51. Behavioral Adaptation of *Paramecium caudatum* to Environmental Temperature

The Effect of Cell Density

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Paramecium is a single cell organism which swims by beating a large number of cilia on its cell surface. When put in a temperature gradient, paramecium cells accumulate in a region of a specific temperature at which they were cultured.¹⁾ The cells cultured at 25°C gather to 25°C and those cultured at 30°C gather to 30°C. In a vessel, of which one end is fixed at a temperature T_1 higher than the culture temperature T_0 and the other end at T_2 lower than T_0 , cells swimming toward the region of T_0 from both sides show a smaller frequency of discontinuous changes of swimming direction than those swimming away from the region of T_0 . Such behaviors result in accumulation of the cells in the region of T_0 .^{2),3)} The cells swimming in a temperature gradient sense a change of the environmental temperature with time. The behaviors of the cells in a temperature gradient can be explained based on their responses to the temperature change with time.^{2),3)}

Thus, paramecium cells retain some memory of the culture temperature and regulate the response to the temperature change to cause accumulation at the culture temperature. Such memory of the culture temperature is expected to be established in the process of adaptation to the environmental temperature. In this work, the process of adaptation has been followed by observing the swimming behaviors of paramecium cells after transferring them from the culture temperature to a different temperature. An effect of the cell density on this process has been found.

Specimen of *Paramecium caudatum* (Ksy 1 of mating type V) have been kindly supplied by Professor Hiwatashi of Tohoku University. They were cultured in a hay infusion containing 2–3 mM Tris-maleate buffer of pH 7.0 with *Aerobacter* in a water bath kept at a constant temperature.

In the first experiment, paramecium cells cultured at 25°C for a few days were transferred into a vessel kept at 30°C. As shown

in Fig. 1, immediately after the transfer, the cells showed a very large frequency of directional changes because of the sudden temperature change. As mentioned already, such an increase of the frequency is an important response for the cells to accumulate at the culture temperature in a temperature gradient. A few minutes after the transfer to 30°C, however, the frequency decreased and attained a stationary value which was maintained for about 40 min. Then, the frequency decreased again to a final value.

Fig. 1. The change of the frequency of directional changes after paramecium cells cultured at 25°C were transferred to 30°C. The transient response immediately after the transfer and the change of the frequency in the stationary or final state are shown in different time scales.

Fig. 2. The relation between the frequency of directional changes in the stationary state and the temperatures. Paramecium cells were cultured at 25°C (○) and 30°C (●), respectively.

Similar behaviors, a transient increase of the frequency followed by a decrease to a stationary value, were observed when the cells were transferred from 25°C to different temperatures T . The frequency in the stationary state depended on T to which the cells were transferred. As shown in Fig. 2, the frequency was low when $T \leq 25^\circ\text{C}$ and increased with rising temperature when $T > 25^\circ\text{C}$. In the case of the cells cultured at 30°C, the frequency in the stationary

state was low when $T \leq 30^\circ\text{C}$ and increased when $T > 30^\circ\text{C}$. The influence of the culture temperature is preserved in the relation between the frequency of directional changes and the temperature T .

Fig. 2 indicates that in the stationary state the frequency of directional changes of paramecium cells cultured at 25°C and then transferred to 30°C is larger than that of the cells cultured at 30°C . However, as shown in Fig. 1, the frequency in the stationary state began to decrease again about 40 min after the transfer to 30°C . The final value of the frequency was equal to that of the cells cultured at 30°C for a few days.

If, after the decrease of the frequency to the final value, the cells were returned to 25°C , their frequency showed a transient increase. This means that the cells adapted to the new temperature, 30°C . Actually, they could accumulate at 30°C if put in a temperature gradient. The experiment in Table I shows the correlation between the frequency in the stationary or final state and the transient response to sudden temperature changes. At various times after the transfer of the cells from 25°C to 30°C (column 1 of Table I), the frequency of directional changes was measured at 30°C (column 2). Then, a temperature change from 30°C to 25°C was given and the transient response in the frequency was observed (column 3). After a few minutes, the temperature was returned to 30°C and the transient response was observed again (column 4). The switching of the transient response (columns 3 and 4) took place in parallel to the decrease of the frequency in the stationary state to the final value (column 2).

In the course of the above experiment, it was found that the time necessary for establishment of adaptation to a new temperature depended on the cell density (the number of cells per unit volume). As shown in Fig. 3a, the decrease of the frequency in the stationary state to the final value occurred faster at higher cell densities. The

Table I. Changes of the frequency in the stationary or final state and the transient response after transfer of paramecium cells from 25°C to 30°C

Time after transfer (hr)	Stationary or final frequency at 30°C (sec^{-1})	Transient changes of frequency	
		30°C to 25°C	25°C to 30°C
0	0.20	—	+
0.5	0.20	+	0
1	0.12	+	—
24	0.10	+	—

The procedure of the experiment is described in the text. +, increase of frequency; —, decrease of frequency; 0, no change of frequency.

time at the midpoint of the frequency decrease, i.e., the half time τ was inversely proportional to the square root of the cell density, as plotted in Fig. 3b.

Fig. 3. a: The effect of the cell density on the process of adaptation. Suspensions of paramecium cells of different cell densities in the same medium were prepared from a culture at 25°C and transferred to 30°C at time zero. The cell density in number/ml was 75 (○), 150 (△), 550 (□) and 1000 (●), respectively. (The initial transient response is not shown in this figure.) b: The relation between the half time of adaptation and the cell density obtained from the data of (a).

A possible mechanism of facilitation of adaptation by high cell density may be that the cells transferred to a new temperature would release to the medium some substance which promotes adaptation. Therefore, the effect of the medium was examined. A suspension of paramecium cells cultured at 30°C was transferred to 25°C. After incubation at the new temperature 25°C for 1–2 hrs, the suspension was gently centrifuged to remove the cells. To the supernatant obtained, other paramecium cells cultured at 25°C were added and the mixture was transferred to 30°C. Immediately after the transfer, the frequency of directional changes of the cells began to decrease and within 30 min, it attained a final value which corresponded to that of the cells cultured at 30°C. On the other hand, the supernatant obtained from a suspension incubated at 25°C for 4 hrs had no effect to promote adaptation of other cells. This result suggests that the effective substance released to the medium has a limited life time and the cells stop to release it after establishment of adaptation.

The same supernatant was equally effective to promote adaptation of other cells to any new temperature in a range from 20°C to 30°C, and the supernatant obtained from suspensions of the cells transferred to various temperatures showed the same effect to promote adaptation of other cells.

In order to describe the process of adaptation, let us denote the total number of cells as N and the number of cells which have adapted to a new temperature as $n(t)$, a function of time t . After the transfer to a new temperature, each cell releases a substance X the concentration of which, x , increases in proportion to the number of cells which have not yet adapted, $N-n$. The adaptation of each cell is facilitated by X , so that the rate of increase of the number n is proportional to the product of x and $N-n$. That is,

$$dx/dt = \alpha(N-n) \quad (1)$$

$$dn/dt = \beta x(N-n) \quad (2)$$

where α and β are constants. The integration of these equations with the initial condition, $n=0$, $x=0$ at $t=0$, gives

$$\ln((\sqrt{N} + \sqrt{n})/(\sqrt{N} - \sqrt{n})) = \sqrt{2\alpha\beta} \sqrt{N} t \quad (3)$$

Therefore, if τ is defined by $n(\tau) = N/2$,

$$\tau = \gamma / \sqrt{\alpha\beta} \sqrt{N} \quad (4)$$

which agrees with the result of Fig. 3b. ($\gamma = \sqrt{2} \ln(\sqrt{2} + 1)$.) The function $n(t)$ of (3) can express approximately the result of Fig. 3a.

Thus, it is very likely that some substance is released from paramecium cells to the medium in the course of adaptation and works as a kind of catalyst for adaptation. The fractionation of an effective component from the medium is not yet successful.

The adaptation of paramecium cells to the environmental temperature was exhibited by the frequency of directional changes which is regulated by fluctuation of the membrane potential.^{4),5)} Therefore, the observed adaptation may be primarily associated with some structural change in the membrane, and the released substance may be a catalyst for such a structural change.

References

- 1) Mendelssohn, M. (1895): Pflüger's Arch. Ges. Physiol., **60**, 1.
- 2) Nakaoka, Y., and Oosawa, F. (1977): J. Protozoology, **24**, 575.
- 3) Oosawa, F., and Nakaoka, Y. (1977): J. Theor. Biol., **66**, 747.
- 4) Majima, T. (1980): Biophys. Chem., **11**, 101.
- 5) Naitoh, Y., and Eckert, R. (1974): Cilia and Flagella (ed. by Sleight, M.). Acad. Press, New York, 305 pp.