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Induction of cyst formation by low temperature in the dinoflagellate *Gonyaulax polyedra* Stein: dependence on circadian phase and requirement of light

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Abstract. Encystment, which at a temperature of 15 °C is photoperiodically controlled in *Gonyaulax polyedra* , can also be induced by a decrease of temperature, from 20 to 10 or 8 °C in the absence of photoperiodic signals. The cyst-inducing capacity of the decrease in temperature depends on the circadian phase: in constant light, the maximum of sensitivity was found at the beginning of subjective night. In a light/dark cycle, however, cyst formation was reduced during dark phase, indicating that light is required for the process of encystment. A similar light dependence was seen in the effect of the physiologically occurring cyst inducer 5-methoxytryptamine, but not in the encystment response to the protonophores monensin and nigericin.
Key words. Circadian rhythms; cysts; dinoflagellates; *Gonyaulax*; 5-methoxytryptamine; melatonin; protonophores.

In the dinoflagellate *Gonyaulax polyedra*, the formation of the resting stage asexual cyst can be induced by short photoperiods[1–3]. This effect depends, however, on a decreased temperature (15 °C instead of the normal rearing temperature of 20 or 18 °C). Encystment can also be induced by the indoleamine melatonin, which occurs in *Gonyaulax* in high quantities (up to 2.5 ng/mg protein). Melatonin exhibits a circadian rhythm in concentration, with a prominent nocturnal maximum[4,5], and its cyst-inducing capacity shows the same temperature dependence as that of short days[1–3]. Intracellular signalling of the photoperiodic information is thought to involve the conversion of melatonin to 5-methoxytryptamine by aryl acylamidase, and cytoplasmic acidification due to proton translocation from an acidic vacuole under the control of 5-methoxytryptamine[3,6]. This non-acetylated indoleamine, which is formed by *Gonyaulax* cells in highest concentrations during the second half of night[5], represents the most potent inducer of cyst formation among the physiologically occurring substances tested to date[1–3,6].

The decrease of temperature which leads to the sensitization of cells to photoperiodic stimuli or to melatonin has been interpreted to result in an accumulation of 5-methoxytryptamine[3,6]. Therefore, I investigated the possibility that cysts may also be induced by further lowering temperature. Moreover, a large temperature step-down may act directly at the level of proton translocation by altering the membrane properties of the acidic vacuole. In order to distinguish a potential effect of low temperature from an interaction with photoperiodic cyst induction, experiments were carried out either in long-day conditions (light/dark cycle of 16:8 h = LD 16:8) or in constant light (LL); for reasons of comparison, an additional series of experiments was conducted under short-day conditions (LD 10:14).

Material and methods

Gonyaulax polyedra was grown in a long day (LD 16:8), at 20 °C, in a modified f/2 seawater medium (for details see ref. 7). All experiments were carried out in samples derived from a single stock culture. Cells either remained in LD 16:8 or were transferred to LL or LD 10:14, at an experimental light intensity of 400 lux. At various circadian phases, samples (50 ml) were subjected to a temperature drop to 10 or to 8 °C, at which they remained throughout the experiments. Cyst formation was evaluated 1 and 3 days after the temperature shifts.

For pharmacological cyst induction, cells were kept in LD 12:12, from which aliquots were transferred to constant darkness (DD). Stock solutions of agents were prepared directly before use in the following solvents: 5-methoxytryptamine 0.2 M in DMSO; monensin 0.5 mg/200 µl ethanol; nigericin 0.5 mg/200 µl DMSO/ethanol 1:1. Solutions were further diluted with culture medium to give the desired molarities. In the concentrations applied, neither DMSO nor ethanol influenced cyst formation.

The circadian phase of *Gonyaulax* subjected to different lighting regimes was determined by measuring the circadian rhythm of spontaneous bioluminescence (for details see ref. 8). Cyst formation was quantitatively evaluated by calculating the ratio of cyst to total cell number. In order to decide whether the encystment had occurred, the presence of a clearly visible cyst wall was used as the most unambiguous criterion.

Results and discussion

A decrease in temperature from 20 to 8 °C can induce quantitative encystment in a culture of *Gonyaulax* cells, even if the cells are kept in a constant light (fig. 1). After the first day of treatment, the percentage of en-

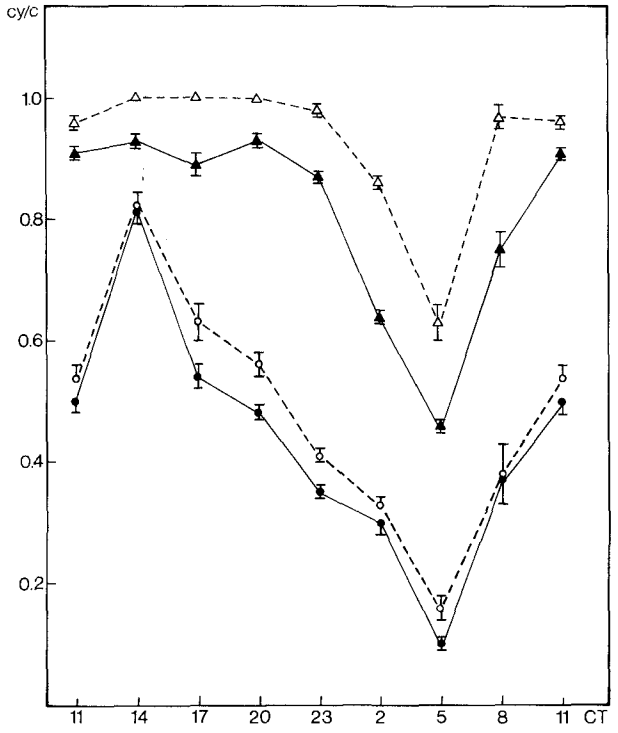

Figure 1. Circadian rhythms in the cyst-inducing capacity of temperature steps from 20 to 10 °C (circles) or 20 to 8 °C (triangles), as demonstrated in constant light (LL). Full symbols: evaluation after 1 day; open symbols: evaluation after 3 days at low temperature. CT: circadian time (h); cy/c: ratio of cysts to total cell number. Vertical lines: SEM.

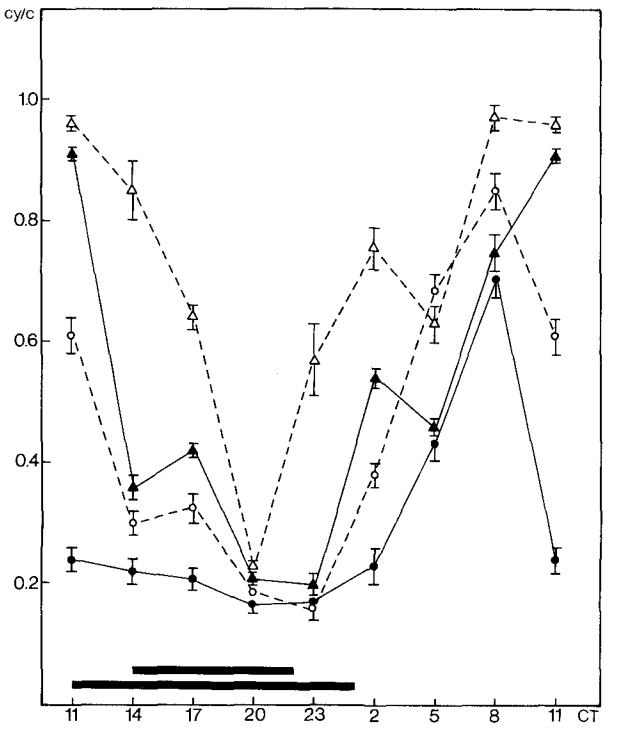

Figure 2. Effects of steps from 20 to 8 °C in light/dark cycles. Circles: LD 10:14; triangles: LD 16:8. Black horizontal bars: scotophases in respective LD cycles. Other details as in Figure 1.

cysted cells is higher than in the case of photoperiodic induction (cf. ref. 2). In contrast to cyst formation elicited by short-days, cells did not always behave in an all-or-none fashion. Especially in the middle of subjective day, only part of the cell population encysted (fig. 1). In these experiments, encystment is obviously triggered by the temperature jump, not by the low temperature per se, since otherwise all cells should form cysts within several days of cold exposure. Moreover, cells do not pass through all circadian phases after transfer to cold, because the oscillator is held at the low temperature[9–11].

This circadian rhythm in responsiveness to low temperature was even more pronounced when steps from 20 to 10 °C were applied because the average rate of encystment was lower. Again, the minimum of encystment occurred in the middle of subjective day, whereas the maximum was found during early subjective night (fig. 1). It is worth noting that this latter circadian phase corresponds to that of the normal maximum of melatonin concentration in *Gonyaulax* kept in LD 12:12 (ref. 4) or in DD[5], although melatonin may not be present in high amounts during exposure to LL. The peak in cyst formation may, therefore, represent a phase of maximal sensitivity to cyst-inducing signals. Since the rhythm in the encystment response occurred in LL, i.e., in lighting conditions counteracting short-day effects, the phase of highest sensitivity to low temperature may not reflect a direct action of melatonin (cf. refs 1–3, 6), but rather an influence of cold on a later step of the signal transduction pathway, e.g., proton transfer from the acidic vacuole[3,6].

Although the rhythm in the cold response indicates a periodic variation in sensitivity at a particular step of the intracellular signalling pathway leading to encystment, this does not mean that cells really encyst preferentially during night under short-day conditions. As shown in figure 2, darkness inhibits encystment, even upon application of strong temperature signals (steps from 20 to 8 °C). The suppression of cyst formation by darkness was seen both in a long day (LD 16:8) and in a short day (LD 10:14). The short photoperiod applied is sufficient to provoke encystment in the entire cell population at 15 °C[1,2].

At first glance, the results of figure 2 are surprising because 1) rates of encystment are mostly lower at 8 than at 15 °C, and 2) cyst formation is not immediately associated with the nocturnal maxima of cyst-inducing indoleamines, melatonin and 5-methoxytryptamine (cf. refs 4, 5). The first point is explained by the observation that photoperiodic cyst induction in *Gonyaulax* seems to require an operating circadian oscillator[12]. Below 11.5 °C, however, the *Gonyaulax* oscillator is held[9–11].

As to the second point, one should distinguish between cyst-inducing signals and the encystment response per se. This becomes particularly obvious when applying

Requirement for light in pharmacological cyst induction at 20 °C.

Cyst inducer	Concentration (M)	Lighting regimen	Cysts/total cells (±SEM)
5-Methoxytryptamine	10^{-5}	LD 12:12	1.00 ± 0.00
5-Methoxytryptamine	10^{-5}	DD	0.14 ± 0.03
5-Methoxytryptamine	2×10^{-5}	LD 12:12	1.00 ± 0.00
5-Methoxytryptamine	2×10^{-5}	DD	0.22 ± 0.04
Monensin	3.3×10^{-7}	DD	1.00 ± 0.00
Nigericin	4×10^{-7}	DD	1.00 ± 0.00

Cyst-inducing agents were added 2 h before the onset of darkness in LD or in the corresponding circadian phase in DD.

the physiologically occurring cyst inducer 5-methoxytryptamine (table). The effect of this indoleamine is also inhibited by darkness. However, this does not imply that cells are generally incompetent to form cysts in the absence of light, since they can be forced to encyst in DD by making the acidic vacuole leaky by application of the electroneutral protonophores monensin and nigericin (table), which are also potent cyst inducers in other conditions[3, 13]. Hence, the inhibition of cyst formation by darkness should be regarded as the consequence of a cellular physiological control mechanism, which may be of biological importance. When encysting under natural conditions, the cells have to prepare themselves for a longer resting period and, therefore, have to accumulate reserve carbohydrates; this has already been demonstrated in an investigation of ultrastructural changes during encystment[14]. Thus, the process of cyst formation itself should take place during photophase, even though signals eliciting the photoperiodic encystment response can be associated with a long scotophase.

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