Thalassomyxa australis Rhythmicity II. No Entrainment by Light-Dark-Cycles and Temperature Cycles?

by

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ABSTRACT

The rhythmic change between an active phase and a resting phase of the plasmodial rhizopod *Thalassomyxa australis* sustained on the diatome *Amphiprora*, is not synchronized by a 12:12 h light-dark-cycle. Likewise, 12:12 h temperature cycles of 8°C difference such as 23°/15°C, 25°/17°C, 27°/19°C are not entraining this rhythm.

INTRODUCTION

In the first publication of this series (Silyn-Roberts et al., 1986) we reported a rhythmic change in shape of an amoeboid rhizopod, *Thalassomyxa australis.* Unlike typical circadian rhythms, this rhythm lacked temperature compensation of its period length, and it was speculated that this might represent an early evolutionary stage of circadian rhythms (Silyn-Roberts and Engelmann, 1986).

Another characteristic feature of circadian rhythms is synchronization by 24-h time cues such as light-dark-cycles and temperature cycles. We were wondering whether *Thalassomyxa australis* also lacked the ability to entrain to external Zeitgeber.

MATERIALS AND METHODS

Thalassomyxa australis was kindly supplied by Grell and reared in Petri dishes of 9 cm or 5.5 cm diameter filled to 10 mm with sea water. The diatom *Amphiprora* served as a food organism. The experiments were conducted in a temperature

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controlled room. Ten white fluorescence tubes (Osram L65W/25) served as light sources and the light intensity at the level of the cultures was 1800 lux. The number of animals in the rounded-up resting phase ρ and in the active phase α was determined under the microscope at a magnification of 100 times. There were usually between 50 and 300 animals per dish, and about 3 to 8 dishes were used per experiment. From the raw data the percentage of animals in the active phase α was determined and plotted as exemplified in Figure 1. From these plots the middle of active phase (α_M, ▲) or the middle of resting phase (ρ_M, ▼) was determined (see Fig. 2). Since we did not count during the night, we had to use either the middle of the active phase (▲) or the middle of the resting phase (▼).
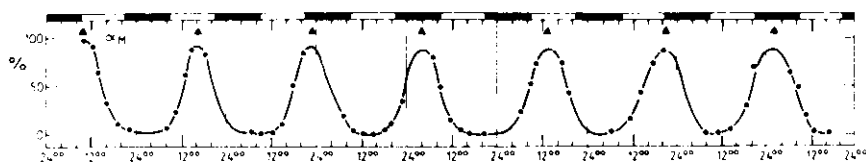

Figure 1a: Middle of active phase (α_M, ▲) of *Thalassomyxa australis* as a function of time. Nine days in 12:12 h light-dark-cycle (see schedule on top of figure). Temperature 18°C throughout the experiment. Data are derived from curves in which the percentage of amoeba of a culture in the active phase was plotted against time. Period length during the light-dark-cycle (30.8 hours) is the same as during continuous light LL.
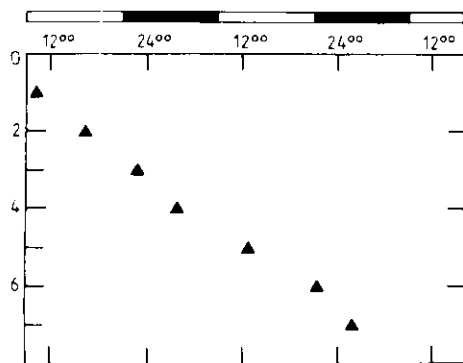

Figure 1b: Same as figure 1a, however, only middle of active phase (α_M, ▲) plotted as function of time of day (horizontal axis) during successive days (vertical axis). 12:12 h light-dark cycle shown on top of figure.

New cultures were obtained by allowing diatoms as well as *Thalassomyxa australis* to settle on cover slips, which were then transferred to new Petri dishes containing seawater. In later experiments we removed *Thalassomyxa australis* in the active phase from the Petri dishes by directing a jet of seawater from a 10 ml pipette onto the culture from which the old seawater had been removed before.

The density of food was regulated by adding seawater if too high or by adding *Amphiprora* if too low.
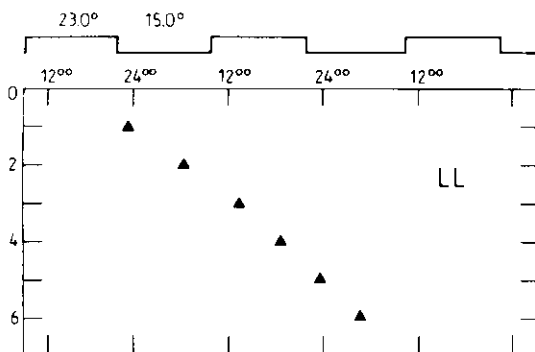
Figure 2: Example for free run of a culture of *Thalassomyxa australis* under 12:12 h temperature cycle of 23°/15°C (schedule see top of figure). Period length τ_{TC} = 30.0±0.7 h in this particular case and 30.5±0.3 h as a mean of 8 cultures. This corresponds to the period length of the rhythm of cultures kept at 19°C (τ_{19} = 30.0 hours). 19°C is the mean temperature of the 23°/15°C cycle applied.

RESULTS

1. Behaviour of *Thalassomyxa australis* under light-dark cycles

Cultures of *Thalassomyxa australis* were kept under 12:12 h light-dark-cycles at a constant temperature of 18°C. Figure 1a shows the percentage of amoeba in the active phase as a function of time. The light-dark-cycle is indicated by white and black bars on top of the graph. From Figure 1b it can readily be seen that the change in shape is rhythmic, but not synchronized by the light-dark-cycle. The period length is 30.8 h and corresponds to the one obtained at the same temperature under continuous light (Silyn-Roberts et al., 1986).

2. Behaviour of *Thalassomyxa australis* under temperature cycles

Having failed to entrain *Thalassomyxa australis* by light-dark-cycles, we tried to synchronize with 12:12 h temperature cycles of different degrees. In Figure 2 the times are indicated by ▲ at which the middle of the active phase was reached when a culture was kept in a 12:12 h cycle of 23°C alternating with 15°C. Obviously the rhythm was not synchronized by the temperature cycle, but showed an endogenous rhythm of 30.0±0.7 h in this particular case. The mean period length of measurements in 8 cultures was 30.5±0.3 h (mean and standard error).

Under a 12:12 h temperature cycle with the same 8°C temperature difference, but 2°C higher temperatures (25 alternating with 17°C) again no clear entrainment was found, as shown by the example in Figure 3. Period length was 25.5±0.4 h in this particular case and amounted to 25.0±0.3 h as a mean of 9 experiments. In 3 experiments period length under the same conditions was, however, 29.0 h.
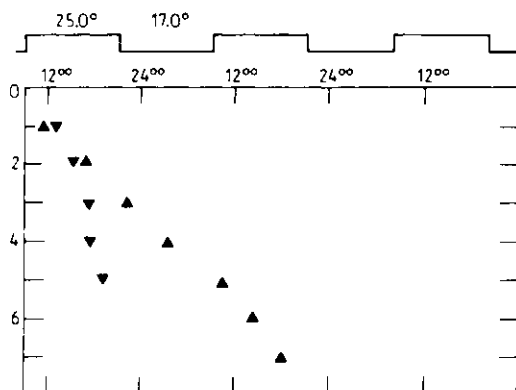
Figure 3: Same as figure 2, however temperature cycle of 25°/17°C. Period length of 3/4 of the cultures exhibit a period length τ_{TC} 25.0±0.3 h (n=9), which corresponds to the period length of the rhythm of cultures kept at 21°C (τ_{21} = 25.0 h). 21°C is the mean temperature of the 25°/17°C cycle applied. The example illustrated is marked with ▼ (in this particular case the middle of the resting phase is shown). 1/4 of the cultures show a period length of τ_{TC} = 29.0±0.7 h (n=3), and an example is shown in the figure marked with ▲ (in this particular case the middle of the active phase is shown).

Still further 2°C higher temperatures (27°C) alternating with 19°C did also fail to synchronize the cultures in their rhythmic change of shape to the 24 h cycle of the applied temperature rhythm, as exemplified in Figure 4. Period length in this particular case was 26.7±0.4 h, and the mean period for 10 cultures 27.6±0.4 h.

We have increased the temperature change from 8 to 12.5°C by using 12:12 h temperature cycles of 25.5°C alternating with 13°C and found an endogenous rhythm of 28.4±0.7 h in the particular case illustrated in figure 5, and a mean period length of 28.7±0.5 h for 3 experiments. In 3 other experiments the rhythm was more or less synchronized to the temperature cycle.

DISCUSSION

Circadian rhythms are widespread among eukaryots (Bünning, 1973) and recently have also been reported to occur in prokaryots (Mitsui et al., 1986). Characteristic features of these rhythms are the temperature compensation of the period length (Pittendrigh and Bruce, 1959) and the entrainability by external 24-h time cues such as the light-dark-cycle and the temperature cycle of the environment. The rhythmic change in shape in *Thalassomyxa australis* has been shown to lack temperature compensation and we now report also a lack of synchronization by light-dark- and temperature-cycles.
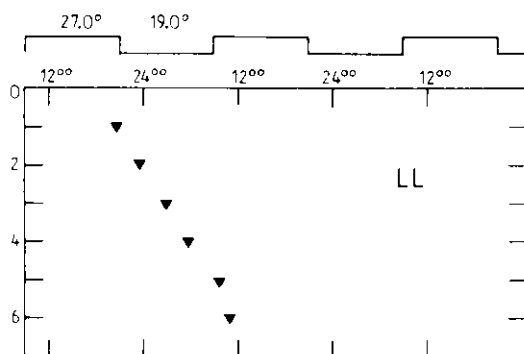
Figure 4: Same as figure 2, however temperature cycle of 27°/19°C. Period length τ_{TC} = 26.7±0.8 h in this particular case and 27.6±0.4 h as a mean of 10 cultures. This is longer than the period length at a constant temperature corresponding to the mean temperature of the 27°/19°C cycle applied (τ_{23} = 21.4 h).
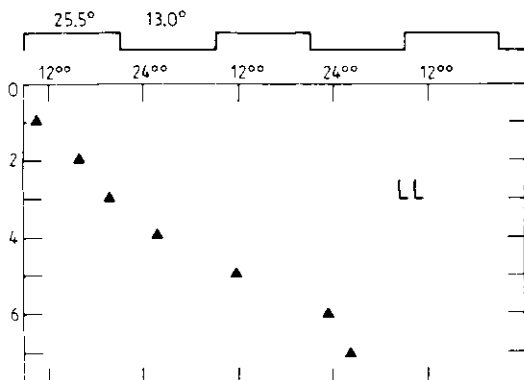

Figure 5: Same as figure 2, however temperature cycle of 25°/13°C. Period length τ_{TC} = 28.4±0.7 h in this particular case and 28.7±0.5 h (n=3), which is shorter than the period length of the rhythm of cultures kept at 19°C (τ_{19} = 30.0 h). 19°C is the mean temperature of the 25°/13°C cycle applied.

If the rhythmic change between α and p has any adaptive value in respect to the time structure of the environment, we would have expected entrainment by either the 24 h or the tidal time cues. An organism with a strong synchronization to environmental cycles could do without temperature compensation of its endogenous oscillatory system, especially if the temperature differences of the marine environment are moderate as is the case in the place where *Thalassomyxa australis* was found (Hodgkin and Phillips, 1969). However, under most conditions tried by us, synchronization was lacking. There are two conditions where some of the cultures showed indications of entrainment.

1. If the temperature cycle was chosen in such a way that the mean temperature was 21°C, only 3 of the 9 cultures showed clear free running periods. The remaining 6 cultures showed indications of synchronization after a few days of transient behaviour or signs of relative coordination. The period length at this temperature (21°C) under continuous light conditions is close to 24 h (Silyn-Roberts et al., 1986), and this might allow entrainment.
2. If the temperature differences of the temperature cycle were 12.5°C instead of 8°C, about 50% of the cultures were synchronized.

Thus, a strong temperature cycle or a temperature cycle close to 24 h might lead to some entrainment. It is unlikely, that themperature changes in the marine habitat of *Thalassomyxa australis,* the sea coast of west Australia, are as large as 12°C and therefore might not play a role as a synchronizing Zeitgeber. However, the mean temperature might be such that the period length is close to 24 h and then entrainment by temperature cycles could result.

Since under field conditions *Thalassomyxa australis* is exposed to a number of different time cues such as light-dark-cycles, temperature cycles and tidal rhythms, synchronisation might depend on a combination of these. This has been studied recently (Förster and Engelmann, 1988). Furthermore, the type and amount of food organism available might be of importance for entrainment (Silyn-Roberts, unpublished). There are indications that the density of *Thalassomyxa australis* and the fusion of individuals to a larger plasmodial syncitium during active phase is important for synchronization to external cycles (unpublished observations of A. Smietanko and C. Förster).

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