feron than those produced in similar cultures stimulated with antigen. Despite the apparent lack of relation between the degree of blastogenesis and interferon production, our data suggest that only those concentrations of PHA or antigen which stimulate blastoid formation are effective inducers of interferon. Maximum synthesis of antigen-induced interferon occurred between 4 to 7 days in culture, while maximum production of PHA-induced interferon occurred within the first 4 days in culture. These intervals correlate with the appearance of blastoid cells in these cultures.

A previous study with viruses has indicated that immune recognition mechanisms may generally function as an enhancer of interferon production (*4*). Our study with nonviral antigens shows that such mechanisms may also function as inducers of interferon. It has not yet been shown that this mechanism is also operative in vivo. Our observations, however, indicate that interferon induction is part of the immune response. These results suggest that the immune induction of interferon may also have a role in vivo.

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Cell Division: A Second Circadian Clock System in Paramecium multimicronucleatum

Abstract. *Light-dark cycles entrain a pattern of division in a population of individually isolated cells cultured in excess nutrients at 14°C. This pattern persists for at least 8 days in continuous dim light. Both clones which do and which do not express a circadian rhythm of mating-type reversals can be entrained. The phase is a clonal characteristic.*

Acyclic cells in *Paramecium multimicronucleatum*, syngen 2, are homozygous for a recessive allele (*c*) and possibly represent a mutant without a circadian clock. They do not express the circadian rhythm of mating-type reversals that cyclic cells with the dominant allele (*C*) express (*1*). Because circadian rhythms of cell division have been reported in a number of Protista (*2*), an assay for a rhythm of cell division in this system was developed. Cell division is used as a second circadian rhythm to help delimit the point of the genetic block.

Cells were assayed by making periodic observations on a fixed population of 118 cells individually isolated in excess food and at a constant temperature (14°C). Temperature, which limits the number of divisions to less than one a day but has no influence over time of division, was controlled by constantly circulating water through a miniature plexiglass waterbath from a reservoir monitored by a thermoregu-

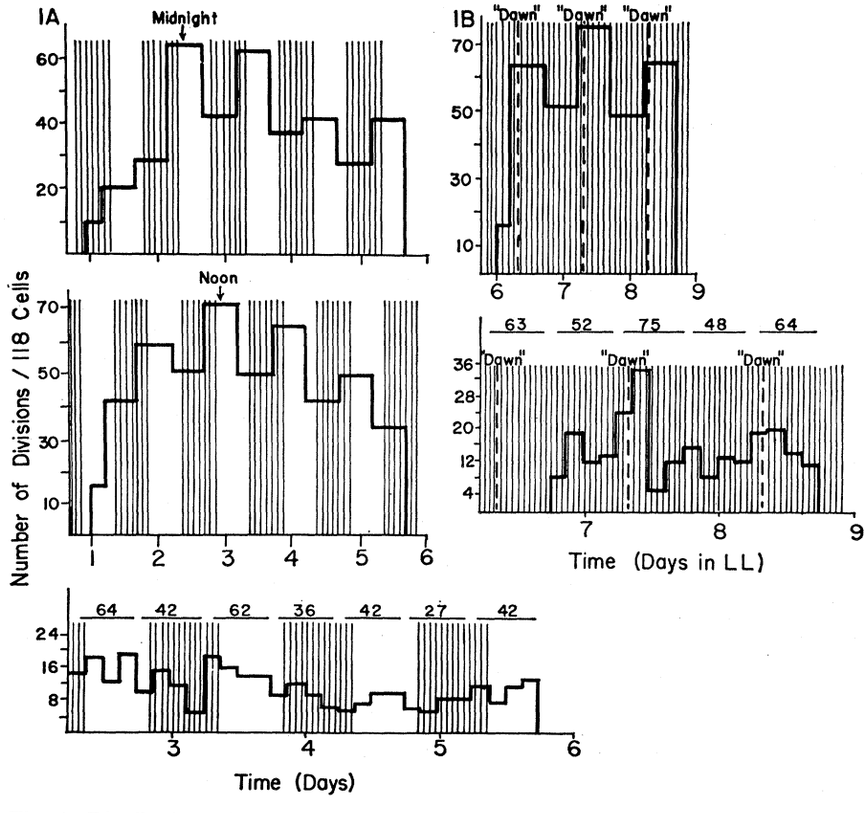

Fig. 1. Distribution of cell divisions in cyclic clone d2-1 of *P. multimicronucleatum* growing in excess nutrients at 14°C. Cells which had been growing in mass culture on the light–dark (LD) regime were individually distributed into 118 wells starting at 1400 (EDT). Counts were made, and one daughter cell of each fission was removed every 3 hours. White represents daylight (< 600 lux), and shading represents the night-light (< 60 lux). (A) Entrainment response in two regimes of LD 12 : 12 (600 lux : 60 lux) which differ by 12 hours in the time of the daily onset (1000, center; or 2200, upper chart) of daylight. The 3-hour counts, totaled from 0800 to 2000 and from 2000 to 0800, are summarized in the top two charts. The bottom chart shows the actual 3-hour counts and the 12-hour total (above the bar) for the top chart. (B) Circadian rhythmicity of cell division for the last 3 of 8 days' growth in constant dim (60 lux) light (LL) after the entrainment seen in A (center). Counts plotted as in (A).

lator. Cells were counted in place through a binocular microscope mounted so it could travel either along the columns or the rows of wells. Population size was kept constant by removal of one daughter cell at each fission. A "night light" mounted underneath the waterbath was on continually, and clock-controlled daylamps were mounted overhead in water-cooled jackets (*3*). The only difference in two setups was that "dawn" (when overhead lamps came on) occurred at 1000 (EDT) in one box and at 2200 in the other. Because a light-dark cycle acts as an entraining agent (zeitgeber) in circadian clock-controlled systems (*4*), cells were exposed to repeated cycles of bright and dim light to see if this would influence the time of division.

Cells homozygous for the cyclic allele do entrain (Fig. 1A). After the initial effect which remains unexplained but is usually seen after any transfer, the cells divided more during their subjective day than they did during their night in each light box. In the observer's day, the count in one series was high whereas the count in the other was low. This indicates that the cells do respond to the zeitgeber. The entrained pattern of peaks of division during the subjective day persists without the day-night cues while cells are growing in continuous dim light (Fig. 1B). The individual 3-hour counts for one of the light–dark (LD) and one of the continuous dim-light (LL) series are also shown. Clearly, without refinements, this is not a method for obtaining synchronous cell divisions because cells are dividing at all times. Because this assay is tedious, it is not optimum for clock studies. Cyclic cells (those homozygous for the cyclic allele) do, however, entrain, and the rhythm persists in continuous light so the assay is adequate to test the acyclic mutants for a second rhythm.

Next, three clones, acyclic for mating-type reversals, were tested for the rhythm of cell division (Fig. 2A), and they too entrain. The first several days in continuous dim light in one of these clones are also shown. After the transfer effect has been expressed, the entrained pattern emerges. Thus cells termed "acyclic" for mating-type reversals show a circadian cyclicity in cell division.

Unlike cyclic clone d2-1, all three acyclic clones have peaks of division at night. To determine if being "day-active" is a characteristic of clone d2-1 or of the cyclic genotype, two other cyclic clones were tested and d2-1 was retested a month later with the result (Fig. 2B) that only clone d2-1, among the six clones tested, shows the higher rates during the subjective day.

Two different physiological systems (cell division and mating-type reversals) in *P. multimicronucleatum* can be clock-controlled but these are mutually exclusive since only nondividing cells (slightly starved) become sexually reactive. Many physiological processes may under appropriate, but normal, conditions fall under the control of the clock. Other cellular-control processes may override such control (for example, fission rates under optimum nutrient and temperature conditions).

That some cells can express the cell division rhythm but not the rhythm of mating-type reversals suggests either (i) that, if the block is in the clock, each clock-controlled system in a single cell is a unique system, and there is no "master clock" or (ii) that the genetic block is not in the clock but is at the link between clock and mating-type expression.

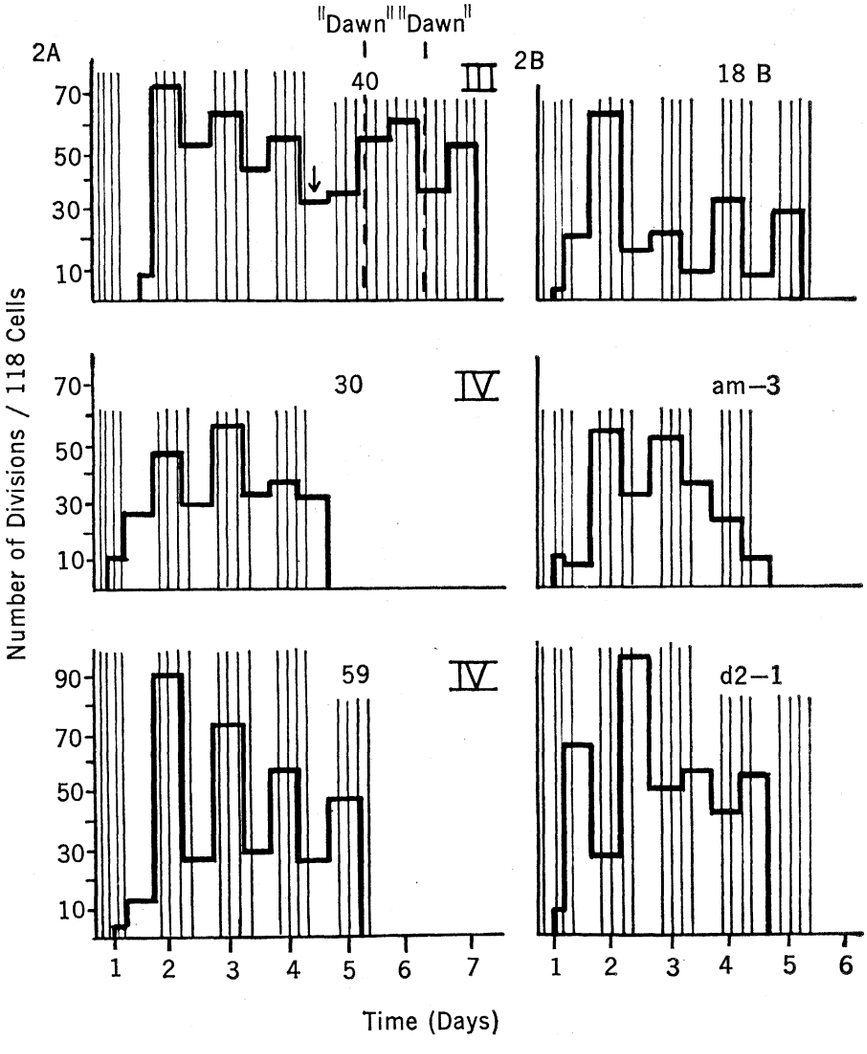

Fig. 2. Distribution of cell divisions in six different clones of *P. multimicronucleatum* growing in excess nutrients at 14°C. Cells were distributed at 1400 (EDT). They were exposed to LD 12 : 12 (600 lux : 60 lux) with daylight beginning at either 1000 or 2200. Total population was kept constant by removal of one daughter cell after each fission. Twelve-hour summary counts are plotted. (↓) Represents the time of transfer to new trays and fresh nutrient. (A) Cells are homozygous (*c*/*c*) for the acyclic allele (do not express the circadian rhythm of mating type reversals). Each plot indicates clone number and mating type (Roman numerals). (B) Cells are homozygous (*C*/*C*) for the cyclic allele (do express the rhythm of twice daily reversal of mating type). Clone numbers are given.

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Leishmania braziliensis Isolated from Sloths in Panama

Abstract. *Two edentates, the two-toed sloth* Choloepus hoffmanni *and the three-toed sloth* Bradypus infuscatus, *infected with* Leishmania *were found in Panama. The rates of infection were 14.1 and 1.3 percent in* Choloepus *and* Bradypus, *respectively.* Leishmania braziliensis sensu lato *was cultured from skin, blood, spleen, liver, or bone marrow of 13 sloths often from two or more tissues from the same animal. This strain is indistinguishable from* Leishmania *strains isolated from humans in Panama.*

As part of a study of human cutaneous leishmaniasis in the Republic of Panama, the local mammalian fauna has been investigated in the search for reservoir hosts. Until now natural infections of leishmaniasis have been reported (*1*, *2*) in six different genera of forest mammals belonging to the orders Rodentia, Carnivora, and Primates. Natural leishmanial infections have been demonstrated with relative frequency in sloths (order Edentata).

The first case found was a three-toed sloth, *Bradypus infuscatus* Wagler, collected in an area from which human cutaneous leishmaniasis has not been reported for several years. A promastigote (leptomonad) flagellate was cultured from the heart blood, liver, spleen, and from one out of three samples of skin. All four cultures, both original isolations and transfers at the third passage, were intradermally inoculated on the noses of 18 golden hamsters. One died within a few days; the others became infected. Within 2 to 3 weeks after inoculation, a swelling typical of experimental cutaneous leishmaniasis in these animals appeared at the site of inoculation (Fig. 1), from which the tissue stage or amastigote form of the parasite (Leishman-Donovan body) was demonstrated in great numbers. Both swelling of the hamsters' noses and morphology of the parasite in stained smears were indistinguishable from those obtained under similar experimental conditions with Panamanian strains of *Leishmania braziliensis sensu lato* isolated from humans.

From November 1967 to June 1968, 162 wild sloths were examined. This includes 77 (38 females, 39 males) *B. infuscatus* and 85 (31 females, 54 males) two-toed sloths *Choloepus hoffmanni* Peters from central Panama.

Usually within a week of their capture, sloths were bled from the heart in order to make blood cultures; at this time thick and thin blood smears were made in most cases; part of the blood was kept without anticoagulant in order to obtain clot cultures later. After bleeding, 2 or 3 samples of skin were obtained by biopsy and cultured (*2*). The nose, jaw, and ears were the main sites of skin biopsies; ear biopsy involved the edge of the pinna as well as skin. During autopsy, cultures were made from liver and spleen, and in certain cases, also from bone marrow. Every strain of hemoflagellate cultured was examined with phase-contrast illumination with attention given especially to the morphology and motility of parasites. At least two hamsters were inoculated on the nose with about 15×10^6 cultural forms of each strain, or substrain, in cases where more than one positive culture was obtained. After they were inoculated, hamsters were observed during a period of a few weeks for swelling on the nose. Skin smears were made from animals with such swellings about day 20, and later in those hamsters that showed no gross indications of infection; in these cases, several skin smears were usually made. No animals with negative skin smears were considered to be uninfected until one or two skin cultures were examined from the site of inoculation.

From 24 *C. hoffmanni* and three *B. infuscatus*, promastigote flagellates were cultured in 13, 11, 9, 8, and 2 animals from blood, spleen, liver, skin, and bone marrow, respectively. Positive cultures made from different tissues in the same animal were frequent. Among the 27 isolates we can distinguish the following: (i) A rather polymorphic promastigote flagellate that infects hamsters when it is inoculated intradermally on the nose was isolated from 13 sloths, 12 out of 85 *C. hoffmanni* (14.1 percent) and one out of 77 *B. infuscatus* (1.3 percent). This parasite is considered to be *L. braziliensis s. lat.*, since all 13 isolates are indistinguishable, both morphologically and by their infectivity in the hamster, from the etiological agent of human cutaneous leishmaniasis in Panama. The other 14 isolates, which failed to infect hamsters, and which seem to include at least two different parasites, are still under study. (ii) One of them, isolated from 11 sloths (10 *C. hoffmanni*, 1 *B. infuscatus*) and mostly from blood, is a promastigote somewhat larger than *L. braziliensis*. Some strains frequently show in culture individuals with a long slender elongation of the posterior end. A similar feature was described by McConnell (*3*) in connection with promastigotes cultured from the gut of Panamanian phlebotomine sandflies. (iii) The three remaining strains were cultured only from blood clots. One, from *C. hoffmanni*, is morphologically similar to *Leishmania*; another flagellate from a specimen of each sloth species is markedly different morphologically from any of the other promastigotes isolated from sloths.

The sloths found naturally infected with *Leishmania* were of both sexes and different ages. Although in no case was there any gross sign of infection, the skin of 8 out of 13 sloths yielded positive cultures. In one case the parasite was isolated not only from liver and spleen, but also from all three samples of skin that were cultured; similarly, in another animal results were positive in two skin cultures, as well as in the spleen culture. In two more animals, two out of three skin cultures were positive.

Fig. 1. Characteristic swelling of hamster's nose as a result of intradermal inoculation with a sloth strain of *Leishmania braziliensis s. lat.*