Cell cycle events in the green alga *Chlamydomonas eugametos* and their control by environmental factors

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Summary

A procedure for routine synchronization of large amounts of the unicellular green alga *Chlamydomonas eugametos* in liquid culture by alternating light and dark periods is described. The synchronized populations were grown at various light intensities and temperatures. The effect of these variables on the lengths of parts of the cell cycle and the number of daughter cells per cell division was followed. The cell cycle of *C. eugametos* started with a period in which the cells increased in size only (precommitment period). The length of this period was dependent on both the light intensity and the temperature. At the end of this period, a key point of the cell cycle (called commitment point) was attained. From this point, the cell were committed to divide and cell reproduction was triggered. The following period (postcommitment period), during which daughter cells were formed, could be traversed without supply of external energy, and without further growth of the cells. However, if sufficient energy was supplied during this period, the cells were able to attain more commitment points, leading to a higher number of daughter cells. The postcommitment period was fairly constant within a certain range of light intensity. At light intensities leading to more commitment points, however, this period was prolonged. No evidence was found for circadian rhythms or endogenous factors of “Zeitgeber” type playing a role in the control of growth and reproductive sequences in the cell cycle of *C. eugametos*.

Key words: cell cycle, *Chlamydomonas eugametos*, commitment to division, cell cycle length, precommitment periods, postcommitment periods, light intensity, temperature.

Introduction

*Chlamydomonas eugametos* has often been used for studies of gametogenesis and cell-cell interactions (Tomson et al. 1986; Homan et al. 1987; Musgrave and Ende, 1987; for review, see Harris, 1989). For example, it was found recently that the sexual cycle is tightly coupled to the vegetative cell cycle, in the sense that newly born cells can be mating competent during the first part of the G1 phase (Zachleder et al. 1991). However, little attention has been paid to the cell cycle of *C. eugametos* in comparison with that of the related *C. reinhardtii* (Lien and Knutsen, 1979; Spudich and Sager, 1980; Donnan and John, 1984; Donnan et al., 1985). John and his collaborators found, in correspondence with previous findings in the alga *Scenedesmus quadricauda* (Setlik et al. 1972; Zachleder and Setlik, 1988, 1990), that the cell cycle of a green alga can be separated into two periods, a precommitment and a postcommitment period. At the end of the precommitment period, a commitment point is reached that is equivalent to the transition point “start” in yeast cells (John et al. 1989). This point plays a key role in the regulation of the cell cycle, because from this point on the cell is committed to undergo cell division. In green algae, an additional feature is that several commitment points can follow each other, which results in multiple cell divisions. The mechanism controlling the number and timing of the consecutive commitments is of particular interest and is addressed in this paper.

Synchronized cultures are the best tool for this type of research. The only attempt to synchronize *C. eugametos* was published by Demets et al. (1985), which did not result in a detailed analysis of the cell cycle. In the present paper, completely synchronized cultures were used to study the effect of light intensity, light/dark regime and temperature on the length of the cell cycle and on cell proliferation in *C. eugametos*.

Materials and methods

Organism

The UTEX 10 strain of *C. eugametos* (mt-) was obtained
from the Algal Collection kept at the University of Texas, Austin, USA.

Culture equipment and conditions
Cells were cultivated batchwise in 1,200 ml plate-parallel vessels (18 mm in width) at 30°C, illuminated by two HgMIF 400/D lamps (Tungsram, Budapest, Hungary). Details of the culture equipment were the same as those described by Doucha (1979). The light intensity at the surface of the culture vessels was approximately 70 W m⁻² of photosynthetically active radiation (400 to 720 nm). The CO₂ concentration in the gas mixture by which the culture was aerated was 2% (v/v). The composition of the mineral nutrient solution was as described by Kates and Jones (1964): 1.0 g l⁻¹ KNO₃; 0.74 g l⁻¹ KH₂PO₄; 0.136 g l⁻¹ MgSO₄.7H₂O; 0.05 g l⁻¹ CaCl₂.2H₂O; 0.14 g l⁻¹ K₃HPO₄; 0.025 g l⁻¹ FeEDTA, and included 1 ml l⁻¹ of solution of trace elements (Zachleder and Setlik, 1982).

The synchronization procedure
Flooded cells from a 3-week-old agar plate were used to inoculate the batch culture that was to be synchronized. Under the conditions described above, cell division started at about the 16th hour and the cells divided mostly into eight daughter cells. The cells were grown for one whole cell cycle and at the beginning of the next light period they were diluted to the initial density (1×10⁶ cells ml⁻¹). The synchronization itself was carried out by alternating light/dark periods, the lengths of which were chosen according to the growth parameters of the cells. The optimal time for darkening the cells was when they started their first protoplast fission. The length of the dark period was chosen to allow all cells of the population to release their daughter cells. For the first two or three cycles, the culture was observed by light microscopy to set the correct length for both the light and dark periods. Once the culture was synchronous, the length of the light and dark periods was kept constant.

Assessment of commitment curves
To determine when the cells were committed to divide, samples taken every 2 h were spread on agar (2% in nutrient medium) in Petri dishes (10 cm in diameter) and incubated in the dark at 30°C. Under these conditions every cell eventually divided if it had passed the commitment point (Setlik and Zachleder, 1984). About 30 h after the beginning of the light period, such cells had formed small colonies in which the number of released daughter cells per mother cell was counted. By this method, the percentage of the cells that had divided into 2, 4, 8, 16 or more daughter cells was determined. The sigmoidal "commitment curves" were obtained by plotting the cumulated number of daughters as a percentage of total cells against time of sampling.

Measurement of light intensity
A quantum/radiometer-photometer (LI-COR, Inc., USA) was used. Adjustment of the light intensity was achieved by inserting metal screens between the light source and the culture vessel. For exact adjustment of the required light intensity the distance of the vessel from the light source was varied.

Results

The synchronization procedure
The most convenient conditions for synchronizing a C. eugametos culture were those optimal for cell growth, i.e. an increase in cell size, and for cell proliferation, i.e. increase in cell number. They were: 30°C, light intensity 50-70 W m⁻², and aerating using 2% of CO₂ in air. It appeared to be advantageous to place the initial cell suspension in the dark for about 15 h. During this period most cells divided. Consequently, the population, which was subjected to the following synchronization procedure, was already partially synchronous (all cells were in the precommitment period). Under the conditions described above, cell division started at about the 16th hour in the light. At that time the cells had rounded off, were immotile and displayed the first protoplast division (Fig. 1, 16 h). Most of the cells divided more or less synchronously into two, four, eight or 16 daughters. If the cells were then transferred to darkness, daughter cells were released without further increase of cell size (Fig. 1, 0 h). The length of the dark period was chosen to allow all cells to release their daughters. The daughter cells were grown further under optimal conditions until protoplast fission was again observed. If the cells were then put in the dark a population of daughter cells was produced that were homogeneous in size and that divided more synchronously. In this procedure, the lengths of the imposed light and dark periods were adjusted to the length of the cell cycle under the given circumstances, rather than the cell cycle being forced into the framework of a diurnal regime. The advantage of this procedure is that it could be used to test the effect of different conditions on the duration of the cell cycle and its components. In the following experiments, a synchronous population, grown for several cell cycles under standard conditions (30°C, 70 W m⁻², 16:10 h), was divided into subcultures at the beginning of the light period and grown for three cycles at four different light intensities (7.5, 15, 35 and 70 W m⁻²) or temperatures (20, 25, 30 and 35°C). By diluting with fresh medium, the cell density of the subcultures at the beginning of each cell cycle was kept at 5×10⁶ cells ml⁻¹ in all experimental variants.

Length of the pre-commitment period
As can be seen in Fig. 2 and Fig. 4 (curve 3), there was an inverse relationship between the length of the precommitment period (measured as the distance between the beginning of the cell cycle and the midpoint of the first commitment curve) and light intensity. This supports the idea that the main (if not only) factor determining the timing of the commitment to divide is the growth rate, which is determined by the rate of photosynthesis (Spudich and Sager, 1980). It can be assumed that the cells that had attained the first commitment point (curve 1 in Fig. 2) at different light intensities were all at the same cell cycle stage, because they all divided without an additional increase in size, implying that all conditions for cell duplication had been fulfilled.

Similarly, the length of the precommitment period was inversely related to the temperature (from 35°C to 20°C) as long as the light intensity was not limiting (Fig. 3, Fig. 5, curve 2). In Fig. 6, the reciprocal values of the
length of precommitment periods are plotted against light intensity (Fig. 6A) or temperature (Fig. 6B). It can be seen that the effects of temperature and light intensity cannot be judged separately. At low light intensities, the energy supply limited growth at higher temperatures (Fig. 6B, curve at 7.5 W m$^{-2}$). Consequently, the length of the precommitment period did not change at various temperatures, giving the impression that the length of the cell cycle is temperature-insensitive. On the other hand, at low temperatures (below 20°C), growth processes were so slow that even low light intensities were sufficient to saturate their photosynthetic demands. So the length of the cell cycle became seemingly light-insensitive (Fig. 6A, curve at 20°C). We conclude that the length of the precommitment period is only dependent on the rate of assimilation, and is not determined by endogenous timing mechanisms.

**Length of the postcommitment period**

The events after the first commitment point do not require external energy, because they can be performed in the dark and are typically temperature-dependent (Fig. 3B,C,D). The length between the first commit-
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Fig. 3. Time courses of commitments to nuclear and cellular divisions and termination of these processes in synchronous populations of *Chlamydomonas eugametos* grown at various temperatures. (A) 35°C; (B) 30°C; (C) 25°C; (D) 20°C. Curves 1, 2, 3, 4, 5, percentage of the cells that attained commitment for the first, second, third, fourth and fifth nuclear divisions, respectively. Curve 6, percentage of the cells that released their daughter cells. Light and dark periods are indicated by white and black strips above panels and separated by vertical lines.

The present results strongly suggest that the number of commitments is only determined by the assimilative activity during this period, which is favoured by high light intensity and temperature. No evidence was found for the idea that the number of commitments is determined by the size of the cells, at the time of commitment, which is determined by synthetic activity during the precommitment period. For example, a high light intensity during the precommitment period, which favoured cell growth, did not necessarily result in an increase in the number of consecutive cell divisions.

Length of the total cell cycle

The length of the total cell cycle can be considered as the sum of the lengths of the pre- and postcommitment periods, and thus shows a complex dependence on external variables, such as light (Figs 2, 5) and temperature (Figs 3, 5). For example, a temperature of...
30°C was found to be optimal for both growth and cell proliferation, resulting in the shortest cell cycle duration and the highest number of daughter cells (Figs 2A, 3B). A higher temperature slowed down cell proliferation, without affecting growth (Fig. 3A). As a result, the cell cycle length was increased (Fig. 3A, Fig. 5, curve 1) and very large cells were formed.

**Discussion**

This paper shows that the length of the precommitment period in *C. eugametos* is only dependent on the rate of assimilation. When the input of energy is limiting, the length of this period is independent of the temperature. On the other hand, its length is light-independent at low temperatures. Thus the growth rate of the cells is the only determining factor for this part of the G1 phase, as was shown earlier by Spudich and Sager (1980) for *Chlamydomonas reinhardtii* and by Zachleder and Šetlík (1990) for *Scenedesmus quadricauda*. These results are, however, in contrast to those of Donnan and John (1983), who observed a constant precommitment period at different growth rates in *Chlamydomonas reinhardtii*. Only when energy limitation slowed growth was the period extended. The authors therefore postulated that commitment to divide is under the control of a temperature-compensated timer. We have also been unable to confirm another conclusion of Donnan and John (1983), namely that the size of the cell at commitment determines the number of consecutive divisions within each cycle. While it is clear that entry into mitosis can only take place when cells have attained a critical mass, and the rate at which a cell accumulates that mass determines the overall timing of the cell cycle, the occurrence of more commitments to divide is only dependent on the input of energy after the first commitment. It seems as if *Chlamydomonas* then rapidly executes a number of cell cycles with a very short G1 phase, which nevertheless require energy for completion of each cycle. It has been noted (Donnan et al., 1985) that in *C. reinhardtii*, the commitments that determine multiple cell divisions lie close together, whereas in *Scenedesmus* (Šetlík and Zachleder, 1984) and *C. eugametos* they are more widely spaced. This may be the reason why in *C. reinhardtii* the first commitment point seems to be the major point at which the cell cycle is controlled in response to cell size and external conditions (as in *S. cerevisiae*; Forsburg and Nurse, 1991), while in *Scenedesmus* and *C. eugametos* this control is distributed over later stages of the cell cycle (as in fission yeast). The phenomenon of multiple commitments and cell divisions in the reproduction of a single cell is reminiscent of the post-fertilization wave of cell divisions in metazoan cells. Therefore, the cell cycle of *Chlamydomonas* remains worthy of further investigation.

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**References**


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