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# Cell Cycle Controls in Phytoplankton

## Comparative Physiology and Ecology

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### INTRODUCTION

Phytoplankton is the generic term for unicellular microalgae, most of which are obligate photoautotrophs, freely floating in the aquatic environment. The diversity of species belonging to this group of organisms (there are roughly 300 genera) is extraordinary in view of the simplicity of their growth requirements and the apparent uniformity of the aquatic environment in which they thrive. As photoautotrophs, these cells require only $CO_2$, light, vitamins, and essential inorganic nutrients to grow and reproduce. When grown on 24 hr photocycles, the cell division cycles of most species of phytoplankton become entrained; that is, division is phased in populations with doubling times greater than 1 day or synchronized in populations doubling about once per day. Although the latter might be considered induction synchrony in the strict sense of the term (Prescott, 1976; Zeuthen, 1974), it is a unique type of induction synchrony in that the periodic inducing stimulus (light) is a natural and ubiquitous one and is essential to cell survival. As such, the responses of the cells to this stimulus must have been shaped by natural selection.

A basic premise of this chapter, then, is that the cell division patterns of phytoplankton populations grown on light-dark cycles reflect an alignment of two periodic phenomena that is "optimized" for the long-term reproduction and survival of the population (Cohen and Parnas, 1976). Since the phase angle of this alignment, and its strictness, varies among phytoplankton species (Chisholm, 1981), we deduce that the requisites of the cell cycle and its controlling mechanisms must also vary. By analyzing in detail the similarities and differences between the growth patterns of various species under iden-

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tical conditions, one should be able to make deductions about the regulation of their cell cycle progression and, ultimately, the adaptive significance of this regulation in the different species. A thorough descriptive review of the patterns of cell division in populations of phytoplankton entrained to photocycles can be found in Chisholm (1981). Our purpose here is to provide a brief review and to focus (admittedly, a bit myopically) on certain aspects of these patterns in a few species, which we believe reveal fundamental differences in cell cycle controls.

## PATTERNS OF MACROMOLECULAR SYNTHESIS OVER THE CELL CYCLE

Surprisingly little is known about the sequences, timing, and regulation of cell cycle events in phytoplankton, relative to the advances that have been made with other eucaryotic cell types. The most comprehensive recent review of the subject can be found in Puiseux-Dao (1981). Much work has been done on the cell cycle of *Chlorella* (Tamiya, 1964, 1966; Morimura, 1959; Setlik et al., 1975; Wanka and Mulders, 1967), but extrapolation from this genus to others is difficult because of the large number of daughter cells produced in one generation. In fact, it is a general source of frustration in compiling data for this type of discussion to find that much of the cell cycle research on algae has been done on genera that produce multiple daughter cells, such as *Platymonas* (Ricketts, 1977, 1979; Tanoue and Aruga, 1975), *Chlamydomonas* (Mihara and Hase, 1971; Lien and Knutsen, 1972; Kates et al., 1968), and *Scenedesmus* (Setlik et al., 1972). For simplicity here, we shall try to restrict ourselves to cell types that produce only two daughter cells. We recognize, however, that the species that produce multiple daughter cells may be of particular interest in terms of the optimization of growth rate under the constraints of strict cell cycle alignment (Bruce and Bruce, 1982).

Patterns of macromolecular synthesis in light-dark synchronized *Dunaliella* and *Euglena* show clear similarities. In both groups, mitosis occurs almost exclusively during the dark period in 24 hr photocycles except in very long photoperiods (Eppley and Coatsworth, 1966; Edmunds, 1965a; Marano et al., 1978). DNA synthesis begins in the middle of the light period and continues until the onset of mitosis, which in this case corresponds with the beginning of the dark period. In *Euglena* total RNA per cell increases continuously throughout the $G_1$ and S phases of the cell cycle, as do soluble protein, dry weight, and photosynthetic pigments (Edmunds, 1965b). In *Dunaliella,* increases in RNA and protein per cell are reduced during late S phase (Marano et al., 1978). Since most of the cellular RNA is ribosomal RNA and a significant fraction of this is in the chloroplasts (Puiseux-Dao, 1981; Cook, 1966b), consideration of the events surrounding plastid replication is important in interpreting patterns of biosynthesis in the whole cell (Ledoigt and Calvayrac, 1979; Puiseux-Dao, 1981).

Generalizations regarding macromolecular synthesis over the cell cycle in dinoflagellates are difficult to make. This group of species has several characteristics atypical of eucaryotes, including: (1) low acid-soluble protein content (and therefore histone content) in the chromatin (Rizzo and Nooden, 1973); (2) membrane-attached chromosomes and absence of a mitotic spindle (Bouligand et al., 1968; Kubai and Ris, 1969; Leadbeater and Dodge, 1967; Soyer, 1969); and (3) permanent condensation of chromosomes throughout the cell cycle (Loeblich, 1976). DNA synthesis has been reported to be continuous throughout the cell cycle in dinoflagellates (Dodge, 1965, 1966) as in procaryotic organisms, but more recent evidence has shown that a distinct S phase does exist in several species and it is synchronous for both nuclear and plastidal DNA (Loeblich, 1976, 1977; Galleron and Durrand, 1979). In *Cachonina niei,* synchronized on a LD: 12,12

cycle, for example, cell division begins at the end of the dark period and is completed within the first 4 hr of the light period. DNA synthesis begins later in the light period and is completed within the first 7 hr of the dark period (Loeblich, 1976, 1977). In *Amphidinium carteri,* cell division begins before or at the beginning of the dark period on most photocycles and is completed before the onset of light (Chisholm and Brand, 1981; Galleron and Durrand, 1979). The rate of DNA synthesis, determined as the rate of thymidine incorporation, is maximal immediately following cell division, suggesting a very short $G_1$ period (Galleron and Durrand, 1979). It should be noted that in *A. carteri* (Galleron and Durrand, 1979), *Prorocentrum micans* (Filfilan and Sigee, 1977), and possibly *C. niei* (Loeblich, 1976, Fig. 1b), residual DNA synthesis occurs outside the true S phase of the cell cycle, the nature of which is not as yet understood. This residual synthesis is probably what mistakenly was identified as a continuous S phase in the earlier studies.

## PATTERNS OF CELL DIVISION PHASING BY LIGHT-DARK CYCLES

In most species of phytoplankton, cell division is most pronounced during the dark period when cultures are maintained on 24 hr photocycles (Nelson and Brand, 1979; Chisholm, 1981). Both the degree of phasing and the timing of the onset of the division burst appear to vary between the species and can be influenced by photoperiod (Paasche, 1968; Eppley and Coatsworth, 1966) and other growth conditions (Chisholm et al., 1975), but a general trend of division onset in the dark predominates (Chisholm, 1981). For all these species there is a significant portion of the photocycle during which mitosis does not occur.

The dinoflagellates, in general, display some of the "tightest" cell cycle phasing, although heterotrophic forms appear to be exceptions (Weiler, 1978). In the well-characterized genera, *Gonyaulax* (Sweeney and Hastings, 1958, 1964), *Ceratium* (Weiler and Eppley, 1979), and *Pyrocystis* (Sweeney, 1982), mitosis is restricted to a 4-6 hr gate in the dark or at the dark-light transition, regardless of the mean generation time of the population. In all cases, the mechanism regulating this tight phasing has been attributed to coupling of the cell cycle to a circadian oscillator. We note here, however, that all three of these genera are typically slow-growing dinoflagellates with maximum growth rates of less than one doubling per day. In species with high intrinsic growth rates, such as *A. carteri* (discussed below), division patterns are more like those of other classes of phytoplankton, characterized by a fairly broad division burst but with a significant proportion of the burst occupying the dark period (Nelson and Brand, 1979; Chisholm and Brand, 1981; Chisholm, 1981). Typical examples are shown in Fig. 1. It has been our observation that cell populations belonging to species that display these types of patterns do not lose rhythmicity in division in the fast or "ultradian" growth mode, as suggested by Ehret and Wille (1970). The mechanism controlling the resultant patterns is most easily interpreted as one fitting a cell cycle "block point" model rather than a circadian clock (see below).

Diatoms (*Bacillariophyceae*) as a group are notably exceptional in their division patterns on photocycles (Nelson and Brand, 1979; Chisholm and Costello, 1980; Chisholm et al., 1980; Yoder et al., 1982). In this group, division patterns in populations grown on all but the shortest photoperiods are not phased, regardless of the growth rate (Chisholm and Costello, 1980). Population rhythmicities with a dominant 24 hr frequency have similar characteristics in both fast (ultradian) and slow (infradian) growing populations (Fig. 2). A typical pattern consists of a major burst of division at the beginning of the light period followed by one or more additional peaks that may extend into the dark period.

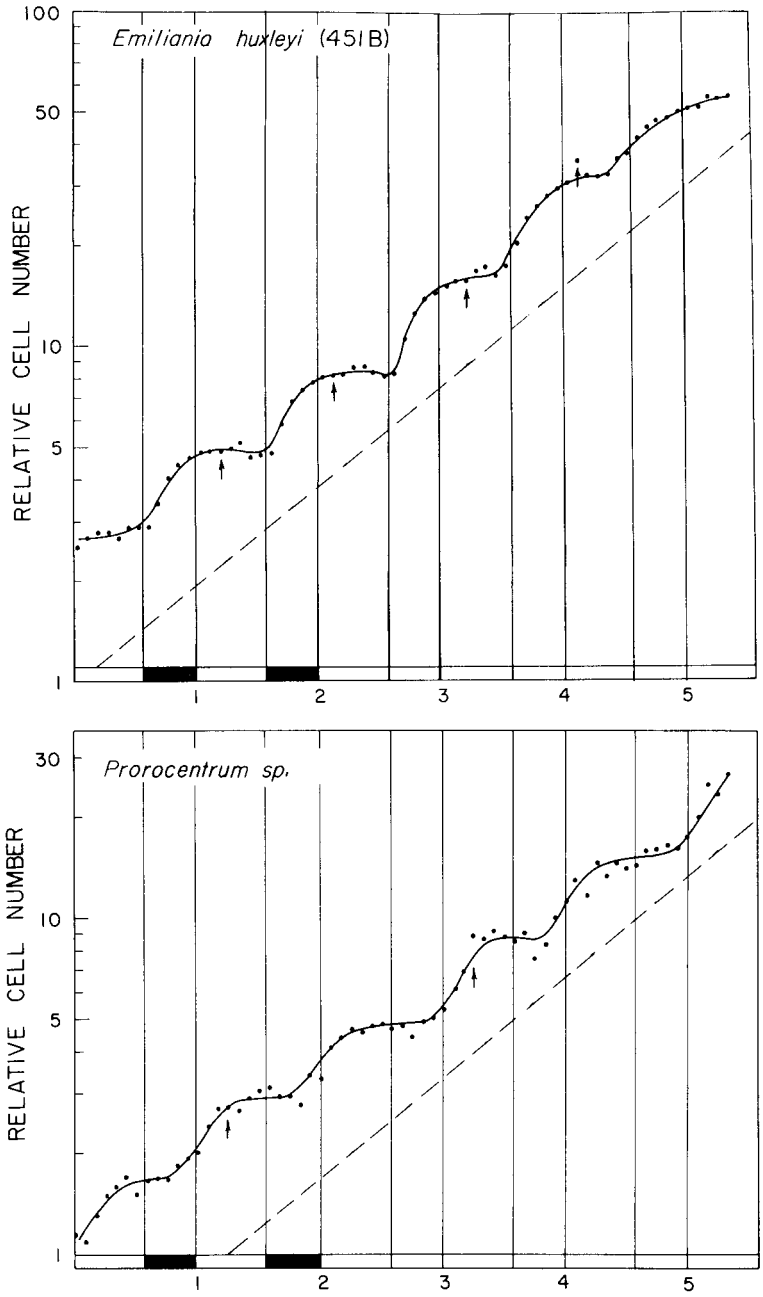



**FIGURE 1** Photocycle-induced cell division patterns of two species of marine phytoplankton grown in semicontinuous batch culture. Arrows indicate times when the cultures were diluted and dashed line has a slope of one population doubling per day. Note that division phasing persists for at least 3 days in continuous light and the average population growth rate does not change. (Adapted from Chisholm and Brand, 1981.)

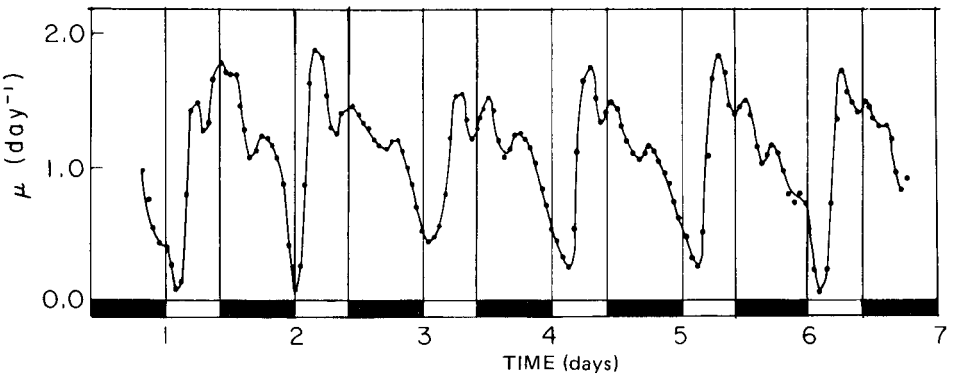


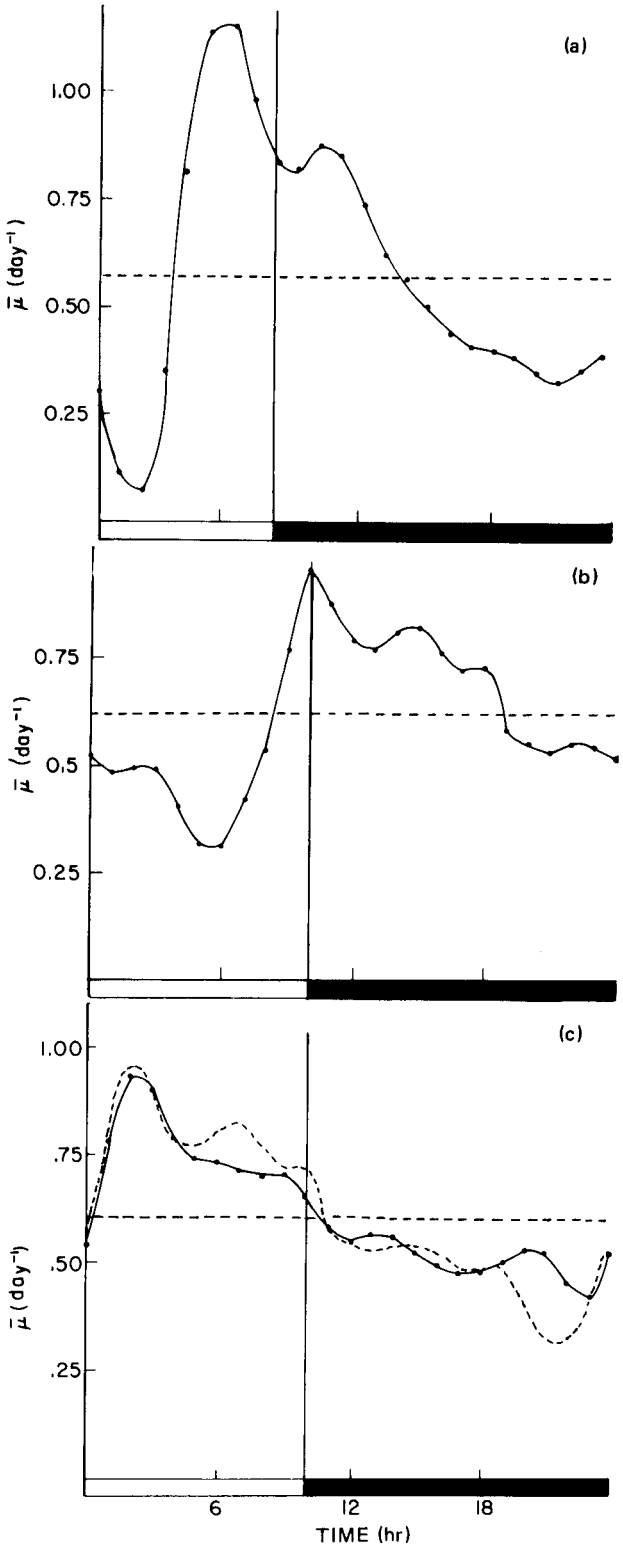

**FIGURE 2** Specific division rate as a function of time in the marine diatom *T. weissflogii* grown in a cyclostat on LD: 10,14. The specific division rate is the derivative of a smoothed function fitted to the cell concentration changes in the vessel (Corrected for dilution). The average growth rate of the population was 1.06 per day. (Adapted from Chisholm and Costello, 1980).

The precise timing and, to some extent, the magnitude of these peaks is conserved between photocycles for a given growth condition but assumes different "fingerprint" patterns when growth rate is limited by different factors, such as nutrients or temperature (Fig. 3). The latter is true, even if the average population growth rates of the cultures are the same, and regardless of whether they are greater than or less than one doubling per day. In all cases, some cells are dividing throughout the photocycle; i.e., there is no division gate.

It could be argued that the division rate periodicities observed in these diatom populations, which are calculated from rates of change of cell numbers, do not reflect underlying cell cycle events, but simply a rhythmicity in cytokinesis or cell separation. Recently, we have examined this possibility using flow cytometric techniques to distinguish between $G_1$ and $G_2$ cells in the population over the 24 hr photocycle (Olson et al., 1983). As can be seen in Fig. 4, the correspondence between the proportion of cells in $G_1$ and $G_2$ and the cell division patterns is quite good; thus, the periodicities we have seen in cell number do indeed reflect cell cycle events.

The resistance of the diatom populations to strict phasing by the light-dark cycle, regardless of the population growth rate, suggests that the cell cycle of these species is not coupled (or very loosely coupled) to an endogneous clock. We may also deduce that the duration of light exposure required for a cell to complete the cell cycle is much shorter than typical daily photoperiods, since in some cases two generations of cells are produced in a single photoperiod. Finally, we conclude that in general, cell cycle progression is not rigidly controlled in diatoms. Even when the average doubling time of the population is 1 day, there is no strictly preferred alignment of mitosis relative to the light-dark cycle. Thus, the change in the age distribution as a function of time in diatom populations entrained to light-dark cycles must differ distinctly from that of species in which division is phased or synchronized.

The division patterns displayed by diatom populations are difficult to interpret in terms of most existing models of cell cycle regulation. The multipeaked nature of the patterns suggested to us that generation times in these populations might be "quantized"


(a)
1.00
0.75
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(c)
1.00
.75
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6
12
18
TIME (hr)

and regulated in a manner similar to that proposed by Klevecz (1976) for mammalian cell lines. Using the Klevecz model as a basis, and superimposing a light requirement on the "$G_q$ loop," one can indeed simulate population division patterns similar to those observed experimentally with diatom populations (Chisholm et al., 1980). We have not been successful, however, in demonstrating directly that the distribution of generation times in these populations are truly quantized. Results of experiments using time-lapse video analysis to measure generation times of developing populations have yielded suggestive, but certainly not conclusive, evidence of quantization in two species of diatoms (Fig. 5). Since the concordance of our model with the data (Chisholm et al., 1980) does not at all imply uniqueness, we consider it still a hypothesis. We *can* conclude, however, that the cell division patterns of the diatom species examined thus far are not characteristic of species in which cell cycles are coupled to a circadian clock. We can also conclude (see below) that in most cases the diatom division patterns cannot be explained on the basis of the simple block-point theory of Spudich and Sager (1980).

Before completing this discussion it should be recognized that some species of diatoms can be synchronized using classic methods of induction synchrony, such as extended dark periods (Lewin et al., 1966), short (e.g., LD: 5,7) photocycles (Darley et al., 1976), and 24 hr photocycles with short photoperiods of high light intensity (Paasche, 1968; Eppley et al., 1967; Chisholm et al., 1980). Also, since diatoms have a silicified cell wall that is replicated only after mitosis and cytokinesis (Lewin et al., 1966), silicon starvation can be used to induce synchrony (Lewin et al., 1966; Busby and Lewin, 1967). In the absence of silicic acid in the medium, the cell cycle is blocked between cytokinesis and division (Lewin et al., 1966; Sullivan, 1977). When silicon is added to the medium, the cells begin synchronous development, but this synchrony decays completely in the subsequent cell cycle; i.e., exponential growth is resumed within a few hours after the synchronous cell wall formation (Busby and Lewin, 1967). This is consistent with our view that diatom populations express an unusual degree of nongenetic variability in generation times when grown in uniform environments (Chisholm et al., 1980).

## DIVISION PATTERNS OBSERVED IN SITU

What limited data exist on the division patterns expressed by phytoplankton species in nature confirm the observations from laboratory cultures; for detailed review see Chisholm (1981). Dinoflagellates divide almost exclusively during the late night-early morning hours (Swift and Durbin, 1972; Elbrachter, 1973; Heller, 1977; Weiler and Chisholm, 1976; Weiler and Eppley, 1979; Pollingher and Zemel, 1981), and diatoms exhibit a wide range of patterns, depending on the species and environmental conditions (Smayda, 1975; Lewin and Rao, 1975; Chisholm et al., 1978; Williamson, 1980). The only other group of

**FIGURE 3** Average specific division rate as a function of time for *T. weissflogii* populations grown in cyclostats: (a) on LD: 8,16 at 20°C, (b) on LD: 10,14 at 15°C, and (c) on LD: 10,14 at 20°C under phosphorus-limited conditions. The $\bar{\mu}(t)$ patterns in each case are an average of at least 5 days of data. The average population growth rates were similar in all three cultures and are indicated by the horizontal dashed line. The function described by the dotted line in (c) is the $\bar{\mu}$ (t) pattern from (b) shifted by 8 hr for comparison. (Adapted from Chisholm and Costello, 1980.)

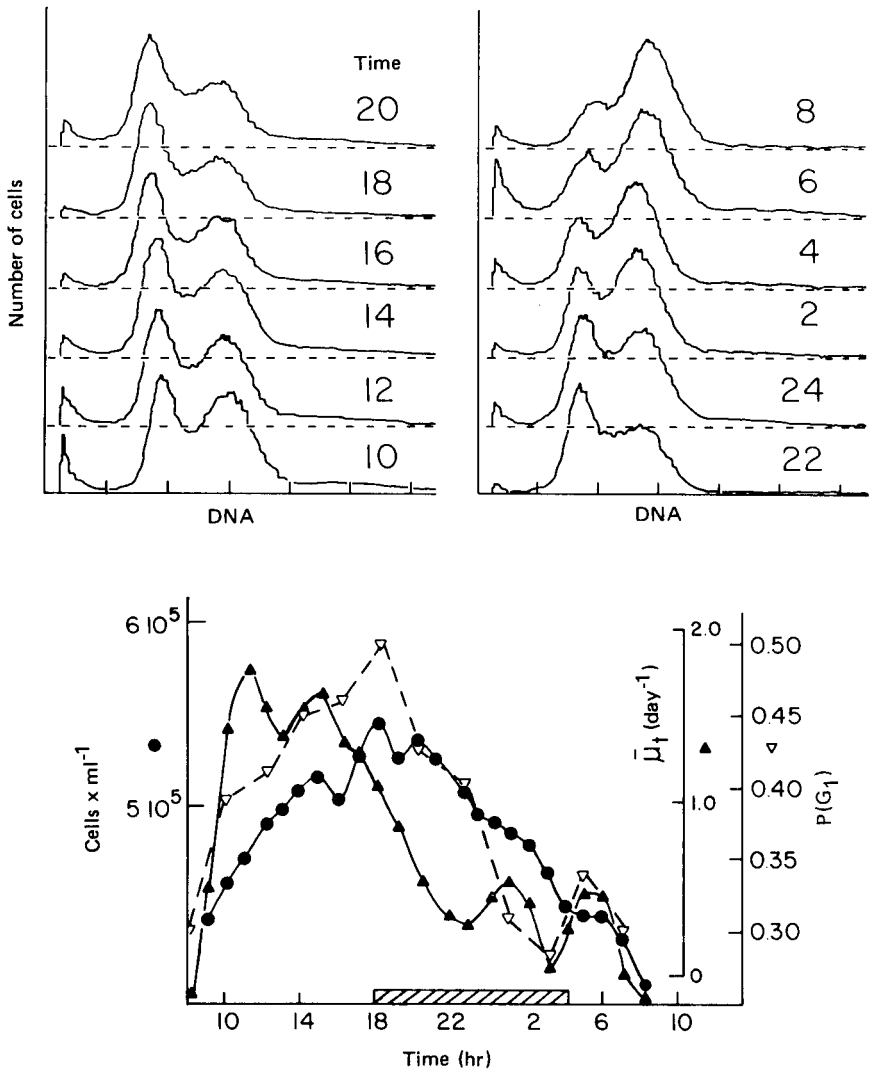


**FIGURE 4** (Lower panel) Cell density, instantaneous cell division rates ($\bar{\mu}_t$), and proportion of cells in $G_1$ phase [$P(G_1)$] over a 24 hr period for a nutrient-replete culture of *T. weissflogii* grown in a cyclostat on LD:14, 10. The average population growth rate was 0.98 per day. Values for $\bar{\mu}_t$ were obtained by averaging data from 5 consecutive days of hourly cell density samples (as described in Chisholm and Costello, 1980). (Upper panel) Relative amounts of DNA in cell populations taken from the culture shown in the lower panel at the times indicated. Hours 18-4 correspond to the dark period. The proportion of cells in $G_1$ at each time point was calculated from the DNA distributions as described in Olson et al. (1983).

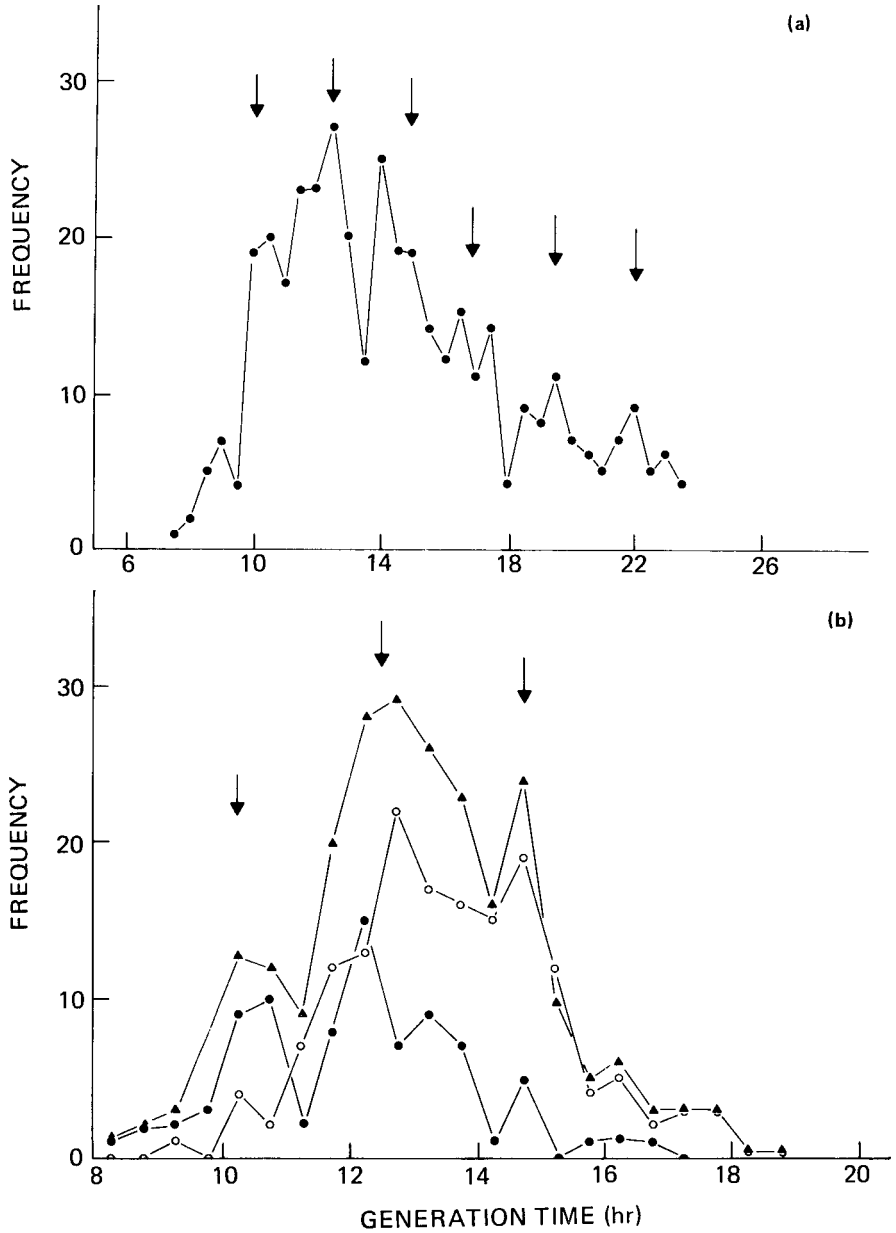


**FIGURE 5** Frequency distributions of diatom generation times, measured using a time-lapse video recording system. Light intensity 100 $\mu$Ein/m$^2$ per sec. (a) *Biddulphia aurita* grown at 18°C in a Palmer-Maloney cell (0.1 ml) filled with seawater medium and sealed with Vasoline. Data are pooled from several experiments. (b) *T. weissflogii* grown at 20°C on 1% agar made with enriched seawater. A thin layer of agar was laid down in a plastic culture dish which was sealed with Parafilm after inoculation. Data are from a single experiment in which two successive generations were followed. Solid circles = first generation observed, open circles = second generation observed, and triangles are the sum of the two. Arrows indicate hypothesized quantized generation times (see text).

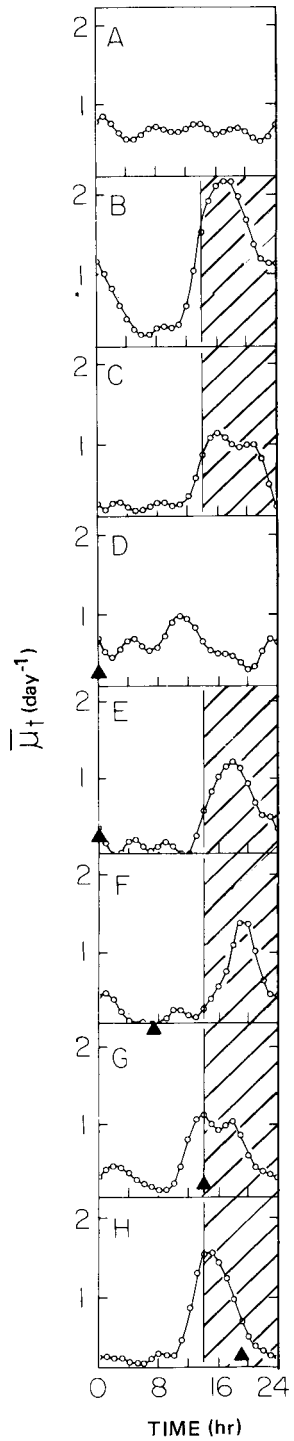


Figure 6

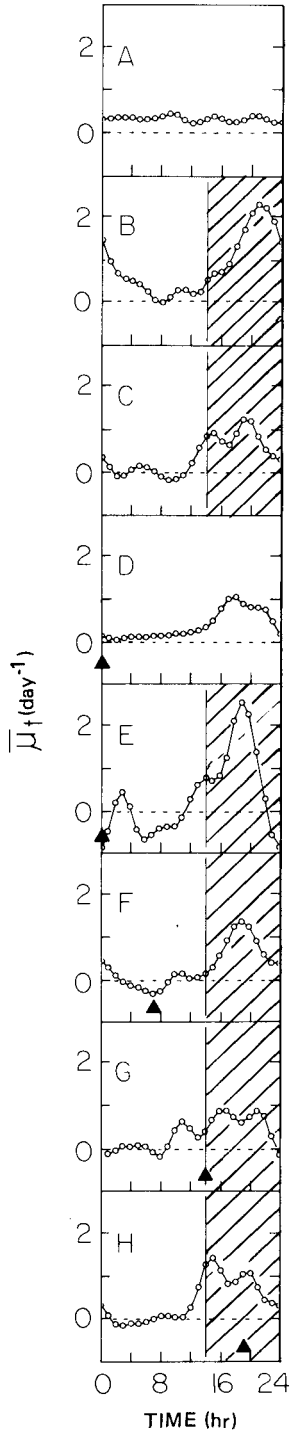


Figure 7

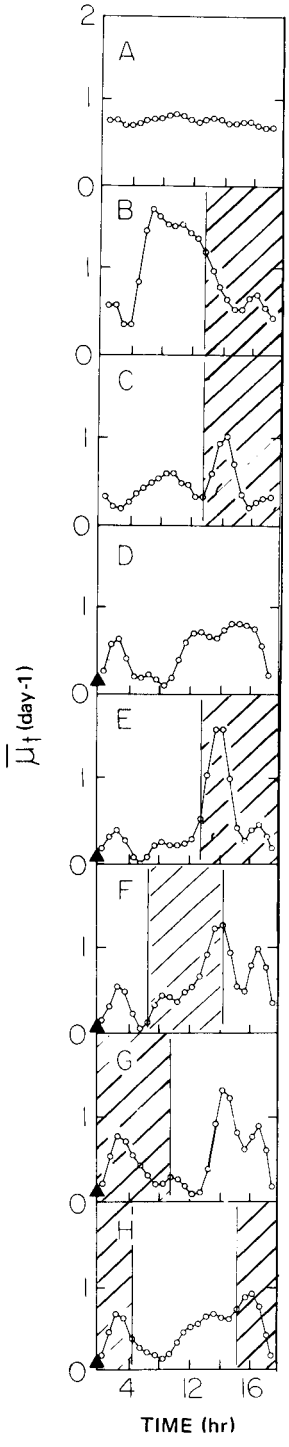


Figure 8

species that has been examined in this regard are members of the green algae in which cell division has been observed to be restricted to the dark period (Stayley, 1971; Simmer and Sodomkova, 1968), as is true of these species in laboratory cultures.

## CELL CYCLE PHASING BY NUTRIENT PULSES

The growth of phytoplankton cells in their natural environment is often (if not usually) limited by the supply of nitrogen (in the sea) or phosphorus (in freshwater). There is growing evidence that a major source of supply of these nutrients to the cells on a microscale is rapid remineralization by heterotrophic organisms, particularly in oligotrophic waters. Moreover, it is argued that the cells may experience this nutrient supply as micropatches (effectively pulses) of high concentration that are transported rapidly by the cells over a short interval relative to their generation times (McCarthy and Goldman, 1979; McCarthy, 1981; Lehman and Scavia, 1982). Cell cycle dependency of nutrient assimilation is reflected by the observation that pulses of limiting nutrients are known to synchronize populations of many cell types (Yoder et al., 1982; Hansche, 1969; Franke, 1970; Thomas et al., 1980). Since the individual phytoplankton cells can be subjected to periodic supplies of energy (light) and essential nutrients, and since the periodicity in nutrient supply may be irregular relative to the photocycle, it is of interest to us to understand to what degree these two stimuli compete in regulating the progression of the cell cycle.

To probe this question, we examined the division patterns of three taxonomically diverse phytoplankton species grown on 24 hr photocycles with periodic supplies of nitrogen (Olson and Chisholm, 1984). In these experiments nitrogen was supplied to cyclostat cultures (Chisholm et al., 1975) as an $NH_4^+$ pulse, once during each 24 hr period. The nitrogen supply was adjusted to be growth limiting and was introduced at various times during the light or the dark period. The responsiveness of the three species, *Hymenomonas carteri* (a coccolithophore), *A. carteri* (a dinoflagellate), and *T. weissflogii* (a diatom) to the competing periodic stimuli revealed distinct differences in terms of cell cycle phasing. In the coccolithophore, division was tightly phased by the light-dark cycle when nitrogen was supplied continuously, and only weakly phased by nitrogen pulses in continuous light. Not surprisingly, when growth conditions included both photocycles and nitrogen pulses the photocycle completely dominated as the phasing stimulus (Fig. 6).

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**FIGURE 6** Average specific division rate as a function of time for *H. carteri* grown in a cyclostat at 20°C on the indicated LD cycle (where darkness is indicated by hatched areas) and with the indicated ammonium supply regime. (A) Control: continuous light and continuous supply of limiting ammonium. (B) LD: 14,10; nutrient replete. (C) LD: 14,10; continuous supply of limiting ammonium. (D) Continuous light; pulse of limiting ammonium at time indicated by arrow (From Olson and Chisholm, 1984).

**FIGURE 7** Average specific division rate as a function of time for *A. carteri.* Details as in Fig. 6.

**FIGURE 8** Average specific division rate as a function of time for *T. weissflogii.* Details as in Fig. 6.

In the dinoflagellate, division was strongly phased by both the photocycle and the nitrogen pulse. The photocycle alone tightly aligned division with the dark period, and the nitrogen pulse alone produced a regular division burst about 14 hr after the introduction of the pulse. When both periodic stimuli are supplied simultaneously, however, the photocycle almost totally dominated the nitrogen pulse as the phasing agent regardless of the phase angle between the two; i.e., the division burst was restricted to the dark period (Fig. 7).

Finally, in the diatom, typical multipeaked division rhythmicity was induced by both the photocycle and the nitrogen pulse when supplied independently, but when this species was grown with nitrogen pulse and photocycle imposed simultaneously, the nitrogen pulse completely dominated as the forcing stimulus. The main division burst always occurred 18 hr after the nitrogen pulse regardless of whether the pulse was given in continuous light or in any phase of the photocycle (Fig. 8).

The different responses of these three species to a periodic supply of nitrogen superimposed on the photocycle must reflect differences in the regulation of cell cycle progression. In other words, the phase angle of cell cycle alignment to the two stimuli should reflect an optimal "strategy" for the cells in "processing" these two growth-limiting substances. In an attempt to arrive at a more mechanistic explanation for the observed patterns the kinetics of nitrogen assimilation were examined in the three species by monitoring the changes in the intracellular pool sizes of $NH_4^+$ and free amino acids after the introduction of the nitrogen pulse (Wheeler et al., 1984). In all three species, the rate of $NH_4^+$ uptake and assimilation into free amino acids and the rate of disappearance of the free amino acid pool were essentially independent of the time in the photocycle at which the nitrogen pulse was introduced. The overall rate of metabolism of the nitrogen pulse in these three species, however, was inversely related to the relative strength of the nitrogen pulse in overriding photocycle entrainment. Large pools of free amino acids accumulated in the diatom (which has a large vacuole) and persisted for a significant portion of the photocycle, whereas in the other two species, assimilation of the $NH_4^+$ into free amino acids and the disappearance of this pool was much faster.

Although not conclusive, these results suggest that the assimilation of the limiting nutrient is the rate-limiting step for cell cycle progression in the diatom, whereas for the other two species the fulfillment of an energy requirement is controlling the progression. This is consistent with the observation that diatom cell cycles are not strictly phased by light-dark cycles except under very short photoperiods (Chisholm et al., 1980).

## MODELS OF CELL CYCLE ENTRAINMENT BY PHOTOCYCLES: CLOCKS VERSUS BLOCK POINTS

In order to explain the coupling between a periodic environmental forcing stimulus (the photocycle) and the growth patterns of algal cell populations, one has to hypothesize some kind of interaction between the forcing variable and the cell cycle. One of the most widespread theories assumes the existence of an internal cellular clock with a period close to 24 hr to which the cell cycle is coupled through gated division (Edmunds and Adams, 1981). A change in the environmental parameter (e.g., light-dark transition) resets the clock, aligning it with the forcing period, and as a consequence aligning the timing of mitosis with the same forcing period. Recently, Sweeney (1982) has shown that the timing of cell cycle stages other than mitosis also appear to be under circadian regulation. This view of cell cycle regulation involves coupled oscillators in the causal sequence:

Forcing stimulus → Clock → Cell cycle

An extensive vocabulary (Ehret and Wille, 1970) and elaborate conceptual models (Edmunds and Adams, 1981) have been developed to characterize the clock and its coupling, but no quantitative model has emerged to account for the growth dynamics of algal populations regulated by such a mechanism. The complexity of the conceptual models for the coupling mechanism is a strong deterrent for the formulation of useful quantitative models because of the multiplicity of parameters required.

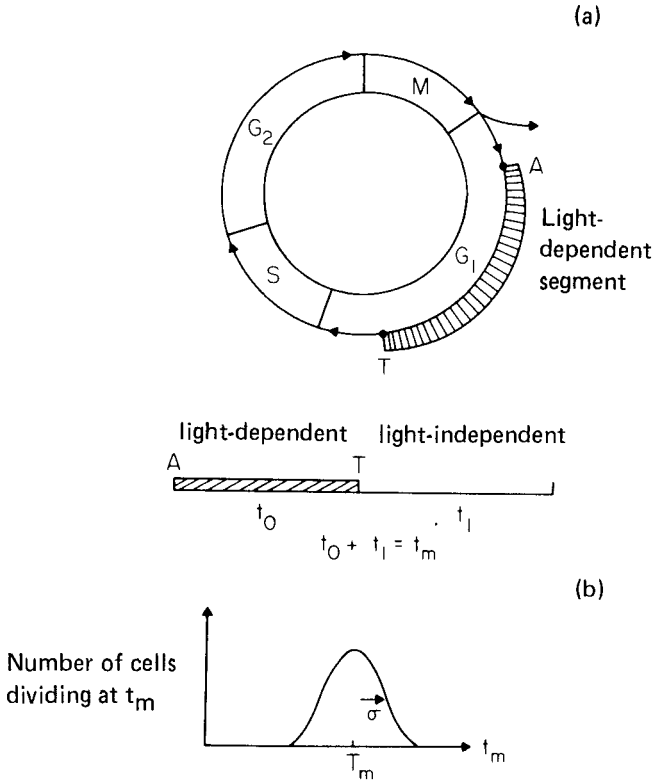


**FIGURE 9** The cell cycle with a light-dependent segment according to Spudich and Sager (1980). The cell cycle is viewed as a succession of four segments; $G_1$, S, $G_2$, and M; S corresponds to DNA synthesis and M to mitosis. Spudich and Sager postulated the existence of a light-dependent segment in $G_1$ from A to T (see text). (b) Stochastic model for entrained populations adapted form the Spudich and Sager model. Individual cell cycles are composed of two segments: one which is light dependent of length $t_0$ (the arrest point A has been placed at the beginning of $G_1$ for simplicity), one which is light independent of length $t_1$. The total length of the cycle is $t_m$ ($= t_0 + t_1$). $t_0$ and $t_1$ are random variables normally distributed among the population $[N(T_0\sigma_0)]$, implying a normal distribution for $t_m$: $N(T_m, \sigma)$. For simplicity the variability has been concentrated in the light-independent segment ($\sigma_0 = 0$, $t_0 = T_0$); thus $\sigma = \sigma_1$ and $T_m = T_0 + T_1$. $T_0$ is called the mean light requirement and $T_m$ the mean generation time. The time evolution of such a stochastic population has been numerically simulated with a technique similar to the age transfer method used by Bronk et al. (1968).

The periodic nature of the cell cycle makes it analogous to a clock or, more appropriately, to an hourglass (Winfree, 1980). This invites the simple hypothesis that the cell cycle is directly entrained by the periodic forcing stimulus, reducing the causal sequence to:

Forcing stimulus → Cell cycle

Spudich and Sager (1980) have recently provided evidence that the regulation of the cell cycle in *Chlamydomonas reinhardtii* may be described by this simple model. By measuring the progression of *C. reinhardtii* through its cell cycle, both by its DNA content and by its light-scattering (cell volume) characteristics, they demonstrated the existence of a light-dependent segment in the cell cycle. This segment is bounded by two points: the primary arrest point A, where the cell cycle is blocked in the absence of light, and a transition point T, where a cell is committed to progress through the cycle to division (Fig. 9). After overcoming T, a cell does not require any more light energy to divide. The link between this segment of the cycle and photosynthesis was further demonstrated by the use of the photosystem II inhibitor DCMU, which mimicked the effect of darkness on division. One could easily extend this hypothesis and postulate the existence of other block points throughout the cell cycle that are overcome by the fulfillment of specific requirements, such as proteins and RNA (Alberghina and Sturani, 1981), cell size (Fantes, 1977), and mitogen levels (Cooper, 1982).

The relative simplicity of the block-point model, and its conduciveness to hypothesis testing, motivated us to build from it a quantitative model to stimulate phytoplankton population growth patterns under a variety of photocycle regimes. Our goal was to compare the results of simple "experiments" obtained using the block-point model with experimental results we have obtained with various species, and with results that would be predicted according to the clock-coupling hypothesis. We have not attempted to fit precisely experimental data by adjusting parameters, but have chosen parameters that are "realistic" based on our experience. The model is simple in construction, and is mechanistically similar to that described in Chisholm et al. (1980). In the model the cell cycle of each cell is composed of two segments (Fig. 9) similar to those described by Spudich and Sager (1980). Progression through the first segment (bounded by A and T) is light dependent, but once the cell has fulfilled its prescribed light requirement ($T_0$) and arrived at the transition point T, progression through the rest of the cycle is light independent. A stochastic component is inserted in the light-independent segment to allow for generation time variability. The precise form of this internal variability is not crucial to the present discussion. We have used a simple gaussian distribution, but recognize that other forms for the variability could be justified (Smith and Martin, 1973; Klevecz, 1976; Cooper, 1982). The evolution of the population itself is stimulated by following the progression of individual cohorts of cells along the cell cycle. This progression is regulated by the amount of light cells have received since the last round of division and, to a lesser degree, by the stochastic component (see Fig. 9 for details).

In deciding what "experiments" would be most useful to conduct with the model, we used the following conceptual framework. A typical entrainment experiment consists of submitting an algal population to a light regime characterized by three parameters: the photocycle length P, the photoperiod length L, and the light intensity I. Quite often two or more different photocycle regimes are applied in a row. For each regime one can distinguish two phases in the population response. (1) an initial transient response, which is dependent upon the initial conditions (and thus the previous regime) during which

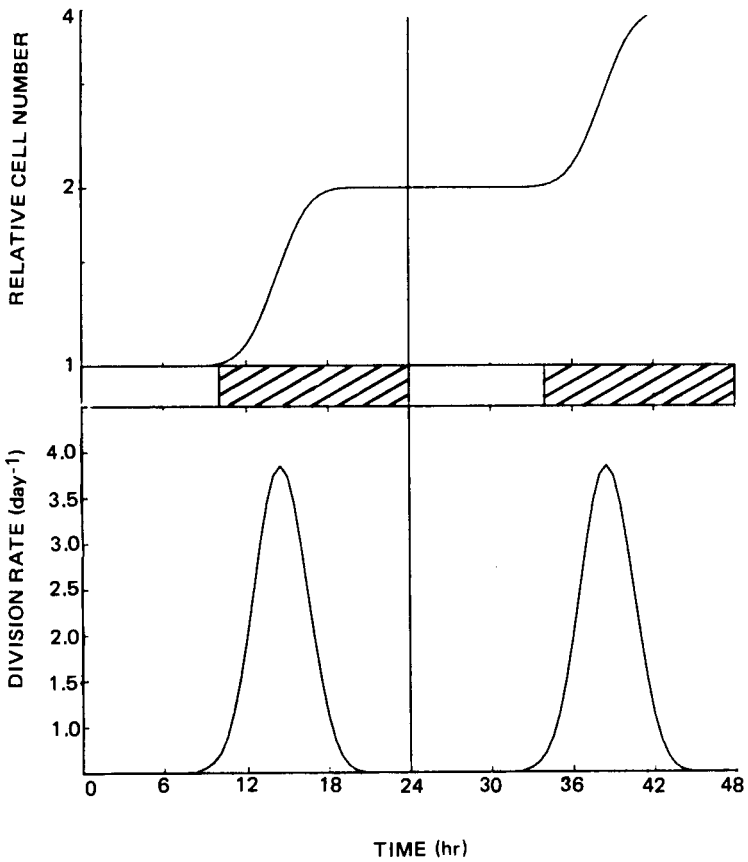


**FIGURE 10** The mechanism of entrainment according to the block-point hypothesis in a population growing at a rate near one doubling per day. Model parameters were $T_0$ = 7 hr, $T_1$ = 8 hr, $T_m$ = 15 hr, and $\sigma$ = 2 hr. The population was entrained to a LD: 12,12 photocycle for 10 days and its average growth rate is 0.68 per day. Cells from the generation born on the first day are synchronized by the imposition of the dark block and are released when the light is on again. They divide at the beginning of the next dark period.

the population adapts to the new conditions (e.g., Edmunds, 1966; Figs. 1 and 2); and (2) a long-term cyclic steady-state response, which is independent of the initial conditions. Of particular interest is the behavior of the population in the regime where P = L (continuous illumination), which is the "free-running" condition often used as diagnostic for clock control.

Unfortunately, in much of the literature describing population growth patterns on light-dark cycles, results are usually expressed as cell number as a function of time in batch cultures. Since an exponential trend is always present, precise frequency analyses are impossible; thus, comparisons with the model results are sometimes difficult. To ease this comparison, we have expressed the model results in two ways: the population division rate $\mu(t)$ as a function of time and the corresponding increase in cell number. The former is directly comparable with cyclostat data (neglecting mortality), and the latter with results from batch culture studies.

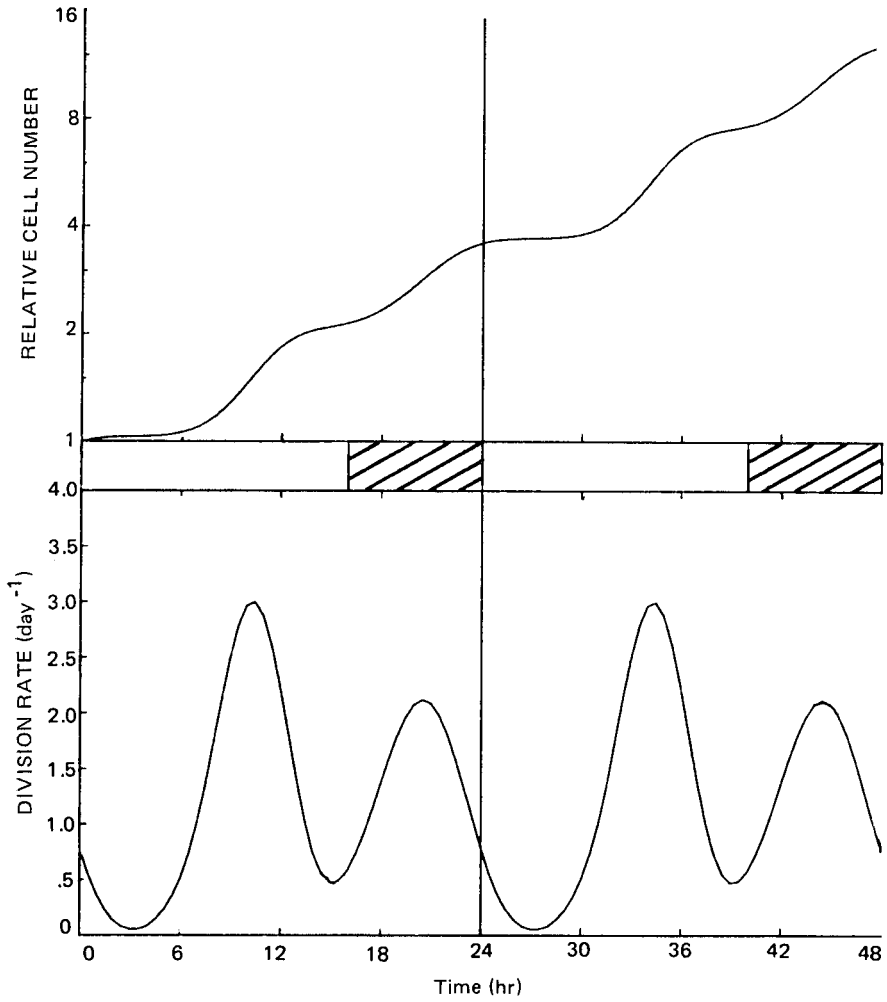


**FIGURE 11** The mechanism of entrainment according to the block-point hypothesis in a population doubling more than once per day. Model parameters were $T_0 = 4$ hr, $T_1 = 7$ hr, $T_m = 11$ hr, and $\sigma = 2$ hr. The population was entrained to a LD: 16,8 photocycle for 10 days and its average growth rate is 1.25 per day. Cells from the generations born on the first day are blocked and divide in the middle of the next day as in Fig. 10, but they are able to see enough light to divide a second time within the same day.

Let us consider first the steady-state population response to growth on 24 hr photocycles. We can see from the model simulation in Fig. 10 that the block-point model generates population growth patterns that are quite similar to "typical" experimental data from *Euglena* (Edmunds, 1965a) and various marine phytoplankton species (see Fig. 1). Cells from the generation born on the first day are synchronized by the imposition of the dark block and are released at the onset of light. They divide again at the beginning of the next dark period. These same results can easily be interpreted in the context of clock coupling, where it is hypothesized that at one of the transitions (presumably the dark-light transition), the clock is reset. Thus, if each cell divides at most once during

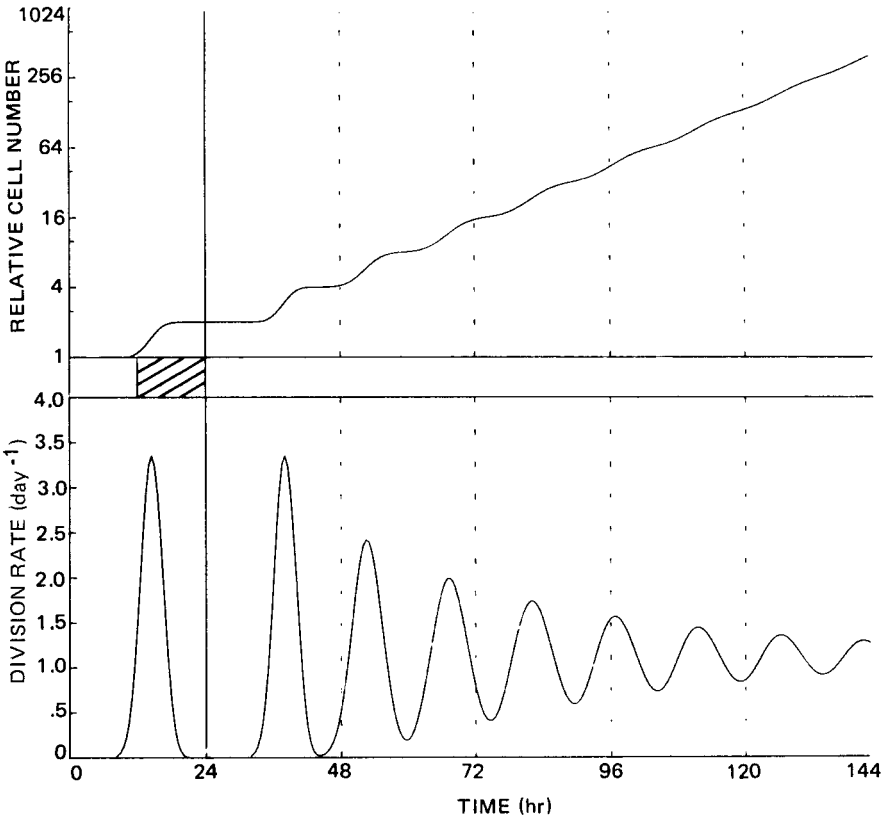


**FIGURE 12** Synchronized population allowed to free run, showing rapidly decaying oscillations. Parameters were $T_0 = 7$ hr, $T_1 = 8$ hr, $T_m = 15$ hr, and $\sigma = 2$ hr as in Fig. 10 (coefficient of variation = 0.13). The population, previously synchronized by a LD: 12,12 photocycle, is released into continuous light at time t = 24 hr. Rapidly damped oscillations with a period close to 15 hr ($T_m$) are observed.

each photocycle, the clock will be reset once a day, inducing a 24 hr periodicity in $\mu$. The maximum division rate should always occur at a fixed phase relative to the resetting signal.

Let us now extend the photoperiod so that the average population growth rate is greater than one doubling per day (Fig. 11). The model predicts a population response with a 24 hr period, but with two division bursts. In this case, the cell cycle is short enough and the light period is long enough that some cells are able to see enough light to divide a second time within the same 24 hr period, giving rise to a second division peak (Fig. 11). This multipeaked pattern is not unlike some we have observed for the diatom *T. weissflogii* and other fast-growing marine phytoplankton species (Chisholm and Costello, 1980). Moreover, Edmunds and Funch (1969) imply that such patterns might also be expressed by *Euglena* grown under long photoperiods of high light intensity. These types of experimental results are difficult to interpret with a clock-coupled cell cycle model because it is difficult to conceptualize how the cell division gating occurs. In theory (Ehret and Wille, 1970), the clock should be completely uncoupled from the cell cycle in this fast growth mode, and aperiodic exponential growth should ensue.

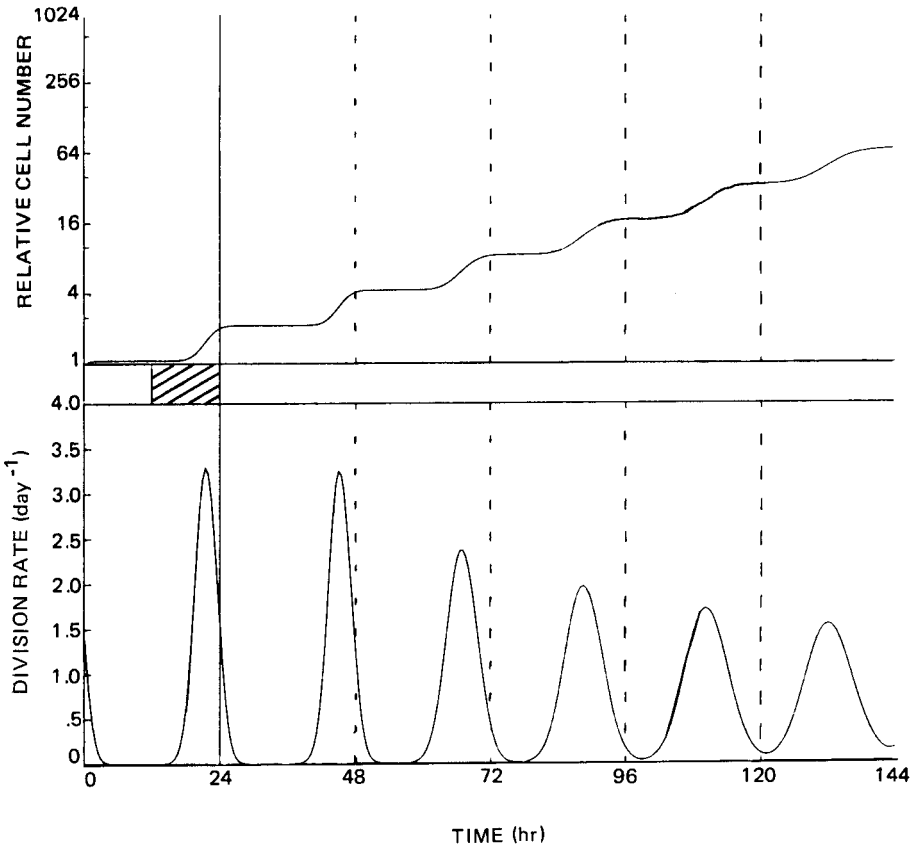


**FIGURE 13** Synchronized population allowed to free run showing slowly decay oscillations. Model parameters as in Fig. 12 except for $T_1$ = 15 hr, $T_m$ = 22 hr (coefficient of variation = 0.09). The oscillations have a period close to 22 hr ($T_m$).

Consider now what the block-point model would predict for a population of cells synchronized as in Fig. 10 and released into continuous light (Fig. 12). Our results concur with the general conclusions of Bronk et al. (1968). The population growth rate exhibits decaying oscillations that have a period close to the mean generation time $T_m$ that would be expressed by the population in steady-state exponential growth under continuous light. $T_m$, in this example, is 15 hr. The loss of synchrony can be characterized by a damping time $T_d$ given by:

$$\frac{T_d}{T_m} = \frac{1}{2\pi^2}\left(\frac{T_m}{\sigma}\right)^2 \tag{1}$$

where $T_m$ is the mean generation time defined above and $\sigma$ is the standard deviation of the generation times (Bronk et al., 1968). The more tightly the generation times are distributed, the longer the transient periodicity will last. For example, if the coefficient of variation (given by $\sigma/T_m$) is 13%, as in Fig. 12, $T_d/T_m$ is equal to 3, but if the CV is lowered to 9%, $T_d/T_m$ is doubled (Fig. 13). Thus, both the period length of the free-running oscillation and the time it takes for it to damp out are strongly dependent on the

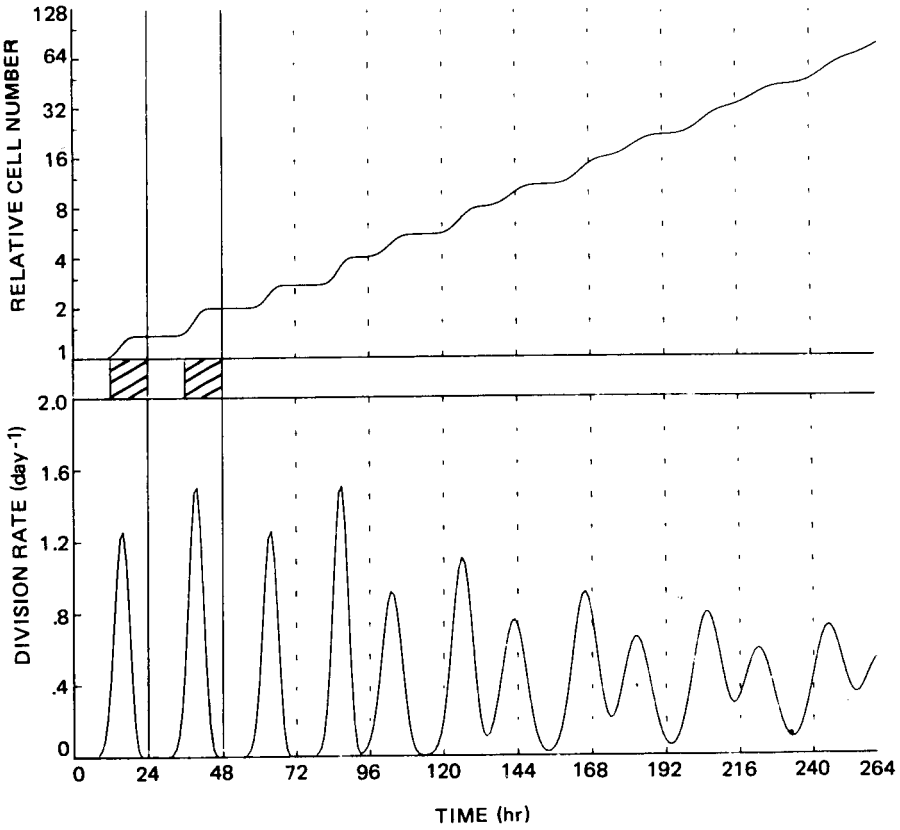


**FIGURE 14** Phase population allowed to free run. Model parameters were $T_0$ = 10 hr, $T_1$ = 30 hr, $T_m$ = 40 hr, and $\sigma$ = 3 hr. The population was previously phased by a LD:12, 12 photocycle and released into continuous light at time t = 48 hr. Damped oscillations of period 40 hr (= $T_m$) are observed. These oscillations have two components, 24 hr out of phase.

values of $T_m$ and the coefficient of variation of the generation times. For a species with a tight generation time distribution and a $T_m$ value close to 24 hr, the free-running patterns generated by the block-point model can appear as circadian oscillations (Fig. 13). We should point out here that population division rhythmicities under clock control are expected to damp out in free-running conditions, presumably because the free-running period lengths of the clocks of individual cells are not identical. The characteristics of decay of the rhythmicity, however, should differ from those of populations controlled by cell cycle block points. In theory, one could quantify these differences if the degree of variability in individual cellular clocks were measurable.

The situation gets more complicated when we consider entrained populations exhibiting phased cell division in which only a portion of the cells divide during each 24 hr period. This growth pattern can be simulated quite readily using the block-point model by choosing appropriate parameters (Fig. 14). When such population (with $T_m$ = 40 hr)

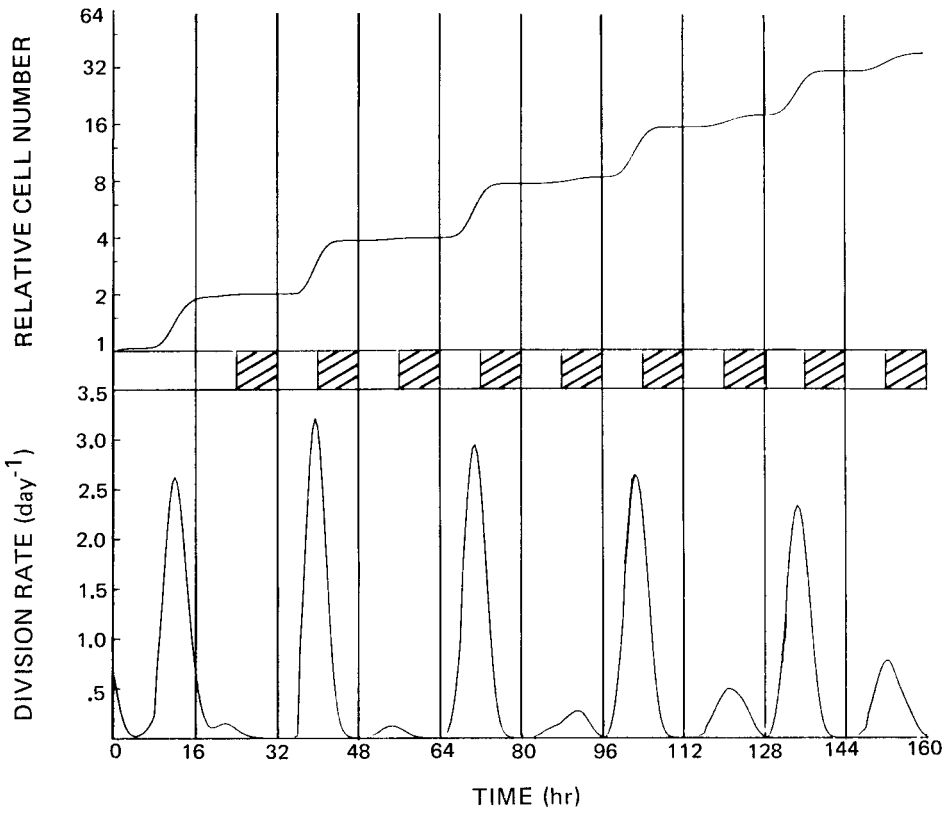


**FIGURE 15** Transients occurring when the light period length L is shorter than the light requirement $T_0$. Model parameters were $T_0$ = 12 hr, $T_1$ = 4 hr, $T_m$ = 16 hr, and $\sigma$ = 2 hr. An initially synchronized population was submitted to a LD photocycle at time t = 16 hr. An additional transient with period 32 hr (two photocycles) is observed, while the long-term behavior exhibits a 16 hr (one photocycle) periodicity.

is released into continuous light, we find that the overall free-running transient pattern has a 40 hr period as our theory would predict, but it contains two components dephased by 24 hr, which could easily be mistaken for a 24 hr circadian rhythm for several cycles.

Finally, consider an example where the light requirement is longer than one light period, but shorter than two light periods. In this case, each cell will need to be exposed to light during two consecutive days before fulfilling its light requirement. Here we find the period length of the major division burst during the initial transient to be twice that of the photocycle, or 32 hr in our example (Fig. 15). More generally, if the light requirement ($T_0$) is larger than (n - 1)L and less than nL, where L is the length of the light period and n is an integer, the transient will have a period of mean lenght nP. This same general response is illustrated by data from *Euglena* where, whenever the photoperiod is shorter than the light requirement (estimated as 10-14 hr; Chisholm, 1981), the period length of the division rhythm is always an integer multiple of the photocycle length (Table 1).

This modeling exercise serves to demonstrate that some of the qualitative properties of population growth that have been attributed to circadian regulation can be reproduced

**TABLE 1** Relationship Between the Period of the Entrained Division Rhythm and the Period of the Entraining Photocycle in *Euglena*[a]

| Light intensity (lux) | Photocycle length = P (hours) | Photoperiod length = L (hours) | Division rate period = $T_\mu$ (hours) | $n = \frac{T_\mu}{P}$ | Light received before dividing = n × L (hours) | Reference |
|---|---|---|---|---|---|---|
| 8000 | 24 | 14 | 24 | 1.0 | 14 | Edmunds (1965) |
| 8000 | 20 | 10 | 20 | 1.0 | 10 | Edmunds and Funch (1969) |
| 8000 | 16 | 8 | 33 | 2.1 | 16 | Edmunds and Funch (1969) |
| 8000 | 10 | 5 | 32 | 3.2 | 15 | Edmunds and Funch (1969) |
| 7500 | 6 | 3 | 30 | 5.0 | 15 | Edmunds et al. (1982) |

[a] Note than n is always close to or equal to an integer and that $n \times L \geq 10$ hr, the estimated required light exposure for *Euglena* under these conditions (see text).

using a block-point model. We do not assert that cell cycles are not clock coupled, but rather that long-term experiments with precise period length measurements are in some circumstances necessary to distinguish between these alternative hypotheses for cell cycle control. Moreover, the block-point model is useful for generating hypotheses that can be tested easily experimentally. It is likely that in its present form it will prove inadequate to describe precisely the growth patterns of many cell types under various conditions, but we see this approach as a fruitful methodology for probing the mechanisms underlying the observed division patterns. In the end, it is probable that a combination of the block-point model and the clock-controlled model will be required to adequately describe the growth patterns of many species (Bruce and Bruce, 1982).

## ADAPTIVE SIGNIFICANCE OF CELL CYCLE PHASING

In the absence of a precise understanding of the mechanisms that control cell cycle progression in the various groups of phytoplankton, it is difficult to postulate why cell cycle phasing might have (or have not) evolved in the different groups. If the timing of mitosis is regulated by a circadian clock, we might hypothesize that there is a selective advantage for each cell to divide at a certain time of day or for genotypically identical cells to divide simultaneously. It is equally plausible, however, that the timing of division per se has no adaptive significance and that natural selection has operated solely upon the coordination and timing of events leading up to division such that phased division is simply the end product of these precisely timed events (Sweeney, 1982). Even so, the clock coupled control mechanism implies that there is an optimal timing of events relative to the photocycle, the selective advantage of which is so strong that it is maintained even in the absence of the entraining stimulus.

If, on the other hand, the division patterns we observe are a result of a light-requiring block point in the cell cycle, any rhythmicity or phasing observed is simply a reflection of the periodic energy supply; i.e., it is a driven or forced rhythm, totally dependent on exogenous periodicity for its maintenance. In this case, the phase angle between the photocycle and the burst in cell division or the absence of division phasing can tell us something about the energetics of the cell cycle, but ecological interpretations can be misleading.

Recognizing the dangers involved in any discussion regarding "the adaptive significance of . . . ," we will attempt here to review briefly some hypotheses that have been put forward to explain photocycle-induced phytoplankton division patterns. The hypotheses fall into two categories: (1) those that consider the energetics of cell cycle progression and optimal alignment of biosynthetic (growth) and reproductive (division) processes, and (2) those that consider selective pressures imposed by the planktonic community (interspecific competition for resources, and mortality from zooplankton grazing) as important in dictating the timing of division. Let us consider the latter case first because it is rather easily dismissed for lack of supporting evidence.

The apparent asymmetry between the assimilatory and reproductive processes of phytoplankton and zooplankton has been noted for years (Wimpenny, 1938; Fleming, 1939). Grazing by zooplankton on phytoplankton ("assimilation") is generally believed to be most intense at night either because of vertical migration patterns or periodicity in actual grazing rates of individual animals (Hart, 1977; Singh, 1972; Arashkevich, 1978; Enright and Honeggar, 1977). *In general,* phytoplankton display a tendency to divide

("reproduce") at night and assimilate during the day. We can make reasonable hypotheses regarding the selective advantage of nocturnal grazing by zooplankton including (1) maximal caloric content and cell size of the phytoplankton, (2) maximal concentration of the phytoplankton crop, and (3) reduced vulnerability to visual predators while in the surface waters. Symmetrical arguments regarding the advantage of nocturnal division of phytoplankton, however, are difficult because dividing (large) cells are often selectively grazed upon (Richman and Rogers, 1969). Moreover, one could argue that there should be a selective advantage for phytoplankton cells to divide during the day (before the interval of most intensive grazing) to mazimize the probability of perpetuating the genotype.

Williams (1971) suggested that intraspecific synchrony combined with interspecific asynchrony in phytoplankton communities could allow temporal partitioning of the demand for limiting nutrients between species and thus reduce competition. This hypothesis has reappeared in several contexts (Eppley et al., 1971, Stross et al., 1973; Weiler and Chisholm, 1976) and is quite easy to support theoretically (Chisholm and Nobbs, 1976), but there is no evidence that such a mechanism is operational in nature.

Given that these synecological hypotheses (i.e., those involving selective pressures imposed by other species) regarding the adaptive significance of the timing of cell division are untenable at present, we might best focus our energies on examining the question in the context of optimal design and the autecology of individual species. Shuter (1979), following Rosen (1967), casts the framework elegantly:

> Given that the realized design of a particular biological structure is optimal in the context of natural selection, then it is also optimal in the sense that it minimizes some cost function which reflects differences in both the intrinsic cost (a measure of the energy required for production and maintenance) and the extrinsic cost (a measure of the selective advantage varying inversely with fecundity) associated with different designs. . . .

Accepting this premise, we conclude that the population growth rates expressed by unicellular algae under various environmental conditions reflect a minimization of this cost function for that condition. The various division patterns (and their degree of flexibility with changing conditions) displayed by different species of algae grown on light-dark cycles, then, must to some extent reflect "strategies" of cost minimization.

Cohen and Parnas (1976) have done a theoretical and experimental analysis of the optimal policy for the production and metabolism of storage materials in *Chlamydomonas reinhardi.* They hypothesized that storage materials are synthesized according to future requirements in algae grown on photocycles, and not simply as a result of an excess supply of energy over growth requirements during the day (Cook, 1966a). They were able to demonstrate that phototrophs produce storage materials during the day (even when energy limits growth) and divide at night because this maximizes the number of daughter cells produced per day. They also showed that cells adjust their biosynthetic and storage patterns according to the energy input. In their experiments, starch synthesis began earlier in the photocycle at low light intensities than at high light intensities, and cells subjected to darkness for 5 days began starch synthesis immediately upon illumination at rates higher than those existing prior to the extended dark exposure. These high levels of synthesis were maintained for at least 3 days, and the beginning of protein synthesis and cell division was delayed by 1 day in these cells.

Although there is a certain amount of teleology in the interpretation of these results, they do support the general tenet that the growth and division patterns of algae grown on light-dark cycles reflect an optimization of resource utilization based on past history designed to maximize long-term growth rate and/or survival of the population. If we compare the *Chlamydomonas* patterns with the division patterns of the diatom *Thalassiosira* (see Fig. 3), it is clear that the optimization strategy of these two species is distinctly different. The adaptive mechanisms dictating the predominance of daytime division in the diatoms are not at all clear at present. Nelson and Brand (1979) hypothesized that the energetic demands of silicification (which is tightly coupled to cell division) may favor daytime division in these cells, but evidence in support of this hypothesis is lacking.

We end this discussion with an anecdotal but intriguing (and testable) hypothesis regarding the selective advantage of the timing of mitosis in certain dinoflagellate species. It has been recognized for some time that dinoflagellate species are particularly sensitive to turbulence. Continuously stirred cultures generally die, or grow at reduced growth rates (White, 1976; Tuttle and Loeblich, 1975; Galleron, 1976). Recently, Pollinger and Zemel (1981) hypothesized that turbulence interferes specifically with the process of nuclear division in these species and showed that *Peridinium* cultures subjected to shaking during the premitotic and mitotic phases of the cell cycle suffered much higher mortality than those shaken during the other phases (i.e., during the light period of the photocycle). Since, on the average, turbulence is significantly reduced in lake surface waters during the night, there should be some selective advantage in nighttime division for these organisms. It is further noteworthy that the many dinoflagellate species actively migrate out of the mixed layer into even less turbulent deeper waters at night and have been observed to undergo division in the mud (Eppley et al., 1968).

## CONCLUSIONS

It should be clear from this review that the issues of cell cycle control mechanisms and the adaptive significance of division periodicity in phytoplankton populations are complex, and our information base is limited. In one sense these cells are not the organism of choice for cell cycle studies because well-developed genetic systems are lacking. They are, however, well suited for studies of population kinetics and analyses of cell lineages and the growth of single cells. Furthermore, the diversity of species with identical growth requirements but distinct growth patterns provides a unique system for comparative studies.

Based on our present knowledge, we must view the photocycle as playing a dual role in regulating cell cycle progression in these populations. By entraining the circadian clock, the photocycle can serve to temporally coordinate cellular processes that lead up to mitosis, which may or may not depend directly upon light energy. In some populations the photocycle also appears to regulate directly cell cycle progression in a manner that can be described using a simple cell cycle block-point model. Because of this dual role of the photocycle in regulating the growth of these organisms, deducing cell cycle control mechanisms from population division patterns can be quite difficult. As Bruce and Bruce (1982) have wisely pointed out, it is likely that multiple control mechanisms are operative within each species, which become relatively more or less important under different environmental conditions. From the point of view of the phytoplankton ecologist, it is important to understand which control mechanisms dictate population growth responses in a given environment and whether these mechanisms vary from species to species.

## ACKNOWLEDGMENTS

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