environment, it is clear that they are not the direct result of cell cycle variation; rather, they are induced by periodicity in the environment. Although others have attempted to dissect these two factors using other experimental designs (6, 13, 29) this study is the first in which changes over the cell cycle were measured in cells with no prior entrainment, *i.e.* no 'memory' of an entraining L/D cycle. Moreover, the populations compared were identical in all regards except for past light history. Even so, we have not sorted out completely the three forces that can affect diel changes in physiology: the cell cycle, the circadian clock, and the simple response to a changing environment. More specifically, our experiments were not designed to address the role of the clock in photosynthetic periodicity.

In addition, our results do not shed light on the intermediate physiological mechanisms causing diel variation in photosynthesis, about which there is much controversy (14). It is agreed by most that electron transfer activity through PSI is constant in cells that exhibit diel variation in photosynthesis (15, 18,

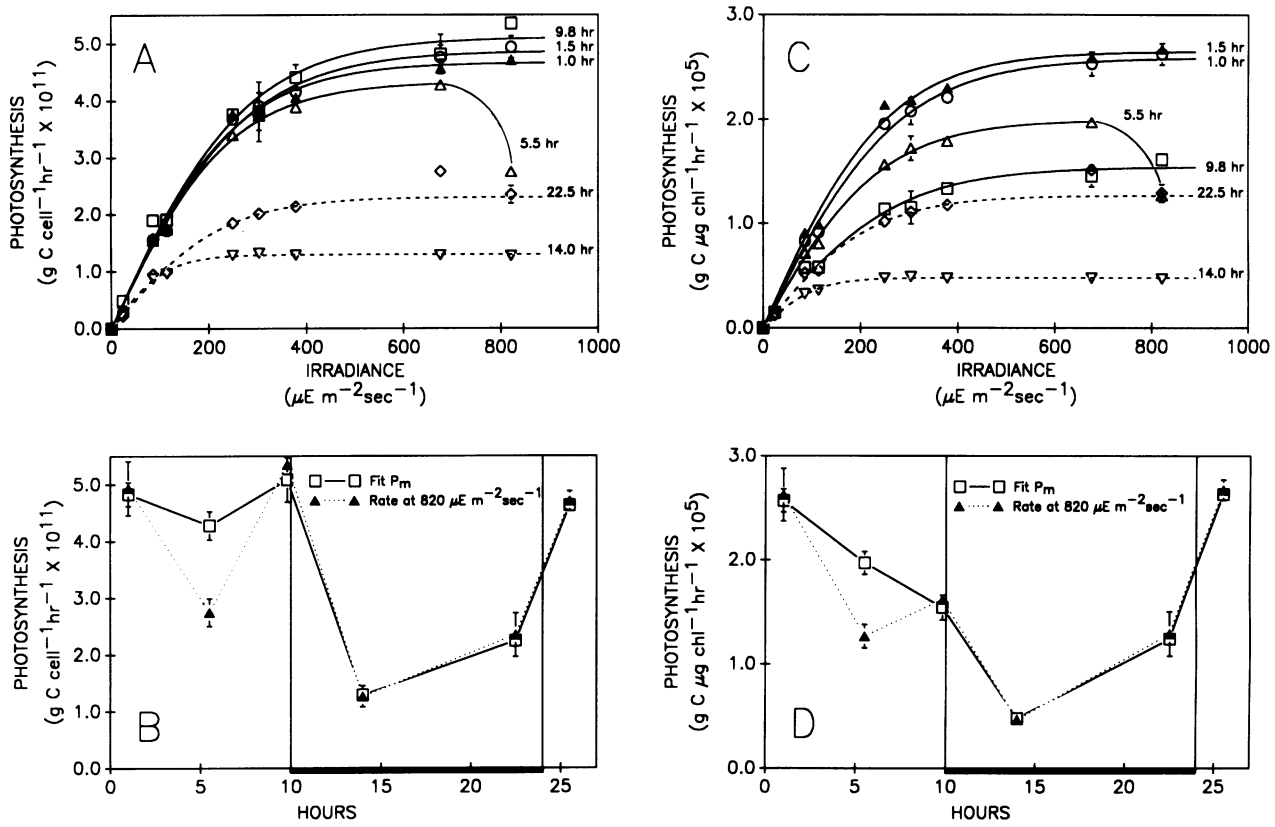

Figure 4. A, Photosynthetic rate per cell as a function of irradiance in samples taken from the culture shown in Figure 2. Solid lines represent samples collected during light period at the times indicated. Broken lines are for samples taken during the dark period. Error bars show the standard error. B, Light saturated photosynthesis (P_m) and photosynthesis at 820 μE m^{-2} s^{-1} from (A) *versus* time. Error bars are the 95% confidence interval. C, Photosynthetic rate per Chl content *versus* irradiance in samples as in (A). D, Light saturated photosynthesis (P_m) and photosynthesis at 820 μE m^{-2} s^{-1} from (C) *versus* time.

27), as is photosynthetic unit size (21, 22). There is disagreement, however, on the behavior of PSII under conditions in which a diel periodicity in photosynthesis is expressed (15, 17, 21, 27). The consensus is that the enzymes in the Calvin cycle are not periodic (14, 15), and thus are not the cause of the periodicity in P_m.

The maximum rate of photosynthesis of cells maintained in constant light in our experiments was approximately 60% that seen in the cells maintained in the periodic light regime. This enhanced photosynthetic performance in the L/D grown cells allowed them to maintain the same daily population growth rate despite different daily rates of photon flux, reflecting an acclamation of the cells physiological response to the photocycle. A similar phenomenon has also been reported by Humphrey (9) for this same species.

A very intriguing phenomenon observed in our cells when maintained on a periodic L/D cycle was the midlight period photoinhibition. Midday photoinhibition has been routinely observed in natural phytoplankton populations but is commonly attributed to bottle effects and damage of the photosynthetic apparatus at the higher light intensities characteristic of midday (7). Neither of these could play a role in our experiments; thus, we conclude that the midday depression has some basis intrinsic to the physiology of the cells on this L/D regime. We cannot shed light on the issue from the results of our constant light experiment because elutriated fractions lack sufficient resolution to discern this transient change in photosynthetic performance. We can only conclude that this is an interesting response worthy of further analysis.

Closer analysis of the data reveals another physiological change which has no apparent environmental correlate. In cells grown on the L/D cycle, Chl per cell began to increase in the middle of the dark period, suggesting increased synthesis long before the onset of the light period. In the absence of evidence for cell cycle control over this phenomenon, we must hypothesize some form of endogenous control (13).

Although we have clearly demonstrated that changes in photosynthetic performance do not occur over the cell cycle in *Amphidinium carteri* unless the cells are subjected to a diel L/D cycle, the possibility remains that cell cycle progression is necessary but not sufficient for the maintenance of a photosynthetic rhythm. For example, it is possible that the observed minimum in photosynthetic capacity observed in cells grown on a L/D cycle might only occur in the G_2 + M stage. Manipulation of the population growth rate so that the cell cycle takes much longer than 24 h while maintaining the culture on a 24 h photocycle, would allow one to separate the influences of light history and cell cycle stage.

LITERATURE CITED

1. Beckman Publication No. JE6B-IM-2 (1981) The JE-6B elutriation system and rotor. Palo Alto, CA, pp 19–22
2. **Edmunds LN** (1984) Circadian oscillators and cell cycle controls in algae. *In* P Nurse, E Streiblova, eds, The Microbial Cell Cycle. CRC Press, Boca Raton, FL, pp 231–252
3. **Gerath MW** (1988) Analysis of cell cycle specific physiology in marine phytoplankton by centrifugal elutriation. MS thesis, Massachusetts Institute of Technology
4. **Grdina DJ, Meistrich ML, Meyn RE, Johnson TS, White RA** (1986) Cell synchrony techniques: A comparison of methods. *In* JE Gray, Z Darzykiewicz, eds, Techniques in Cell Cycle Analysis. Humana, Clifton, NJ, pp 367–402
5. **Grumbach KH, Lichtenthaler HK, Erismann KH** (1978) Incorporation of $^{14}CO_2$ in photosynthetic pigments of *Chlorella pyrenoidosa*. Planta **140:** 37–42
6. **Harding LW, Prezelin BB, Sweeney BM, Cox JL** (1981) Diel oscillations in the photosynthesis-irradiance relationship of a planktonic marine diatom. J Phycol **17:** 389–394
7. **Harris GP, Piccinin BB** (1977) Photosynthesis by natural phytoplankton populations. Arch Hydrobiol **80:** 405–457
8. **Howell SH, Posakony JW, Hill KR** (1977) The cell cycle program of polypeptide labeling in *Chlamydomonas reinhardtii*. J Cell Biol **72:** 223–241
9. **Humphrey GF** (1979) Photosynthetic characteristics of algae grown under constant illumination and light-dark cycles. J Exp Mar Biol Ecol **40:** 63–70
10. **Jassby AD, Platt T** (1976) Mathematical formulation of the relationship beween photosynthesis and light for phytoplankton. Limnol Oceanogr **21:** 540–547
11. **John PCL, Donnan L, Harper JDI, Rollins MJ, Keenan CA** (1983) Control of cell division in *Chlorella* and *Chlamydomonas*. *In* J Chaloupka, A Kotyk, E Streiblova, eds, Progress in Cell Cycle Controls. Czechoslovak Academy of Sciences, Prague, pp 81–95
12. **Krumlauf R, Birnie G** (1981) Analysis of cell-cycle expression of a mouse H4-histone gene by centrifugal elutriation. Biochem Soc Trans **9:** 593–594
13. **Laval-Martin DL, Shuck DJ, Edmunds LN** (1979) Cell cycle-related and endogenously controlled circadian photosynthetic rhythms in *Euglena*. Plant Physiol **63:** 495–502
14. **Lonergan TA** (1984) Regulation of the photosynthetic light reactions by the biological clock. Photochem Photobiol **40:** 789–793
15. **Lonergan TA, Sargent ML** (1979) Regulation of the photosynthesis rhythm in *Euglena gracilis* II. Involvement of electron flow through both photosystems. Plant Physiol **64:** 99–103
16. **Maltese MA, Sheridan KM** (1986) 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cells isolated in specific phases of the cell cycle by centrifugal elutriation. J Lipid Res **27:** 565
17. **Marcus Y, Schuster G, Marcus A, Kaplan A** (1986) Adaptation to CO_2 level and changes in the phosphorylation of thylakoid proteins during the cell cycle of *Chlamydomonas reinhardtii*. Plant Physiol **80:** 604–607
18. **Mell V, Senger H** (1978) Photochemical activities, pigment distribution, and photosynthetic unit size of subchloroplast particles isolated from synchronized cells of *Scenedesmus obliquus*. Planta **143:** 315–322
19. **Mitchison JM** (1984) Dissociation of cell cycle events. *In* LN Edmunds, ed, Cell Cycle Clocks. Marcel Dekker, New York, pp 163–171
20. **Mitsui A, Kumanzawa S, Takahashi A, Ikemoto H, Cao S, Arai T** (1986) Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically. Nature **323:** 720–722
21. **Mushkind M, Mauzerall D, Beale SI** (1979) Diurnal variation *in situ* of photosynthetic capacity in *Ulva* is caused by a dark reaction. Plant Physiol **64:** 896–899
22. **Myers J, Graham JR** (1975) Photosynthetic unit size during the synchronous life cycle of *Scenedesmus*. Plant Physiol **55:** 686–688
23. **Olson RJ, Chisholm SW** (1986) Effects of light and nitrogen limitation on the cell cycle of the dinoflagellate *Amphidinium carteri*. J Plankton Res **8:** 785–793
24. **Olson RJ, Frankel SL, Chisholm SW, Shapiro HM** (1983) An inexpensive flow cytometer for the analysis of fluorescence signals in phytoplankton: Chlorophyll and DNA distributions. J Exp Mar Biol Ecol **68:** 128–144
25. **Parsons TR, Maita Y, Lalli CM** (1984) A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon, Oxford, pp 107–109, 115–120
26. **Ricketts TR** (1977) Changes in cell concentrations of various constituents during synchronous division of *Platymonas striata* Butcher (*Prasinophyceae*). J Exp Bot **28:** 1278–1288
27. **Samuelsson G, Sweeney BM, Matlick HA, Prezelin BB** (1983) Changes in photosystem II account for the circadian rhythm in photosynthesis in *Gonyaulax polyedra*. Plant Physiol **73:** 329–331
28. **Spudich JL, Sager R** (1980) Regulation of the *Chlamydomonas* cell cycle by light and dark. J Cell Biol **85:** 136–145
29. **Sweeney BM** (1982) Interaction of the circadian cycle with the cell cycle in *Pyrocystis fusiformis*. Plant Physiol **70:** 272–276
30. **Vaulot D, Olson RJ, Chisholm SW** (1986) Light and dark control of the cell cycle in two marine phytoplankton species. Exp Cell Res **167:** 38–52