consisted of distension of the gallbladder and urinary bladder by injection of normal saline through implanted cannulae, and in some experiments, by balloon distension of the descending colon and rectum. Somatic stimulation consisted of mechanical manipulation of the extremities and body wall. Unit recordings were started between 6 and 30 hours after spinalization.

A unit was classified as a direct ascending fiber if a receptive field could be found and if it responded with a direct or antidromic spike in response to stimulation at C_1 (Fig. 1A). The criteria for establishing direct or antidromic activation included one spike for each stimulus (a following rate of at least 250 per second), invariant latency as stimulus frequency and intensity were varied, and, when present, collision between a spontaneously occurring spike and one evoked by C_1 stimulation. Units driven by stimulation at C_1, for which no receptive fields could be found and which exhibited no spontaneous activity were not classified since they could be either ascending, or more likely, descending fibers. Most units encountered, however, did have identifiable receptive fields but were not antidromically activated by cervical stimulation (*3*). These units are probably propriospinal.

Twenty-two units with visceral components in their receptive fields could be antidromically activated from C_1. These units were all spontaneously active and all responded to both somatic and visceral inputs (Fig. 1B).

Adequate receptive field studies were carried out in 18 of the direct somatovisceral fibers; 17 had bilateral and 13 had heterosegmental somatic inputs. The somatic receptive fields consisted of discrete, discontinuous components including cutaneous and deep structures. We found both excitatory and inhibitory fields, but we did not observe any systematic relation of these components. For example, the unit shown in Fig. 1B was inhibited by distension of both gallbladder and urinary bladder as well as by deep pressure over the middle third of the tail and lightly bending hairs in an area (1 by 2 cm) on the ventral metatarsal surface. This same unit was also excited by distension of the sigmoid colon, light touch over the sternum, light touch to the dorsal aspect of the ipsilateral thigh, and bending of hairs in an area (9 cm^2) on the contralateral thigh.

No units were found with purely inhibitory somatic inputs. Five had only excitatory somatic inputs; of these, two had inhibitory visceral inputs and three had excitatory visceral inputs. In six of the nine units that responded to both, the inputs from gallbladder and urinary bladder were of opposite sign. All six of these units had both excitatory and inhibitory somatic field components.

The average conduction velocity for 21 of the direct somatovisceral fibers was 68.6 ± 6.7 m/sec, with a range of 50 to 95 m/sec. These notably fast conduction velocities may not be representative inasmuch as our recording technique probably does not sample fibers conducting slower than about 30 m/sec (*4*, *5*). However, near the ventromedian fissure (Th 3), fiber diameters show a bimodal distribution (*6*). Whereas most fiber diameters are less than 7 μm, there is a secondary peak at 10.5 μm. This diameter peak corresponds to a velocity of 63 m/sec (*7*), a value consistent with our findings. A group of fibers that have a similar range of conduction velocities and demonstrate widespread convergence of somatic inputs has been found in the ventral part of the lateral funiculus (*5*); however, visceral inputs were not tested in these fibers.

The direct somatovisceral fibers tended to be near the ventromedian fissure (Fig. 2). The anatomical location, however, gives little clue as to the location of the pathway by which these fibers ascend the neuraxis. Although gross evoked potential and unit responses to visceral inputs have been found throughout the ventral white matter (*1*, *2*, *8*), most known ascending tracts are located in the dorsal or lateral funiculi. We have been unable to document an ascending pathway in the medial part of the ventral funiculus.

The above results demonstrate that there is a direct ascending pathway for visceral input. A highly convergent somatic input also projects to the fibers of this pathway. The presence of somatovisceral convergence at spinal neurons has been used as evidence to support the convergence projection theory of referred visceral sensation (*9*). Although the fibers we found do not ascend in classical somatosensory pathways, at least some fibers with both somatic and visceral inputs ascend to supraspinal structures.

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Persisting Circadian Rhythm of Cell Division in a Photosynthetic Mutant of Euglena

Abstract. *A persisting, free-running, circadian rhythm of cell division in a heterotrophically grown mutant of* Euglena gracilis *var.* bacillaris *having impaired photosynthesis is obtained upon placing a culture that has been previously synchronized by a 10,14 light-dark cycle into continuous darkness at 19°C (but not at 25°C). A similar persisting rhythm is initiated in exponentially increasing cultures (growing in darkness at 19°C) by a single "switch-up" in irradiance to continuous bright illumination. The results implicate an endogenous biological clock which "gates" the specific event of cell division in the cell developmental cycle.*

Entrainment of the cell division rhythm in *Euglena* by light-dark cycles or, perhaps, by temperature cycles and its persistence, after removal of the synchronizing regime, are thought to result from the functioning of an endogenous circadian clock that is intricately involved in the control of the cell cycle (*1*). We have assumed (*2*, *3*) that appropriate *Zeitgeber* entrain the cell division rhythm of individual cells in a population; under free-running condi-

tions, divisions would be "gated" by a biological oscillation operating through molecular and biochemical control mechanisms to intervals of approximately 24 hours (or to integral multiples thereof). Substantial evidence supporting this hypothesis has been advanced for *Euglena* (*2–4*), as well as for other microorganisms (*5–7*). The actual mechanism of the clock, however, remains unidentified.

Synchronization of cell division in *Euglena*, or in any photoentrainable, photosynthetic microorganism, has thus far been achieved only in cases where a minimal salt medium is employed. The cells, therefore, must be given a minimum amount of light energy for photosynthesis in order for any divisions to occur. This requirement of light for autotrophic culture imposes limitations on the study of light-induced synchrony. Thus, the determination of the effect of a single light pulse upon the phase of a cell division rhythm is confounded by the dual use of light by the cell for both phase resetting and for photosynthesis. This problem would not be entirely eliminated even if heterotrophically growing *Euglena* could be synchronized by light-dark (LD) cycles (that is, mixotrophic culture) since the cells might still utilize the light for photosynthesis, or photoenhancement of some other related process might occur. Similarly, studies of the effects of high frequency cycles, "random" LD regimes, and "skeleton" photoperiods (*3*) are also complicated by the minimum light requirement for cell growth and division in autotrophically grown *Euglena*.

These problems and others can be effectively eliminated by utilizing a light-synchronizable, nonphotosynthetic microorganism. Cell division rhythms in *Tetrahymena* (*7*) and *Paramecium* (*6*), both obligate heterotrophs, have already been shown to be capable of entrainment by LD cycles or transitions. In this report we describe a light-entrainable cell division rhythm in a heterotrophically grown mutant of *Euglena* having impaired photosynthesis, which persists with a circadian period upon removal of the synchronizing regime.

Cultures of the P_4Z ultraviolet-induced mutant (*8*) of *Euglena gracilis* var. *bacillaris* strain Z (Pringsheim) were used. Although this mutant (hereafter designated P_4ZUL) has normal amounts of the reductive pentose phosphate cycle enzymes and somewhat reduced levels of both chlorophyll and carotenoids, it is unable to carry out the Hill reaction owing to a block at or near photosystem 2 in the photosynthetic electron transport chain (*9*). Consequently, it is unable to grow autotrophically on minimal salt medium. Batch cultures were grown (*1*, *10*) axenically and heterotrophically in 4-liter serum bottles at either 19° or 25°C (±0.5°) on a low *p*H medium (*11*) containing malic and glutamic acids. In some experiments thioglycollic acid ($5 \times 10^{-5} M$) was added in an attempt to improve the synchrony; the observed effects, however, were negligible. The cultures were aerated with 575 to 625 ml of air per minute and were magnetically stirred at a rate sufficient to counteract phototactic and other motility responses (*12*), thereby insuring a homogeneous cell distribution. The serum bottles were maintained in Hotpack environmental chambers whose sides were furnished with banks of six fluorescent bulbs (Westinghouse, 20-watt, cool-white), which provided an incident illumination of about 5000 lux. The light regimes were controlled by an Intermatic 24-hour timer. A miniaturized fraction collector, a Brewer pipetting machine, and a Coulter electronic particle counter were routinely used to automatically monitor the cell concentration (*3*, *10*).

The effects of a 10,14 light-dark cycle (*LD*: *10*,14) upon cultures of the mutant grown at two different temperatures are represented in Fig. 1. At 25°C the culture grew exponentially with a generation time (GT) of about 10 hours, which represents the "ultradian" mode of growth (*7*). If the temperature of the culture was lowered to 19°C, however, the results were quite different (curve B): the overall GT increased to approximately 24 hours—the "circadian-infradian" growth mode (*7*)—and the division rhythm was entrained by the imposed LD cycle. Cell division was confined primarily to the dark periods. The average period (τ) of the population rhythm, taken as the time interval between successive onsets of division, was 24.0 hours, matching that of the driving *Zeitgeber* cycle. The average step size, defined as the ratio of the cell concentration immediately after a division "burst" in the population to that just prior to the onset of the burst, was 1.96 for this particular experiment, indicating that most cells divided once, and only once, during each cycle.

The average duration of the successive fission bursts was 15.5 hours, which is somewhat longer than that found in light-induced division synchrony of the wild type (*E. gracilis* Klebs, Z strain) grown autotrophically on a minimal salt medium in *LD*: *10*,14 (*3*, *10*). Neither case, of course, represents an ideally synchronized culture where the fission time observed in the population equals the average fission time of the individual cell.

But the observation of synchronization by LD cycles does not in itself provide conclusive evidence for the endogenous theory of entrainment or demand a self-sustaining circadian oscillation. It is conceivable that the alternation of light and dark periods reversibly enhances or inhibits the photoassimilation of organic substrate from the surrounding medium, or cell division itself, or even both, thereby resulting in synchronization of the culture (that is, a passive oscillating system capable only of forced oscillations).

That this does not appear to be the case is demonstrated in Fig. 2. If a culture, grown at 19°C, is synchronized by a *LD*: *10*,14 cycle and then is subsequently placed in continuous darkness, the cell division rhythm persists for at least 6 days before it damps out owing to the onset of the stationary growth phase where nutrients are limiting. Furthermore, the rhythm free runs with a $\bar{\tau}$ of 22.4 hours in this experiment (that is, significantly shorter than that of the prior entraining cycle). On the basis of the reduced step sizes (average step size was 1.64) and the average GT of about 31 hours in the population, the period of the individual cell cycles can be inferred to be considerably longer than 24.0 hours and is given by the expression $n\tau$, where n is a variable integer, and τ is the free-running period observed in the population (*3*). Thus, not all the cells in the population divide during each circadian cycle, but those that do are "gated" to a restricted time period during that cycle.

To obtain still further evidence in support of the endogenously controlled clock-gating hypothesis, the effects of a single change in illumination were examined (Fig. 3). If a culture of the mutant that has been growing exponentially (with a GT of 26 hours) in continuous darkness at 19°C is suddenly exposed to continuous bright (5000 lux) light (that is, a "switch-up" in irradiance by a single transition of dark to light), synchronous cell di-

vision ensues with a free-running $\bar{\tau}$ of 23.0 hours and an overall GT of 26 hours. Once again, not all cells divide during any one circadian cycle in the population, since the average step size is only 1.86. This value, however, includes the first step size of 1.76, which may be lower because of some initial photoinhibitory effect of the continuous light. Although in this experiment the overall GT in continuous light of about 26 hours was slightly shorter than that observed in continuous darkness (Fig. 2), the difference is probably not significant. Indeed, the GT's of cultures growing exponentially at 19°C in either continuous light or in continuous darkness were almost identical.

It would appear, therefore, that either (i) the hypothesized endogenous oscillations in the individual cells were themselves asynchronous but running in the prior continuous darkness and subsequently reset and synchronized by the dark-to-light transition; or (ii) the circadian oscillations were absent or damped out in continuous darkness and subsequently initiated or released when the switch-up occurred (*1*, *3*). What is surprising is that a free-running rhythm was observed in continuous bright illumination of an intensity that frequently causes rhythms in other

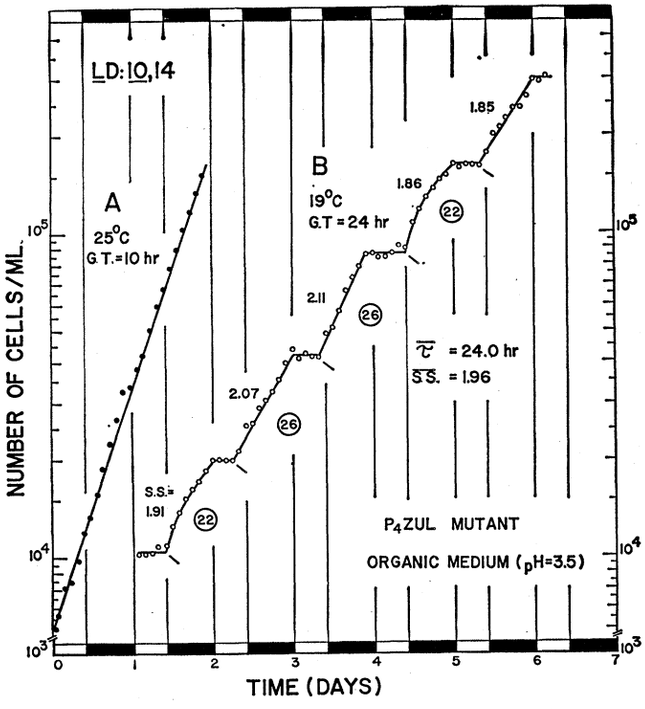

Fig. 1 (top left). Population growth of a photosynthetic mutant (P_4ZUL) of *Euglena gracilis* var. *bacillaris* strain Z (Pringsheim) grown on defined heterotrophic medium in *LD*: *10*,14. Curve A: exponential increase in cell number (GT of 10 hours) at 25°C. Curve B: entrainment of the cell division rhythm at 19°C. Step sizes (ratio of the number of cells per milliliter after a division burst to that just before the onset of divisions in the culture) are indicated for the successive division bursts. The period of the rhythm is also given in hours (encircled to the right of each burst). The average period ($\bar{\tau}$) of the rhythm in the population was 24.0 hours, and the average step size (ss) was 1.96 yielding a generation time (GT) of about 24 hours.

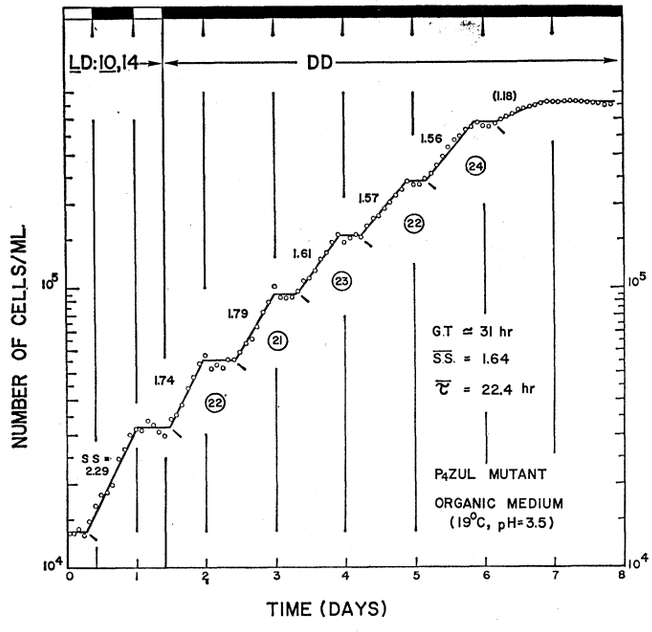

Fig. 2 (top right). Persisting, free-running, circadian rhythmicity in the cell division rhythm of cultures of the P_4ZUL mutant of *Euglena* grown heterotrophically at 19°C in continuous darkness. The culture had been previously entrained to a 24-hour period by a *LD*: *10*,14 regime. Other labels as for Fig. 1. The average free-running period ($\bar{\tau}$) of the population rhythm is 22.4 hours. The average step size (ss) of the successive fission bursts is substantially less than 2.00, indicating that not all cells divide during any one circadian cycle.

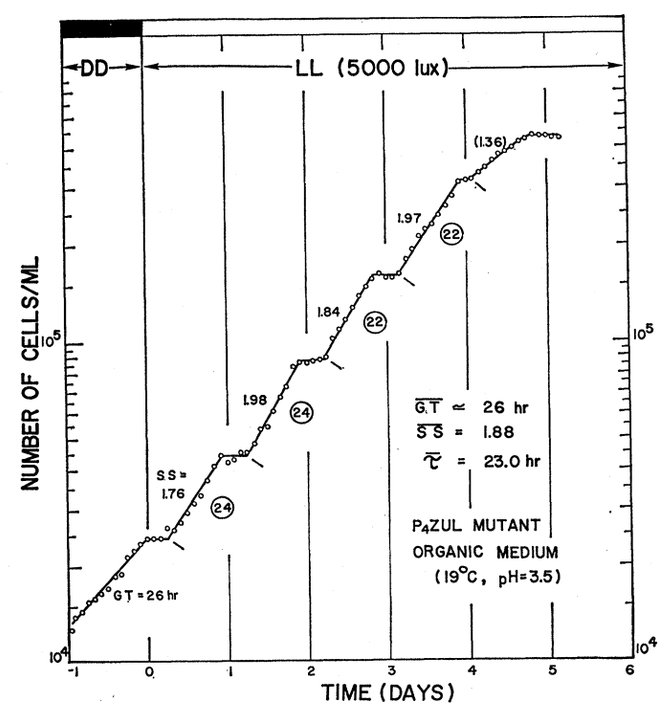

Fig. 3 (bottom right). Initiation of a persisting, free-running, circadian rhythm of cell division by a single "switch-up" transition (continuous dark to continuous light) in cultures of the P_4ZUL mutant of *Euglena* grown at 19°C on an organic medium. The culture had been growing exponentially with a GT of about 26 hours during the preceding period of continuous darkness (DD). Other labels as for Fig. 1. The average free-running period ($\bar{\tau}$) of the population rhythm is 23.0 hours, and the average step size (ss) of the successive fission bursts is 1.88.

circadian systems—and in autotrophically grown wild type *Euglena* (*2*)—to damp out.

The fact that the P_4ZUL mutant can be synchronized by LD cycles when grown at 19°C (Fig. 1, curve A), but not when grown at 25°C (Fig. 1, curve B), leads to some interesting theoretical possibilities. One of the consequences of the chronon model (*13*) for circadian clocks is that only and all eukaryotic cells possess regulatory capacities for circadian timekeeping, but that these capabilities can be expressed only when the cells are in the circadian-infradian growth mode (GT $\geqq$ 24 hours). This phenomenon has been termed the GET effect (*7*) after the *Gonyaulax* (*5*), *Euglena* (*2*), and *Tetrahymena* (*7*) systems, since in each of these organisms a single transition from bright to dim illumination (switch-down) occurring during the infradian growth phase of an exponentially increasing culture is sufficient to elicit a free-running, circadian rhythm of cell division.

Our data for the mutant further support (although they do not demand) this hypothesis: as predicted, entrainment did not occur at 25°C when the GT was only 10 hours (ultradian mode), but was observed at 19°C when the overall GT was increased to about 24 hours (Fig. 1). Furthermore, a single switch-up in irradiance (Fig. 3) was sufficient to generate a persisting rhythm of cell division in cultures that had been exponentially increasing in the circadian-infradian mode (*7*) at 19°C.

It may well be that the paucity of reports of light-induced synchrony in heterotrophically grown unicells may be due to the fact that at commonly used laboratory temperatures (21° to 25°C) cell division may be taking place so rapidly that the rhythm cannot be expressed. Indeed, we have found that the rhythm can be entrained by a *LD*: *10*,14 cycle in the wild type of *E. gracilis* Klebs (Z strain) when the cells are grown heterotrophically at 19°C (but not at 25°C) on either the low *p*H medium or on a 0.02*M* sodium acetate medium (*14*); the synchrony persists for at least several cycles in continuous darkness. Similarly, Mitchell reports (*15*) LD-induced synchronization of an ultraviolet-bleached mutant of this same strain also maintained on acetate, although it is not yet clear whether the observed synchronous cell division in this case will persist under constant conditions.

In addition, reports from our laboratory (*10*, *16*) concerning the breakdown of division synchrony in autotrophic cultures of wild type *Euglena* maintained on a minimal salt medium in LD at 25°C (GT of 24 hours) by either exposure to continuous illumination (at 3500 lux) or by the addition of 0.025*M* sodium acetate, 0.006*M* ethanol, or 0.34*M* L-glutamic acid to the medium are also consistent with the circadian-infradian "rule" (*7*) that the source of constant illumination or the introduction of utilizable, exogenous carbon resulted in a decrease in the GT to 13 to 15 hours (that is, constituting a switch to the ultradian growth mode), which, in turn, obliterated the cell division rhythm.

In conclusion, our results with the P_4ZUL mutant corroborate our thesis that a cellular circadian clock in some manner periodically gates cell division in *Euglena* grown under appropriate conditions which allow for the expression of this rhythm. Of course, this hypothesis is at best only a superficial description of what is surely a complex phenomenon; we do not yet know the nature or location of the photoreceptor system that is implicated, the identity of the molecular components of the postulated underlying biological clock, or the manner in which information regarding period and phase is transduced from this circadian oscillator to the observed rhythm of cell division. It is not clear to what extent interaction among individual cells in a population of oscillators may contribute to the extended persistence of the rhythm under constant conditions (*1*, *17*). Nevertheless, we believe that the photosynthetic mutant, as well as other strains of *Euglena* that lack chlorophyll and plastids, provides useful experimental systems.

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Senescence and Genetic Load: Evidence from Tribolium

Abstract. *After 40 generations in which young adults were killed shortly after the onset of reproduction, strains of* Tribolium castaneum *with significantly decreased median longevity evolved. These findings support the hypothesis that the longevity of a species is controlled by genetic factors, and represents a compromise between selection for longer reproductive period and the limit set by environmental hazards.*

Edney and Gill (*1*) have discussed possible reasons behind differences in longevity (onset of senescence) among organisms. Elaborating on earlier models (*2*) they reason that the intrinsic, genetically controlled longevity of each species must have developed as a compromise between the forces tending to prolong it (for increased reproduction) and hazards tending to curtail it (random accidents and environmentally induced senescence, including mechanical deterioration of the organisms). This hypothesis could be tested in two ways—rearing organisms in an environment in which hazards are reduced should eventually result in longer lived populations, although this may take some time in view of the genetically programmed life-span