29. B. E. Bursten, P. A. Cotton, J. C. Green, E. A. Seddon, G. G. Stanley, *ibid.* **102**, 4579 (1980); M. B. Hall, *ibid.*, p. 2104.
30. See references in R. H. Summerville and R. Hoffmann, *J. Am. Chem. Soc.* **101**, 3821 (1979).
31. J. Lauher, M. Elian, R. H. Summerville, R. Hoffmann, *ibid.* **98**, 3219 (1976).
32. W. Siebert and K. Kinberger, *Angew. Chem.* **88**, 451 (1976); *Angew Chem. Int. Ed. Engl.* **15**, 434 (1976); G. E. Herberich, J. Hengesbach, U. Kölle, G. Huttner, A. Frank, *Angew. Chem.* **88**, 450 (1976); *Angew Chem. Int. Ed. Engl.* **15**, 433 (1976).
33. See references in D. L. Thorn and R. Hoffmann, *Inorg. Chem.* **17**, 126 (1978).
34. See references in B. E. R. Schilling and R. Hoffmann, *J. Am. Chem. Soc.* **101**, 3456 (1979); *Acta Chem. Scand. Ser. B* **33**, 231 (1979).
35. J. R. Fritch, K. P. C. Vollhardt, M. R. Thompson, V. W. Day, *J. Am. Chem. Soc.* **101**, 2768 (1979); H. Yamazaki, Y. Wakatsuki, K. Aoki, *Chem. Lett.* **1979**, 1041 (1979).
36. A. J. Deeming and M. Underhill, *J. Chem. Soc. Dalton Trans.* **1974**, 1415 (1974); *J. Organomet. Chem.* **42**, C60 (1972); *Chem. Commun.* **1973**, 277 (1973).
37. S. Komiya, T. A. Albright, R. Hoffmann, J. Kochi, *J. Am. Chem. Soc.* **98**, 7255 (1976); D. L. Thorn and R. Hoffmann, *ibid.* **100**, 2079 (1978); H. Berke and R. Hoffmann, *ibid.*, p. 7224.
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Clocked Cell Cycle Clocks

Leland N. Edmunds, Jr., and Kenneth J. Adams

Summary. The cell division cycle of both mammalian cells and microorganisms, which apparently has both deterministic and probabilistic features, is a clock of sorts in that the sequence of events that comprise it measures time under a given set of environmental conditions. The cell division cycle may itself be regulated by a programmable clock that, under certain conditions, can generate circadian periodicities by interaction with a circadian pacemaker. These clocks must insert time segments into the cell division cycle in order to generate the observed variability in cellular generation times.

Our understanding of the cell cycle has come a long way since Howard and Pelc (*1*) more than 25 years ago divided it into four consecutive intervals—G_1, S, G_2, and M—where G_1 and G_2, respectively, designated the gaps in time between the completion of cell division and the onset of DNA synthesis, and between the end of replication and the onset of mitosis (M). Advances have not been confined, however, to a mere filling in of these gaps and subdividing them into smaller steps. Rather, recent experimental and theoretical work has emphasized the mechanisms controlling the cell cycle and, indeed, has indicated that a single "cell cycle" may be somewhat of a misnomer. Finally, perhaps there is no such thing as the cell division cycle; the final event would be merely the end of a sequence of events and the beginning of nothing (*2*).

The cell division cycle—a clock in the sense that it measures time under a given set of environmental conditions—may be governed by an underlying oscillatory mechanism or timing device, which is itself a part of the clocked division cycle in the sense that the timer is replicated with each round of division. After reviewing some of the major notions of the cell division cycle and different types of cell cycle oscillators, we examine the void between cell cycles and circadian clocks (*3*, *4*). Finally, we address the basic problem of variability in cell cycle generation times, a major challenge in constructing models for cell cycle regulation, and explore new molecular approaches for the insertion of time segments into the cell cycle.

Models of the Cell Cycle: Deterministic and Probabilistic

Attempts to describe the cell division cycle (CDC) have been (i) deterministic and (ii) indeterminate or probabilistic. Within the former, there are two possible types of mechanism for ordering a fixed sequence of cell cycle events relative to each other (*5*, *6*). There may be a direct causal connection between one event and the next so that it would be necessary for the earlier event in the CDC to be completed before the following could occur. Hartwell and co-workers (*7*) have referred to this notion as the "dependent pathway" model and have analyzed the "circuitry" of the CDC of the budding yeast, *Saccharomyces cerevisiae*, using temperature-sensitive mutants whose CDC is blocked at various stages. In contrast to this sequential type of approach, there is the possibility that no direct causal connection exists between any two events but that they are ordered by some master timing mechanism that operates on one or more key events ("control points") of the CDC, such as the initiation of DNA synthesis or mitosis. In this "independent pathway" model (*7*), the accumulation of a mitogen or other substance, the completion of a "division protein" structure, or the attainment of a critical ratio of DNA to mass or nuclear volume may initiate a new CDC state. Obviously, it is possible, even likely, that the CDC is controlled by a combination of these types of mechanism.

Such deterministic models, however, do not adequately account for the large variances commonly observed in generation time (as great as 20 percent of the mean in mammalian cell systems), rendering timekeeping relatively imprecise and leading to the rapid decay of synchrony in phased cultures (*8*). In an effort to explain this variability, other attempts to characterize the cell cycle traverse have considered a portion of the CDC to be indeterminate (*9*) or have turned to probabilistic descriptions (*10*–*12*). Thus, Smith and Martin (*11*), observing that the number of cells that have divided as time progresses decreases exponentially, have suggested that the S and G_2 portions of the CDC (which they

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term the B phase), are deterministic and invariant, while other parts are probabilistic (the A state, or "waiting" phase, of G_1). According to this transition probability model, newly divided cells enter the A phase in which their activity is no longer actively directed to proliferation; they then have a certain degree of probability of again entering the B phase wherein DNA synthesis and mitosis occur once more. Gilbert (*12*) has taken a similar theoretical approach, giving particular attention to various perturbations that may trigger the transition of the cell from the quiescent state into the more highly dynamic one of replication and the possible relation to differentiation and cancer.

Finally, and more recently, Klevecz (*13*) has proposed a quantal subcycle, G_q, for mammalian cells, whose traverse time is about 3 to 4 hours. This cycle would be appended to the deterministic $S+G_2+M$ pathway at a point *i*. The exit of a cell from G_q would be probabilistic in the sense that there could be an indefinite number of G_q cycles, depending on environmental conditions (such as cell density, nutritional variables, and mitosis-stimulating factors). This model has the virtue of explaining the heterogeneity of G_1 (and hence, of the generation time of individual cells) as arising from the "gated entry of cells into the S phase" (*13*) and gains credence from two sets of observations: (i) the distribution of possible generation times in populations of mitotically selected cells taken from synchronous cultures or from randomly dividing cultures, as observed by time-lapse videotape microscopy, does not appear to be continuous but rather is quantized in multiples of 3 to 4 hours (*13*); and (ii) the activity of a number of enzymes that have no obligatory relation with other periodic events, such as DNA synthesis, oscillate with periods also of 3 to 4 hours, even if DNA and RNA synthesis are inhibited (*14*). In a sense, then, the G_q subcycle would constitute a cellular clock (*13*) with a basic period of about 3 to 4 hours (at least in cells whose cycle time is less than 24 hours), and CDC times would be multiples of this fundamental period (increasing, for example, at higher cell densities or lower temperatures).

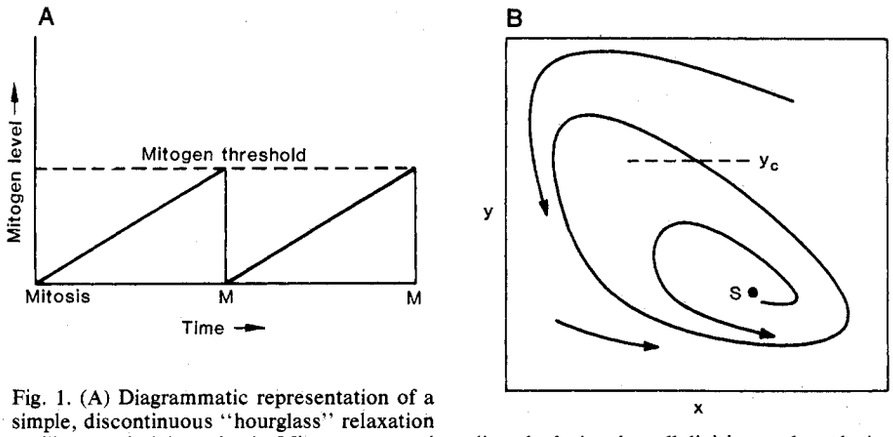

Fig. 1. (A) Diagrammatic representation of a simple, discontinuous "hourglass" relaxation oscillator underlying mitosis. Mitogen accumulates linearly during the cell division cycle and triggers mitosis at a critical threshold level; it is then destroyed, the "clock" is reset, and it reaccumulates again during the next cycle. [Redrawn from Tyson and Kauffman (*97*); courtesy of *Journal of Mathematical Biology*] (B) Diagrammatic representation of a continuous biochemical oscillator exhibiting limit cycle behavior. Two interacting substances (X and Y) autonomously fluctuate; mitosis occurs when one of them (Y) attains a threshold level (Y_c). S denotes the point of singularity, which is unstable with respect to small perturbations. Trajectories spiral out to the closed curve, the limit cycle. [From (*24*); courtesy of the *Journal of Theoretical Biology*]

Cell Cycle Oscillators:

Relaxation and Limit Cycle Models

Inasmuch as mitosis is a periodic event of short duration relative to the total length of the CDC, it is not surprising that various types of oscillatory systems, or biological "clocks" (*15*) have been proposed to underlie the CDC. Indeed, even if the CDC is merely a linear array of discrete metabolic states, each causing the next, and if it is controlled simply by the sequential transcription and subsequent translation of genes linearly ordered on the chromosomes (*16*), an oscillator could be formulated by invoking a recycling component that would initiate another time-metering, transcriptional cycle (*17*).

Several biochemical oscillators have been hypothesized to control the CDC, for example, those that underlie the high degree of natural mitotic synchrony which occurs every 10 to 12 hours in the nuclei (as many as 10^8) within the syncytial plasmodia of the myxomycete *Physarum polycephalum*. Plasmodia of different stages, or phases, of the CDC can be fused; their nuclei and cytoplasm intermingle; and the fused pair then undergoes mitosis at some intermediate phase (*18, 19*). These facts have led Sachsenmaier and co-workers (*18*) to characterize the timing mechanism of mitosis as an "hourglass" or discontinuous, extreme relaxation oscillator (*20-22*), in which mitotic initiator molecules ("mitogen") are formed more or less continuously and proportionately to the increase in plasmodial mass during G_2. These molecules would be counted by combining stoichiometrically with a given number of nuclear receptor sites. Mitosis would be started at a critical ratio of initiator to nuclei, and the clock would be reset by each nuclear division (or by a related, obligatory event), at which time the number of nuclear sites would also double in a stepwise manner (Fig. 1A). In this model, the event M is an essential feature of the oscillatory system.

Given the same set of facts, Kauffman and Wille (*19*) have put foward an alternative model, namely, that the timing mechanism for mitosis is a continuous limit cycle oscillator (*20-22*), analogous to those proposed for circadian rhythms, which would gate mitosis and DNA replication. On this hypothesis, two (or more) interacting components (X and Y) fluctuate autonomously, and mitosis would be triggered if one of them (an initiator) reached a threshold level (Fig. 1B). The critical distinctions between this model and Sachsenmaier's hourglass model are that in the limit cycle (i) mitosis does not function as an essential component of the oscillator system, and, thus, under certain conditions, the system may continue to oscillate at a subthreshold level even if mitosis is blocked and does not occur; and (ii) although the amplitude and period do not depend on initial conditions (that is, the limit cycle is stable, resisting and recovering from most perturbations), a perturbation given at a singularity point (*23*) results in a phaseless, timeless (motionless) state. Although the experimental evidence obtained thus far does not rigorously exclude either hypothesis in *Physarum*, one critical study (*24*) favors the discontinuous relaxation oscillator.

Finally, there is the possibility that Klevecz's G_q quantal subcycle (*13*) might itself constitute a limit cycle oscillator having a 3- to 4-hour period [whereas that of Kauffman and Wille (*19*) would have a period equal to that of the CDC]. Consistent with this notion is the recent finding (*25*) that Chinese hamster cells

with an 8.5-hour CDC yield a biphasic phase-response curve when high concentrations of serum are given in pulses so that both advances and delays occur in the timing of cell division.

Circadian Clocks and Cell Cycles

Thus far, we have arbitrarily discussed cell division cycles having periods in the neighborhood of 4 to 16 hours. These rhythmicities fall between the high frequency (ultradian) biochemical oscillations (*26*) in the various components of the glycolytic pathway and the circadian periodicities that are ubiquitous throughout the plant and animal kingdoms at every level of eukaryotic organization (*27–29*). These latter rhythmicities typically can be synchronized by imposed diurnal light and temperature cycles to precise 24-hour periods and can be predictably phase-shifted by single light and temperature signals, yet they are able to free run for long time spans under conditions held constant with respect to light and temperature with a natural period close to but seldom exactly 24 hours. Further, the free-running period is compensated for changes in temperature within the physiological range, as might be expected in order for it to function as an accurate biological clock. We now consider the extent to which endogenous, light-entrainable, self-sustaining circadian oscillators may underlie the CDC (*3*, pp. 370–371).

There is abundant evidence that the CDC's of unicellular algae, fungi, and protozoans (*30*), as well as mammalian cells in situ (and perhaps in cell culture), exhibit persisting circadian rhythms of cell division (or "hatching") (*31–33*). The algal flagellate, *Euglena gracilis* Klebs (Z strain), an extensively studied model system in this regard (*31, 32*), serves as the basis for subsequent discussion (Figs. 2 to 5).

Photoautotrophically grown cultures of *Euglena* can be synchronized by appropriate repetitive, 24-hour cycles of light and darkness (*34–36*) so that cell division is confined almost entirely to the dark intervals; typical synchrony obtained in *L*D: *10*,14 (*37*) is shown in Fig. 2. In this case, the population doubles ($\overline{ss} \simeq 2.0$) at each step (*37*), the period ($\bar{\tau}$) of the rhythm in the population is exactly 24 hours [matching the period T of the imposed LD cycle], and, by inference (Fig. 6B), the length of the *individual* CDC's also must average 24 hours (the rate of cell death is insignificant). If one reduces the total duration of the light interval (for example, *L*D: *8*,16) within a 24-hour framework, the amplitude of the rhythm of cell division in the population is proportionately reduced ($\overline{ss} < 2.0$), but the culture continues to be synchronized (Fig. 3) in the sense of event simultaneity (*31–33*). The average individual CDC is now lengthened, however, to approximately 36 hours ($\overline{ss} \simeq 1.68$), and the culture is no longer developmentally synchronous to the extent of a one-to-one correspondence between the CDC stages of all the constituent cells (see Fig. 6C). Nevertheless, cell divisions, when they do occur, do so during the dark span only, at intervals of 24 hours, and the rhythmicity observed in *L*D: *8*,16 (and *L*D: *10*,14) (*37*) stands in sharp contrast to the asynchronous, exponential growth curve obtained in LL (see Fig. 6A), where the minimum doubling time is 12 to 14 hours (*34*).

Although the synchronization of the rhythm of cell division by diurnal, full photoperiod LD cycles is consistent with the notion that a putative circadian clock is entrained by the imposed light regime and, in turn, phases or "gates" cell division to a period of 24 hours (perhaps by

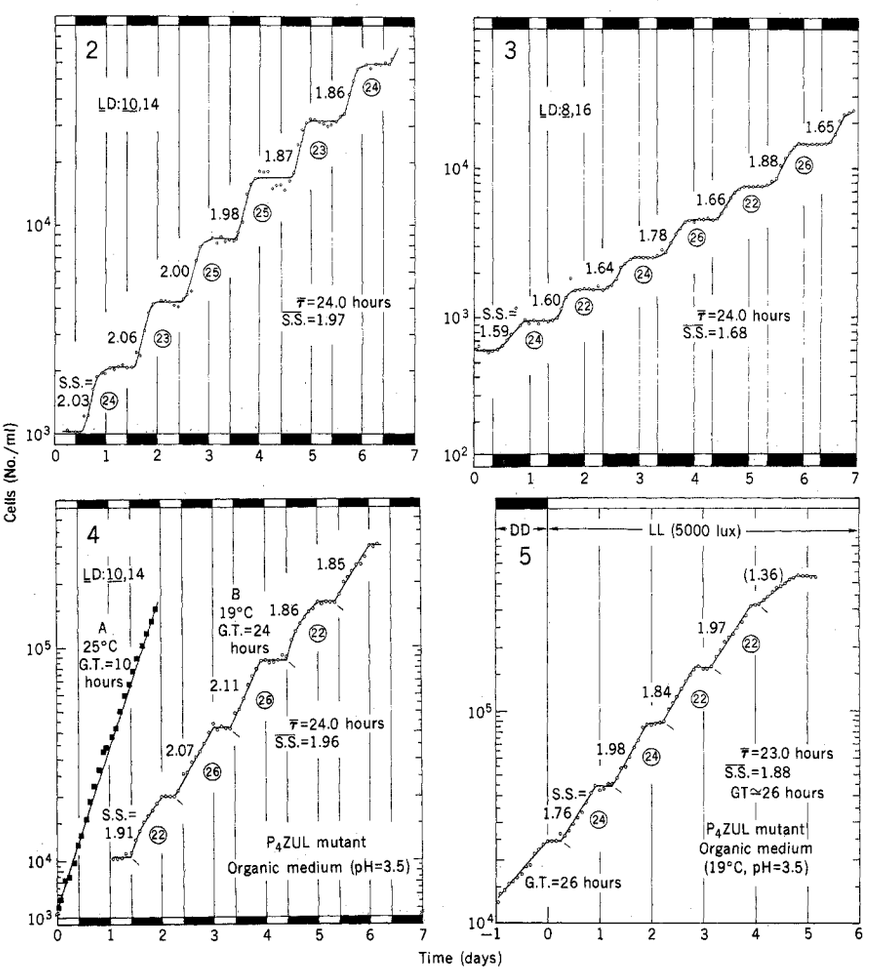

Fig. 2. Entrainment of the cell division rhythm in a population of *Euglena* grown photoautotrophically at 25°C in *L*D: *10*,14. Stepsizes ($\overline{ss}$, ratio of number of cells per milliliter after a division burst to that just before the onset of divisions) are indicated for successive steps; the period of the oscillations is also given in hours (encircled just to the right of each division step). The average period ($\bar{\tau}$) of the rhythm in the culture is essentially identical to that of the synchronizing LD cycle, and a doubling of cell number ($\overline{ss} \simeq 2.00$) usually occurs every 24 hours. [Adapted from (*35*)] Fig. 3. Entrainment of the cell division rhythm in a population of *Euglena* grown photoautotrophically in *L*D: *8*,16. (Labels as for Fig. 2.) Although $\bar{\tau}$ of the rhythm is precisely that of the synchronizing LD cycle, $\overline{ss}$ of the successive fission bursts is substantially less than 2.0, an indication that not all of the cells divide during any one cycle. [Adapted from (*35*)] Fig. 4. Population growth of a photosynthetic mutant (P_4ZUL) of *Euglena* grown photoorganotrophically on a defined medium containing glutamate and malate in *L*D: *10*,14. (Curve A) Exponential increase in cell number (generation time, 10 hours) at 25°C. (Curve B) Phasing of the cell division rhythm at 19°C (generation time, 24 hours). Other labels as for Fig. 2. [After (*36*)] Fig. 5. Initiation of a persisting, free-running, circadian rhythm of cell division by a single transition from darkness to continuous light in cultures of the P_4ZUL photosynthetic mutant of *Euglena* grown at 19°C on glutamate-malate medium. The culture had been increasing exponentially with a generation time of about 26 hours during the preceding interval of darkness (*DD*). Other labels as for Fig. 2. [After (*42*)].

acting on one or more key control points of the CDC), it does not demand it. Light (or darkness) could be acting by directly inhibiting (or promoting) division, and periodic shifts between light and dark would synchronize the culture. A number of other observations in a variety of experimental organisms, however, render this seemingly straightforward hypothesis unlikely.

These lines of evidence (*31, 32*) for our illustrative *Euglena* system include: (i) entrainment by LD cycles having T not equal to 24 hours (for example, LD: *10*,10) may also occur within certain limits (*36, 38*); (ii) appropriate temperature cycles (*18°*, 25°C: *12*,12 or *28°*, 35°C: *12*, 12) will entrain the rhythm maintained in LL (*39*); (iii) "skeleton" photoperiods comprising the framework of a normal, full-photoperiod cycle (for example, LD: *3*,6,*3*,12) will also entrain the rhythm to a precise 24-hour period (*36*); (iv) high frequency (LD: *1*,3) LD cycles (*36*) and even "random" illumination regimes (*35, 36*) induce circadian division periodicities; and (v) rhythmic cell division will persist for a number of days ($\bar{\tau} \simeq 24$ hours) in the autotrophically grown Z strain batch-cultured under dim LL (*40*). This last series of experimental results was probably the most definitive but was restricted by the low light intensities (800 lux) that had to be used (*41*). Nevertheless, although the division bursts were relatively small [generation time (g) $\simeq$ 5 days], those cells that did divide did so during their subjective night at the times that they would have experienced darkness had the entraining LD cycle previously imposed been continued.

Even more conclusive evidence for the implication of a basic circadian oscillator in the control of the CDC has been obtained by utilizing photosynthetic mutants of *Euglena* (obligate heterotrophs), thereby circumventing the problem of the dual use of light as an energy source for photosynthesis, on the one hand, and as a timing cue for the circadian clock on the other (*31, 32*). Representative results for the ultraviolet light-induced P_4ZUL mutant (*42*) grown on a medium containing glutamic and malic acids as carbon sources under LD: *10*,14 are shown in Fig. 4. Entrainment to a 24-hour period was not possible with cultures in the fast-growing ultradian ($g < 24$ hours) growth mode (*43*); at 25°C, the doubling time of the exponential growth curve obtained was about 10 hours. If the growth temperature was reduced to 19°C [yielding an exponential curve with $g \simeq 24$ to 26 hours in DD or LL (not shown)], the culture was synchronized. In this infradian ($g > 24$ hours) growth mode (*43*), divisions were set back or delayed for 8 to 10 hours at 24-hour intervals. Furthermore, the rhythmicity persisted with a circadian period (22 to 23 hours) in DD (not shown) for as long as 5 days in batch culture (*42*) and for at least 10 to 14 days in continuous culture in DD or in LL (*44*). The period of the oscillation is temperature-compensated over a range of at least 7°C within physiologically "permissive" conditions (allowing measurable cell division to occur but not encroaching upon the ultradian domain); thus, batch cultures of the P_4ZUL mutant synchronized by LD at 14°C displayed a persisting rhythm having a $\bar{\tau}$ of 22.9 hours, although the amplitude was reduced ($\overline{ss}$ = 1.34) as expected (*44*). Even a single transition of D to L (*37*) was sufficient to induce rhythmicity, which then persisted under LL with $\bar{\tau}$ = 23.0 hours (Fig. 5). These results have been extended both to the naladixic acid-induced Y_9ZNalL photosynthetic mutant of *Euglena* and to the white, heat-bleached W_6ZHL strain that totally lacks chloroplasts (*31-33, 45*); the results are consistent with the data of Mitchell (*46*) for the pale green nitrosoguanidine-mutagenized P_7ZNgL mutant and the white, ultraviolet-bleached W_nZUL strain.

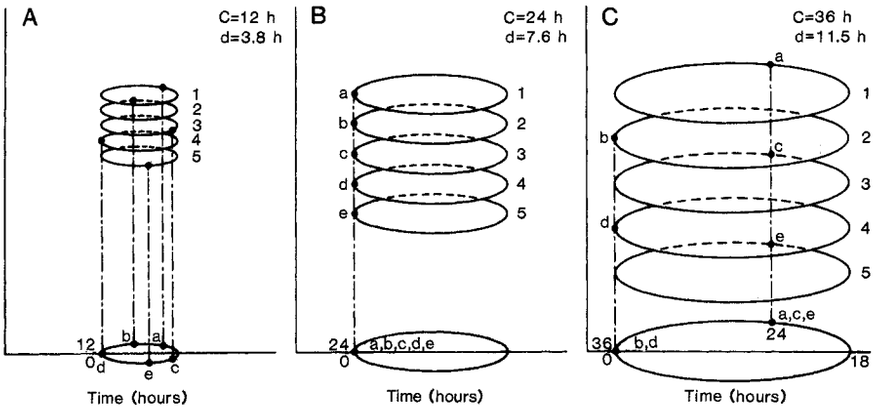

Fig. 6. Diagrams of developmentally asynchronous and developmentally synchronous cultures in various modes of growth. The stacked "doughnuts" depict individual cell division cycles (CDC); solid points indicate the landmark of cell division that terminates the CDC. The circumference (C) of the cycles (projected as ellipses) is equal to the generation time (g), and is calculated as πd (where d is diameter). (A) Exponentially growing culture in ultradian growth mode ($g < 24$ hours); the population is developmentally asynchronous, with divisions occurring continuously. (B) Synchronized (entrained) culture in circadian growth mode ($g \simeq 24$ hours); the population is developmentally synchronous, with divisions being confined to relatively narrow intervals ("windows" or "gates") 24 hours apart. (C) Culture in infradian growth mode ($g > 24$ hours); the population is developmentally asynchronous but is synchronized, nevertheless, with regard to the circadian oscillations in each cell since divisions—when they do occur—are clustered at intervals of approximately 24 hours. The lengths of the individual CDC's here only *average* 36 hours (and actually may be discontinuously distributed), and the ellipses should vary in size to reflect the variance (see text). [Adapted from (*32*)]

Finally, we have observed (*45*) that cultures of W_6ZHL and P_4ZUL may gradually lose both their capacity to exhibit division synchrony in LL or DD and even to be entrained by imposed LD cycles, reverting to random exponential growth ($g > 24$ hours). These properties could be restored, however, by the addition of certain sulfur-containing compounds (such as cysteine or methionine) to the medium at the onset of the experiment. If these substances were added at various times to an arrhythmic P_4ZUL culture in LL (after prior exposure to LD: *10*,14), periodic division was likewise induced whose phase was precisely that predicted on the assumption that the underlying clock had been running undisturbed (but unexpressed) throughout the experiment, merely having been uncoupled from division itself until the sulfur compounds were added (*45*).

These results, then, taken together with those of numerous other systems (*31-33*), implicate a master timer—in this case a circadian oscillator—that, while entrainable by appropriate LD (or temperature) cycles, can itself modulate the CDC and phase or "gate" cell division (and probably other marker events) to intervals of approximately 24 hours. The overt rhythmicity can be abolished by changing the environmental conditions so that the overall g of the culture is less than 24 hours (ultradian growth mode) as, for example, by raising the temperature in organotrophic cultures, or by increasing the intensity or duration of illumination or by introducing utilizable organic carbon sources into photoautotrophic cultures (*31-33*). Presumably, the circadian clock mechanism is operating at a higher frequency matching that of the fast-cycling CDC (with a lower limit

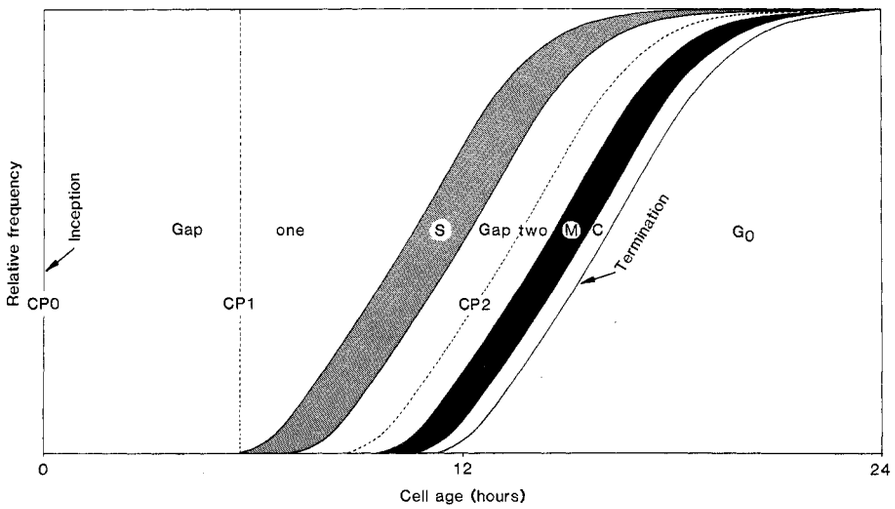

Fig. 7. Typical population phase-duration map for an exponentially increasing culture of *Euglena gracilis* (Z) synchronized by a LD cycle at the inception of the CDC. The mean durations of chromatin replication (*S*) (1 to 2 hours), gap two (2 to 3 hours), mitosis (*M*) (60 minutes), and cytokinesis (*C*) (40 minutes) for a given cell age are independent of the duration of gap one. Gap one is shown subdivided into a common phase (left) and a variable phase (right) by the hypothetical control point locus CP1.

equal to that of the minimum possible g for a species—about 8 hours for *Euglena* cultures. Alternatively, the oscillator may be operating and merely uncoupled from the CDC, or perhaps "stopped" or even absent (*31-33, 43*). In any case, circadian rhythms would not be (and, indeed, have never been) observed in cultures of microorganisms in the ultradian growth mode (*47*).

Conversely, as g exceeds 24 hours (by lowering the temperature or by nutritional limitation), the periods of the basic oscillator and that of the CDC start to diverge in the other direction (*30-32, 42*). In the limiting case, where g approaches infinity (very slowly dividing, stationary cultures), low-amplitude division bursts occur at circadian intervals in the population (but CDC > 24 hours), along with numerous other cyclic physiological and biochemical events [such as motility (*48*), photosynthetic capacity (*49*), and oscillations in enzymatic activity (*50*)] that are not necessarily related to the CDC (*33*).

Cell Cycle Variability

Thus far we have considered the CDC as a time-measuring process and its modulation by a circadian clock that appears to lie outside of the CDC itself. Implicit in such a discussion is the notion of some degree of precision and accuracy on the part of the basic oscillators or timing sequences, yet we are faced paradoxically with a disturbing amount of variability in the length of the CDC of most cells. This variability in traverse time of the complete CDC has been observed with bacteria (*51*), yeasts (*52*), algae (*53*), and cultured animal cells (*10, 54*) and has been extended to other CDC stages, such as the entry of mammalian G_1 cells into S (*55*). How, then, can this dilemma be resolved?

The synchronous cell division observed in populations of *Euglena* (Fig. 2) entrained by LD cycles, as well as of other microorganisms, is far from perfect (*34*), despite the fact that a doubling of cell number occurs every 24 hours in *LD*: *10*,14 : divisions occur over a time span of 8 to 12 hours in the culture with some cells dividing almost at the beginning of darkness and others several hours later. A similar situation was found for the reduced steps in *LD*: *8*,16 (Fig. 3) as well as for the P_4ZUL photosynthetic mutant in *LD*: *10*,14 (Fig. 4), where divisions seemed to be delayed 8 to 10 hours every day and then took place over a relatively long 12- to 14-hour interval. This "spread" might be anticipated given the fact that individual cells, grown photoautotrophically on minimal medium with CO_2 as the sole carbon source, show a variation in g from 8 to 24 hours, and from about 10.5 to 22 hours on proteose peptone-supplemented medium, although pairs of daughter cells appear to have closely related values of g (*53*). The decay in synchrony on removal of the synchronizing regime, at least in bacterial and mammalian cell cultures, has been attributed to this variance in CDC lengths (*8*), presumably arising from cellular heterogeneity or stochastic processes (*9-11, 13*). Indeed, Smith and Martin (*11*) conclude that perfect synchrony can never be attained because of the probabilistic component (the A state) that they hypothesize to be a part of G_1.

That this variability in the CDC of *Euglena* is not merely a proportional extension of all the constituent CDC stages is clear from the results of phase distribution studies (*56, 57*); a typical "population phase-duration map" is shown in Fig. 7. Thus, the duration of mitosis is independent of the rest of the CDC and averages approximately 1 hour. Over a wide range of cell concentrations the duration of cytokinesis is also independent of the lengths of the earlier stages of the CDC, averaging 30 minutes to 1 hour (*58*). Furthermore, studies on the accumulation of total cellular DNA in the population across the CDC (*57, 59*) indicate that neither the duration of S nor the intervening G_2 phase can account for the observed variation in generation times (Fig. 7). As was concluded for other cell types, it would seem, therefore, that the differences in the lengths of the CDC among individual cells of a population must result primarily from variation in the classically defined (*1*) G_1 phase or some substage such as A, G_q, or even G_0 (sometimes called proliferative rest).

The preceding discussion deals only with low level variability in the length of the CDC. We are confronted with a higher level of variability, however, in slowly growing, "synchronously" dividing cell populations where not every cell divides at each step (that is, where $\overline{ss} < 2.0$) but where the steps themselves occur at periodic (for example, circadian) intervals with virtually no division taking place between the steps. An individual cell may take several days (during which it undergoes several LD cycles) to complete the CDC, and yet it will somehow be programmed to complete the terminal acts of mitosis and cytokinesis at night. Since in the fast-growing (ultradian) mode it would normally be expected to complete its CDC within the range from approximately 8 to 24 hours, some sort of mechanism, coupled in some manner to the LD cycle, must stretch the CDC successively day by day to account for these very long infradian cell cycles. Just such a situation can be observed in photoautotrophic cultures of *Euglena* entrained by *LD*: *8*,16 (Fig. 3), where the doubling time for the population is about 36 hours ($\overline{ss} \simeq 1.68$), as well as for the longer interdivision intervals in high-frequency cycles (*36*), random illumination

regimes (*35, 36*), dim LL (*40*), and other conditions in which $g >> 24$ hours, but bursts of cell division in the population occur predictably (*60, 61*) with circadian periods. Finally, in *LD*: *12*,36, where a doubling occurs during the first 8 to 10 hours of darkness every 48 hours (effectively yielding $g = 48$ hours), long plateaus of some 36-hour duration occur during which no cell number increase is observed (*61*).

In contrast, the phased division in *LD*: *10*,14 for the P_4ZUL mutant (and others) cultured at 19°C (Fig. 4) yielded rather different growth curves. In this case, although divisions were set back by 8 to 10 hours (plateaus), the increases in cell number, when they did occur, took place at a rate ($g \simeq 14$ to 16 hours) greater than that found for asynchronous, exponential growth (not shown) in LL or DD ($g \simeq 24$ to 26 hours), as reflected in the increased slopes of the growth steps, which were log-linear rather than sigmoidal, as obtained with photoautotrophic cultures of the Z strain (Figs. 2 and 3). The net result of this compensatory process was that a doubling of cell number ($\overline{ss} \simeq 2.0$) still occurred every 24 hours, and cell concentration attained approximately the same value as it would have by uninterrupted growth in DD or LL (without prior entrainment), at the same time conserving the developmental asynchrony (*62*) normally characterizing such exponential growth. This conservation of developmental asynchrony rules out any simple model employing a localized block or transition point in a determinate sequence (*5*, p. 220) behind which cells would be expected to accumulate in synchrony, and the paradox can be resolved only if it is assumed that a simultaneous prolongation of the individual CDC's by 8 to 10 hours occurs once every circadian cycle and is accompanied by the reduction or complete suppression of any G_1 variability. A similar analysis can be made for the persisting rhythm observed in LL (or DD) following a transition from D to L (Fig. 5), as well as for curves found for low-temperature pulses (13°C) of various durations imposed on exponentially increasing, photoautotrophic cultures of *Euglena* maintained in LL at 25°C (*57, 63*).

All of these observations on synchronized cultures of *Euglena*, then, appear to demand that individual CDC's be either delayed (prolonged) or advanced (shortened), or both, in order to cluster cell divisions (and probably other events of the CDC) at periodic intervals separated by longer time spans during which no cell division takes place. Since this gating is observed for many days under conditions held constant with respect to illumination (LL or DD), temperature, and other variables (*64*), the master timer or oscillator hypothesized to underlie the CDC (at least in the infradian growth mode) must perform this function, even on probabilistic models for CDC control.

Insertion and Deletion of Time Segments in Cell Cycles

We now consider how these shortenings or lengthenings of individual cell division cycles could be generated by the master (circadian) clock at the biochemical or molecular level. It would appear almost as if *Euglena* (and other microorganisms) has a programmable "clock for all seasons," or at least for a variety of sets of specified values for illumination, temperature, and nutritional conditions (*57*). Although the term "programmable" smacks of deterministic sequences, one can couple various stochastic or probabilistic processes to sequential-type mechanisms, generating any desired degree of variance in the overall control system for the CDC (*9–13*).

It is perhaps a matter of personal preference (in our current state of knowledge) as to the degree which one focuses on a tight genetic control, as, for example, by sequential tape-reading of a segment of DNA [such as the chronon of Ehret and Trucco (*17*)] or RNA template; or, alternatively, whether one assumes that the genetic program is relatively remote from the actual biochemistry of the CDC. In any case, the evidence reviewed in earlier sections formally demands that a clock of some sort (at least sometimes circadian in nature) predictably inserts time segments into, or deletes them from, the CDC.

On the view that time dilation or contraction has an immediate molecular basis, one can envisage two ways by which such time segments could be added or subtracted from the CDC by a master oscillator. An indeterminate, variable number of traverses of Klevecz's G_q subcycle (*13*) could generate the necessary variance in g values. The fundamental period of 4 hours hypothesized for mammalian cell cultures in the ultradian growth mode would either have its analog in a circadian oscillator, which would then generate CDC's whose lengths would be integer multiples of 24 hours, or would be somehow "transformed" by a circadian clock into a longer period subcycle. The former notion implicates a multiplicity of clocks (a "clockshop"); the latter requires frequency transformation by another control system or by an internal modification of a versatile, "pliable" oscillator. Unfortunately, the G_q quantal cycle thus far is only a descriptive notion without molecular basis, although concomitant oscillations in enzyme activity have been observed (*14*) in mammalian cell cultures.

Alternatively, there is no reason to suppose that only one G_q subcycle is possible and that the duration of G_1 is to be accounted for solely by summation of a variable number of rounds of G_q. Other subcycles of different lengths and functional roles might exist from which the cell could choose. For example, one way that the CDC might be programmed would be for a collection of timing loops of different lengths to couple in various combinations to form a flexible timer (the whole of which we term the cytochron) as diagrammed in Fig. 8. This scheme is sufficiently generalized to apply to any eukaryotic cell cycle, although it was originally devised to summarize experimental findings in *Euglena*. In this model, the physiology of the cell is held in a steady state by feedback reactions, save for perturbations induced by environmental fluctuations, and the cell grows and accumulates reserves, or performs some steady-state functional role, in an indeterminate sequence. The cytochron, however, is to be regarded as a separate entity—a clock (*15*) or timer—that meters time with the primary function of giving order and temporal separation to the key events (black bars) that trigger or initiate (small arrows) the determinate phases (S, M, and C) of the mitotic cycle, and possibly also the sequence of events leading to differentiation in multicells.

The cytochron (Fig. 8) is shown schematically to have a basic circular track which can be modified by the insertion (or deletion) of a variety of time loops of different lengths. These loops can be selected either at random (variable segments) to generate G_1 variability, or specifically (compensator segments) by the cell's circadian clock to manipulate the duration of CDC phases so that they coincide with appropriate time slots in the day-night cycle. The time track can be envisaged as being marked out in hours such that, if a *Euglena* cell followed the main track and bypassed all the lateral loops, its total traverse would require 8 to 10 hours, the minimum cycle time (organotrophic growth at 25°C) (see Fig. 4A). In *Euglena* grown photoauto-

trophically in LD regimes, however, the CDC (and by implication the cytochron) operates in a noncyclic mode (Fig. 9). Once mitosis and cytokinesis are completed, the daughter cells do not proceed to another CDC unless illumination is maintained, and in darkness they appear to be held in an indeterminate or untimed G_0 state. At 25°C, as for *Chlamydomonas* (*65*), a minimum of about 6 hours of light is required to prime a population for subsequent cell division in darkness under optimum conditions, a value that coincides with the shortest duration for G_1 (Fig. 7), suggesting that the extra variable segments, introduced into the cytochron tracks of other cells in the population to generate G_1 variability, may be put in at the end of a common light-driven segment.

It would appear, thus, that temporal loci exist along the cytochron track where the addition or deletion of time loops, or stopping or starting, is programmed. To avoid semantic problems involved in the use of terms such as threshold or transition point (often applied to temporal loci blocked by quite unphysiological agents), these apparently natural branching points in the CDC program are referred to here as control points (CP). Control point zero (CP0) represents both the inception and termination locus for the cytochron timer, and the specification to set the timer running is made at this locus. Illumination seems to be a requirement in photoautotrophic *Euglena* populations since the timer stops at and starts from CP0 on either side of a dark interval, only running in a truly cyclic mode in LL. Given an organic carbon source such as ethanol (*34*), however, the cytochron immediately adopts the cyclic mode and programs successive mitotic cycles, apparently without regard to any LD regime. This stop-start mechanism serves to synchronize the cytochrons (and CDC inception) in LD-cycled populations dependent on chloroplast-fixed carbon (Fig. 9).

A second control point, CP1, tentatively located at the inception of S in cells with minimal cycle times, is the point at which variability is inserted into the G_1 segment and is probably the last locus along the cytochron track at which the sequence of events can be blocked or adjustments made before the cell irretrievably goes into DNA replication and chromatin duplication. Although it would appear that selection of individual variable segments is random, the range may be modulated, nevertheless, by environmental conditions, longer segments being made available in the infradian growth mode brought about by lower temperatures, low light intensities, or a poor nutrient status. Indeed, it seems probable that alternative time loops are also available for the CP0-to-CP1 and the CP1-to-CP2 segments to suit different environmental regimes. Certainly, in *Euglena* additional options are available in the CP2-to-CP0 segments to permit the multiple rounds of S that occur in the stationary phase of batch cultures, while cell division is suppressed, and which lead to an increase in chromosome number from the usual 21 pairs to more than 80 pairs (*56*), this being followed on inoculation into fresh medium by successive rounds of M and C, which rapidly restore ploidy to the exponential-phase norm.

Therefore, it begins to look as though the CP0-to-CP1, CP1-to-CP2, and CP2-to-CP0 segments may also be loops, and that the three control points may each lie at the junction of a complex of loops (the cytochron) (Fig. 8). Such a state of affairs would also help to explain how it is that the circadian clock can apparently insert its delaying compensator loops (that under adverse conditions stretch the CDC from one circadian cycle to the next) at any locus along the cytochron-CDC track, with the exception of the post-CP2 segment (Fig. 9). We have precisely located this last control point (CP2) at 1 hour (25°C) prior to mitosis; it may represent the locus at which the key events (four black bars) take place that

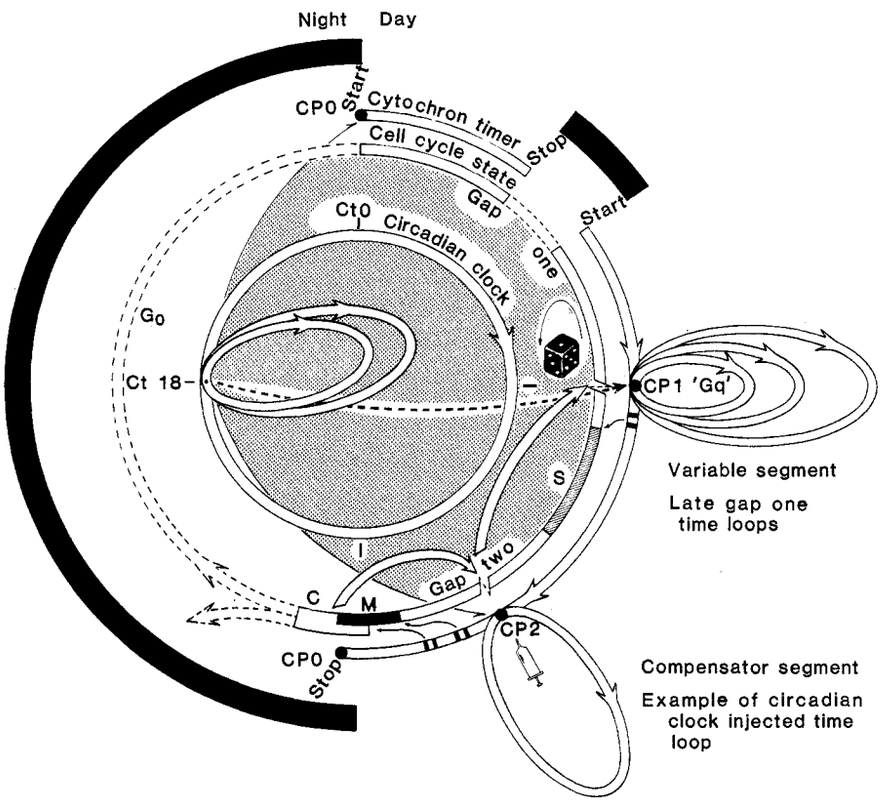

Fig. 8. A model for the insertion and deletion of time segments in the track of the cytochron (cell cycle clock) hypothesized to program the events of the cell division cycle of *Euglena*. Cytochrons in each cell start up synchronously at dawn and meter time (unless interrupted by a dark pulse) around to the CP0 control point, triggering sequentially (black bars) the events leading to chromatin replication (S), mitosis (M), and cytokinesis (C). In photoautotrophic cultures in LD regimes they stop (noncyclic mode) at CP0 in the dark, having triggered M and C, leaving cells in an untimed G_0 state until dawn restarts the cycle. In LL, or on certain organic substrates, however, the cytochron is cyclic and runs on through CP0 (that is, there is no G_0). At CP1, the addition of one or more variable-segment time loops by a random selector (dice symbol) generates variability in the duration of gap one and disperses (desynchronizes) subsequent cell divisions across the next dark period. A circadian clock, entrainable by LD zeitgeber, can couple to the cytochron at a unique circadian time (Ct 18) and inject (syringe symbol) a determinate compensator-segment time loop into the cytochron track anywhere within the arc CP0 to CP2 (stippled), so that the cell cycle is extended until a subsequent circadian cycle. Thus divisions are phased in "bursts" or "clusters" at 24-hour intervals, the scatter within each "cluster" being generated by the gap 1 variable-segment time loops, dawn synchronization of the cytochrons ensuring that each pulse is confined to the dark period in each LD cycle. The circadian clock can also apparently delete gap one variable loops and reduce cell cycle variation under some regimes (dashed arrow). Finally, division is suppressed in cells approaching the infradian ("stationary") phase of population increase, but multiple rounds of S raise the ploidy level. When conditions improve, ploidy is reduced by successive rounds of M and C. Anticlockwise arrows represent these temporary loop closures.

Therefore, it begins to look as though the CP0-to-CP1, CP1-to-CP2, and CP2-to-CP0 segments may also be loops, and that the three control points may each lie at the junction of a complex of loops (the cytochron) (Fig. 8). Such a state of affairs would also help to explain how it is that the circadian clock can apparently insert its delaying compensator loops (that under adverse conditions stretch the CDC from one circadian cycle to the next) at any locus along the cytochron-CDC track, with the exception of the post-CP2 segment (Fig. 9). We have precisely located this last control point (CP2) at 1 hour (25°C) prior to mitosis; it may represent the locus at which the key events (four black bars) take place that

trigger M and C. It could be that the cytochron runs only as far as CP2 and that the events of mitosis and cytokinesis, once initiated, are self-propelling and run to completion of their own accord. The cytochron-CDC, however, can be blocked at this locus by a variety of environmental shifts; therefore, it must be distinct from CP0, although it must lie very close to it.

The CP0, CP1, and CP2 control points in the postulated *Euglena* cytochron are reminiscent of the G_1 and G_2 loci at which the CDC can be arrested in higher plant meristems and released by subsequent hormone action (*66*), and by analogy, the equivalent of the CP0 control point in mammalian cells may represent the locus at which cycling and therefore unrestricted cell division, is suppressed. Indeed, if the cytochron is fundamentally (primitively) a cyclic clock that can be stopped at CP0 by a variety of natural control mechanisms, it is perhaps hardly surprising that the transformation of mammalian cells can be accomplished by a single protein coded by a virus such as SV40 (*67*).

Problems and Prospects

We have argued that the sequential events of the CDC, although in themselves a timer of sorts, cannot constitute the chronometer controlling the temporal spacing of these events by a simple stopping and restarting of the sequence, either in response to environmental shifts or to commands from an endogenous circadian oscillator. At least in an ordinary sense, the cell division cycle is not the clock. A separate programmable entity (which we term the cytochron) must interpret the environmental signals and insert, or delete, appropriate time loops of specified sign and duration into the CDC (selected from a finite library of available loops), thereby controlling the time at which each change in cell cycle state is triggered. For example, at 19°C it appears that an 8-hour delay loop is inserted into the CDC program of *Euglena* in response to cues from a circadian clock, whereas at 25°C the loop length is 14 hours (Fig. 9). In this example, loop length is apparently selected to compensate for a change in temperature—the higher the temperature the longer the loop—so that the thermally induced speeding up of cellular metabolism does not advance the timing of cell division.

That the cytochron also must be functionally independent of the circadian clock (although not necessarily an entirely separate mechanism) is apparent from the way in which the two can be uncoupled in the P_4ZUL mutant (*45*) when it is deprived of certain sulfur compounds. Such cells continue to divide asynchronously, implying continued cycling of the cytochron, yet the addition of cysteine, for example, reinstates insertion of a delay loop at circadian intervals in phase with a D-to-L transition made several days prior to the addition. The circadian clock, therefore, must have been synchronized and have been cycling independently of the uncoupled cytochron. The persistence of circadian rhythmicity in "stationary" phase metabolism (infradian growth) in the absence of cell division (*31–33*) also supports the concept of a functionally separate CDC.

We have yet to address ourselves to the identity of these time segments formally demanded by our data. Although it is obviously beyond the scope of this article to assess all the evidence for the role of genes and their expression in the operation of the CDC and circadian chronometers (*68*), the concept of a discrete programmable cytochron, with optional time loops, and interacting with a resettable circadian clock, does suggest, however, a possible molecular basis of a kind not previously proposed. Several years ago one of us (K.J.A.), while attempting to account for the complexity and apparent programmability of the

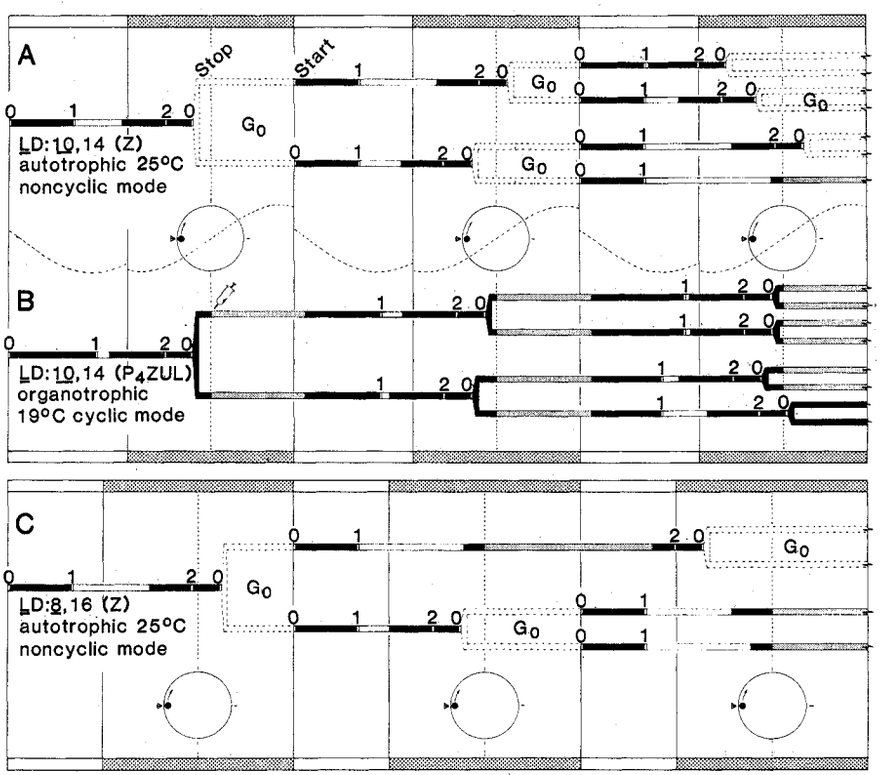

Fig. 9. Generation of the two types of variance observed during synchronous or phased cell division by the cytochron and circadian clocks. Time tracks for cytochron clones in wild-type *Euglena* (strain Z) for photoautotrophic growth at 25°C in (A) *LD:10*,14 and (C) *LD:8*,16 cycles; and for the photosynthetically incapacitated mutant P_4ZUL (B) on organic medium in *LD:10*,14 cycles at 19°C. Open bands (0,1,2,) denote control points CP0, CP1, and CP2. Determinate segments CP0 to CP1, S to CP2, and CP2 to CP0 (black) are interspersed with variable segments (open track), selected randomly from a range modulated by the LD regime; and compensator segments (fine stipple), injected at circadian intervals by the entrained circadian clock (dials) into the cytochron tracks at the midnight point in each cycle. In photoautotrophic cells (A and C), cytochrons operate in the noncyclic mode, stopping in darkness at CP0 after initiating cell division, and restart in synchrony at dawn. Random choice of variable segments disperses cell divisions across each dark period, but the daughter cells are resynchronized as they collect in the untimed G_0 phase for a common start at the next dawn. Organotrophic growth (B) switches the cytochrons into the cyclic mode, in which they remain asynchronous and oblivious to LD cycles, but once a day the LD-entrained circadian clocks simultaneously inject an 8-hour compensator segment (time delay) into the tracks of all the cytochrons, regardless of phase (except for the minority in the CP2-to-CP0 segment), giving rise to a stepped log-linear growth curve (see Fig. 4B). In (A), synchronization of the cytochrons at dawn ensures that most cells reach CP2 by midnight, permitting a population doubling every cycle, since the circadian clock cannot couple to G_0 or post-CP2 segment cells. In (C), the shorter light periods increase the range of available variable segments, so that many of the cells fail to reach CP2 by midnight of the first cycle, and the circadian clocks inject 14-hour (at 25°C) compensator segments that carry them over to complete division in the subsequent dark period (see Fig. 3).

CDC clock, concluded that the information content required to operate the mechanism (i) exceeded that likely to be available from any simple membrane-based oscillator (*69*, *70*) and (ii) favored direct readout of a nucleotide sequence as a basis for both the timing and the programming functions of the proposed cytochron. This model (Fig. 10) permits the hypothesis that a small segment of chromosomal DNA involving a hundred or so transcriptional units is folded into loops by bridging cross-links (of protein or RNA) at genetically defined loci to form a three-dimensional network of anastomosing loops. The entire complex would constitute a giant functional gene of up to 3000 kilobase pairs that is capable of metering periods as long as 24 hours by the incorporation of 30 to 40 (*71*) or as few as 19 (*72*) nucleotides per second, as originally proposed in the chronon model of Ehret and Trucco (*17*).

Our model differs radically from the chronon model, however, in proposing that (i) the transcription of one loop in the sequence triggers transcription of another specific loop somewhere else within the giant gene without the in-

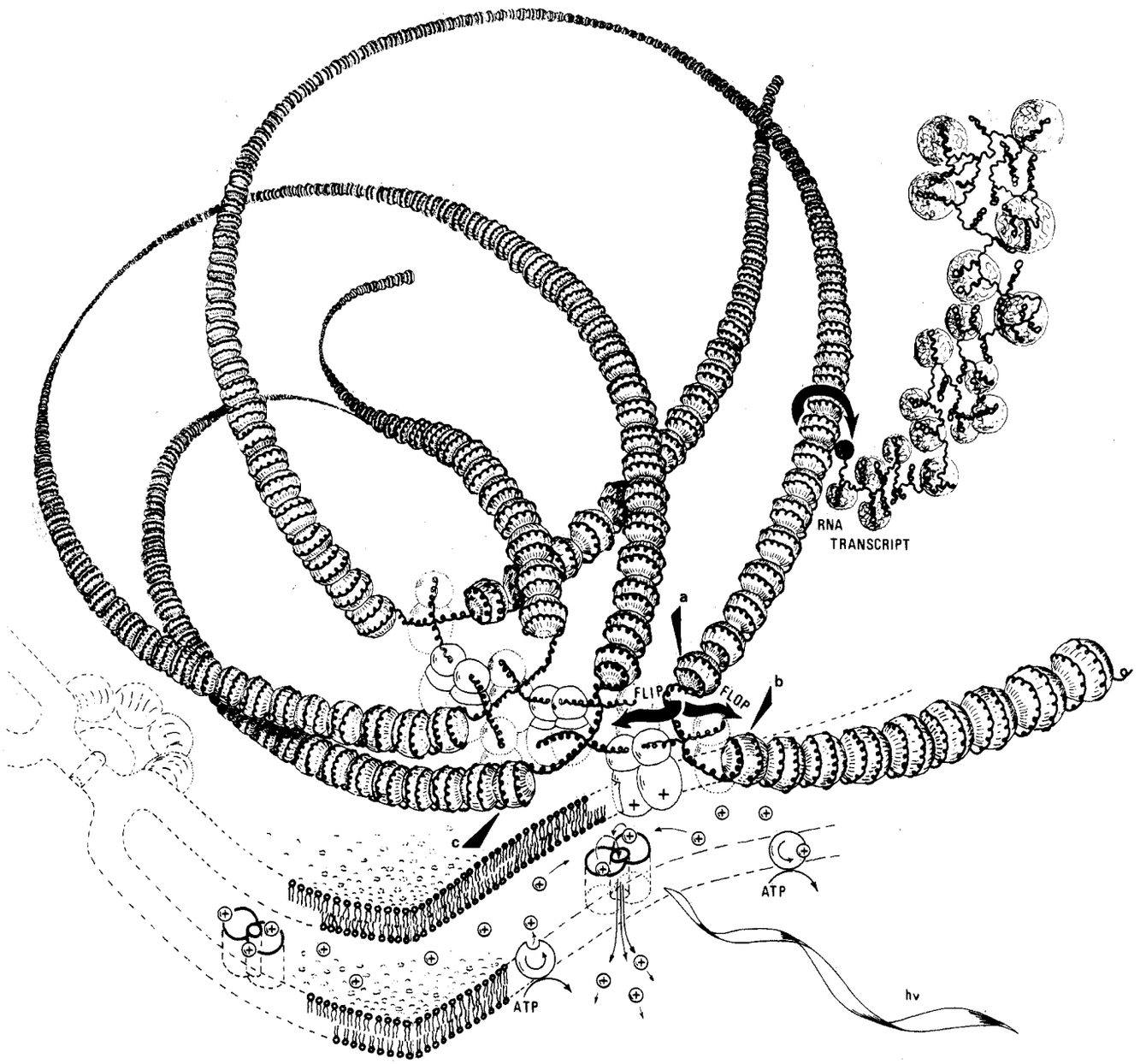

Fig. 10. Diagram of a chronogene segment, one possible molecular basis for metering time in longer period cellular oscillations. Transcriptional units of chromosomal DNA, wound around their nucleosomes, loop out from protein complexes that anchor them by their inverted-repeat cruciforms to the nuclear envelope and cross-link them to other units at paired genetic loci. At dawn, light-absorbing pigments in the membrane trigger the opening of ion gates that collapse a membrane potential accumulated by ATP (adenosine triphosphate)-driven pumps; the resulting transient change in electric field reprograms the time-metering transcriptional sequence by switching all the protein links to the flip mode. Upon completion of transcription of the top loop (*a*), transcription is initiated on the adjacent unit (*c*) in response to a (torsional?) signal transmitted across the link rather than on the next unit (*b*) in the tandem sequence (as it would in the flop mode). As the membrane potential restabilizes, the flip mode decays so that the only effective switching is mediated by the link at the end of the transcriptional unit being actively transcribed at the time that the transitions between light and dark occur. In this way, long segments of tandemly arranged units are either inserted into or are deleted from a coupled transcriptional circuit at dusk or dawn to generate the advancing or delaying adjustments that serve to sychronize the clock with the earth's rotation. The genetically programmed siting of the links ensures that the precise loop lengths required to effect these phase shifts occur at the right places in the multiple-path transcriptional circuit.

volvement of a translation step; and that (ii) the sequence of transcription of the units is programmable. As a transcription complex nears the end of a transcription unit (Fig. 10), the initiator site on a loop joined to it by a cross-link opens up, and transcription of this second unit begins in response to a signal [possibly torsional (*73*)] transmitted across the link. To permit programming of the network, each bridging cross-link [perhaps a protein dimer linked to the hairpins of two distant inverted-repeat cruciforms (*74*)] would exist in a passive (flop) or an active (flip) mode. In the flop mode, the bridge cannot transmit a signal across to a distant loop; instead, as the transcription complex nears the end of the unit and approaches the link, an initiator site opens up on the next unit in tandem sequence along the DNA tape. When the bridge switches to the flip mode, however, the completion of transcription of a unit triggers transcription of the distant unit coupled to the other side of the bridge, instead of the one in genetic sequence. Modulation of the program by accessory proteins that change modes would permit time-metering loops (groups of transcriptional units) to be added or deleted from the cyclic program (*75*), thus generating instant advances, or delays, or serving to compensate the cycle time for thermal effects on transcription rate. Some of the bridges may be attached to complexes embedded in the nuclear envelope and could be switched from the flop to the flip mode by the collapse of a membrane potential generated across the nuclear membrane (for example, by charge accumulation in the perinuclear space). We suggest that this collapse could occur in response to the opening of ion gates linked to light-sensitive pigments (and temperature sensors) in the envelope (*61*), the magnitude of the resulting advance or delay being dependent on the position of the time-metering transcriptional complexes in the transcription circuit at the time of the collapse and on the position of the next available bridge in the programmed topology of the network.

In this model, the majority of the RNA transcripts have no coding function and are never capped or processed to serve as message, being produced solely to meter time in between key structural genes, and possibly derive from some of the highly mutated, "rusting hulks" of DNA that persist in eukaryotic genomes. Small RNA segments transcribed from critically spaced loops in the temporal program would serve to trigger events such as S, M, and C at the appropriate times, either by being processed and translated into an enzyme, or in a more subtle way [as for example, by forming the recently discovered small nuclear RNA's that are thought to hold the ends of intervening-sequence loops in place (*76*)], so that splicing enzymes can process the messenger RNA precursors. Chronogenes may even be modulated by the products of successive cycles to generate long-term programs that control sequential development in multicells.

Such a molecular model originally developed to account for the cytochron might also account for the complex light- and temperature-resetting patterns (embodied in their phase response curves, or PRC's) of circadian clocks (*77*). In fact, transformation of PRC data onto a folded template map suggests a mechanism that could account for the many different PRC shapes, the gradual buildup of resetting amplitude with increasing irradiance, and the advancing transients (*78*). This type of model, invoking a discrete clock gene, or chronogene (*78*), similar to that proposed for the cytochron is consistent with (i) observations that clock double mutants exhibit additivity (*79, 80*) and map at a small number of genetic loci (*79–81*); and (ii) the demonstration of protein cross-linked, 30- to 90-kilobase-pair DNA loops (*82*), apparently radially arranged (*83*), in eukaryotic chromosomes (*84*).

Although earlier reports of circadian rhythmicity in mature (anucleate) erythrocytes (*85*) have not been confirmed (*86*), this class of transcriptional model nevertheless appears to be in conflict with results of experiments with enucleated *Acetabularia* and those with actinomycin D and rifampicin which suggest that neither nuclear nor chloroplastic DNA is essential for the expression of a circadian rhythm or its resetting by light or dark (*68, 87, 88*). The requirement for protein synthesis in the *functioning* of circadian clocks (*68, 89*), particularly for production of some key component of the clock itself (*89, 90*), is by no means certain inasmuch as inhibitors such as puromycin and cycloheximide may affect circadian oscillations by changing membrane properties (*68*). Further, *Acetabularia* is a gigantic cell and, when enucleated, it can even differentiate a cap structure by somehow utilizing the large cytoplasmic pool of long-lived messenger RNA's (*91*). This suggests the possibility that, in order to solve the problem of long-range intracellular communication (messenger RNA's take several days to reach the cap) (*91*), this cell may have developed a mechanism for sequential translation of messenger RNA's to control its differentiation and possibly its many circadian clocks, which must be independently associated with each chloroplast in order to synchronize the photosynthetic rhythm in these widely dispersed organelles. The recent discovery of small circular multi-RNA complexes in eukaryotic cells that are thought to permit continuous cyclic translation (*92*) gives some credence to the possibility that *Acetabularia* may have developed a giant RNA-based circadian clock metering time by translation as an analog of the chronogene (*93*).

Alternatively, these results could be interpreted as indirectly supporting a nucleic acid–independent, membrane-based clock (*69, 70, 89*); as Scott and Gulline (*94*) have pointed out, however, the possibility of membrane-based oscillations in some organisms does not preclude different mechanisms in others, or even in the same cell, because if rhythmicity confers a selective advantage on biological systems, various evolutionary strategies may have been adopted independently. One possibility here is that cytochrons may be based on chronogenes, and the circadian clocks on membrane-based devices requiring the nucleus only for a supply of parts.

On the other hand, the uncanny accuracy with which double mutants of *Neurospora* (*79*) and *Chlamydomonas* (*80*) add and subtract time segments from the circadian cycle, as if they were rails in a toy train circuit, is suggestive of a template clock read at a constant speed, to and from which loops can be added and deleted by, for example, mutant bridge-protein genes. Although the cytochron and circadian clock of *Euglena* might, or might not, comprise, as we propose, a DNA or RNA template with a branching network of tracks and binary switching points, it would seem in principle that they could have a similar molecular basis, or at least that they share certain elements or pathways in common, as has been suggested (*95*). In fact, we suspect that there is but one programmable clock, a veritable on-board computer, that can plot mid-course corrections in response to real-time variables and ensure its survival against most possible odds. Until we can establish the existence of a single clock with a multiplicity of functions, however, any attempt to unravel its mechanism must take into account the evidence for two functionally separate cellular clocks, both clearly outside the passive sequence (or network) of metabolic states that we recognize as the cell division cycle.

At present, we do not know enough about the detailed mechanism underlying

either circadian rhythms or the proposed cytochron in *Euglena* to be more mathematically precise (concerning, for example, the modes and constraints of clock coupling, even assuming limit cycle dynamics of the circadian oscillator) or more explicit in molecular terms. (We also do not know whether our late-G_1, variable-segment time loops are quantized or generated instead by an entirely probabilistic mechanism.) Rather, we consider this to be a "thought" model having several possible solutions, arising from the need to explain the insertion and deletion of the finite time segments in cell division cycles formally demanded by their empirically observed variability.

Note added in proof: For a recent review of temporal patterns of cell division in unicellular algae, see (*96*).

References and Notes

1. A. Howard and S. R. Pelc, *Heredity* **6** (Suppl.) 261 (1953).
2. S. Cooper, *Nature (London)* **280**, 17 (1979); C. E. Helmstetter, S. Cooper, O. Pierucci, E. Revelas, *Cold Spring Harbor Symp. Quant. Biol.* **33**, 809 (1968).
3. J. W. Hastings and H.-G. Schweiger, Eds., *The Molecular Basis of Circadian Rhythms* (Dahlem Konferenzen, Berlin, 1976; available from Verlag Chemie International, Inc., Plaza Center Suite E, Deerfield Beach, Fla.).
4. J. M. Mitchison, G. Gerisch, F. C. Kafatos, S. A. Kauffman, H. R. Matthews, W. Sachsenmaier, E. Zeuthen, in (*3*), p. 361.
5. J. M. Mitchison, *The Biology of the Cell Cycle* (Cambridge Univ. Press, London, 1971).
6. _____, in *Cell Cycle Controls*, G. M. Padilla, I. L. Cameron, A. Zimmerman, Eds. (Academic Press, New York, 1974), p. 125.
7. L. H. Hartwell, J. Culotti, J. R. Pringle, B. J. Reid, *Science* **183**, 46 (1974).
8. J. Engelberg, *J. Theor. Biol.* **20**, 249 (1968).
9. H. Temin, *J. Cell. Physiol.* **78**, 161 (1971).
10. H. E. Kubitschek, *Exp. Cell Res.* **26**, 439 (1962); F. J. Burns and I. F. Tannock, *Cell Tissue Kinet.* **3**, 321 (1970).
11. J. A. Smith and L. Martin, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1263 (1973).
12. D. A. Gilbert, *BioSystems* **5**, 197 (1974); *ibid.* **10**, 227 and 235 (1978); *S. Afr. J. Sci.* **70**, 234 (1974).
13. R. R. Klevecz, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4012 (1976).
14. _____, *J. Cell Biol.* **43**, 207 (1969).
15. We use the word "clock" as a generic term denoting a timekeeping device, without any bias as to the underlying mechanism. This definition was adopted at the Dahlem Conference on the Molecular Basis of Circadian Rhythms in 1975 [see (*3*, *20*)].
16. For evidence relating periodic enzyme synthesis to the order of transcription in bacteria, see M. Masters and A. B. Pardee, *Proc., Natl. Acad. Sci. U.S.A.* **54**, 64 (1965); in yeast, see P. Tauro, H. L. Halvorson, R. L. Epstein, *ibid.* **59**, 277 (1968).
17. C. F. Ehret and E. Trucco, *J. Theor. Biol.* **15**, 240 (1967).
18. H. P. Rusch, W. Sachsenmaier, K. Behrens, V. Gruter, *J. Cell Biol.* **31**, 204 (1966); W. Sachsenmaier, U. Remy, R. Plattner-Schobel, *Exp. Cell Res.* **73**, 41 (1972); W. Sachsenmaier, in (*3*), p. 409; *Arzneim.-Forsch.* **28**, 1819 (1978).
19. S. A. Kauffman and J. J. Wille, Jr., *J. Theoret. Biol.* **55**, 47 (1975); in (*3*), p. 409.
20. For a brief, easily grasped mathematical background relative to biological rhythms, see J. J. Tyson, S. G. A. Alivisatos, F. Grün, T. Pavlidis, O. Richter, F. W. Schneider, in (*3*), p. 85.
21. B. Chance, E. K. Pye, A. K. Ghosh, B. Hess, Eds., *Biological and Biochemical Oscillators* (Academic Press, New York, 1973)
22. T. Pavlidis, *Biological Oscillators: Their Mathematical Analysis* (Academic Press, New York, 1973).
23. A. Winfree, *J. Theor. Biol.* **28**, 327 (1970); in *Lectures in the Life Sciences*, M. Gerstenhaber, Ed. (American Mathematical Society, Providence, R.I., 1970), vol. 2, p. 110; in (*21*), p. 479.
24. J. Tyson and W. Sachsenmaier, *J. Theor. Biol.* **73**, 723 (1978).
25. R. R. Klevecz, J. Kros, S. D. Gross, *Exp. Cell Res.* **116**, 285 (1978).
26. B. Chance, A. Ghosh, J. Higgins, P. K. Maitra, *Ann. N.Y. Acad. Sci.* **115**, 1010 (1964). Provocatively, however, J. Das and H. G. Busse of the University of Kiel (Federal Republic of Germany) have reported (Roundtable on "The Molecular Basis of Cellular Circadian Clocks," 26 to 28 April 1980, Max-Planck-Institut für Zellbiologie, Ladenburg b. Heidelberg) that glycolytic oscillations having longer periods—even a single circadian cycle—can be observed in homogenized yeast cultures in which the protein concentration has been decreased.
27. "Biological Rhythms," *Cold Spring Harbor Symp. Quant. Biol.* (1960), vol. 25 (entire volume); L. E. Scheving, F. Halberg, J. E. Pauly, Eds., *Chronobiology* (Igaku Shoin, Tokyo, 1974); *Proceedings of the 12th International Conference of the International Society for Chronobiology* (Casa Editrice "Il Ponte," Milano, 1977).
28. J. Aschoff, Ed. *Circadian Clocks* (North-Holland, Amsterdam, 1965).
29. M. Menaker, Ed., *Biochronometry* (National Academy of Sciences, Washington, D.C., 1971).
30. To name a few: *Candida, Chlamydomonas, Chlorella, Dunaliella, Euglena, Gonyaulax, Gymnodinium, Paramecium, Pyrocystis, Saccharomyces, Tetrahymena* [reviewed in (*31-33*)].
31. L. N. Edmunds, Jr., *Colloq. Int. C.N.R.S.* **240**, 53 (1975).
32. _____, in *Aging and Biological Rhythms*, H. V. Samis and S. Capobianco, Eds. (Plenum, New York, 1978), p. 125.
33. L. N. Edmunds, Jr., and F. Halberg, in *Neoplasms—Comparative Pathology of Growth in Animals, Plants and Man*, H. E. Kaiser, Ed. (Williams & Wilkins, Baltimore, 1981), p. 105.
34. L. N. Edmunds, Jr., *J. Cell. Comp. Physiol.* **66**, 147 (1965).
35. _____ and R. R. Funch, *Science* **165**, 500 (1969).
36. _____, *Planta* **87**, 134 (1969).
37. Abbreviations: *LD*: *10*,14, a repetitive light cycle (period T = 24 hours) consisting of 10 hours of light (L) followed by 14 hours of darkness (D); LL, continuous light; DD, constant dark; D/L, a single transition from D to L; $\bar{\tau}$, the free-running period of the rhythm of cell division (onset to onset) in a population in LL or DD; $\overline{ss}$, stepsize, or factorial increase in cell concentration (plateau to plateau) after a synchronized or phased "burst" (step) of division; $\bar{g}$, average generation time (doubling time) of a population of cells.
38. G. Ledoigt and R. Cavayrac, *J. Protozool.* **26**, 632 (1979).
39. O. Terry and L. N. Edmunds, Jr., *Planta* **93**, 106 (1970).
40. L. N. Edmunds, Jr., *J. Cell. Physiol.* **67**, 35 (1966).
41. Because of the requirement of light for photosynthesis, one cannot use DD; nor can one impose LL of those moderate intensities (3500 to 7000 lux) normally used in the entraining LD cycle, for the population reverts to asynchronous, exponential growth (*40*).
42. R. M. Jarrett and L. N. Edmunds, Jr., *Science* **167**, 1730 (1970).
43. C. F. Ehret, J. C. Meinert, K. R. Groh, K. W. Dobra, G A. Antipa, in *Growth Kinetics and Biochemical Regulation of Normal and Malignant Cells*, B. Drewinko and R. M. Humphrey, Eds. (William & Wilkins, Baltimore, 1977), p. 49; L. N. Edmunds, Jr., in *International Cell Biology 1980-81*, H.-G. Schweiger, Ed. (Springer-Verlag, Heidelberg, 1981), p. 831.
44. L. N. Edmunds, Jr., L. Chuang, R. M. Jarrett, O. W. Terry, *J. Interdiscip. Cycle Res.* **2**, 121 (1971).
45. L. N. Edmunds, Jr., *et al.*, *Arch. Microbiol.* **108**, 1 (1976).
46. J. L. A. Mitchell, *Planta* **100**, 244 (1971).
47. Ultradian cultures, however, can be phased (if not truly synchronized) by LD cycles whose period is also less than 24 hours. Using *LD*: *6*,6 (T = 12 hours), G. Ledoigt and R. Calvayrac (*38*) have phased *Euglena* cultures growing (g = 12 hours) on lactate so that a doubling of cell number occurred every 12 hours during darkness. For a possible exception to this generalization, see S. W. Chisholm, F. M. M. Morel, W. S. Slocum, *Brookhaven Symp. Biol.*, in press.
48. K. Brinkmann, *Planta* **70**, 344 (1966).
49. W. G. Walther and L. N. Edmunds, Jr., *Plant Physiol.* **51**, 250 (1973); D. L. Laval-Martin, D. J. Shuch, L. N. Edmunds, Jr., *ibid.* **63**, 495 (1979).
50. F. M. Sulzman and L. N. Edmunds, Jr., *Biochem. Biophys. Res. Commun.* **47**, 1338 (1972); *Biochim. Biophys. Acta* **320**, 594 (1973).
51. A. L. Koch and M. Schaechter, *J. Gen. Microbiol.* **29**, 435 (1962); H. E. Kubitschek, *Cell Tissue Kinet.* **4**, 113 (1971).
52. P. Nurse and P. Thuriaux, *Exp. Cell Res.* **107**, 365 (1977); P. Fantes and P. Nurse, *ibid.*, p. 377.
53. J. R. Cook and B. Cook, *ibid.* **28**, 524 (1962).
54. T. Fox and A. B. Pardee, *J. Biol. Chem.* **246**, 6159 (1971); D. F. Peterson and E. A. Anderson, *Nature (London)* **203**, 642 (1964); J. E. Sisken and L. Morasca, *J. Cell Biol.* **25**, 179 (1965).
55. A. Yen and A. B. Pardee, *Science* **204**, 1315 (1979).
56. K. J. Adams, thesis, University of London (1966).
57. _____ and L. N. Edmunds, Jr., in preparation.
58. Stirring rate, however, as well as other culture conditions, may have a significant effect on the time required for the separation of pairs of daughter cells.
59. L. N. Edmunds, Jr., *Science* **145**, 266 (1964); *J. Cell. Comp. Physiol.* **66**, 159 (1965); K. Adams and L. N. Edmunds, Jr., unpublished data.
60. The timing of the bursts can be calculated from the expression $t_d = k + n\bar{\tau}$, where t_d is the midpoint of the observed fission burst, k is a constant (empirically determined to be about 12 hours after the onset of light in the prior entraining LD cycle), n is an integer denoting the nth free-running (or entrained) circadian cycle in LL or DD (or LD), and $\bar{\tau}$ is the free-running (or entrained) period of the cell division rhythm as exhibited by the population (*31-32*).
61. L. N. Edmunds, Jr., in (*29*), p. 608.
62. In this case, the phased division observed in DD would not be truly "synchronous" in the customary sense, despite the discontinuous doubling patterns.
63. K. J. Adams, *Br. Phycol. J.* **8**, 204 (1973).
64. The fact that synchrony of cell division (and of other events in nondividing populations) does not decay, in fact, for extended time spans in LL (or DD) in the absence of all obvious *Zeitgeber*, itself presents an intriguing problem (*61*). Either the periods of the individual cellular oscillators comprising the population must be rather precise and have relatively small interindividual variance (offset, perhaps, by a "random walk" type of variation), or some sort of intercellular communication must occur that maintains the synchrony within the population of self-sustaining oscillators [R. Wever, *Z. Vgl. Physiol.* **51**, 1 (1965); A. T. Winfree, *J. Theor. Biol.* **16**, 15 (1967); T. Pavlidis, *ibid.*, **22**, 418 (1969)].
65. J. L. Spudich and R. Sager, *J. Cell Biol.* **85**, 136 (1980).
66. J. Van't Hof, in *Cell Cycle Controls*, G. M. Padilla, I. L. Cameron, A. Zimmerman, Eds. (Academic Press, New York, 1974), p. 77.
67. A. Durham, *New Sci.* **77**, 860 (1978).
68. For a relatively up-to-date, state-of-the-art summary, see M. L. Sargent, in (*3*), p. 295; V. G. Bruce, in (*3*), p. 339.
69. D. Njus, F. M. Sulzman, J. W. Hastings, *Nature (London)* **248**, 116 (1974); B. M. Sweeney, *Int. J. Chronobiol.* **2**, 25 (1974); L. N. Edmunds, Jr., and V. P. Cirillo, *ibid.*, p. 233; Group report 4 [A. Keynan, rapporteur, in (*3*), p. 239]; B. M. Sweeney, in (*3*), p. 267; D. Njus, in (*3*), p. 283; L. N. Edmunds, Jr., in *Chronobiology: Principles and Applications to Shifts and Schedules*, L. E. Scheving and F. Halberg, Eds. (Sijthoff and Noordhoff, Alphen aan den Rijn, Netherlands, 1980), p. 205.
70. W. Engelmann and M. Schrempf, in *Photochemical and Photobiological Reviews*, K. C. Smith, Ed. (Plenum, New York, 1979), p. 49.
71. M. M. Lamb and B. Daneholt, *Cell* **17**, 835 (1979).
72. D. M. Anderson and L. D. Smith, *Dev. Biol.* **67**, 274 (1978).
73. M. Spencer, *Nature (London)* **281**, 631 (1979).
74. D. A. Wilson and C. A. Thomas, *J. Mol. Biol.* **84**, 115 (1974).
75. Only a single bridge would need to be changed in order to break the circuit and convert the cytochron into a linear clock to achieve suppression of cellular proliferation. It is equally easy to envisage how the cytochron could be recyclized by the action of an endogenous environmental sensor, a hormone, or a transforming virus. Viral DNA coding for a new bridge protein or the integration of viral DNA into the cytochron gene capable of binding existing bridge protein subunits to form a new bridge that closes the gap are possible explanations of the transformation phenomenon based on this type of model.
76. M. R. Lerner, J. A. Boyle, S. M. Mount, S. L. Wolin, J. A. Steitz, *Nature (London)* **283**, 220 (1980).

77. C. S. Pittendrigh, in (*28*), p. 277; A. T. Winfree, *J. Theor. Biol.* **28**, 327 (1970).
78. K. Adams, unpublished data; in (*70*), pp. 60 and 69.
79. J. F. Feldman and C. A. Atkinson, *Genetics* **88**, 255 (1978); J. F. Feldman, G. Gardner, R. Denison, in *Biological Rhythms and their Central Mechanism*, M. Suda, O. Hayaishi, H. Nakagawa, Eds. (The Naito Foundation, Elsevier/North-Holland, Amsterdam, 1979), p. 57.
80. V. G. Bruce, *Genetics* **77**, 221 (1974).
81. R. Konopka and S. Benzer, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2112 (1971).
82. J. R. Paulson and U. K. Laemmli, *Cell* **12**, 817 (1977).
83. M. P. F. Marsden and U. K. Laemmli, *ibid.* **17**, 849 (1979).
84. A chronogene-based clock would be susceptible to three types of period-length mutation: (i) changes in the sequence of genes coding for the protein cross-links; (ii) sequence changes, or transposon-mediated positional changes, in the DNA segments at the bridge protein-binding loci; and (iii) duplications or deletions of, or within, the transcriptional units. Eukaryotes with large genomes could afford several circadian chronogenes containing relatively few peptide-coding sequences, in which case types B and C mutants would be expected to map in linked clusters, whereas type A mutants might map at scattered loci unless derived from a family of tandemly duplicated, bridge protein-coding genes. In lower eukaryotes with but a few millimeters of DNA, a single chronogene embracing a high proportion of the genome (possibly linking several chromosomes) is more likely and would have to incorporate many peptide-coding genes within its transcriptional circuit. This would be tantamount to sequential reading of the genome, though not necessarily in genetic (or even fixed) sequence; however, it would make unrelated genes on clock loops susceptible to positional effects in clock mutants.
85. Y. E. Ashkenazi, H. Hartmann, B. Strulowitz, O. Dar, *J. Interdiscip. Cycle Res.* **6**, 291 (1975).
86. L. Peleg, A. Dotan, I. E. Ashkenazi, *Chronobiologia* **6**, 142 (1979); J. W. Hastings, personal communication.
87. B. M. Sweeney and F. T. Haxo, *Science* **134**, 1361 (1961); E. Schweiger, H. G. Wallraff, H. G. Schweiger, *ibid.* **146**, 658 (1964); M. W. Karakashian and H. G. Schweiger, *Exp. Cell Res.* **97**, 366 (1976).
88. T. Vanden Driessche, S. Bonotto, J. Brachet, *Biochim. Biophys. Acta* **224**, 631 (1970).
89. The related and particularly provocative "coupled translation-membrane model" of Schweiger and Schweiger [H.-G. Schweiger and M. Schweiger, *Int. Rev. Cytol.* **51**, 315 (1977)] for circadian rhythms posits the synthesis of "essential" proteins on 80S ribosomes and their assembly, insertion (loading) into membranes, and subsequent disassembly (unloading and degradation) as the key elements of the oscillatory system.
90. M. W. Karakashian and H. G. Schweiger, *Exp. Cell Res.* **98**, 303 (1976); *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3216 (1976).
91. J. Brachet, in *Differentiation and Control of Development in Plants—Potential for Chemical Modification of Function*, E. C. George, Ed. (British Plant Growth Regulation Group, Monograph No. 3, Agricultural Research Council Letcombe Laboratory, Wantage, Oxfordshire, 1979), p. 29.
92. M. T. Hsu and M. Coca-Prados, *Nature (London)* **280**, 339 (1979).
93. It is perhaps ironic that in the one cytoplasmic organelle with a genome large enough to operate a circadian chronogene, the *Acetabularia* chloroplast, inhibitor experiments (*88*) seem to rule out the possibility. In contrast, the enormous size of the genome suggests that it is perhaps rather primitive and still has much in common with its prokaryotic ancestor.
94. B. I. H. Scott and H. F. Gulline, *Nature (London)* **254**, 69 (1975).
95. J. J. Wille, Jr., in *Biochemistry and Physiology of Protozoa*, M. Levandowsky and S. Hutner, Eds. (Academic Press, New York, ed. 2, 1979), vol. 2, p. 67.
96. S. W. Chisholm, in *Physiological Phytoplankton Ecology*, T. Platt, Ed. (published for the NATO Advanced Study Institute by Lipari, Sicily, in press).
97. J. Tyson and S. Kauffman, *J. Math. Biol.* **1**, 289 (1975).
98. Supported by NSF grants PCM76-10273 and PCM78-05832 (to L.E.), by a Science Research Council Fellowship (Queen Mary College, London) and grants from the University of London Central Research Fund (to K.A.). L.E. thanks the Laboratoire du Phytotron, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France, and the Centre International des Étudiants et Stagiares for permitting a sojourn conducive to the writing of this article; some of these ideas were presented (by L.E.) at a conference on Biological Rhythms and Aging, 13 to 15 April 1977, St. Petersburg Beach, Fla.

Instrumentation Needs of Research Universities

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Instruments are the tools with which researchers expand scientific understanding of the properties of nature. Their importance to the progress of science is indicated by the number of Nobel Prizes awarded for the development of instruments or methods of measurement. Within the past three decades, for example, Nobel Prizes in physics have been awarded for the discovery of nuclear magnetic resonance (NMR), the phase-contrast microscope, the transistor, the Cerenkov counter, the bubble chamber, the maser and laser, and holographic imagery.

Traditionally, research universities have played an integral role in the conception, development, and innovative use of instruments. For example, in 1928 Ernst Ruska, a beginning graduate student, began work on the first electron microscope. A half-century of development, based to a large extent on university research, has established the electron microscope as a powerful tool for the investigation of structure down to the atomic level. The development of instruments such as the flow cytometer has provided methods for conducting precise analyses of the chemical constituents of individual cells. As was true of the electron microscope, the flow cytometer was developed through a convergence of technologies.

Due to the rapid pace of instrument development, many instruments purchased only a few years ago are now obsolete. The 1960 Nobel Prize in physics was awarded for the development of the bubble chamber; today this technique has largely been replaced by electronic detectors such as drift chambers and wire chambers. F. Block and E. M. Purcell developed NMR in 1945 and 1946; enhancements of the technique (Fourier transform, signal-averaging methods, superconducting magnets) have produced an approximately 10,000-fold increase in speed and a 100-fold increase in sensitivity over the best equipment available only 10 years ago.

However, the cost of many new instruments threatens to make them inaccessible to many university researchers. The cost of multinuclear, high-field NMR spectrometers is approaching $500,000; flow cytometers cost up to $175,000. But without such instruments, the capacity of researchers to work at the frontiers of knowledge would be greatly impaired, and opportunities to develop superior instruments and expand their uses would be lost.

To assess the present capacity of universities to acquire necessary instruments, the authors, under the sponsorship of the Association of American Universities, conducted a study for the National Science Foundation (NSF) on the scientific instrumentation needs of research universities. The study examined the current status of scientific instruments in major research universities and sought to identify factors that facilitate or impede their acquisition, use, and development.

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