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Evidence for a circaseptan and a circasemiseptan growth response to light/dark cycle shifts in nucleated and enucleated *Acetabularia* cells, respectively

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ABSTRACT **Nucleated as well as enucleated *Acetabularia mediterranea* cells were subjected to 14 different patterns of shifts in a regimen of 12 hr of light alternating with 12 hr of darkness in four 30-day long experiments. With one exception, which might be due to a circannual modulation, these experiments showed that nucleated cells had maximal growth rates when a shift was performed every 7th or 15th day. In enucleated cells, maxima were observed on shift schedules that were about 3–4 days rather than about 7 days apart. The results indicate that in the unicellular green alga *Acetabularia* a rhythm of about 7 days (circaseptan) exists and that removal of the nucleus results in a circaseptan frequency multiplication.**

A large number of organisms exhibit circadian (≈24-hr), (1–4), circatrigintan (≈30-day) (5, 6), and circannual (≈1-year) (6, 7) rhythms. Within the past decades, evidence has accumulated that there is also a built-in circaseptan (≈7-day) rhythm. This evidence comes from various sources (6, 31–43).

Observations over a full decade on the urinary 17-ketosteroid excretion, by a healthy man, revealed that the excretion of these hormonal metabolites peaked consistently on Wednesdays or Thursdays. Following the use of testosterone suppositories, which may have desynchronized the adrenal from the pineal gland, these about weekly peaks in 17-ketosteroid excretion recurred in slightly less than a week (6). This ≈7-day period has no known environmental counterpart: it is likely endogenous.

Supporting evidence for ≈7-day rhythmicity induced by a single stimulus is the rejection of murine kidney or heart or human kidney allografts that occurs at intervals of about 7 days (e.g., on post-operative days 7, 14, 21, 28, etc.) whether the operations are done on Mondays, Wednesdays, or Fridays (8, 42). A similar cycle was observed for the mortality of mice infected with malaria. Such mice would preferentially die on the 7th, 14th, or 21st day (9).

Circaseptan rhythms are also observed with the so-called frequency responses that are demonstrated by an environmental 24-hr cycle, such as a regimen of alternating 12-hr light and dark intervals or a regimen of optimal and suboptimal environmental temperature, that is manipulated by prolonging a single span, e.g., of light or optimal temperature from 12 to 24 hr and by implementing this manipulation at various intervals ranging from 2 to >15 days. Under these conditions, the life spans of the face fly *Musca autumnalis* (10) or the springtail *Folsomia candida* (11) are markedly longer when shifts are carried out at intervals that are 7 days apart. A circaseptan rhythm is also found in *Gonyaulax polyedra* as a feature of cell-to-cell communication (12, 36).

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Thus, it was of interest to see whether a circaseptan rhythm also existed in the marine green alga *Acetabularia* and, in particular, to determine the effect of enucleation on any circaseptan rhythm identified. *Acetabularia* is an organism of choice in cell biology since many of its functions, including its circadian rhythms, can be studied easily before and after simple enucleation (13–16).

MATERIALS AND METHODS

The unicellular siphonous marine green alga *Acetabularia (A.) mediterranea* was grown in an artificial medium as described (17) and cells were used when they were ≈30 mm long. Enucleation was performed by cutting off the rhizoid (18). All of the cells survived this surgery.

Four experiments were performed with nucleated and with enucleated cells of *A. mediterranea*. The experiments were begun in April 1982, in August 1982, in April 1983, and in September 1983 under similar conditions, except for the season.

Each experiment was performed on sets of nucleated and enucleated cells. Each set was composed of 15 groups; each group contained 30 cells. Each group was in a separate jar at 21 ± 3°C containing artificially defined sea water.

Each group was exposed to a regimen of alternating 12-hr light and dark intervals. In a control jar (jar 1), this regimen was continued for the entire duration of the experiment. Jars 2–15 from each set were exposed to a 12-hr (180°) shift of the light/dark cycle implemented by extending the dark cycle by 12 hr that was alternated at the next shift by extending the light cycle by 12 hr. During 30 days, such shifts occurred at regular intervals that varied from every 2nd to every 15th day (Table 1), resulting in 14 different shifting frequency schedules (one set of nucleated and enucleated cells was included in each shift frequency). The length of the cells was measured before the start of each experiment and at days 10, 20, and 30 in the first experiment and at or near days 20 and 30 in the next three experiments.

During the light phase the cells were illuminated with a fluorescent lamp at an intensity of about 2500 lux.

Growth measurements were made during the light phase. The total duration of these measurements was about 5 hr. Any handling effects remain unevaluated.

All changes in length are based on groups of cells differing only in their light/dark cycle regimen. Intergroup differences in growth increment were analyzed for fit with a 7-day period by single-cosinor (19), linear-least-squares rhythmometry (20) bracketing this period, and by the concomitant fit of both cosines and linear trends (21) in the spectral region examined. In addition, nonlinear-least-squares analyses (20) were performed to find the best-fitting periods with the 95% confidence limits.

Table 1. Scheme of schedule shifts of the light/dark regimen

Interval between shifts, days	Experimental day																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Control	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL
2	DL	DD	LD	LL	DL	DD	LD	LL	DL	DD	LD	LL	DL	DD	LD	LL	DL	DD	LD	LL	DL	DD	LD	LL	DL	DD	LD	LL	DL	DD
3	DL	DL	DD	LD	LD	LL	DL	DL	DD	LD	LD	LL	DL	DL	DD	LD	LD	LL	DL	DL	DD	LD	LD	LL	DL	DL	DD	LD	LD	LL
4	DL	DL	DL	DD	LD	LD	LD	LL	DL	DL	DL	DD	LD	LD	LD	LL	DL	DL	DL	DD	LD	LD	LD	LL	DL	DL	DL	DD	LD	LD
5	DL	DL	DL	DL	DD	LD	LD	LD	LD	LL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LL
6	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LL	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LL	DL	DL	DL	DL	DL	DD
7	DL	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LD	LL	DL	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LD	LL	DL	DL
8	DL	DL	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LD	LD	LL	DL	DL	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LD
9	DL	DL	DL	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LD	LD	LD	LL	DL	DL	DL	DL	DL	DL	DL	DL	DD	LD	LD	LD
10	DL	DL	DL	DL	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DD
11	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LL	DL	DL	DL	DL	DL	DL	DL	DL
12	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LL	DL	DL	DL	DL	DL	DL
13	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LL	DL	DL	DL	DL
14	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LL	DL	DL	
15	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DL	DD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LD	LL

D, 12 hr of dark. L, 12 hr of light.

RESULTS

In the first study, performed in April 1982, not only did some groups of cells grow faster than others (Table 2), but the differences in growth increment as a function of the interval between shifts also showed a periodic (rather than a linear or random) pattern. Data from the first study of a population of intact *Acetabularia* cells, maintained under controlled conditions (Table 1), showed a circaseptan rhythm in growth response to schedule shifts (Fig. 1). A linear trend that is statistically significant remains unexplained. The variability around the trend is not random but can be represented by a cosine curve best fitting the data.

In a single-cosinor display, the 95% confidence region of the directed line representing the joint amplitude–acrophase estimate does not cover the center of the plot, whereby the assumption of zero amplitude (i.e., no rhythm) is rejected (Fig. 2). By a linear-least-squares analysis, a prominent 7.5-day period is found at 20 and 30 days of the experiment. By further nonlinear-least-squares analysis, 7.4-day and 7.3-day periods, respectively, with a 95% confidence interval extending from 5.8 to 9.6 days or from 5.5 to 10.2 days were found at experimental days 20 and 30, respectively (Table 3).

A striking result was obtained when enucleated *Acetabularia* cells were used. The circaseptan rhythm of the nucleated cells changed to a circasemiseptan rhythm (Figs. 3 and 4) with a period of 3.7 days (Table 3). As with the intact, nucleated cells, the rhythm of the enucleated cells was also statistically significant.

No statistically significant circaseptan or circasemiseptan component was apparent in the second experiment begun on August 4 and 5, 1982, for nucleated and enucleated cells, respectively. This study was to serve as a replicate of the first experiment. Although there were no differences at the start, the results were not the same. Linear-least-squares analysis suggests a period of about 11 days for the enucleated cells.

Although this length of time is included in a definition of circaseptan, such an infradian (low frequency, ref. 31) component will have to await scrutiny with more extensive data from shifts not only at the intervals studied here but also at longer intervals, to cover at least two such infradian periods.

The first "Spring" experiment was repeated in April 1983. The results of this third experiment supported the data from

Table 2. Growth rates of nucleated and enucleated cells of *Acetabularia* during 30 days on various light regimen shifts (experiment 1)

Interval between shifts, days	Nucleated cells			Enucleated cells		
	Length, mm		Growth, mm	Length, mm		Growth, mm
	Day 0	Day 30		Day 0	Day 30	
2	25.4 ± 1.2	43.2 ± 5.8	17.8	25.2 ± 1.6	28.6 ± 3.1	3.4
3	25.5 ± 1.4	40.9 ± 7.0	15.4	25.7 ± 1.4	29.4 ± 2.8	3.7
4	24.6 ± 1.4	41.9 ± 6.3	17.3	24.5 ± 1.5	27.4 ± 2.5	2.9
5	25.2 ± 1.6	45.3 ± 7.1	20.1	25.2 ± 1.6	27.8 ± 2.4	2.6
6	24.8 ± 1.6	44.4 ± 7.2	19.6	25.2 ± 1.6	27.9 ± 2.0	2.7
7	24.8 ± 1.3	46.0 ± 8.5	21.2	25.0 ± 1.6	28.7 ± 2.1	3.7
8	24.9 ± 1.3	43.0 ± 8.9	18.1	25.1 ± 1.5	27.8 ± 2.8	2.7
9	24.9 ± 1.3	45.3 ± 8.3	20.4	25.5 ± 1.7	28.9 ± 3.2	3.4
10	24.8 ± 1.2	41.0 ± 7.2	16.2	25.2 ± 1.6	28.8 ± 2.7	3.6
11	25.1 ± 1.5	44.1 ± 6.3	19.0	25.4 ± 1.6	29.3 ± 2.8	3.9
12	24.5 ± 1.4	45.5 ± 8.6	21.0	25.2 ± 1.8	28.5 ± 2.3	3.3
13	24.9 ± 1.5	46.5 ± 7.7	21.6	25.1 ± 1.9	27.0 ± 2.9	1.9
14	24.5 ± 1.4	45.6 ± 7.2	21.1	25.2 ± 1.8	28.2 ± 2.8	3.0
15	24.7 ± 1.2	47.9 ± 9.9	23.2	25.0 ± 1.5	28.2 ± 2.6	3.2
Unshifted control	25.5 ± 1.2	40.5 ± 6.1	15.0	25.1 ± 1.6	28.5 ± 3.0	3.4

The growth rates are given in mm as mean ± SD.

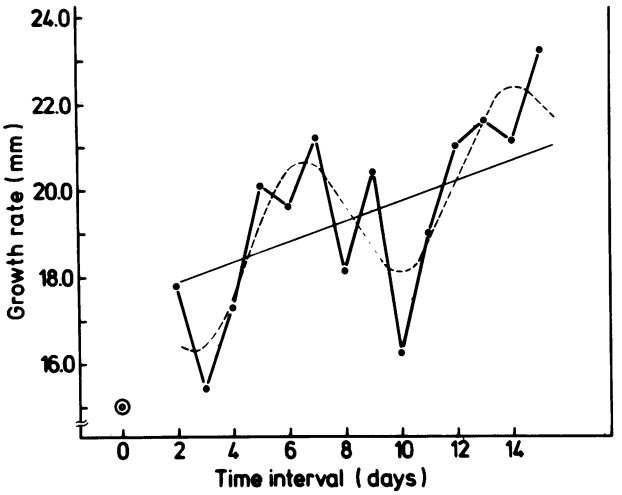

FIG. 1. Growth rates of nucleated *A. mediterranea* cells on various light/dark regimen shifts. The experiment was started in April 1982. The interval between consecutive light-period shifts in days is plotted against the growth rates in mm over the 30-day experiment. Solid circles, experimental (shifted) groups. Open circle, control (unshifted) group. Dashed line, best-fitting cosine curve. Thin line, calculated trend.

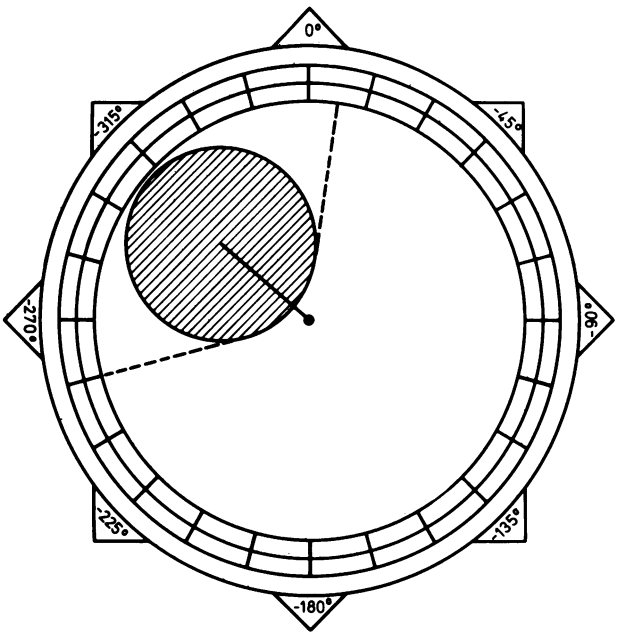

FIG. 2. Single-cosinor treatment of data obtained with nucleated cells (360° ≅ 7.5 days) (see Fig. 1). The hatched area represents 95% joint confidence region (from fitted 7.5 day curve) for amplitude (vector length) and acrophase (vector orientation in relation to circular scale; scale divisions are of 7.5 hr; 0°, day 1 of study). The failure of the confidence region to cover center of display (pole) leads to rejection of "no rhythm" possibility.

April 1982 (Table 3). The linear trend and the cosine curve fit were statistically significant: the no-rhythm assumption was rejected below the 5% level of statistical significance in favor of a circaseptan rhythm for the nucleated cells and a circasemiseptan response for the enucleated cells, as seen by experimental day 30.

For nucleated cells, a linear-least-squares analysis gave the best fit at 180 hr (7.5 days). As in the first experiment, a trend was found. Moreover, this trend had a negative slope in the third experiment as compared to a positive slope for the trend in the first one. This difference notwithstanding, the circaseptan change around the trend was fully replicated. The growth rate fit a 7.5-day cosine curve and allowed us to reject the zero-circaseptan-rhythm possibility.

As in the April 1982 experiment, the enucleated cells in experiment 3 showed a statistically significant circasemiseptan bioperiodicity. This rhythm was statistically significant below the 5% level (Table 3).

A fourth study, performed in the fall of 1983, also revealed a circaseptan growth response in intact *Acetabularia* and a circasemiseptan response in the enucleated cells (Table 3).

DISCUSSION

The unicellular green alga *Acetabularia* exhibits a circaseptan rhythm in its response to illumination-regimen shifts. Since the circaseptan period emerges from the shift intervals independently of the day of the week, the possibility that the rhythmicity is due to an unknown exogenous weekly event is excluded. The endogenous character of the circaseptan rhythm raises the question as to whether it is related to the well-established circadian rhythm in this organism and, if so, in which way.

It is of practical and also of theoretical interest that after enucleation the cells still exhibit maximal growth on light/dark regimens that involve schedule shifts at frequencies half-a-week apart. This finding suggests that the circaseptan rhythm does not depend on the nucleus. It is even more interesting to note that, in the enucleated cells, the maxima appearing about 7 days apart are supplemented by additional maxima after 3.5 days, 10.5 days, etc. Such circasemiseptan rhythms are also found in the glutathione content of the anucleate human platelet reminiscent of the growth of the enucleated *Acetabularia* (22). This might indicate that, due to enucleation, a previously partially suppressed 3.5-day rhythm is desuppressed, but it might also

Table 3. Statistical evaluation of periodicities in growth increment observed in four experiments performed on nucleated and enucleated *Acetabularia* cells

Exp.	Start of experiment (month/year)	Condition	No.	Period, days LLS	NLLS	CI	*P*
1	4/82	Nucleated	14	7.5	7.3	(5.5, 10.2)	0.011
		Enucleated	14	4.0	3.9	(3.5, 4.5)	0.028
2	8/82	Nucleated	14	11.5	11.5	—	0.410
		Enucleated	14	9.5	9.5	—	0.122
3	4/83	Nucleated	14	7.5	7.4	(6.1, 9.3)	0.019
		Enucleated	14	3.5	3.6	(3.3, 4.2)	0.047
4	9/83	Nucleated	14	8.0	8.2	(7.0, 10.1)	0.041
		Enucleated	14	4.0	4.1	—	0.227

LLS, linear-least-squares spectrum; NLLS, nonlinear-least-squares spectrum (17); CI, confidence interval for 95% probability.

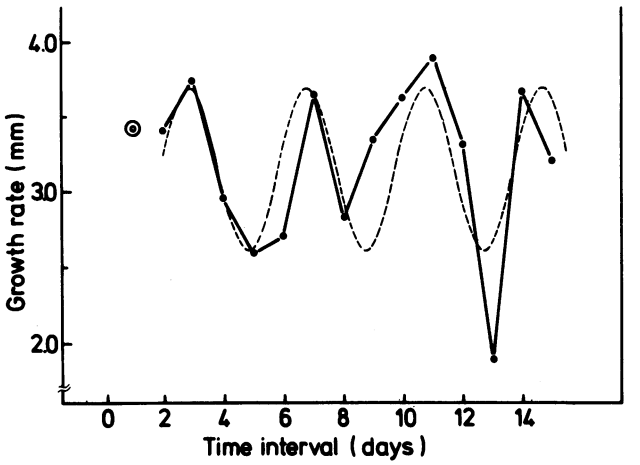

FIG. 3. Growth rates of enucleated *Acetabularia* cells on various light/dark regimen shifts. The experiment was started in April 1982. The interval between consecutive light periods in days is plotted against the growth rates in mm over the 30-day experiment. Solid circles, experimental (shifted) groups. Open circle, control (unshifted) group. Dashed line, best-fitting cosine curve.

mean that in the absence of the nucleus a preexistent circaseptan rhythm is subjected to frequency multiplication. If that is true, it could imply that the suppression is associated with nuclear function (i.e., transcription) since replication can be excluded in an *Acetabularia* cell during the vegetative phase of the life cycle. A suppressive effect of the nucleus on the life-cycle-dependent "regulation" of a dCMP deaminase has also been shown in *Acetabularia* (23).

Of the four experiments performed, one did not show a statistically significant circaseptan rhythm. Perhaps the manifestation of the circaseptan rhythm is subjected to annual variation, i.e., to a circannual rhythm. By no means would this be a surprise since circannual changes of circadian oscillations are well established (24). To the best of our knowledge, however, in *Acetabularia* no major annual variations in the expression of circadian rhythms have been reported.

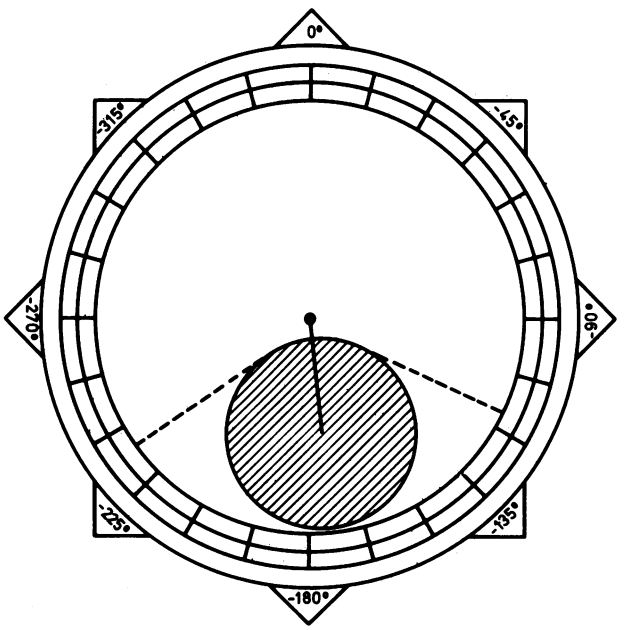

FIG. 4. Single-cosinor treatment of data obtained with enucleated cells (see Fig. 3) (360° ≅ 3.5 days). The circular scale now has divisions of 3.5 hr. Failure of hatched 95% confidence region to cover pole leads to rejection of "no rhythm" possibility (see Fig. 2). The least-squares cosinor method was used, but similar results were obtained with nonlinear-least-squares analysis (20). Data from experiments 1 and 2 were corrected for a linear trend before applying the analytical methods mentioned above.

Some notable exceptions, such as transplant rejection notwithstanding (8), circaseptan rhythms usually do not exhibit a substantial amplitude and, therefore, need thorough statistical evaluation. This is a drawback, particularly if the nature of this rhythmicity is to be attacked in individual cells. One possible experimental approach, however, might be to stably transform *Acetabularia* cells (25) by microinjecting (26) *Drosophila* gene constructions that are known to affect circadian and ultradian rhythms (27–30). The transformed progeny of such *Acetabularia* cells might then be used to determine whether a change in a circadian rhythm would also affect a circaseptan rhythm.

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