ABOUT-WEEKLY CHANGES IN ELECTRICAL POTENTIAL, CHLOROPLAST MIGRATION AND OXYGEN PRODUCTION IN *ACETABULARIA* GROWN UNDER CONTINUOUS EXPOSURE TO LIGHT

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Abstract

The presence of an about-weekly (circaseptan) component was examined in the giant unicellular alga *Acetabularia* and compared with its circadian rhythms. The analysis was based on the archival data collected by the late Hans-Georg Schweiger of Germany. Changes in electrical potentials, chloroplast migration and oxygen production were investigated and related to whether, during cultivation, this algae was exposed to continuous light (LL) or to an alternation of light and darkness at 12-hour intervals (LD 12:12). In LD 12:12 conditions, the circadian rhythm was a prominent spectral component while , in LL conditions, the circaseptan component increased. The results provide further evidence for the ubiquity of circaseptan rhytms and suggest that this component, appearing early in the course of evolution, has become anchored in the genetic makeup of living organisms.

Key words

Circaseptan rhythm, Electrical potential, Chloroplast migration, Oxygen production, *Acetabularia*

INTRODUCTION

Acetabularia has historically been used as a cellular model for experiments concerned with circadian time keeping. The persistence of circadian rhythms under exposure to continuous light (LL) has been regarded as evidence of the existence of a circadian system (*1–6*). The existence of endogenicity of an about-weekly (circaseptan) component shows that (i) the component can be amplified after the application of a single stimulus that carries no 7-day information; this may be mild, such as balneotherapy (*7, 8*), or drastic as in unilateral nephrectomy (9) or organ transplantation (*10*); (ii) it may be desynchronised from a precise 7-day schedule (free-run) under both ordinary life conditions after a heavy dose of androgens (*11*) or isolation from society (*12, 13*). Circaseptan rhythms are often prominent in relation to growth, regeneration and repair (*9, 14, 15*) and are seen prominently early in postnatal life in humans (*16–18*) as well as in rats (*19*), pigs (*20)* and crayfish (*21*). Because *Acetabularia* may have been on earth for some 500,000,000 years (*6*), it constitutes an ideal material for investigating the relative prominence of circaseptan and circadian rhythms occurring in eukaryotic life. It may also complement earlier studies on *Gonyaulax polyedra* (*22, 23*).

MATERIALS AND METHODS

The experiments were carried out at the Max-Planck-Institut für Zellbiologie in Ladenburg, Germany. After standardisation by cultivating *Acetabularia* for a week under a regimen of changing exposure to light and to darkness at 12-hour intervals (LD 12:12), the cells were transferred to LL conditions for a week or longer (up to 2 weeks). During periods of continuous exposure to light, oxygen production, chloroplast migration and/or electrical potentials were measured in each cell. Measurements were made automatically at 30-minute intervals. They were checked for agreement between the automatic record and the protocol. The latter was adjusted, in the case of discrepancies, to the former, with checks by several investigators. Data were analysed by linear-nonlinear least squares rhythmometry (*24–26*). Thus, at least a tentative estimate of the circadian-to-circaseptan amplitude ratio was possible, with the realisation that chronomes (time structures) (*14, 24*) include extracircadian components (*28*), which is an alternative view of considering extracircadian variability to be a noise (*29*).

RESULTS

Our results were based on a total number of 38,578 measurements over an experimental span of 376 days. In the LD12:12 span, the circadian rhythm constituted a principal component, although it was also possible to find statistically significant infradian changes to a variable extent. In the LL span, on the average, extracircadian variability was much more pronounced than it was in LD12:12 and it frequently predominated. Ratios of circaseptan (CS) to circadian (CD) amplitudes (A) were computed separately for each series in two ways: either at trial periods of precisely 168 and 24 hours $(CS/CD)_F$, or at nonlinearly assessed, best-fitting periods in the CS and CD ranges $(CS/CD)_{NL}$.

The CS-A/CD-A ratio in LD12:12 is, in the vast majority of cases, much smaller than one. In LL, this ratio is much larger and, on the average, approximates or exceeds one, often showing an extracircadian-over-circadian dominance. When all series were pooled into a single series (after detrending by fitting polynomials, when needed) and analysed by the least-squares spectrum, the principal component was a circaseptan rhythm. When the circaseptan amplitude was equated to 100%, the second most prominent component was the circadian rhythm, its amplitude reaching 62% of the circaseptan amplitude. The third peak in the spectrum was the circasemiseptan rhythm, with an amplitude barely over 40% of the circaseptan amplitude (*Fig. 1*). These components, validated by nonlinear least-squares rhythmometry, had one-parameter limits that did not cover either 168, 24 or 84 hours.

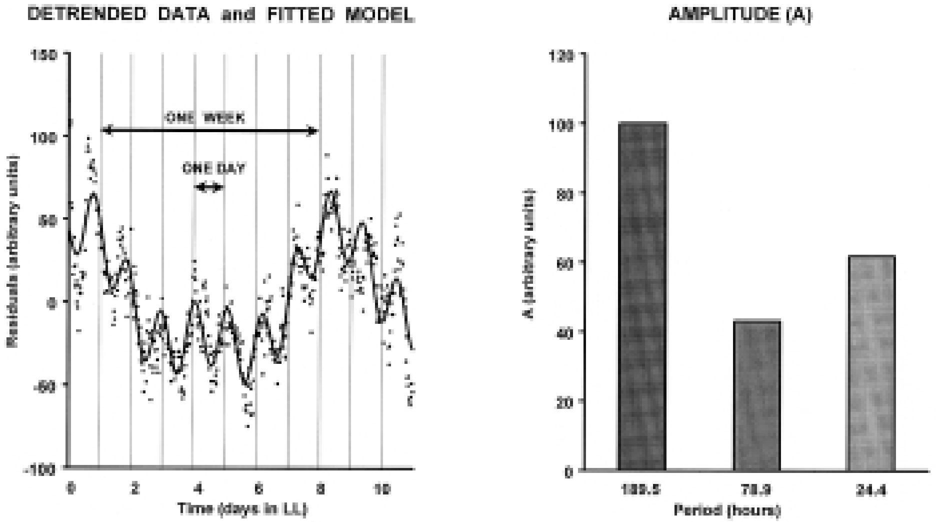

Fig. 1

Circadian and circaseptan rhythms in electrical potentials assessed in the cells of *Acetabularia acetabulum* cultivated under continuous exposure to light. The results are based on signal-averaged data from 20 cells evaluated by nonlinear spectral analysis. Note that the amplitude (A) for the circaseptan component is more prominent than those for the semicircaseptan and circadian rhythms.

DISCUSSION

Although our conclusions were based on a single or a few cycles only, sufficient information was gained from replications across experiments as well as, in some cases, by replications of several 7-day records in the same alga. The relative prominence of circaseptan vs. circadian components shown by *Acetabularia* in LL was in agreement with a similar prominence found in multicellular organisms. A chronobiologic view of a sequence of changes in ontogeny and their relation to phylogeny prompts a modification of the spatial morphological concept formulated by Walter Zimmerman as hologeny, i.e., a sequence of ontogenetic and phylogenetic changes over millions of years (*30*). We may speak of chronobiologic hologeny or „chronohologeny“ when, during the growth phase of an alga that has been on earth perhaps for half a billion years, we found a circaseptan prominence that is commonly encountered in more recent species (*31, 32*). The fact that, in the crayfish, the circaseptan rhythm remains prominent in LD12:12 for up to 6 months of life (*21*) whereas, in humans (*16–18*), mice (*19*) and pigs (*20*), it loses its dominant amplitude approximately at the end of the first month of postnatal life, shows the possibility that circaseptan rhythms are the dominant frequency of growth. The relatively long-lasting circaseptan rhythms in the crayfish may relate to the very slow growth over about 40 years (attributed to one species, *Orconectes,* claimed to live for one hundred years).

A c k n o w l e d g e m e n t s

This study was supported by the U.S. Public Health Service (GM-13981; FH), Dr. h.c. Dr. h.c. Earl Bakken Fund (FH, GC) and the University of Minnesota Supercomputing Institute (FH, GC).

This report is dedicated to Dr. Sigrid BERGER, Dr. Lübbo von LINDERN and Dr. Peter TRAUB, who made these analyses possible by providing the original edited data and by giving helpful advice. This is also to commemorate the late Hans-Georg SCHWEIGER, whom we enjoyed hosting in our Centre as an associate in research and a visiting professor.

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ASI TÝDENNÍ ZMĚNY V ELEKTRICKÝCH POTENCIÁLECH, MIGRACI CHLOROPLASTŮ A TVORBĚ KYSLÍKU PŘI TRVALÉM OSVĚTLENÍ U ŘASY *ACETABULARIA*

S o u h r n

U obří jednobuněčné řasy *Acetabularia* byla zjišťována přítomnost asi týdenní komponenty (circaseptan) a srovnávána s cirkadiánním rytmem.. Analýza byla založena na archivních datech získaných zesnulým Hans-Georg Schweigerem z Německa. Změny v electrických potenciálech, migraci chloroplastů a tvorbě kyslíku byly sledovány v podmínkách kultivace při kontinuálním světle (LL) a při střídání světla a tmy ve 12-hodinových intervalech (LD12:12). Při variantě LD12:12 byl cirkadiánní rytmus dominantní ve spektrální komponentě, v pdmínkách LL circaseptánní komponenta vzrůstla. Výsledky poskytují další důkaz pro jedinečnost cirkaseptánního rytmu a poukazují na to, že tato komponenta, objevující se v časných fázích evoluce, se zakotvila v genetické informaci živého organizmu.

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