

THE CELL CYCLE OF PHYTOPLANKTON : COUPLING CELL GROWTH TO POPULATION GROWTH

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I. INTRODUCTION

The cell cycle is the succession of steps that are completed between the birth of cell and its division into two daughter cells. It plays a central role in cell biology since it encompasses key events such as DNA replication, chromosome segregation and cell division. In less than ten years, there has been a tremendous increase in the knowledge of the fundamental biological mechanisms that drive the cell cycle, in particular at the molecular level. The cell cycle engine appears almost perfectly conserved among all eukaryotes from yeast to man. In contrast cell cycle mechanisms appear more diverse among bacteria although some of the basic controls are probably universal as in eukaryotes.

The cell cycle is of particular relevance to aquatic ecology since cell division translates cell growth into population growth. In response to light, cells take up carbon, and accumulate protein and other key metabolites. As they grow bigger, cells ultimately divide, which results in population increase expressed as the cell division rate or population growth rate $m(\text{day}^{-1})$. Cell metabolism is usually assessed in aquatic systems by monitoring carbon uptake using the classical ^{14}C method. In some respects, carbon uptake measurements are difficult to interpret. First, they do not allow separation between net and gross processes. Second, carbon uptake is obtained as mgC per unit time and is difficult to convert into a true rate (with dimension T^{-1}) since the denominator (i.e. the photosynthetic fraction of the particulate carbon) is not easy to estimate. In contrast, the population growth rate is the net result of all metabolic processes and reflects the actual speed at which a given population is turning over. The measurement of phytoplankton growth rates has challenged aquatic ecologists for a long time (Furnas 1990). Cell cycle-based methods offer an elegant way to circumvent this road block.

I will first briefly review recent advances in fundamental cell cycle research, drawing largely from the excellent book recently written by Murray and Hunt (1993) to which I refer the reader who wants to gain a deeper understanding of the cell cycle. I will then focus on phytoplankton. I will detail cell cycle control by external factors and how we can use this knowledge to interrogate the status of natural populations. Finally I will examine how the natural synchrony of aquatic populations to the diel cycle can be used to estimate their growth rate.

II. THE CELL CYCLE

A. EUKARYOTES

The succession of events that make up the cell cycle contain a few steps that are easily recognizable by classical microscopical observation (Figure 1A), in particular mitosis (segregation of chromosome), karyokinesis (nuclear division) and cytokinesis (cell division). In 1951, Howard and Pelc demonstrated that DNA synthesis was not continuous in eukaryotes but restricted to a specific phase they called S, for synthesis (Figure 1A). This phase is preceded by a first gap (G_1) and separated from mitosis (M) by a second gap (G_2). Gametic cells of diploid organisms undergo a differentiated cycle called meiosis in which a round of DNA replication is followed by two rounds of cell division yielding haploid cells.

In order to study the cell cycle, it is critical to determine the localization of cells in the different cell cycle phases. The method of choice is analysis by flow cytometry of cells stained with a DNA fluorochrome such as propidium iodide, DAPI or Hoechst 33342 (Darzynkiewicz and Crissman 1990). The distribution obtained can be deconvoluted into the different populations G_1 , S and G_2 (Figure 1B). Other cell characteristics such as RNA, protein, or the level of a given antigen can be determined simultaneously to the cell cycle stage.

1. The universal engine

Our current understanding of the cell cycle originates from the merging of two completely different approaches which developed over the past 25 years.

In amphibians and marine invertebrates, eggs are large, very well synchronized and therefore easy to study. Masui and Markert (1971) showed that a frog egg, which is stimulated by progesterone and induced to enter meiosis I, contains a substance they called maturation promoting factor (MPF). MPF induces meiosis I when injected into an unstimulated egg. MPF activity was shown to oscillate regularly in cells with a peak at mitosis and a trough during interphase. In eggs, the oscillation was independent of spindle assembly and of DNA synthesis, demonstrating that it was a key upstream event. Later Evans et al. (1983) discovered a protein named cyclin that was continuously synthesized but periodically destroyed during the cell cycle. This led to the hypothesis that high cyclin levels activate MPF. Since then, different types of cyclin have been found, some specific of M (cyclin B), some of S (cyclin A) and some of G_1 (cyclins C to F).

During the same period, research on both the budding yeast (baker's yeast, *Saccharomyces cerevisiae*) and the fission yeast (*Schizosaccharomyces pombe*) produced a number of temperature-sensitive mutants (i.e. with an aberrant phenotype at 37°C, but not at 25°C) that exhibited defects for the cell cycle. For example in fission yeast, two mutants $cdc2^{ts}$ and $cdc25^{ts}$ failed to divide at the restrictive temperature. In contrast, another mutants $wee1^{ts}$ divided at a smaller size than the wild type. Intriguingly the double mutant $cdc25^{ts}wee1^{ts}$ divided normally. Careful analysis demonstrated that while the $cdc2$ gene was required for mitosis, $wee1$ and $cdc25$ respectively inhibited and stimulated its activity, explaining why double mutants of the later genes recovered the wild phenotype. Cloning revealed that $cdc2$ encoded a 34 kDa protein kinase (often called $p34^{cdc2}$). Genes analogous to $cdc2$ were later found in many organisms ranging from the budding yeast (CDC28) to *Homo sapiens*.

The real breakthrough in cell cycle research came with the purification of MPF (Lohka et al. 1988) from frog eggs. This allowed to establish that MPF is made of two units, one analogous to $p34^{cdc2}$ previously identified in yeast and the other to cyclin B identified in sea urchin, demonstrating

the highly conserved nature of cell cycle components. This allowed the reconstruction of the basic engine of the cell cycle (Figure 2). In brief, MPF is only active when p34^{cdc2} is phosphorylated on a single of its two phosphorylation sites (threonine 161). In this state, it induces key mitotic events such as spindle assembly. Just after mitosis, cyclin B is very rapidly degraded, leading to the destruction of the MPF complex. At the same time, p34^{cdc2} is dephosphorylated. During interphase, cyclin accumulation leads to the building up of new MPF. However, since the two sites of p34^{cdc2} are dephosphorylated, several kinases (wee1) and phosphatases (cdc25) are involved in restoring MPF back to its activated state. Still many details of the cell cycle engine remain obscure: in particular other kinases may be required for its successful functioning.

2. Controls and checkpoints

Both internal and external controls are required for the cell cycle to work correctly. On the one hand, it is crucial for cell integrity that events such as DNA synthesis and mitosis be coordinated: for example DNA synthesis must not occur twice in a row without an intervening round of mitosis. On the other hand, the cell cycle needs to respond to the external environment such that it can slow down or even completely stop under adverse conditions, such as the absence of key nutrients or growth factors, in order to prevent irreversible damages.

The need to prevent re-replication of DNA is a good example of internal control. For this purpose, cells use what is called a licensing factor which is bound to chromosomes and is needed for DNA replication. This molecule is destroyed during replication and can only enter the nucleus when the nuclear membrane is either permeable or absent, a condition that only occurs during mitosis. Therefore in the absence of mitosis, DNA cannot replicate again. Moreover in yeast, the licensing factor appears to be inactivated as long as G₁ is not completed; therefore although the licensing factor is bound to chromosomes, cells cannot replicate DNA right at the beginning of the cell cycle.

External factors do not exert their influence on the whole cell cycle but only on certain of its parts. For example, in both budding and fission yeasts, the G₁ phase contains a critical point called Start (Figure 1), beyond which cells are committed to replicate their DNA and divide. In fission yeast, cells can pass Start only when they reach a critical size. In mammalian cells, the same type of control exists and is called restriction point. Once cells are located beyond the restriction point, they are committed to complete the current cycle even if the necessary growth factors are absent. In contrast, G₁ cells that have not yet reached the restriction point and that are deprived of a growth factor, enter an inactive state called G₀. The details of the pathway that leads cells to pass through the restriction point when stimulated by the presence of optimal conditions are still to be fully worked out. In many cases, protein kinase cascades appear to be involved. A protein kinase becomes active once phosphorylated. In turn, it phosphorylates the next kinase in the cascade. Such cascades probably amplify rapidly the initial triggering signal.

B. PROKARYOTES

The main difference between prokaryotic and eukaryotic cells is the absence of distinct cellular compartments. In particular the chromatin is not enclosed inside the nucleus but is loosely arranged within the cytoplasm, forming what is called the nucleoid (Robinow and Kellenberger 1994). Moreover there is no structure such as the cytoskeleton to move cell components within the cell. However, with respect to the cell cycle, bacteria still require some coordination between the DNA replication cycle and the division cycle in order to ensure that all dividing cells contain at least one copy of the genome. A full discussion of the prokaryotic cell cycle can be found in a recent book by Cooper (1991).

In contrast to eukaryotes, prokaryotes may display a cell division cycle that is shorter than the DNA replication cycle (Helmstetter and Cooper 1968). In *E. coli* at slow growth rates, DNA replication only occupies a fraction of the cell cycle and phases analogous to G_1 , S and G_2 can be delineated (Figure 3). At high growth rates however chromosome replication takes longer than the division cycle and spans therefore over more than one cell generation. For this to be possible, new replication forks are initiated on chromosomes that have not yet finished the previous round of replication (Figure 3). The end of one round of replication triggers chromosome separation and cell division.

Despite these differences, some controls appear similar between prokaryotes and eukaryotes. For example, new replication forks can be initiated only when the ratio of the cell mass to the number of replication forks reaches a given threshold (Bremer and Churchward 1994), a reminder of Start control in yeast.

At the genetic level, some of the key events such as the initiation of DNA replication or cell division are well understood (see review by Donachie 1993). However, the underlying oscillators (potentially analogous to MPF in eukaryotes) are unknown. If such oscillators exist, it is not clear whether they are phylogenetically conserved across all prokaryotes. For example, some of the mechanisms observed in *E. coli* model (such as the synchronous initiation of replication forks, Figure 3) clearly do not apply in other eubacteria, such as the cyanobacterium *Synechococcus* PCC 6301 (Binder and Chisholm 1990).

III. THE CELL CYCLE OF PHYTOPLANKTON

Cell cycle basic mechanisms and regulations have only been investigated in detail in a few unicellular photosynthetic organisms such as *Chlamydomonas*, *Chlorella* or *Euglena* (e.g. Donnan et al. 1985). However these cellular models, with their very special form of division (in particular the potential to form multiple daughter cells: 2, 4, 8, 16...) may not be relevant to species found in natural phytoplankton communities, especially in oceanic waters. Recently the use of flow cytometry has considerably advanced our knowledge of phytoplankton cell cycle both in culture (Olson et al. 1983) and in nature (Boucher et al. 1991).

Within eukaryotic phytoplankton, all organisms investigated to date (see e.g. Table 1) display a typical eukaryotic cell cycle with clearly defined G_1 , S and G_2 phases. In the past, there had been some speculations that dinoflagellates, which have permanently condensed chromosomes, continuously synthesize DNA throughout the cell cycle (Karentz 1983). Recent evidence, especially from studies using single cell DNA measurements, do not support this hypothesis and indicate a clearly defined S phase (e.g. Bhaud et al. 1991). Interestingly, of all phytoplankton species studied to date, only the dinoflagellate *Gyrodinium uncatenum* displays a cell cycle with a very long G_2 phase (Cetta and Anderson 1990).

In photosynthetic prokaryotes, the few cases studied indicate in general the presence of phases analogous to G_1 , S and G_2 , suggesting DNA replication is shorter than the division cycle (Figure 3). However, several *Synechococcus* strains, in particular a freshwater one PCC 6301, display an intriguing behavior. Cells may have more than 2 copies of the genome even at relatively low light-limited growth rates. Moreover copy numbers of completed chromosomes are equal to 1, 2, 3, 4, 5 etc... and do not correspond to powers of two, i.e. 1, 2, 4, 8 etc..., as predicted by the *E. coli* model (Binder and Chisholm 1990, submitted). Such behavior has also been observed in *Prochlorococcus*, where up to 5 copies of the genome may occur in the same cell (Vaultot, unpublished data). The interpretation of these observations is that initiation of new replication origins

is not synchronous and that the DNA replication cycle and the division cycle are loosely coupled in these genera (Binder and Chisholm submitted).

IV. REGULATION OF PHYTOPLANKTON CELL CYCLE BY ENVIRONMENTAL FACTORS

A. CULTURE STUDIES

In marine phytoplankton, several studies have tried to assess how phytoplankton cell cycle responds to its environment. Is the concept of restriction point (see above) applicable to phytoplankton and, if yes, how do the different stimuli map onto the cell cycle? Two methods can be used to address these questions.

One can **starve** cells for a given factor (e.g. maintain cells in darkness) and locate the portion of the cell cycle where cells accumulate. The reversibility of the arrest can be verified by restoring the missing factor (e.g. put cells back into light). In order to map more precisely the location of the restriction point within the cell cycle, selective inhibitors may be used: for example hydroxyurea that prevents DNA synthesis and blocks S phase cells, allowing to distinguish between G_1 and G_2 restriction points (e.g. Vault et al. 1986). Alternatively, one may **limit** cells by decreasing the level of the factor under study without removing it totally (e.g. put cells under low light conditions) and determine the phases which lengths are preferentially increased.

Although starvation and limitation experiments yield in general similar answers, it is not always the case. For example Brzezinski et al. (1990) found that the diatom *Thalassiosira weissflogii* arrests in G_2 after silica starvation, but does not show any increase in G_2 during silica limitation.

Temperature appears to have the most uniform action of all factors investigated (Table 1). When temperature decreases, all phases are lengthened in equal proportions. This suggests that the effect of lowering the temperature on the cell cycle is due to a uniform slowing down of biochemical reactions. Although it has not been studied, temperature stress may have a more specific effect, such as the induction of heat shock proteins (Alexandrov 1994).

The effect of **light** on the cell cycle of *Chlamydomonas* was investigated in detail by Spudich and Sager (1980). They found a restriction point located in mid- G_1 . They also demonstrated that DCMU, an inhibitor of photosynthetic electron transport, had the same effect than darkness, pleading for a direct coupling between reductant pools and the cell cycle. The existence of a light-dependent restriction point was also demonstrated in the diatom *Thalassiosira pseudonana* (Heath and Spencer 1985) and in the green alga *Chlamydomonas eugametos* (Zachleder and Van den Ende 1992). Recent studies using flow cytometry have demonstrated that light limitation and starvation induce phytoplankton cell arrest in G_1 (Table 1). Two groups of organisms, diatoms and cyanobacteria, present a second control point in G_2 . In diatoms, this late control appears to be linked to the light dependence of silica uptake during frustule formation (Vault et al. 1986; Brzezinski et al. 1990). In *Synechococcus*, darkness blocks cell division and prevents the initiation of new replication forks (Mann and Carr 1974; Binder and Chisholm 1990). In *Prochlorococcus*, a genus phylogenetically close to *Synechococcus*, light limitation only affects G_1 but darkness blocks cells in all cell cycle phases including S (Vault, unpublished data).

One key question with respect to the effect of light on the cell cycle concerns circadian rhythms. It is well known that phytoplankton division is synchronized to the light:dark cycle both in cultures and in the natural environment (Chisholm 1981). Many other cellular processes such as

photosynthesis or gene expression are also synchronized. Do this synchrony results from the direct entrainment of the cell cycle by light or is the light signal relayed through a cellular clock? Strong evidences for a circadian clock exist in some eukaryotes such as *Euglena* (Edmunds and Adams 1981), but also in some prokaryotes, such as *Synechococcus* (Kondo et al. 1993). However, the synchrony observed in most phytoplankton species can be explained without resorting to an endogenous clock (Vault and Chisholm 1987).

In all species investigated to date, **nitrogen** supply is only required in G₁ (Table 1) which suggests either that cells do not require nitrogen once DNA synthesis has started (most likely because protein synthesis is not required during the S phase, Ronning and Lindmo 1983), or that they have a control mechanism that prevents them from entering S, when they are nitrogen-depleted. In striking contrast, the only study dealing with **phosphorus** starvation (done on the prokaryote *Prochlorococcus*, Parpais 1994) revealed that not only it induced cells to arrest in all cell cycle phases, but also that cells arrested in S could not resume their cycle once resupplied with phosphorus, while those arrested in G₁ and G₂ could. This behavior may be due to the absence of strong checkpoints in prokaryotes, but comparable work needs to be done in eukaryotes. **Silica** is a requirement specific to diatoms and appears needed both in G₁ and G₂ for most, but not all species (Table 1). The G₁ control is apparently due to a silica requirement for DNA synthesis (Darley and Volcani 1969), while the G₂ control is linked to frustule formation (Volcani 1981).

B. NATURAL POPULATIONS

Only very recently, have natural populations been interrogated with respect to their cell cycle status. Vault and Partensky (1992) found that, in the Mediterranean Sea and in winter, some surface *Prochlorococcus* populations had very few cells in S+G₂. These were found at stations where ambient nitrate concentration was below 0.3 μM. Incubating samples with the addition of 1 μM nitrogen (as nitrates or ammonia) induced a large fraction of cells to enter in S+G₂ after 24 hrs. The cell cycle response decreased with depth, pointing out that light also plays a key role in the cell cycle control of these natural populations. In contrast, we observed that, in summer in the Mediterranean Sea, the cell cycle of surface *Synechococcus* populations did not respond to nitrogen addition. It did respond to phosphorus addition even at very low levels of the order of 20 nM (Vault et al., unpublished). These data suggest an alternance between nitrogen and phosphorus limitation in this region depending on the time of the year.

V. USING THE CELL CYCLE TO ESTIMATE THE GROWTH RATE OF NATURAL POPULATIONS

Phytoplankton populations are naturally entrained to the alternating light:dark cycle. In particular, the percentage of dividing cells fluctuates during the day with a maximum occurring in general at night and a minimum during the day (Chisholm 1981). If cells that are dividing or close to division (e.g. with two nuclei) can be discriminated, then one has a handle on estimating the fraction of the population that will divide every day. Gough as early as 1905 used this method to estimate that the dinoflagellate *Ceratium fusus* divided on average once every two days in the English Channel. As will be seen later, the so-called mitotic index method is not as simple as it seems and suffers severe limitations. Recent refinements using cell cycle measurements should make it more useful and easily applicable.

A. MODELING OF CELL POPULATIONS

The mitotic index method relies on the formalism of age-structured populations (Nisbet and Gurney 1982). A population with cell concentration $N(t)$, is best expressed in terms of its age distribution $N(t,a)$ such that $N(t,a).da$ is the number of cells between ages a and $a+da$ at time t :

$$\int_0^{\infty} N(t,a).da = N(t)$$

When the sole source of mortality is cell division, "conservation of cells" can be expressed as:

$$\frac{\partial N}{\partial t} + \frac{\partial N}{\partial a} + m(t,a).N = 0 \quad (\text{Eq. 1})$$

with a boundary condition, expressing that cells divide into two new-born cells of age 0:

$$N(t,0) = 2 \int_0^{\infty} N(t,a).m(t,a).da = 2m(t) \quad (\text{Eq. 2})$$

$m(t,a)$ is the age-dependent division rate and $m(t)$, the population division rate such that:

$$\frac{1}{N} \frac{dN}{dt} = m(t)$$

If the dynamics of the population do not depend on age but on another cellular variable such as cell size or the concentration of a given protein, it is easy to express Eqs. 1 and 2 as a function of the driving cellular variable (see Vaultot and Chisholm 1987)

As an example, one can solve easily Eqs. 1 and 2 if conditions are constant (e.g. continuous light) and if one assumes that all cells divide at a given age t_g , also called the generation time (in fact, this hypothesis is never strictly realized, since there is always some cell-to-cell variability, Vaultot and Chisholm 1987). Then $m(t,a) = \delta(a-t_g)$, δ being the Dirac function. Using the normalized age density such that:

$$n(t,a) = \frac{N(t,a)}{N(t)}$$

Then simple calculus gives the equation:

$$\frac{\partial n}{\partial t} + \frac{\partial n}{\partial a} = -m(t).n(t) \quad (\text{Eq. 3})$$

with boundary condition:

$$n(0,t) = 2n(t, t_g) = 2m(t) \quad (\text{Eq. 4})$$

Since the hypothesis was made of constant conditions, then $n(t,a)$ and $m(t)$ do not depend on t and solution to Eqs. 3 and 4 is:

$$n(a) = 2m \exp(-ma) \quad (\text{Eq. 5})$$

$$m = \ln(2) / t_g$$

Eq. 5 can be used to estimate the percentage of cells in each cell cycle phase as a function of the stage duration under steady state conditions (Slater et al. 1977). For example, if the duration of $G_2 + M$ is t_{G_2+M} and the fraction of cells in $G_2 + M$ is f_{G_2+M} then:

$$f_{G_2+M} = \int_{t_g - t_{G_2+M}}^{t_g} n(a) \cdot da = \exp(-m t_{G_2+M}) - 1$$

Under non-steady state conditions (i.e. when $n(t,a)$ is a function of both a and t), such as light:dark cycles, Eq. 1 and its boundary condition, Eq. 2, are in general not solvable in closed analytical form. Two alternatives can be used to investigate solutions:

1. Monte-Carlo simulations. In this case, each individual cell is followed as it responds to variations in external conditions (e.g. in the dark, cells progress in their cell cycle only if they are in S or in $G_2 + M$ but not if they are in G_1). The population behavior is reconstructed by summing the behavior of a statistically significant number of cells.
2. Numerical solution. It is possible, for example, to solve Eqs. 1 and 2 along characteristics (Vaultot and Chisholm 1987; Chang and Carpenter 1990).

B. THE MITOTIC INDEX METHOD

This method has been formalized by McDuff and Chisholm (1982) and Vaultot (1992). The idea is to transform the curve of the frequency of cells observed in an easy-to-recognize terminal event of the cell cycle into the division rate curve (Figure 4). Mathematically, this can be expressed as deducing $\mathbf{m}(t)$ from $f(t) = \int_{t_g - t_d}^{t_g} n(t, a).da$, where t_d is the duration of a terminal phase of the cell cycle, such as the phase between mitosis and division. Two conditions are necessary for the method to apply. First the phase chosen must be terminated by cell division. Second, its duration must be constant for a given set of conditions. For example if cells are under light:dark conditions, light-born cells must not spend 2 hours between mitosis and division and dark-born cells 8 hours.

Under the condition that t_d is invariant, use of Eqs. 1 and 2 yields (McDuff and Chisholm 1982):

$$\ln[1+f(t)] = \int_t^{t+t_d} \mathbf{m}(t) dt \quad (\text{Eq. 6})$$

If $f(t)$ is periodic, as observed in nature, one can find an approximate equation :

$$\mathbf{m}_p \approx \frac{1}{mt_d} \sum_{i=1}^m \ln(1 + f_i) \quad (\text{Eq. 7})$$

where m is the number of samples collected at fixed intervals during a given entraining period, t_p (usually 24 h), and \mathbf{m}_p the average growth over the entraining period.

Vaultot (1992) established further that:

$$\mathbf{m}_p > \frac{1}{t_p} \ln\left(\frac{1 + f_{\max}}{1 + f_{\min}}\right) = \mathbf{m}_{\min f_{\max}} \quad (\text{Eq. 8})$$

where f_{\min} and f_{\max} are respectively the maximum and the minimum of the fraction of cells $f(t)$ during a daily cycle. In most case, $f_{\min} = 0$, and then Eq. 8 reduces to:

$$\mathbf{m}_p > \frac{1}{t_p} \ln(1 + f_{\max}) = \mathbf{m}_{\max} \quad (\text{Eq. 9})$$

\mathbf{m}_{\max} was used by early investigator as an estimate of \mathbf{m}_p (Swift et al. 1976; Weiler and Chisholm 1976). However, it may severely underestimate the actual division rate. This is easily explained by the fact that only considering the maximum value of $f(t)$ may cause to miss cells that have already left the terminal phase before this maximum or will enter it afterwards. Underestimation increases both as population asynchrony increases and as the duration of the terminal phase chosen is shorter (Vaultot 1992).

In contrast, Eq. 7 is clearly more accurate and has been used in more recent studies (e.g. Braunwarth and Sommer 1985). The major problem associated with Eq. 7 lies in the estimation of t_d , which has to be extrapolated from culture work (e.g. Campbell and Carpenter 1986). However, first, the cell cycle of natural populations may be regulated by factors not tested in culture and that induce unexpected change in the duration of t_d . Second, natural populations may be genetically different from cultured populations and exhibit different t_d duration. To make matters worse, in some

cases such as the dinoflagellate *Gyrodinium aureolum*, the duration of the post-mitotic phase is not constant but linearly related to the generation time: in such case Eq. 7 is useless (Videau and Partensky 1990).

A derivative of the mitotic index method, called the frequency of dividing cells method (FDC, Hagström et al. 1979), has been used to estimate the division rate of natural assemblages of bacteria. It consists in establishing, usually under controlled conditions, a relation between the fraction of bacterial cells in division (i.e. those exhibiting a septum) and cell division rate. This empirical relationship (that should have the form of Eq. 7, with f_i constant if cell division is asynchronous) is then used for field populations. As pointed out by Newell et al. (1986), the FDC method has very severe flaws. In particular, Eq. 7 is only valid for a genetically homogeneous population in which all cells behave similarly. This is obviously not the case for natural bacterial populations (e.g. Giovannoni et al. 1990).

C. THE CELL CYCLE METHOD

In order to circumvent the problems of the mitotic index method, and in particular the need to make assumptions on t_d , Carpenter and Chang (1988) came up with the elegant idea to use cell cycle data to compute directly t_d from field data. Instead of one terminal event (e.g. mitosis), they considered two cell cycle events namely S and $G_2 + M$. Using a simple population model, they demonstrated that under periodic conditions (e.g. light:dark), t_{S+G_2+M} is equal to twice the difference between $t_{G_2+M_{max}}$ and $t_{S_{max}}$, the times at which f_S and f_{G_2+M} are respectively maximum:

$$t_{S+G_2+M} = 2(t_{G_2+M_{max}} - t_{S_{max}})$$

Moreover, t_S and t_{G_2+M} can be computed using the following equation, where i and m have the same meaning than in Eq. 7:

$$\frac{t_S}{t_{G_2+M}} = \frac{\sum_{i=1}^m (1 + f_S + f_{G_2+M})}{\sum_{i=1}^m (1 + f_{G_2+M})} - 1$$

One can then estimate m from Eq. 7 using either $G_2 + M$ or $S + G_2 + M$ as the terminal phase (e.g. replacing t_d by t_{G_2+M} and f by f_{G_2+M}). Moreover, Eq. 9 can be used to obtain a lower bound to the division rate.

In the original work of Carpenter's group, $f_S(t)$ and $f_{G_2+M}(t)$ were determined by microscopic image analysis of cell nuclei stained with the DNA fluorochrome DAPI. The cell cycle method has been validated both using population models (Chang and Carpenter 1990; Chang and Dam 1993) and dinoflagellate cultures (Chang and Carpenter 1988; Antia et al. 1990; Chang and Dam 1993). The following remarks apply:

1. The use of a longer terminal events (i.e. $S + G_2 + M$ instead of $G_2 + M$) gives more accurate estimates.
2. A 2 h sampling interval provides a good compromise between a reasonable number of samples and a good accuracy.
3. Grazing does not influence the estimates unless it is strongly cell cycle stage specific (e.g. if grazers only prey on the larger cells that are close to division).

Initial field applications using image analysis were realized for large species (diatoms, dinoflagellates) both in coastal (Chang and Carpenter 1991) and oligotrophic waters (Chang and Carpenter 1994). The estimated rates were in good agreement with those obtained from changes in cell number in caged cultures, but much larger than those obtained by the mitotic index method, especially in the

case of oligotrophic waters. The failure of the latter method is probably due to the fact that mitosis is too short for these dinoflagellates (Vaultot 1992; Chang and Carpenter 1994).

Recently, flow cytometry has been used to obtain time series of cell cycle distributions of the marine photosynthetic prokaryotes *Prochlorococcus* and *Synechococcus* in several oligotrophic areas including the Pacific (Vaultot et al.; Campbell et al. unpublished data), Atlantic (Partensky et al. unpublished data) and Indian Oceans (Veldhuis et al. unpublished data) as well as the Mediterranean Sea (Vaultot et al. unpublished data). Advantages of flow cytometry over image analysis include increased accuracy of cell cycle stage determination, larger number of cells analyzed, and higher speed. These data revealed that these prokaryotes are highly synchronized to the daily light cycle with division rates of the order of 1 doubling per day (0.69 day^{-1}). Such tight synchrony of some of the key photosynthetic producers in oligotrophic waters may echo throughout the whole food web, as suggested by the synchronous division of tintinnines observed off Hawaii by Heinbokel (1987).

Widespread application of this technique should allow oceanographers to obtain highly accurate estimates of division rates of dominant phytoplanktonic populations as well as to better understand how the cell cycle is regulated by the daily light cycle in nature.

VI.PERSPECTIVES

As should be now evident from reading this chapter, still very little is known on the mechanisms and regulations of phytoplankton cell cycle. However, cell cycle analysis of both cultured and natural populations is very informative about their environmental status and their absolute division rate. Therefore basic studies are needed to interpret these data.

In this respect, the use of molecular techniques should be very useful, but almost everything has to be done. For example, virtually nothing is known on the basic cell cycle engine in unicellular algae (see however John et al. 1989, 1991; Rodriguez et al. 1993; Lin et al. 1994). Another area of research is the characterization of genes that are activated or de-activated at specific points in the cell cycle (e.g. Gagné and Guertin 1992), as well on how environmental signals are transduced to the cell cycle. Finally, a fascinating topic is the relationship between the cell cycle and the sexual cycle (Armbrust et al. 1990).

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VIII. TABLES

Table 1: Cell cycle dependence on external factors in phytoplankton. Data obtained mainly from starvation experiments. When limitation has a different effect than starvation, it is mentioned (e.g. for silica in diatoms).

Factor	Group	Species	G ₁	S	G ₂	References
Temperature	Diatoms	<i>Thalassiosira weissflogii</i>	+	+	+	Olson et al. 1986
	Prymnesiophytes	<i>Pleurochrysis carterae</i>	+	+	+	Olson et al. 1986
	Dinoflagellates	<i>Amphidinium carterae</i>	+	+	+	Olson et al. 1986
Light	Diatoms	<i>Thalassiosira weissflogii</i>	+		+	Olson et al. 1986; Vaultot et al. 1986
		<i>Cylindrotheca fusiformis</i>	+		+	Brzezinski et al. 1990
		<i>Thalassiosira pseudononana</i>	+		+	Brzezinski et al. 1990
		<i>Minutocellus polymorphus</i>	+		+	Brzezinski et al. 1990
		<i>Chaetoceros simplex</i>	+			Brzezinski et al. 1990
		<i>Chaetoceros muellerii</i>	+		+	Brzezinski et al. 1990
		<i>Phaeodactylum tricornutum</i>	+			Brzezinski et al. 1990
	Chlorophytes	<i>Chlamydomonas reinhardtii</i>	+			Spudich and Sager 1980
	Prymnesiophytes	<i>Pleurochrysis carterae</i>	+			Olson et al. 1986; Vaultot et al. 1986
	Dinoflagellates	<i>Amphidinium carteri</i>	+			Olson and Chisholm 1986; Olson et al. 1986
	Euglenophytes	<i>Euglena gracilis</i>	+			Yee and Bartholomew 1988
	Cyanobacteria	<i>Synechococcus</i> WH8101	+		+	Armbrust et al. 1989
		<i>Synechococcus</i> WH8103	+		+	Binder and Chisholm submitted
		<i>Synechococcus</i> WH7805	+		+	Binder and Chisholm submitted
		<i>Synechococcus</i> PCC6301	+		+ ¹	Binder and Chisholm 1990
<i>Synechococcus</i> WH7803		+		+ ¹	Binder and Chisholm submitted	
Prochlorophytes		<i>Prochlorococcus</i> strain MED	+	+ ²	+ ²	Vaultot, unpublished data
Nitrogen	Diatoms	<i>Thalassiosira weissflogii</i>	+			Olson et al. 1986; Vaultot et al. 1987
	Prymnesiophytes	<i>Pleurochrysis carterae</i>	+			Olson et al. 1986; Vaultot et al. 1987
	Dinoflagellates	<i>Amphidinium carteri</i>	+			Olson et al. 1986
	Prochlorophytes	<i>Prochlorococcus</i> strain MED	+			Parpais 1994
Phosphorus	Prochlorophytes	<i>Prochlorococcus</i> strain MED	+	+ ³	+	Parpais 1994
Silica	Diatoms	<i>Thalassiosira weissflogii</i>	+		+ ⁴	Vaultot et al. 1987; Brzezinski et al. 1990
		<i>Cylindrotheca fusiformis</i>	+		+	Brzezinski et al. 1990
		<i>Thalassiosira pseudononana</i>	+		+	Brzezinski et al. 1990
		<i>Minutocellus polymorphus</i>	+		+	Brzezinski et al. 1990
		<i>Chaetoceros simplex</i>	+		+ ⁵	Brzezinski et al. 1990
		<i>Chaetoceros muellerii</i>	+		+	Brzezinski et al. 1990
		<i>Phaeodactylum tricornutum</i> ⁶				Brzezinski et al. 1990
Vitamin B12	Euglenophytes	<i>Euglena gracilis</i>		+	?	Lefort-Tran et al. 1987

¹ Cells may have more than 2 copies of the chromosome² Light limitation does not affect S and G₂. Darkness induces cells to arrest also in S and G₂³ Arrest in S is irreversible⁴ Only Si starvation, not Si limitation, has effect on G₂⁵ Only Si limitation, not Si starvation, has effect on G₂⁶ Si has no effect on the cell cycle

IX. LIST OF FIGURES

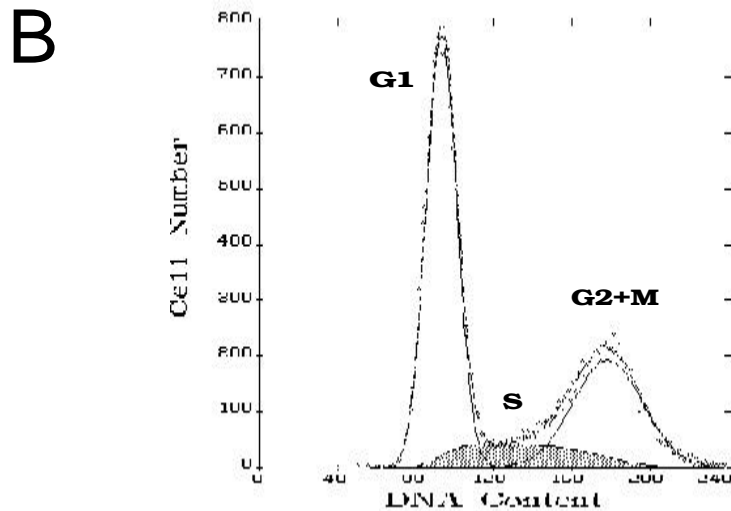
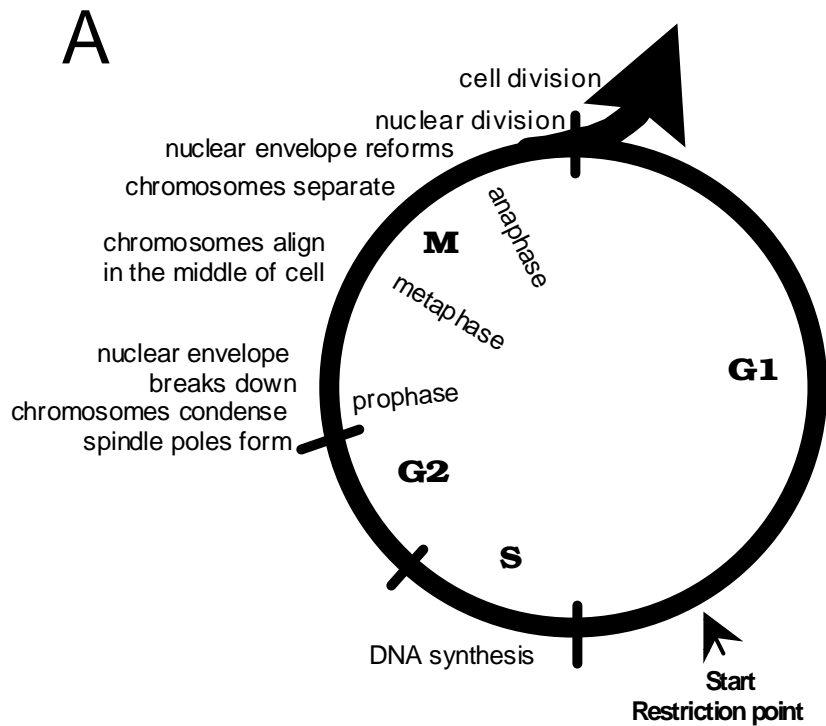


Figure 1: (A) Some of the key events of the eukaryotic cell cycle (see text for details). (B) Use of flow cytometry to determine the cell cycle distribution of a population. X axis corresponds to DNA content and y axis to the number of cells with a given DNA content. The two peaks correspond to cells in G_1 and G_2+M , with the cells in-between belonging to S. Software analysis (e.g. MCYCLE from Phoenix Flow Systems) permits to compute the percentage of cells in each phase.

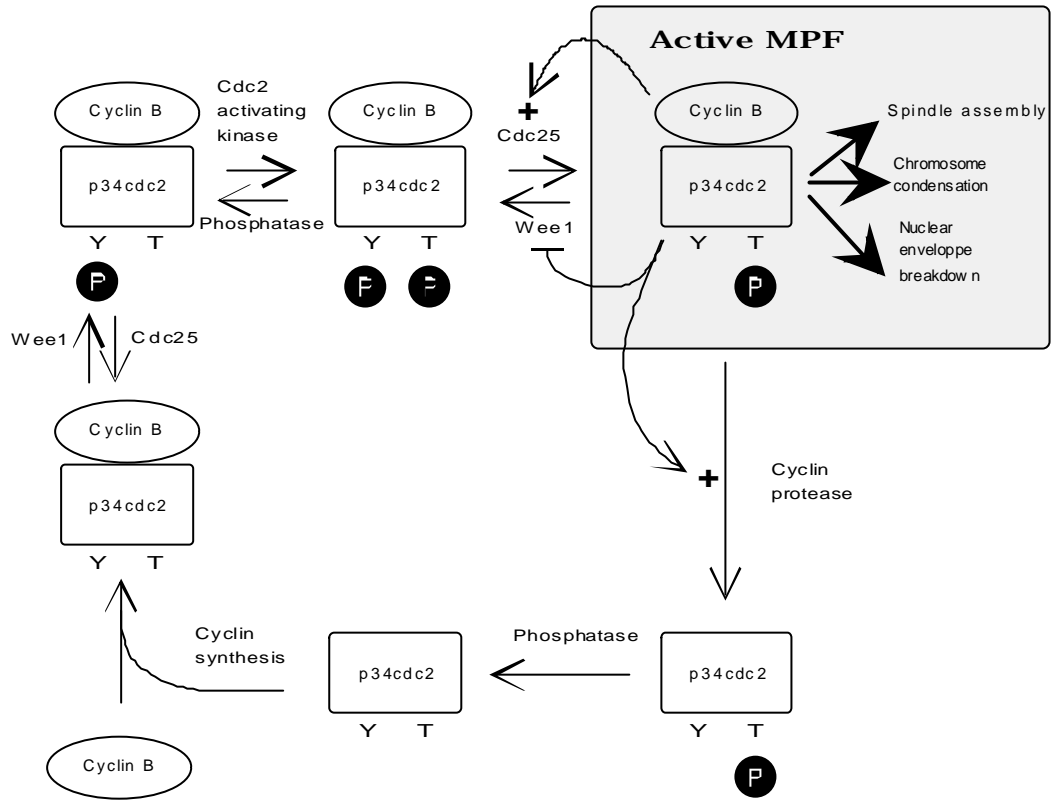


Figure 2: The basic engine of the cell cycle (see text for details, redrawn from Murray and Hunt 1993).

Figure 3: The prokaryotic cell cycle. In slow-growing populations (A), DNA replication is shorter than the division cycle yielding flow cytometric DNA distributions (C) analogous to that of eukaryotes. In fast-growing populations (B), DNA replication is longer than the division cycle. New-born cells have more than one chromosome and DNA replications is continuous, yielding flow cytometric DNA distributions (D) without well-defined peaks (reprinted with permission from Armbrust et al. 1989).

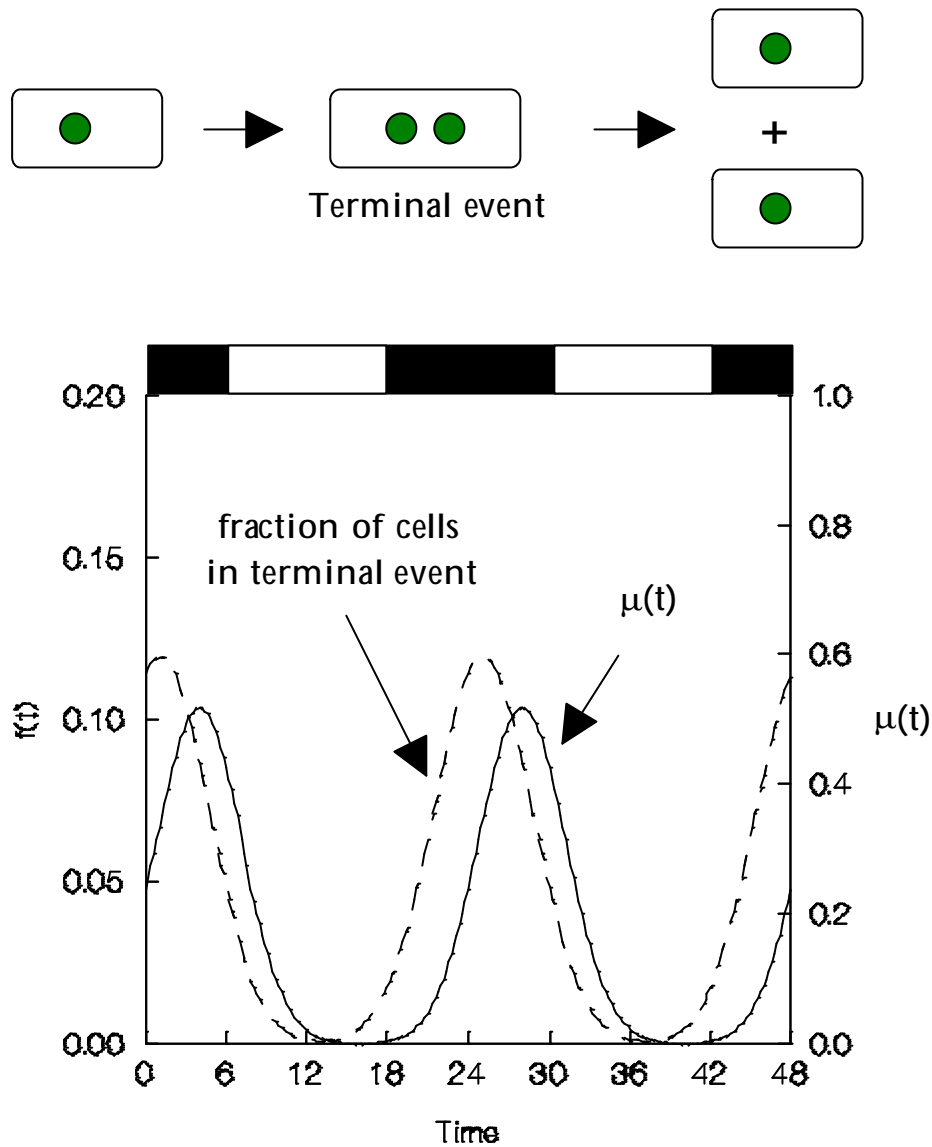


Figure 4: The principle of the mitotic index method. The fraction of cells in a well-recognizable terminal phase of the cell cycle (e.g. post-mitotic cells with 2 nuclei) is counted during a diel cycle [$f(t)$, dashed curve]. From $f(t)$, the average value of the division rate [$\mu(t)$, continuous curve] is estimated (see text).