Experimental Cell Research 167 (1986) 38–52

Light and Dark Control of the Cell Cycle in Two Marine Phytoplankton Species

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The effect of light and dark on growth, DNA replication and cell division of two marine phytoplankters *Thalassiosira weissflogii* (a diatom) and *Hymenomonas carterae* (a coccolithophorid) was investigated using flow cytometry. The two species displayed very differing behavior. When transferred from light to prolonged darkness, all coccolithophorid cells were arrested at the beginning of the G1 stage of the cell cycle. When shifted back into light, they resumed cycling at a rate slightly slower than prior to arrest. In contrast, diatom cells were arrested either in the G1 or G2 stage of the cell cycle in the dark. Upon re-exposure to light, cells which had been dark-arrested in G1 resumed cycling at the same rate as prior to arrest, while cells arrested in G2 cycled much more slowly. These results suggest that in both species, light control of cell cycle progression is effective only over a restricted part of the cell cycle, as has been hypothesized by Spudich & Sager (J cell biol 83 (1980) 136) [38] for *Chlamydomonas*. In the coccolithophorid there is a single light-dependent segment located at the beginning of G1, whereas the diatom appears to have two such segments, one in G1 and the other in G2, corresponding to two different light requiring processes.

Cell populations of numerous phytoplankton species have been shown to be entrained by alternating periods of light and dark. The entrainment regulates the timing of cell division as well as other cellular processes such as photosynthetic capacity and enzyme activity (see [6] for a review). Two kinds of hypotheses have been formulated to account for the entrainment of cell division. The first assumes that the cell cycle is coupled to an internal clock, itself entrained by the forcing photocycle [17, 18, 40]. The second, which has received increasing attention recently [7, 21, 23, 41], proposes that cell division and the cell cycle are directly driven by the forcing photocycle without an intervening clock [3, 38].

The cell cycle can be viewed as a composite of two cycles: the DNA/division cycle, which encompasses the replication and division of genetic material, and the growth cycle, which includes all other macromolecular syntheses [24]. During balanced growth, these two cycles have to be coupled so that, on average (but not necessarily for each individual cell), cell mass doubles during the interdivision time. A potential coupling mechanism for these cycles is the requirement that a

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0014-4827/86 $03.00

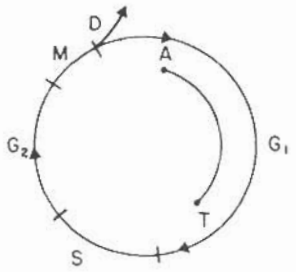

Fig. 1. The light-dependent cell cycle according to the model of Spudich & Sager [38]. The light requirement for cell cycle completion must be fulfilled during the light-dependent segment AT. When cells are placed in the dark, those that are between A and T are arrested in their cell cycles. All the others progress through their cycles until they reach the point A where they are arressted until re-exposed to light.

specific cellular component or characteristic, such as size [19], total protein [32] or total rRNA [14] must reach a critical level in order to trigger the initiation of DNA synthesis. Besides this internal regulation, cell cycle progression is also temporally regulated by a multiplicity of external factors such as nutrients, oxygen, pH or hormones [30, 44]. In mammalian cells the effect of a perturbation in the supply of a given factor depends on the location of a cell in its cell cycle at the time of perturbation. In general, cells which are located beyond a certain point in the cell cycle (called restriction point or transition point) can complete the DNA/division cycle even in the absence of the factor [31]. In contrast, cells perturbed before the restriction point may either stop in their cycle or enter a differentiated quiescent state called G0 [33].

The same type of control has been proposed to operate in the case of phytoplankton and light [38, 7, 21]. According to this model, the cell cycle is divided into a light-dependent and a light-independent segment. A cell must receive a given amount of light energy while in the light-dependent segment in order to progress into the light-independent segment and be committed to divide (fig. 1). It is likely that part of this energy is stored in the form of lipid and carbohydrate reserves [26, 25] and then used later to carry out the necessary biosyntheses for completion of the cell cycle. In prolonged darkness, cells are assumed blocked in the light-dependent segment bounded by two points: an arrest point A and a transition point T (fig. 1). Before A and beyond T, cell cycle progression is light-independent; A represents the last point to which a cell born in the dark can progress in its cycle if maintained in the dark. When re-exposed to light, cells proceed normally in their cycle starting from the location where they were arrested.

In the present study, we examined the kinetics of dark-arrested and dark-released populations of two phytoplankton species, a diatom, *Thalassiosira weissflogii*, and a coccolithophorid, *Hymenomonas carterae*, using flow cyto-

metry to record DNA and protein distributions. These two species have been chosen because they exhibit very different division patterns when grown on 24-h photocycles [5, 7]. In coccolithophorid populations, division is restricted to the dark period, whereas division in diatom populations occurs primarily at the end of the light period, but continues throughout the 24-h cycle. We wanted to test whether the differences in division behavior of these populations could be related to differences in the way light controls their cell cycle. Indeed the data obtained for the coccolithophorid are consistent with the existence of a single light-dependent segment in its cell cycle, whereas the diatom appears to possess two such segments, one in G1 and the other in G2.

MATERIALS AND METHODS

Culture Conditions

Thalassiosira weissflogii clone 'Actin' and *Hymenomonas carterae* clone 'Cocco II' were obtained from the culture collection of Dr R. R. L. Guillard (Bigelow Laboratory, Boothbay Harbor, Maine). Both were maintained in batch cultures at 20°C at a light intensity of 100 $\mu E\ m^{-2}\ s^{-1}$ in f/2 enriched seawater medium [20] filter-sterilized through 0.22 μm pore size filter. For the experiments described here, the cells were grown in 100–500 ml Erlenmeyer flasks in f/2-enriched seawater medium with 0.2 mM N as $(NH_4)_2SO_4$ and 0.2 mM Si as $Na_2SiO_3\ 9H_2O$ for the diatom. Dark conditions were obtained by wrapping the flasks in aluminum foil.

In experiments with *T. weissflogii*, two cell cycle-specific blocking agents (Sigma Co., St. Louis, Mo.) were used: hydroxyurea to inhibit DNA synthesis and colcemid to inhibit mitosis. The agents were used at the lowest concentrations (6.5 and 0.02 mM respectively) required for complete inhibition. This was determined by adding increasing drug concentrations to batch cultures and monitoring the evolution of the DNA distributions using flow cytometry (see below). At these concentrations and for the time scale of interest (30 h for hydroxyurea, and 8 h for colcemid), these agents did not influence transit through the non-inhibited part of the cell cycle (see Results).

Cell Counting and Fixation

Cells were maintained in a refrigerated bath at ~1°C for 0–48 h before being enumerated and fixed for flow-cytometric analysis. Cells sampled from cultures in the dark were maintained in the dark to minimize disturbances. Control experiments established that no noticeable change in the cellular characteristics of interest to us occurred under these storage conditions. Cell number and cell size were determined with a model Z_f Coulter Counter (Coulter Electronics, Hialeah, Fla.) using a 100-μm aperture tube. Cell volume distributions were stored on an Apple 2+ computer for subsequent analysis. 10^5–10^6 cells per sample were fixed in methanol as described in [27], and stored at 4°C for up to six months prior to staining for the flow-cytometric measurements.

Cell Staining

In preparation for staining, fixed cells were rinsed twice out of methanol by centrifuging and resuspended in fresh phosphate-buffered saline (PBS: 10.9 g/l $Na_2HPO_4\ 7H_2O$; 3.9 g/l KH_2PO_4; 5.0 g/l NaCl). Using a modification of the procedure described in Crissman & Steinkamp [12], cellular DNA was stained with propidium iodide (PI, Sigma Co.) in the presence of RNase (RAS-A, Worthington Division, Freehold, N.J.) to eliminate interference from RNA, and cellular protein was stained with fluorescein isothyocyanate (FITC, Sigma Co.). A mixture of PI, FITC and RNase was added to the cells in the buffer (to obtain final concentrations of 5.0, 4.2 and 40.0 mg/l respectively) and allowed to react for 2 h at room temperature prior to the measurements. Under these conditions, RNase was shown to eliminate completely the fluorescence due to the PI–RNA complex. The values of protein per cell were not calibrated with a direct chemical assay; thus the data reported here reflect relative changes in protein content and must be interpreted conservatively.

Flow-Cytometric Measurements

Cells stained for DNA (PI) and protein (FITC), were analysed using a Coulter Epics V flow cytometer/cell sorter (Coulter Electronics, Hialeah, Fla.). The 488-nm laser line was used to excite green fluorescence (FITC–protein) between 515 and 560 nm and red fluorescence (PI–DNA) above 630 nm. Data were transferred to an IBM CS 9000 computer for subsequent analysis.

Aliquots of 10^4–2.10^5 cells were analysed per sample. Instrument accuracy and long-term drift were monitored using fluorescent standard beads (3.79 μm diameter Polybeads, Polysciences, Warrington, Pa.). The measured coefficient of variation of the standard beads was always between 1 and 3%. Drift never exceeded 10% for a series of samples and was usually below 3%.

Microscopic Counts of Doublet Cells

In *T. weissflogii*, as in diatoms in general [39], there is a post-cytokinesis doublet stage devoted to the formation of the silicon frustule, during which the daughter cells remain attached and grow in size. This stage may last from 1–2 h, i.e. up to 25% of the total cell cycle in optimal growth conditions. The doublets are recorded as single cells by the Coulter Counter and cannot be distinguished from cells in the G2+M stage in flow-cytometric measurements of DNA distributions made according to our procedures. Their frequency was thus determined microscopically using a Zeiss epifluorescence microscope. The counts were done on fixed samples stained with the DNA-specific stain Hoechst 33342 [27]. At least 500 cells were examined per sample. Cells in this stage were subtracted from the G2+M peak and assigned to a separate category called D representing a stage between M and G1 in the cell cycle.

DNA Distribution Analysis

DNA distributions obtained by flow cytometry consist of two main peaks, corresponding to G1 and G2+M cells, separated by a region corresponding to cells in the S phase (figs 1, 2, 3). In *T. weissflogii* the second peak also includes cells in the doublet stage D (see above). The G1 and G2+M peaks are assumed to have Gaussian distributions. In our samples, typical coefficients of variation (CV) for the G1 peak were 6–8% for *T. weissflogii* (fig. 2) and 4–6% for *H. carterae* (fig. 3). The analysis of such distributions provides estimates of the proportion of cells in the three phases G1, S and G2+M. In the present case, the S phase was modelled as a sum of rectangles broadened by Gaussian distributions having the same CV as the G1 and G2+M peaks (figs 2, 3). This representation of the S phase is more appropriate for the analysis of synchronous DNA distributions than a Gaussian integral or a broadened polynomial [1]. In order to cover optimally the S phase the number of S peaks was set as inversely proportional to the width of the G1 peak (typically 3 S peaks for a 7% CV and 5 S peaks for a 4% CV). The set of fitting parameters (CV of G1, relative positions of G1 and G2+M peaks, and number of cells in G1, G2+M and each S peak) was determined by a non-linear Macquart algorithm (personal communication of P. Dean).

An anomaly in the data was noted in *H. carterae*. In cell populations stained with either PI (fig. 3) or Hoechst 33342, many DNA distributions had a positively skewed G1 peak. Since the analysis method assumes that the G1 peak has a non-skewed Gaussian distribution, an unusually large number of cells were found in the first S peak (called S1) when such a skew was present (fig. 3). The mathematical analysis of such DNA histograms being necessarily ambiguous [1], it is not clear whether the cells is S1 were actually in early S or in G1. If they were in S, this could reflect slow rates of DNA synthesis in early S [15]. Alternatively, if they were in G1, then the skew in the G1 peak was real and may have been the result of plastid DNA staining [8] or non-specific staining. The fact that *H. carterae* cells were arrested in both G1 and S1, but never in the rest of the S phase (S2) or in G2+M in prolonged darkness (see below) indicates that G1 and S1 have similar properties with respect to light/dark control. We have therefore pooled the DNA phases as G1+S1, S2 and G2+M for the analysis of *H. carterae* data. The basic conclusions of this work, however, are not dependent on the inclusion of S1 cells in either G1 or S.

Transit Times through Cell Cycle Phases

When the experimental conditions are such that there is no incoming flux of cells into one of the DNA phases, but only an outgoing flux, one can compute the average transit time of the outgoing cells through this phase assuming first-order exit kinetics [37]. A typical example is colcemid inhibition of

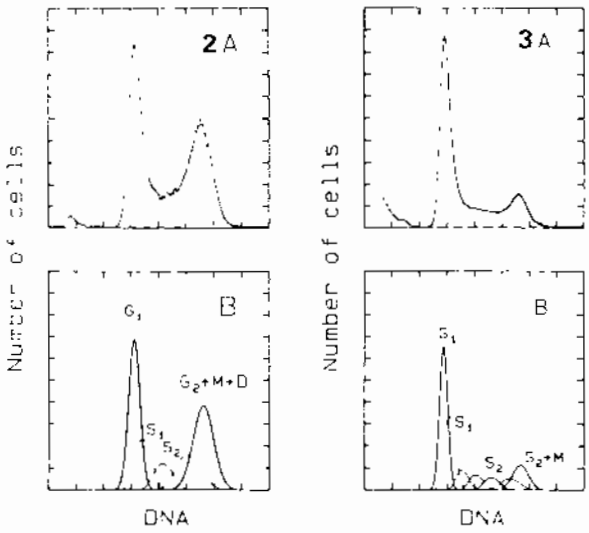

Fig. 2. DNA distribution of an exponentially growing population of the diatom *T. weissflogii*, stained with propidium iodide (*PI*) and analysed by flow cytometry. The DNA distribution was modelled as a sum of broadened rectangles fitted using a non-linear algorithm. The coefficient of variation of the G1 peak was 6.5%. (*A*) Raw data (· · · · ·) and fitted curve (——). (*B*) Decomposition into G1, S1 (first S peak), S2 (all other S peaks) and G2+M+D. D is a post-cytokinesis doublet stage in which daughter cells remain attached.

Fig. 3. DNA distribution of an exponentially growing population of the coccolithophorid *H. carterae*. The coefficient of variation of the G1 peak was 5.2%. (*A*) Raw data (· · · · ·) and fitted curve (— —). (*B*) Decomposition into G1, S1, S2 and G2+M. Note that the S1 peak was more prominent than in *T. weissflogii* (fig. 2).

mitosis, for which there is no flux of newly divided cells into G1. If T_p is the average transit time through phase P, $X_p(t)$ is the total number of cells in phase P at time t, and $t=0$ is the time at which the flux into phase P stops, then:

$$X_p(t) = X_p(0) \cdot \exp\ (-t/T_p) \tag{1}$$

and thus T_p can be computed easily from the slope of the curve:

$$y(t) = \ln\ [X_p(t)] = \ln\ [X_p(0)] - t/T_p. \tag{2}$$

RESULTS

Kinetics of Dark Arrest: T. weissflogii

T. weissflogii cells had an average generation time of about 7.6 h in continuous light under the conditions of these experiments (table 1). The cells spent roughly the same amount of time in each of the four cell cycle stages we have identified in this species. When cells were placed in the dark, cell division within the population continued for 8 h (fig. 4*A*). During this period average volume and protein per cell did not change (fig. 4*C*), but total cell volume and protein per volume of

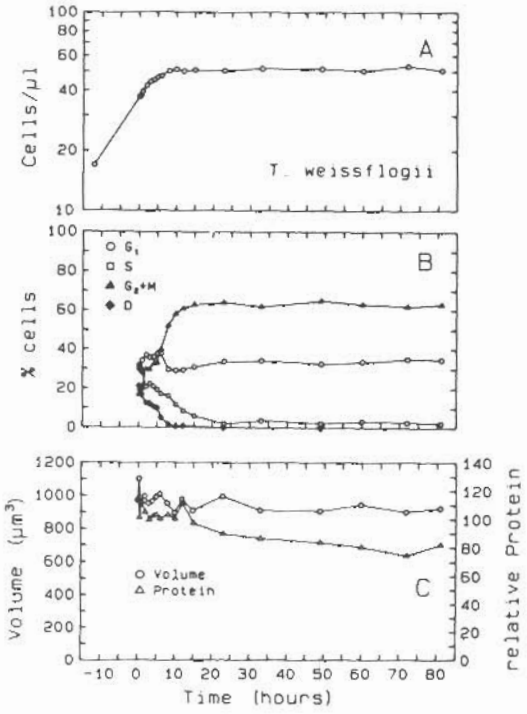

Fig. 4. Dark arrest experiment (*T. weissflogii*). A cell population growing exponentially in continuous light was transferred to dark conditions at $t=0$. (*A*) Cell concentration as a function of time. (*B*) Percentages of the cells in the different DNA phases as a function of time. (*C*) Average cell volume and average cell protein (relative units) as a function of time.

culture (i.e., the product of cell concentration and protein or volume per cell) increased in the dark. The protein accumulation rate was slower in the dark than in the light (0.7 vs 2.1 day^{-1}) as has been observed for natural plankton communities [25, 13].

When division in the population finally ceased in the dark ($t=8$), cells still in S slowly exited to G2+M with an average transit time through S of 6 h (computed

Table 1. *Average duration of the cell cycle phases of T. weissflogii and H. carterae in continuous light (all values in hours)*

	Generation time	Length of cell cycle phases[a]			
		G1	S	G2+M	D[b]
TW	7.6	1.7	2.5	1.2	2.1
		G1+S1[c]	S2[d]	G2+M	
HC	12.9	7.2	3.1	2.7	

[a] DNA phase lengths are computed fromthe mean generation time and the percentage of cells in the corresponding phase, assuming an exponential age distribution [27].
[b] Cells in the D phase of the cell cycle are unseparated post-cytokinesis doublets (see Methods).
[c] S1 is the first peak of S (see fig. 3 and Methods). G1, 4.9 h; S1, 2.3 h.
[d] S2 is the sum of all the S peaks but S1.

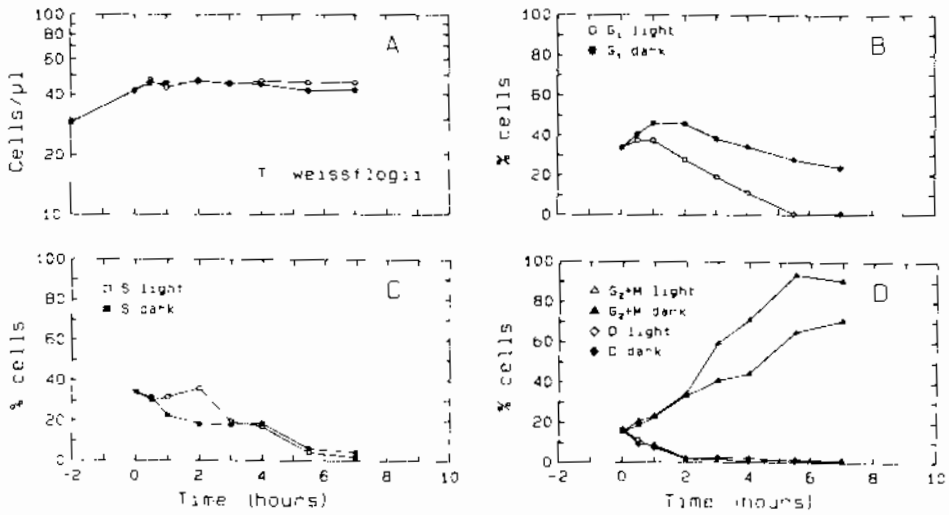

Fig. 5 Dark arrest experiment combined with colcemid block (*T. weissflogii*). A culture in exponential growth was exposed to colcemid (0.02 mM) at $t=0$ and was divided into two aliquots. One was left in the light (open symbols). (*A*) Cell concentration as a function of time. (*B*) Percent of cells in (○) G1. (*C*) S.

applying eq. (1) in Methods to the data of fig. 4), which is twice the average transit time in continuous light (table I). After 25 h in the dark 40% of the cells had accumulated in G1, 60% in G2+M and no cells remained in S or in the post-cytokinesis doublet stage D (fig. 4*B*). In prolonged darkness, protein per cell decreased slowly at a rate of 0.10 day^{-1} (fig. 4*C*).

In this first experiment some cells completed their cell cycle in the dark and re-entered a new one. This created two types of G1 cells in the dark-arrested population having different histories: some had entered G1 in the light and others had entered it in the dark. In order to assess the effect of darkness on those cells which had entered G1 in the light, we performed a second experiment in which mitosis was blocked with colcemid [35, 36] thus minimizing the appearance of new G1 cells in the dark. In this experiment a culture of *T. weissflogii* grown in continuous light was exposed to colcemid (0.02 mM) at $t=0$ and was immediately divided into two aliquots: one was maintained in the light and the other placed in the dark (fig. 5). Colcemid completely inhibited mitosis (fig. 5) but did not affect greatly the rate of cell progression through G1. This conclusion is based on the agreement between the average G1 transit times of cells in continuous light in the absence of colcemid (table I) and in the presence of colcemid (calculated applying eq. (1) to the data of fig. 5*B*). The former was 2.1 h and the latter 3.0 h.

Almost immediately after colcemid addition, the percentages of cells in G1 and S differed between light and dark conditions (fig. 5*B*, *C*), whereas this difference did not appear in the G2+M population until 2 h after colcemid addition (fig. 5*D*). This indicates that cells which were well advanced in their cycle at $t=0$ were not

affected by darkness, whereas some cells located earlier in the cell cycle were blocked. Darkness had no influence on doublet (D stage) separation. At the end of the experiment about 20% of the cells remained arrested in G1 in the dark (fig. 5*B*). Those cells which left G1 in the dark did so after an average transit time of 3 h (eq. (1), fig. 5*B*), which is only slightly slower than the G1 transit time in the light (table 1). In the dark there were still some cells in the S phase after 6 h in the absence of colcemid (fig. 4*B*), while none remained in the presence of colcemid (fig. 5*C*). We deduce that in the absence of colcemid, cells present in S after 6 h in the dark must have been cells which re-entered the cell cycle and initiated DNA synthesis in the dark. Thus some cells could traverse both G1 and S in the dark, while others could not—and remained blocked in G1.

Kinetics of Dark Arrest: H. carterae

H. carterae had a longer average generation time in continuous light than *T. weissflogii* (12.9 vs 9.6 h; table 1). When placed in the dark (fig. 6) the rate of cell division in the population decreased much more gradually than that of *T. weissflogii*, reaching zero after 30 h in darkness (fig. 6). Cells placed in the dark accumulated in G1+S1 (fig. 6*B*) and in contrast to the diatom, essentially no cells arrested in G2+M.

Mean volume and protein per cell declined rapidly until $t=15$ (fig. 6*C*), but total cell volume and protein per volume of culture remained constant. The decrease in volume and protein per cell was thus totally accounted for by cell division. This absence of net accumulation of protein in the early part of dark exposure was in direct contrast to the behavior of the diatom (fig. 4). In prolonged darkness (i.e. after 30 h in the dark) protein decreased at a steady rate of 0.11 day^{-1} (fig. 6*C*), which was very similar to that of the diatom (0.10 day^{-1}; fig. 4*C*).

We were unable to inhibit mitosis with colcemid in *H. carterae*, as we did in *T. weissflogii*.

Kinetics of Dark Release: T. weissflogii

The experiments described thus far have demonstrated that *T. weissflogii* cells can be blocked in both G1 and G2+M in the dark, while *H. carterae* cells are only blocked in G1+S1. We now ask: are blocked cells able to resume cycling immediately and at a normal rate when resupplied with light, or does this arrest bring the cells to a resting state (equivalent to G0 in mammalian cells [33]) from which they re-emerge very slowly?

To address this question, cultures which had been arrested in the dark for 34 h were transferred back into continuous light. (In the process of doing this set of experiments, we learned that when *T. weissflogii* was re-exposed to light after prolonged darkness, part of the population underwent gametogenesis. This aspect is discussed elsewhere [41] and we will only consider the non-gametic part of the population here.) *T. weissflogii* cells blocked in G1 synchronously initiated DNA

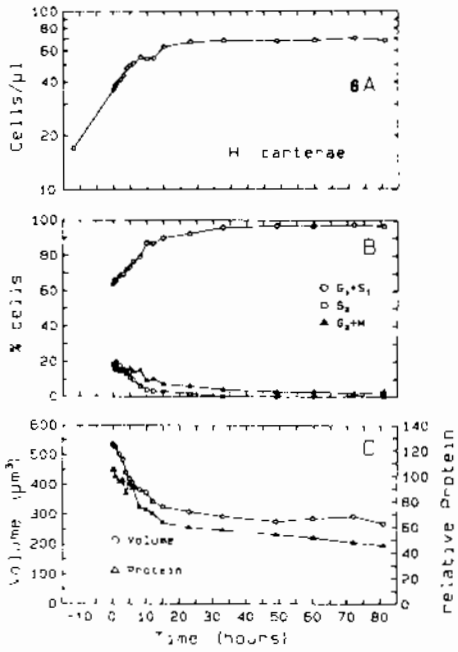

Fig. 6. Dark arrest experiment (*H. carterae*). Caption as in fig. 4.

synthesis less than 2 h after being released from darkness (figs 7, 8). No major increase in protein or volume took place during this short lag phase. The average transit time through S was roughly 2 h, as evidenced by following the synchronous wave of cells going through S (fig. 8). All cells initially arrested in G1 had divided by $t=7$ (fig. 7*A*). The transit times of these cells through the cell cycle compare well with those established in continuous light (G1, 1.7 h, S, 2.5 h, and generation time, 7.6 h; table 1). Thus, we conclude that cells released from G1 were blocked at the beginning of this phase, did not show any signs of slow emergence from a resting state and behaved very similarly to cells in balanced growth.

Is the release behavior of cells blocked in G2+M in the dark different from those blocked in G1? This is difficult to assess from the previous experiment because G1 cells synthesized DNA almost immediately when released (figs 7, 8) and entered G2 to merge with the cells originally blocked in G2+M. To circumvent this problem, we performed another experiment in which we stopped cell flux from G1 to G2+M by inhibiting DNA synthesis with hydroxyurea [33]. When the drug was applied to a population in exponential growth in continuous light, it completely inhibited DNA synthesis, but cells in G2+M and D progressed normally in their cycle (data not shown). No cells remained in these phases by $t=14$ (fig. 9*A*). When the same treatment was applied to a dark-arrested population at the time of release into light, DNA synthesis was also inhibited but a large proportion of cells were still in G2+M after 14 h (fig. 9*A*) and only 40% of the

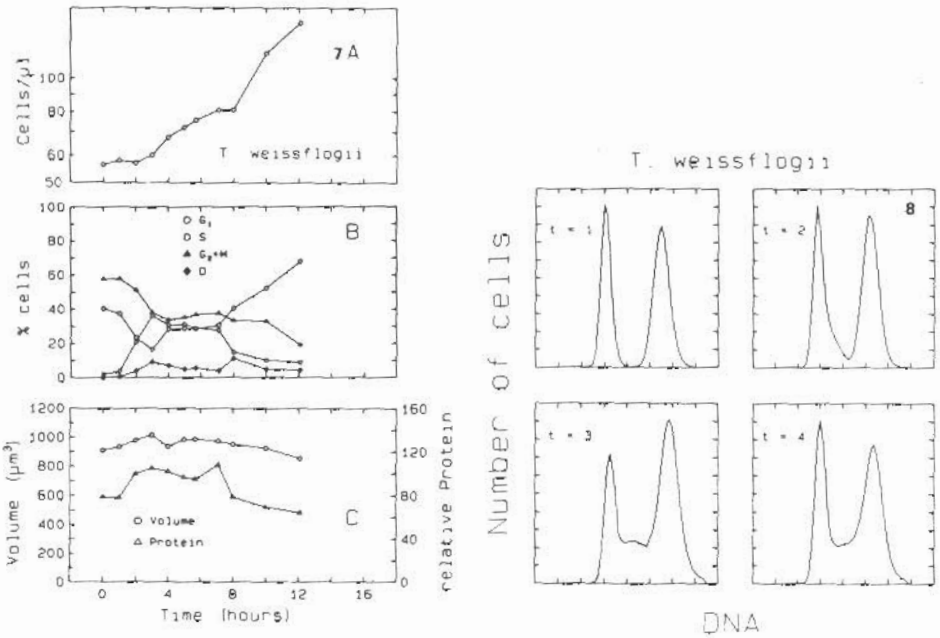

Fig. 7. Dark release experiment (*T. weissflogii*). A cell population held in the dark for 42 h, was re-exposed to light at $t=0$. Caption as in fig. 4.
Fig. 8. Evolution of the DNA distributions over the first 4 h of the dark release experiment described in fig. 7. Note the wave of cells proceeding through S at $t=3$.

cells in G2+M had divided and separated after 27 h (fig. 10). These cells proceeded through G2+M with an average time of 32 h (as computed from eq. (1) applied to fig. 10) vs 1.7 h in continuous light (table 1). Cells arrested in the dark had thus very different behaviors depending on whether they were in G1 or G2+M: cells arrested in G1 resumed cycling at the same rate as before arrest, but cells arrested in G2+M cycled about 18 times more slowly.

Kinetics of Dark Release: H. carterae

In contrast to *T. weissflogii*, *H. carterae* displayed a long lag phase between re-exposure to light and the beginning of cell division (figs 11, 12). Protein accumulation and volume growth took place during this lag such that cell volume (fig. 11 *C*) regained the level it had prior to dark arrest (fig. 4 *C*). DNA synthesis began about 4 h after release from darkness (fig. 12) and the rate of cell entry into S2 was maximal at $t=7$ (fig. 11 *B*). Division began at $t=10$ and half of the dark blocked population had divided by $t=16$. The transit times of an 'average cell' between release and division was thus 16 h. A comparison with transit times in continuous light (table 1) suggests that: (1) all cells were dark-blocked very early in the cycle

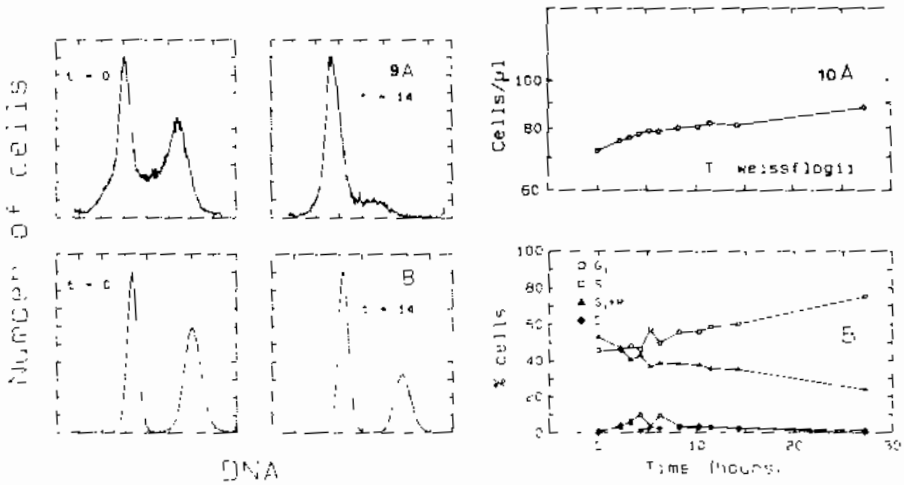

Fig. 9. Effect of hydroxyurea (6.5 mM) on the evolution of DNA distribution in *T. weissflogii* (*A*). At $t=0$, hydroxyurea was added to a population growing in continuous light. After 14 h all the cells in G2+M had divided and cells were either in G1 or in S. (*B*) At $t=0$, a population previously held in darkness for 2 days was released into the light and hydroxyurea was added. After 14 h in the light only a small fraction of the cells in G2+M had divided.

Fig. 10. Dark release experiment combined with hydroxyurea addition (*T. weissflogii*). A population, dark arrested for 2 days, was re-exposed to light at $t=0$ and simultaneously exposed to hydroxyurea (6.5 mM). See also fig. 9*B*. (*A*) Cell concentration as a function of time. (*B*) Changes in percent of cells in the different cell cycle stages as a function of time.

since it took them 7 h to reach the G1+S1/S2 boundary and (2) upon release the cells transited slightly more slowly through their cycle. A similar increase in cell cycle transit time after release from darkness was also observed in cyclostat perturbation experiments [41].

DISCUSSION

In the two species examined, *T. weissflogii* and *H. carterae*, both light-dependent and light-independent segments exist in the cell cycle. This has been postulated for other species [38, 16, 23, 21, 29] and cast in the cell cycle transition point framework by Spudich & Sager [38]. This framework as applied to *Chlamydomonas* [38] stipulates three criteria for the concept of transition point to apply strictly: (1) cells exposed to darkness while they are in the light-independent part of their cell cycle must keep progressing at a normal rate; (2) in prolonged darkness cells must arrest in a discrete and continuous cell-cycle segment (AT, fig. 1); (3) cells released into light must resume cycling at a normal rate from their arrest location. Our results for *H. carterae* deviate from these criteria only to a minor degree; *T. weissflogii*, on the other hand, exhibits some very significant differences. These criteria will be used as a reference for the discussion below.

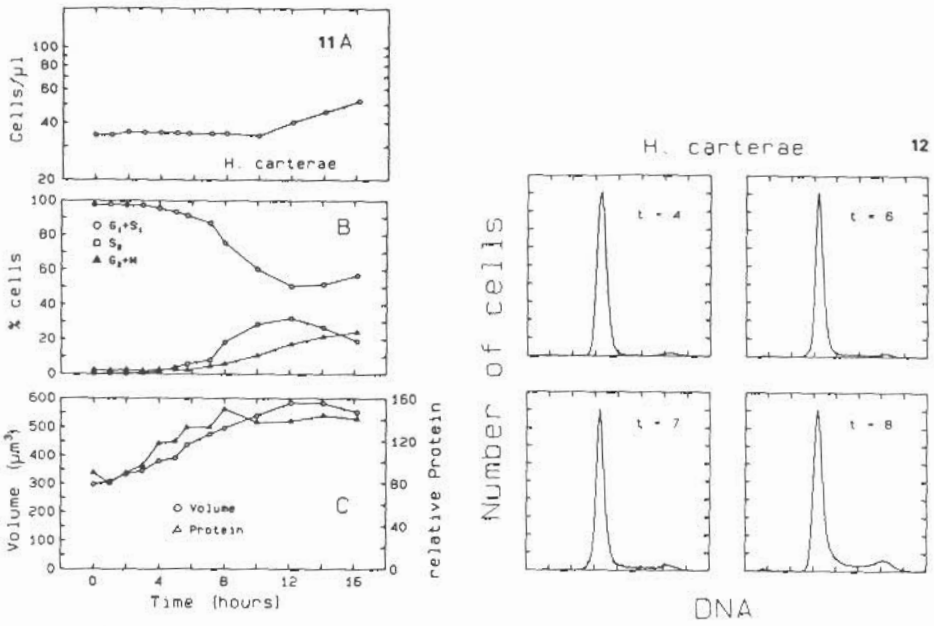

Fig. 11. Dark release experiment (*H. carterae*). A cell population held in the dark for 42 h was re-exposed to light at $t=0$. Caption as fig. 4.
Fig 12. Time evolution of the DNA distributions from the dark release experiment described in fig. 11.

In *H. carterae*, cells released into light after 34 h of dark arrest, progressed through their cycle about 25% more slowly than unperturbed cells. This contradicts the observations of Spudich & Sager [38] for *Chlamydomonas*, in which cells were not retarded after release from 48 h spent in the dark. The increase in cycling time in *H. carterae* was concentrated mostly in the later part of the cell cycle and thus cannot be interpreted as reflecting the slow release of the dark-blocked cells from a resting stage similar to the mammalian G0 [2, 31, 11]. It is likely rather that the prolonged dark exposure depleted metabolic reserves to a point where progress toward division was retarded upon re-exposure to light.

In the diatom *T. weissflogii*, cells which arrested in the dark before completing one cell cycle cycled at a normal rate until the time they were arrested (e.g. cells in G2+M and D, fig. 5). In contrast, cells which completed one cell cycle in the dark and re-entered a new one were slowed down during the second cell cycle traverse (cells in S after $t=10$ in fig. 4). As in *H. carterae*, this conflicts with the first criterion discussed above, i.e. the rate of progression through the light-independent segment of the cycle is not a constant. In addition, cells were arrested *in fine* in both G1 and G2+M (fig. 4) and none of the cells were blocked in S (figs 4, 5). This violates the second criterion; i.e. that the light-dependent segment is uninterrupted. Finally, cells blocked in G1 cycled normally upon release, while

cells blocked in G2+M cycled much more slowly than they would in continuous light, which contradicts the third criterion. Clearly, some additional hypotheses have to be invoked in order to account for these observations. A realistic assumption is to postulate two light-dependent segments in *T. weissflogii*, one in G1, the other in G2+M, which have different physiological underpinnings.

The first light-dependent segment in G1 in *T. weissflogii* is similar to that observed in *H. carterae*, and also in *Chlamydomonas* [38, 23], and most likely results from the energy requirement for the growth cycle. All cells did not possess the G1 requirement however; based on the results from the colcemid experiment, some cells which completed their cell cycle and divided in the dark were able to progress through both G1 and S without receiving any light energy before arresting in G2+M. This could be explained by the inheritance by daughter cells of energy storage compounds (lipids, carbohydrates) from the mother cell such that those cells which have enough reserve at birth do not require any supplementary energy input in order to progress through G1 and S.

The second light-dependent segment located in G2+M in *T. weissflogii* could be unique to diatoms. Diatom cells are enclosed in a silica frustule [42], which is formed after cytokinesis; the required silicon is transported and assimilated very late in the cell cycle [39]. The silicon uptake system appears to be partially dependent on light, as evidenced by the lower uptake rates generally observed in the dark [39, 9]. In one species, *Nitzschia angularis*, it has been demonstrated that the synthesis of the proteins involved in the transport system is interrupted in the dark and that these proteins degrade with a half-life of about 12 h [4]. If this observation applies to *T. weissflogii*, then cells would be able to take up silicon and to complete their cell cycle immediately after transfer to dark conditions, but they would lose this capability gradually, as the silicon transport system degrades, and would remain blocked at the end of the cell cycle. Darkness does not seem to affect the final phase before cell separation (stage D), as demonstrated by the absence of cells blocked in D in the dark (fig. 4) and by the results of the colcemid experiment (fig. 5). The retardation of cell cycle progression when G2+M-arrested cells are released into light could result from the slow restoration of the silicon transport system [4].

Environmentally controlled transition points in S or G2 are extremely unusual in other cell types such as mammalian cells [31] (but for exceptions see [4, 34, 22]). In consequence, G2 is generally viewed as a simple lag between DNA replication and mitosis, which can proceed independently of external conditions. For example, the 'continuum model' of Cooper [10] states that a certain level of an 'initiator' protein triggers irreversibly progression through the sequence S, G2 and M. This is not consistent with our observations on *T. weissflogii*. The slow reversibility of G2+M arrest is reminiscent of that of G0 arrest in mammalian cells [31]. In both cases the cause of the slow reversibility could be identical: a missing cell component has to be synthesized *de novo* before cells can cycle again.

In summary, *H. carterae* possesses only one cell cycle segment controlled by light, located in G1, while *T. weissflogii* has two such segments, one in G1 and the other in G2+M. This confirms a parallel study by Olson et al. [28] in which only G1 was found to expand with decreasing light intensity in *H. carterae*, whereas both G1 and G2+M were found to expand in the diatom. In the present study, the two species also revealed marked differences with respect to their growth behavior in terms of cell volume and protein. While *T. weissflogii* kept accumulating protein immediately after transfer to darkness, *H. carterae* did not. Conversely when diatom cells were released into light very little growth took place during the lag phase, whereas in the coccolithophorid both volume and protein content increased at a faster rate than before arrest. These differences can be related to the differences observed in the cell cycle patterns: continued growth in the dark in *T. weissflogii* may explain the absence of a lag phase upon release into light and conversely the lag phase in *H. carterae* may result from the absence of cell growth in the dark.

CONCLUSION

These experiments have revealed two essential elements which have to be incorporated in a model of the light-dependent phytoplankton cell cycle. First, dark arrest and release is not a simple 'stop or go' mechanism: cycling rates through specific cell cycle stages are altered by darkness and vary from cell to cell. Second, several transition points exist in the cell cycle corresponding to different cellular mechanisms regulated by light. The differences observed in the number of light-dependent segments and in the growth patterns between *T. weissflogii* and *H. carterae* parallel those observed in the division patterns of the two species in light–dark regimes [5, 41]. Since these patterns are typical of the taxonomic groups to which these species belong [6], it is likely that the mechanisms of cell cycle control elucidated here are typical of these groups and are linked to their specific structural and biochemical properties, such as the diatom silicon requirement.

This research was supported in part by NSF grants OCE 8316616, OCE 8121270 and CEE 8211525 and grant N00014-83-K-0661 from the Office of Naval Research. D. V. was supported as a Whitaker Fellow at MIT and with funds from the Education Office of the MIT/Woods Hole Joint Program. We thank Ms Sheila Frankel for technical assistance.

REFERENCES

1. Baisch, H, Beck, H-P, Christensen, I J, Hartmann, N R, Fried, J, Dean, P N, Gray, J W, Jett, J H, Johnston, D A, White, R A, Nicolini, C, Zeitz, S & Watson, J V, Cell tissue kinet 15 (1982) 235.
2. Baserga, R, Multiplication and division in mammalian cells. Marcel Dekker, New York (1976).
3. Bernstein, E, J protozool 11 (1964) 56.
4. Blank, G S & Sullivan, C W, Arch microbiol 123 (1979) 157.
5. Chisholm, S W & Costello, J C, J phycol 16 (1980) 375.

6. Chisholm, S W, Can bull fish aqua sci 210 (1981) 150.
7. Chisholm, S W, Vaulot, D & Olson, R J, Cell cycle clocks (ed L N Edmunds) p. 365. Marcel Dekker, New York (1984).
8. Coleman, A W, J phycol 21 (1985) 1.
9. Coombs, J, Halicki, P J, Holm-Hansen, O & Volcani, B E, Exp cell res 47 (1967) 315.
10. Cooper, S, Nature 280 (1979) 17.
11. — Cell growth (ed C Nicolini) p. 315. Plenum Press, New York (1981).
12. Crissman, H A & Steinkamp, J A, Cytometry 3 (1982) 84.
13. Cuhel, R L, Ortner, P B & Lean, D R S, Limnol oceanogr 29 (1984) 731.
14. Darzynkiewicz, Z, Crissman, H, Traganos, F & Steinkamp, J, J cell physiol 113 (1982) 465.
15. Dean, P N, Dolbeare, F, Gratzner, H, Rice, G C & Gray, J W, Cell tissue kinet 17 (1984) 427.
16. Donnan, L, Carvill, E P, Gilliland, T J & John, P C L, New phytol 99 (1985) 1.
17. Edmunds, L N, J cell comp physiol 67 (1966) 35.
18. Edmunds, L N & Adams, K J, Science 211 (1981) 1002.
19. Fantes, P A, J cell sci 24 (1977) 51.
20. Guillard, R R L, Culture of marine invertebrates, p. 29. Plenum Publishing Corporation, New York (1975).
21. Heath, M R & Spencer, C P, J gen microbiol 131 (1985) 411.
22. Liso, R, Calabrese, G, Bitonti, M B & Arrigoni, O, Exp cell res 150 (1984) 314.
23. McAteer, M, Donnan, L & John, P C L, New phytol 99 (1985) 41.
24. Mitchison, J M, The biology of the cell cycle Cambridge University Press, Cambridge (1971)
25. Morris, I, Can bull fish aquat sci 210 (1981) 83.
26. Myklestad, S, J exp mar biol ecol 15 (1974) 261.
27. Olson, R J, Frankel, S L, Chisholm, S W & Shapiro, H M, J exp mar biol ecol 68 (1983) 129
28. Olson, R J, Vaulot, D & Chisholm, S W, J plant physiol. In press
29 Olson, R J & Chisholm, S W, J plank res 8 (1986) 785.
30. Otto, A M, Ulrich, M & Jimenez de Asua, L J cell physiol 108 (1981) 145.
31. Pardee, A B, Dubron, R, Hamlin, J L & Kletzien, R F, Ann rev biochem 47 (1978) 715.
32. Popolo, L, Vanoni, M & Alberghina, L, Exp cell res 142 (1982) 69.
33. Prescott, D M, Reproduction in eukaryotic cells. Academic Press, New York (1976).
34. Probst, H, Gekeler, V & Helftenbein, E, Exp cell res 154 (1984) 327.
35. Puck, T T & Steffen, J, Biophys j 3 (1963) 379.
36. Sharpless, T K & Schlesinger, F H, Cytometry 3 (1982) 196.
37. Smith, J A & Martin, L, Proc natl acad sci US 70 (1973) 1263.
38. Spudich, J & Sager, R, J cell biol 85 (1980) 136.
39. Sullivan, C W & Volcani, B E, Silicon and siliceous structures in biological systems (ed T L Simpson & B E Volcani) p. 15. Springer Verlag, New York (1981).
40. Sweeney, B M & Hastings, J W, J protozool 5 (1958) 217.
41 Vaulot, D, Cell cycle controls in marine phytoplankton Ph.D Thesis. MIT/WHOI, WHOI-85 10 (1985).
42 Volcani, B E, Silicon and siliceous structures in biological systems (ed T L Simpson & B E Volcani) p. 157. Springer-Verlag, New York (1981).
43. Wotring, L L & Roti-Roti, J L, Cancer res 40 (1980) 1458.
44. Yen, A & Riddle, V G H, Exp cell res 120 (1979) 349.

Received January 13, 1986
Revised version received May 26, 1986