## Replication of DNA and Cell Division in Synchronously Dividing Cultures of Euglena gracilis

Abstract. *Autotrophic* Euglena gracilis *Klebs (strain Z) that are grown axenically on defined, inorganic medium on a regime of 14 hours of light and 10 hours of darkness double their cell number every 24 hours by dividing synchronously during the dark period. The DNA content doubles in a stepwise manner during the last 6 hours of the light period just prior to cell division. This result agrees with those for most higher cells. Although DNA replication is a necessary condition for cell division it is not a sufficient one.*

Synchronized cultures of microorganisms have proved useful tools for elucidating physiological and biochemical aspects of the cell cycle (*1, 2*). In well-synchronized cultures most cells pass through the same stage at the same time, and that which is determined for the entire culture is assumed to obtain for the individual cell. One of the most important aspects of the cell cycle is DNA replication and its interrelations with cellular growth and division. Among others, this parameter has been investigated (*3*) in the unicellular, photosynthetic algal protist, *Euglena*—an organism particularly well suited for metabolic and physiological studies (*4*) by synchronous culture techniques (*5, 6, 7*)—and it appears that doubling of DNA occurs in a step-wise manner during the few hours just prior to cell division, although the doubling of the DNA is a necessary but not sufficient condition for cell division.

*Euglena gracilis* Klebs (strain Z) was grown axenically and autotrophically in 3-liter serum bottles on a modified Cramer and Meyers (*8*) inorganic medium at 25°C (± 0.5°C). The cultures were magnetically stirred (heat compensated) and aerated with 575 to 625 ml of air per minute while being subjected to alternating periods of light (14 hr) and dark (10 hr) by a clock timer. Illumination (3500 lux; saturating) was furnished by a bank of three General Electric 40-watt cool white fluorescent bulbs. The cell number was automatically monitored every 2 hours with a Brewer automatic pipetting machine, a miniaturized fraction collector (*7*), and a Coulter cell counter.

Metabolic events (*3*) were followed by allowing a culture to attain the desired cell concentration (5 to 10 × $10^4$ cells per milliliter) and "harvested" by means of a siphon at a particular time point. Tests were then made upon the portion of culture removed. About 350 ml of culture were allowed to remain in the culture vessel which was then diluted to 3 liters with fresh medium. The entire series of tests could be repeated at the next successive time point when the appropriate cell concentration was attained again a few days later, so that eventually the entire 24-hour cell cycle was scanned.

Deoxyribonucleic acid was extracted from *Euglena* by the method of Smillie and Krotkov (*9*) and estimated by the colorimetric diphenylamine reaction for deoxyribose sugar content (*10*), by phosphorus determination (*11*), and by the determination of absorbance at 260 mμ. The phosphorus values for the nucleic acid fraction had to be corrected for the presence of phosphorus other than nucleic acid phosphorus ("polyphosphate-P"). Measurements of total protein, an indication of cellular growth, were made by a modification of the Folin method (*3, 12*).

Figure 1 presents the repeatedly observed pattern of synchronized cell division in *Euglena* on the 14 : 10 light-dark cycle described. The relative increase in cell number (step size) and the approximate duration of the cell division burst (time during which increase in the cell number occurred) is given for each numbered light-dark cycle of 24 hours. It is evident that an approximate doubling of cell number occurs during each cycle (step size is 2.00), and that this doubling is confined almost completely to the dark period; cell division starts at the end of the 14-hour light period and is completed at the onset of "dawn" in the ensuing cycle. It is interesting that in a 16 : 8 light-dark cycle cell division in *E. gracilis* (Z) commences after about 14 hours of light also, but this point is now 2 to 3 hours before the dark period (*3, 6, 7*). This finding suggests that a certain amount of light is required for cellular growth and is a necessary condition for cell division.

Estimations of the degree of synchrony in the aforementioned *Euglena* populations were made, and average values were obtained for cycles three to nine. The first two cycles were considered to represent a transition state subsequent to inoculation of the culture. Scherbaum's "synchronization index" (*SI*) (*13*) was calculated to be 0.6095. The three parameters investigated by Spencer *et al.* (*13*) characterizing the distribution of cellular division time with respect to the onset of light—standard deviation ($s^*$), coefficient of

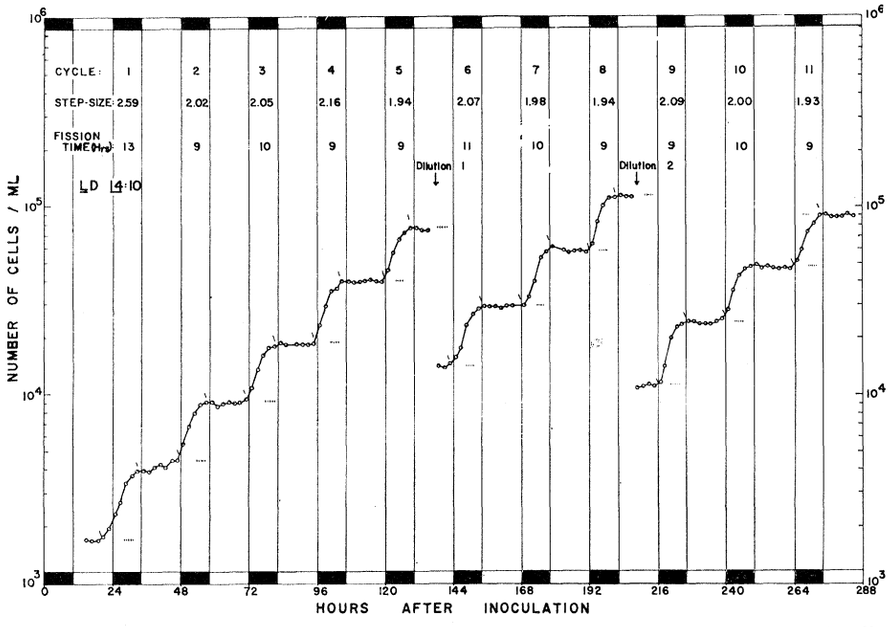


Fig. 1. Light-induced division synchrony in *Euglena gracilis* Klebs (Z); measured cells per milliliter of culture (ordinate) are plotted as a function of time (abscissa, 1 unit of time is 2 hours). Growth conditions are described in the text. The initial inoculum came from stock grown in a 14 : 10 light-dark cycle under similar conditions. Dilutions with fresh medium were made at the points indicated. For each numbered cycle (24-hour duration), fission time (duration of each division burst) and step size (ratio of number of cells per milliliter of culture after cell division to number of cells per milliliter of culture before division) are given.

variation ($C$), and range coefficient ($R_y$)—were found to be 139.8 minutes, 13.00 percent, and 0.4743, respectively, based on cell-number determinations made every 2 hours. The mean of the distribution of cellular division time in *Euglena* is 17.9 hours under the stated conditions. These data indicate that *Euglena* cell division can be sufficiently well synchronized to provide a useful tool for elucidating metabolic parameters of the cycle.

The DNA content (*3*) during the cell cycle is presented in Fig. 2. The amount of DNA present in the average cell is constant essentially for the first 8 to 9 hours of the light period, with an average value of about 2.2 to 2.3 $\mu\mu$g of DNA per cell. Then, approximately 6 hours before dark, DNA replication occurs with a doubling of total DNA by the onset of the dark period, at which time cell division commences and continues throughout the dark. During this period the amount of DNA per cell must necessarily be divided in half since the cell divides to yield two daughter cells; this is shown by the solid-line curve for the dark period; and the shape of the curve reflects partially the population spread. Considered on the basis of micrograms of DNA per milliliter of culture, the DNA content remains essentially constant throughout the dark, indicating that no net synthesis has occurred. The general range of DNA found (2.2 to 4.4 $\mu\mu$g per cell) agrees well with values reported in the literature for DNA in various species of *Euglena* grown under somewhat similar conditions (*6, 9*).

A generalization can be made (*14*) that during the division cycle of higher cells there exists a period after mitosis during which net DNA synthesis does not occur ($G_1$ phase), a subsequent period (S) of DNA synthesis and doubling of the DNA of the interphase nucleus, a phase ($G_2$) during which DNA is not synthesized, and, finally, a period of mitosis (M). These periods vary widely from system to system; thus, in forms such as bacteria with cell cycles of brief duration DNA is sythesized (*15*) throughout most of the cycle. Many protists seem to exhibit the discontinuous step-wise synthesis of DNA prior to cell division cited for higher cells and reported herein for *Euglena*. In particular (*16*), the colorless flagellate, *Astasia longa* (Blum and Padilla); the green alga, *Chlorella pyrenoidosa* (Miyachi and Miyachi; Stange *et al.*); the protist, *Tetrahymena*

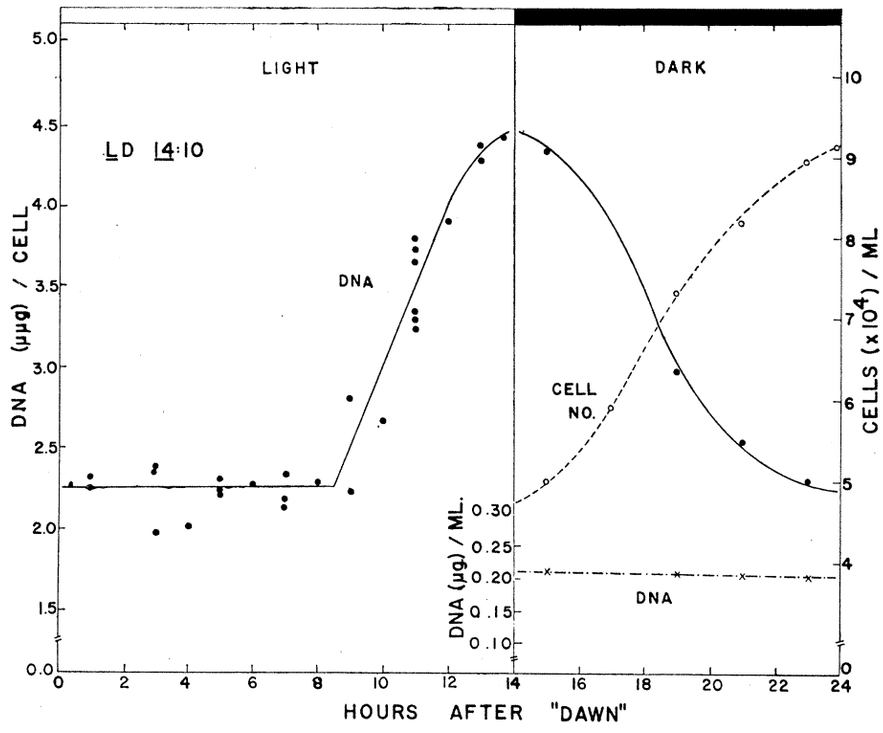


Fig. 2. The DNA content per cell at various points across the light-dark cycle (14 : 10) of synchronously grown *E. gracilis* (Z). Each solid point represents the average $\mu\mu$g DNA per cell determined at a given time in a given experiment. The open circles indicate the typical synchronous doubling of cell number during the dark period; the cell number remains essentially constant during the light period. The DNA content ($\mu$g/ml of culture) throughout the dark period is depicted by the crosses.

*pyriformis* (Prescott); the protist, *Euplotes eurystomus* (Gall); *Paramecium aurelia* (Kimball and Barka); and the fission yeast, *Schizosaccharomyces pombe* (Sando), all seem to synthesize DNA in this step-wise manner, with varying relationships between the various phases, and, indeed, sometimes with the $G_2$ phase almost completely suppressed, as seems to be the case in *E. gracilis* (Z). Cook, however, reported (*6*) that in *E. gracilis* (unnamed strain) synchronized by a 16:8 cycle under slightly different conditions DNA apparently doubled in an approximately linear manner during the entire light period; his data admittedly needed further corroboration because of few samples.

Once the pattern of DNA synthesis during the life cycle is established, the question is immediately raised concerning its relation to cellular growth, cell division, and, in the case of *Euglena*, with the synchronizing light-dark cycle (reference *2* for various aspects of this problem). Cook (*5, 6*) has shown for *E. gracilis* (strain not given) and Edmunds (*3*) for *E. gracilis* (Z) that parameters of cellular growth such as dry weight, total protein, RNA, chlorophyll *a*, and carotenoids of the average cell increase to a doubling in a linear fashion throughout all or most of the light period, but during the dark period (and cell division) cellular growth comes to a standstill with little or no net synthesis of these compounds. Thus, after 14 hours of light, the large *Euglena* cell has doubled its size, replicated its DNA, and is ready for division with the onset of dark. But is cell division in *Euglena* initiated by the onset of dark or by the completion of DNA replication, or both? Is a certain amount of protein synthesis and cellular growth a prerequisite for cytokinesis?

A series of experiments was conducted in an attempt to shed some light on these questions; the results are summarized in Fig. 3. *Euglena* was grown for several cycles under a 14 : 10 cycle until the cells had reached a concentration of about $7.5 \times 10^4$ cells per milliliter. The light of the ensuing cycle (No. 4), however, was reduced from 14 hours to 8 hours, and then the cells were placed in darkness for 24 hours. Cell number, cellular growth (total protein), and DNA were measured throughout most of the cycle. This shortening of the light period by 6

hours was instituted to determine whether DNA replication would still occur at the usual time (hours 8 to 14 after "dawn"), even though now this time would fall within the lengthened dark period, and to see if cell division would occur although the light energy available for cellular growth had been reduced by 6 hours. After the 24-hour dark period, a 6-hour exposure to light was given in an attempt to compensate for the previous loss of 6 hours of light; this 6-hour light period was followed by a normal dark period (10 hours) and then light; the cell number was monitored throughout the experiment.

The results demonstrated that doubling of DNA did indeed occur in the *dark* (2.3 to 4.6 $\mu\mu$g per cell); however, the increase in total protein ceased as soon as darkness began. Cell division did not occur, the concentration of $7.5 \times 10^4$ cells per milliliter being maintained throughout the entire reduced light period and lengthened dark period. Actually, a small increase (5 percent) in cell number did occur after 6 hours of dark, the time in previous cycles that represented the usual light-dark transition and onset of cell division. This almost negligible and perhaps transient increase may reflect the division of those cells which were metabolically "precocious" after only 8 hours of light and required only DNA replication in order to divide. Or perhaps this small increase was caused by sampling errors due to differential cell motility. After the 6-hour exposure to light, however, a substantial division burst occurred with a step size of 1.46 representing a 44 percent cell number increase. These results indicate that 8 hours of light is insufficient for cell division to occur, even though DNA replication has occurred, and that only when 6 hours more of light was obtained after a 24-hour dark period did significant fission take place. That the step size of this last burst was much less than 2.00 suggests that some of the cells had depleted their metabolic pools because of starvation during the extended dark period to such a degree that even the additional 6 hours of light was not enough for normal cellular growth to have been completed. It is also evident that this latter experiment introduced a $G_2$ phase of no net DNA synthesis between the normal period of synthesis (S) and mitosis (M), whereas under the usual 14:10 cycle the $G_2$ phase is almost entirely absent.

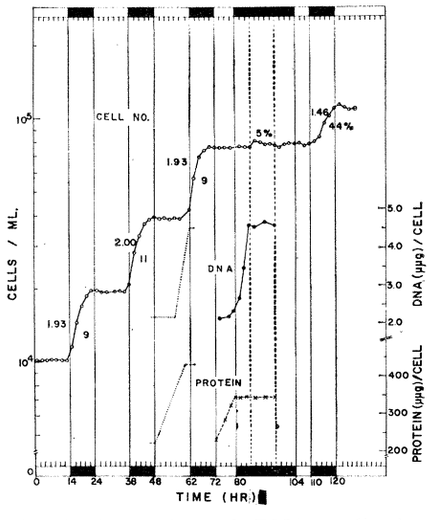


Fig. 3. Separation of DNA replication and cell division by dark interruptions. For the first three cycles *E. gracilis* (Z) was grown in a 14:10 light-dark cycle under described conditions. During the next cycle only 8 hours of light were given, followed by periods of 24 hours dark, 6 hours light, 10 hours dark, and then continuous light. At intervals of 2 to 4 hours, DNA, cell growth (total protein), and cell number were determined across a portion of this extended fourth cycle as indicated. The percentage increase in cell number is given for the last two smaller fission bursts, and step size for all major bursts. The dotted lines represent DNA and protein synthesis during a normal cycle.

A further experiment, in which the light period was reduced to only 2 hours, demonstrated that protein synthesis once more ceased abruptly with the onset of dark, and that *neither DNA synthesis nor cell division occurred*, even after 24 hours of dark. These results suggest that DNA replication is dependent on events occurring within the cell 2 to 8 hours after the onset of light, as, for example, the synthesis of a specific enzyme.

In conclusion, the results corroborate the generalization that DNA replication is a necessary but not sufficient condition for cellular division. The problem of the initiation of DNA replication remains. It may depend on prior protein or RNA synthesis or both and perhaps direct energy input by way of the light (*14*). Alternatively, the onset of light may serve as a phasing synchronizing agent (*17*). It may well be that these aspects of the cell cycle can be attacked profitably through dark blocks and brief intervals or "pulses" of darkness during the cycle of *Euglena* in synchronous division.

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## Automatic Sampling Device for Study of Synchronized Cultures of Microorganisms

Abstract. *A versatile automatic culture-sampling system is described and data are presented from experiments in which this device was used to study growth and synchronized cell division in* Euglena gracilis.

The desirability of synchronized cultures of microorganisms for studying events in the cell cycle has created a need for following the time course of division in the population. This report describes a sampling system designed to fulfill the requirements encountered in a study of growth characteristics of *Euglena gracilis* under varying culture conditions. The device is reliable, ac-