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# Relationship between Intracellular Period Modulation and External Environment Change in *Physarum* Plasmodium

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*ABSTRACT.* **The relationship between intracellular period modulation and external environment change was investigated from the viewpoint of internal information coding in *Physarum* plasmodium. For the external conditions, concentration changes of attractant (galactose) and repellent (KCl) were used, and the internal responses were measured as the thickness oscillation of the plasmodium. (i) Period of the intracellular oscillation decreased when the concentration of attractant was increased and when the concentration of repellent was decreased. (ii) The period increased when the attractant was decreased and when the repellent was increased. (iii) The larger concentration change induced the larger period modulation. (iv) These responses were observed when the change of concentration was greater than a threshold value. From these results, it was clarified that the relative change in environmental condition is encoded on the relative period modulation in intracellular oscillation. This means that the period change does not directly represent the environment itself but represents the change of its condition. Thus, it is further suggested that the plasmodium estimates the environmental condition based on the relationship between the previous external condition and the present one.**

The *Physarum* plasmodium is a large amoeboid cell. Though it has no nervous system, it exhibits highly coordinative tactic behavior even in a complicated environment. How does the organism process the environmental information to achieve such an environment-dependent coordination in the tactic response?

It is widely suggested that the intracellular chemical rhythms play important roles in processing external information. Tension oscillations with a period of about 2 minutes are observed in every part of the organism (16), and have a fixed phase relationship with the concentration oscillations of some chemical substances, such as $Ca^{2+}$ (3, 17), ATP (18), $H^+$ (11) and cyclic nucleotides (1). Environmental stimulation by attractants such as glucose and galactose decreases the period of these oscillations, whereas repellents such as sucrose and ribose increase them (2). Since they can be entrained with each other by $Ca^{2+}$ oscillation (7, 12, 13), a local stimulation can generate a global phase wave propagating between the stimulated site and the other regions, and its direction depends on whether the stimulus is an attractant or a repellent (4, 14), and the migration direction coincides with the phase advance in the wave (6).

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In this report, we focus our attention on the first sensory stage of this information processing. As explained in the above, it has been suggested that the environmental condition is received and encoded on the period modulation of intracellular chemical oscillations. In addition, concentration change of cyclic nucleotides (1) and the response of respiratory chain (5) were clarified to depend on whether the stimulus is an attractant or a repellent. Furthermore, we recently found a very interesting phenomenon associated with the response to the relative change of environmental condition (6). When the concentration of attractant was decreased, the period of intracellular oscillation increased. When the concentration of repellent decreased, the period decreased. This preliminary observation suggests the possibility that the period modulation does not depend on the stimulant itself, such as attractant or repellent, but depends on its relative change. However, it still remains obscure how the external environment information is encoded on the intracellular oscillator system in the plasmodium.

Therefore, the purpose of this report is to experimentally clarify the relationship between the intracellular period modification and the external environment change around the organism in detail. Thus, through measuring the response of intracellular oscillation under vari-

ous changes of the environmental conditions, the internal information coding mechanism of external conditions was analyzed.

## MATERIALS AND METHODS

*Organism.* *Physarum* plasmodium was allowed to migrate on 1.5% agar gel sheet overnight at room temperature (20–23°C) without feeding before use. A sheet of plasmodium about 8 mm in length and 4 mm in width was excised from the tip portion of the migrating plasmodium and used for the experiments.

*Regulation of chemical environment.* The sheet of the plasmodium was first transferred to a cellophane sheet placed on a 1.5% agar gel plate containing a chemical substance. After about 2 hours of this treatment, by carefully sliding the cellophane sheet on which the plasmodium was laid, the entire organism was transferred to another agar gel plate and exposed to another chemical condition. Galactose and KCl were used as the attractant and repellent, respectively. When the concentration of stimulant to which the organism was exposed was increased, the concentration in the first agar gel plate was fixed to 0 mM. Under these conditions, the concentration in the second agar gel plate was increased to 0.01 mM, 0.03 mM, 0.1 mM, 0.3 mM, 1 mM, 3 mM, 10 mM and 30 mM. When the concentration of stimulant to which the organism was exposed was decreased, the concentration in the first agar gel plate was fixed to 30 mM. The concentration in the second agar gel plate was then decreased to 29.99 mM (−0.01 mM), 29.97 mM (−0.03 mM), 29.9 mM (−0.1 mM), 29.7 mM (−0.3 mM), 29 mM (−1 mM), 27 mM (−3 mM), 20 mM (−10 mM) and 0 mM (−30 mM), respectively. These two procedures are summarized in Figs. 1a and b, respectively. The specimen was always kept in humid air in a container at 20°C.

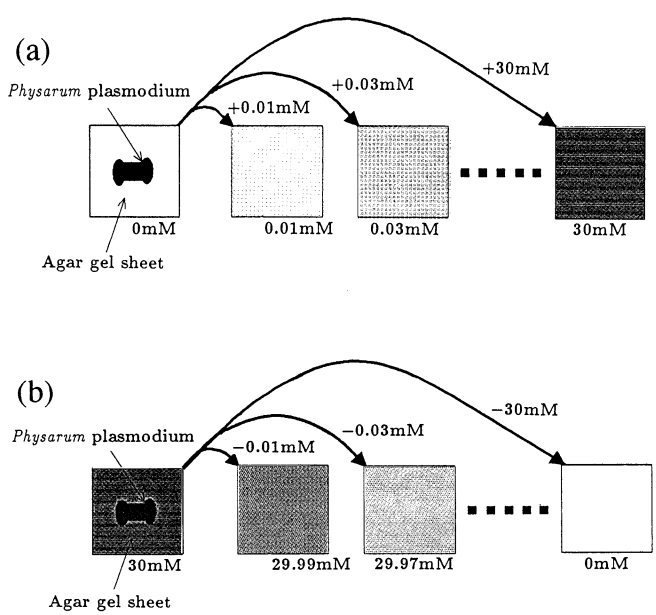


**Fig. 1.** Regulation procedure of chemical environment around the plasmodium. (a) Concentration increase. (b) Concentration decrease.

*Measurement of intracellular oscillation.* By successively processing the optical images of the plasmodium, it was possible to measure its intracellular oscillation without directly touching it. The entire organism was illuminated homogeneously by diffuse white light of about 1,000 lux from above, and its reflected light images were recorded by a video camera (GR-60, Victor). The red component of the image was digitized into 256 levels in each pixel (MT98-CVFM01, Microtechnica) and transmitted to a personal computer (PC-98RX, NEC). Data acquisition was achieved at 2-sec intervals. The digitized image of the organism was divided into each unit square (5 × 5 mm), and these pixel data were averaged in each unit. To avoid counting pixels which did not correspond to a part of the organism, those pixels with intensities below a fixed threshold value were eliminated. The resultant time course of the reflected light intensity was stored in another personal computer (PC-98DX, NEC). Furthermore, by measuring the thickness of cytoplasm using a photointerrupter (GP2L02, Sharp), the reflected light intensity was clarified to oscillate in phase with the thickness change of the plasmodium, and the reflected light intensity was further calibrated to be proportional to the thickness. Under these conditions, the thickness oscillation can be regarded as the tension oscillation.

*Data analysis.* Since the reflected light intensity is proportional to the thickness of the organism in each part, temporal development of the intensity was separated into oscillation and offset components. Then, as shown in Eq. (1), the oscillation component was calculated as the difference between the reflected light intensity and the offset component which was defined as the time average of the reflected light intensity. This value can be regarded as the thickness oscillation in each unit square.

$$\textit{Thickness oscillation } (x,t) = F(x,t) - \frac{1}{T}\int_{t-T/2}^{t+T/2} F(x,\tau)\,d\tau, \qquad (1)$$

where $F(x,t)$ is the reflected light intensity at position $x$ and time $t$, and $T$ is 256 sec. Under these conditions, the thickness oscillation was further analyzed with respect to the period. It was approximately defined as the time interval between the two successive minimal peaks in the same oscillation. These values were obtained in each unit square and averaged over 20-min periods.

## RESULTS

*Response to concentration increase.* Figures 2a and b show examples of the temporal development of the thickness oscillation. When the concentration of galactose to which the plasmodium was exposed was in-

creased from 0 mM to 30 mM, the period of thickness oscillation decreased markedly as shown in Fig. 2a. On the other hand, when the concentration of KCl was increased from 0 mM to 30 mM, the period increased markedly as shown in Fig. 2b.

Furthermore, these results obtained under the same experimental conditions were analyzed and averaged among many samples (n=5). Figures 3a and b show the temporal development of the averaged period observed before and after the change of chemical environment. In each figure, the ordinate shows the relative period normalized by the value observed before the change of environmental condition and the abscissa indicates the time from the change.

When the galactose concentration to which the plasmodium was exposed was increased, the periods of the thickness oscillation gradually decreased and reached minimum values at about 40 to 60 minutes after the environmental change as shown in Fig. 3a. After that, they gradually recovered to the original values. Under this condition, the larger the increase in stimulant concentration, the larger the period of decrease. This relationship was not proportional but nonlinear over the range of concentration changes, i.e., from 0 mM to 0.01 mM, 0.03 mM, 0.1 mM, 0.3 mM, 1 mM, 3 mM, 10 mM and 30 mM.

When the concentration of KCl was increased, the periods gradually increased and reached maximum values at about 40 to 60 minutes after the environmental change. After that, they gradually recovered to the original values as shown in Fig. 3b. Under this experimental condition, it was also observed that the larger the concentration change induced the larger the period modification. However its sign of period modulation was opposite to the above results.

*Response to concentration decrease.* Figures 4a and b show examples of the temporal development of the thickness oscillation, and the responses observed were completely the reverse of those of Figs. 2a and b. When the concentration of galactose to which the plasmodium was exposed was decreased from 30 mM to 0 mM, the period of thickness oscillation increased markedly as shown in Fig. 4a. On the other hand, when the concentration of KCl was decreased from 30 mM to 0 mM, the period decreased as shown in Fig. 4b.

These results obtained under the same experimental conditions were further analyzed and averaged among many samples (n=5). Figures 5a and b show the temporal development of the average period observed after the change of chemical environment. In each figure, ordinate and abscissa are the same as in Figs. 3a and b.

When the concentration of galactose was decreased, the periods gradually increased and reached maximum values at about 40 to 60 minutes after the environmental change as shown in Fig. 5a. After that, they gradually recovered to the original values. Under this experimental condition, it was observed that the larger concentration decrease induced the larger period increase, and this relationship was nonlinear over the range of concentration changes. These properties are similar to the cases in Fig. 3b.

When the KCl concentration was decreased, the periods gradually decreased and they reached minimum values at about 40 to 60 minutes after the environmen-

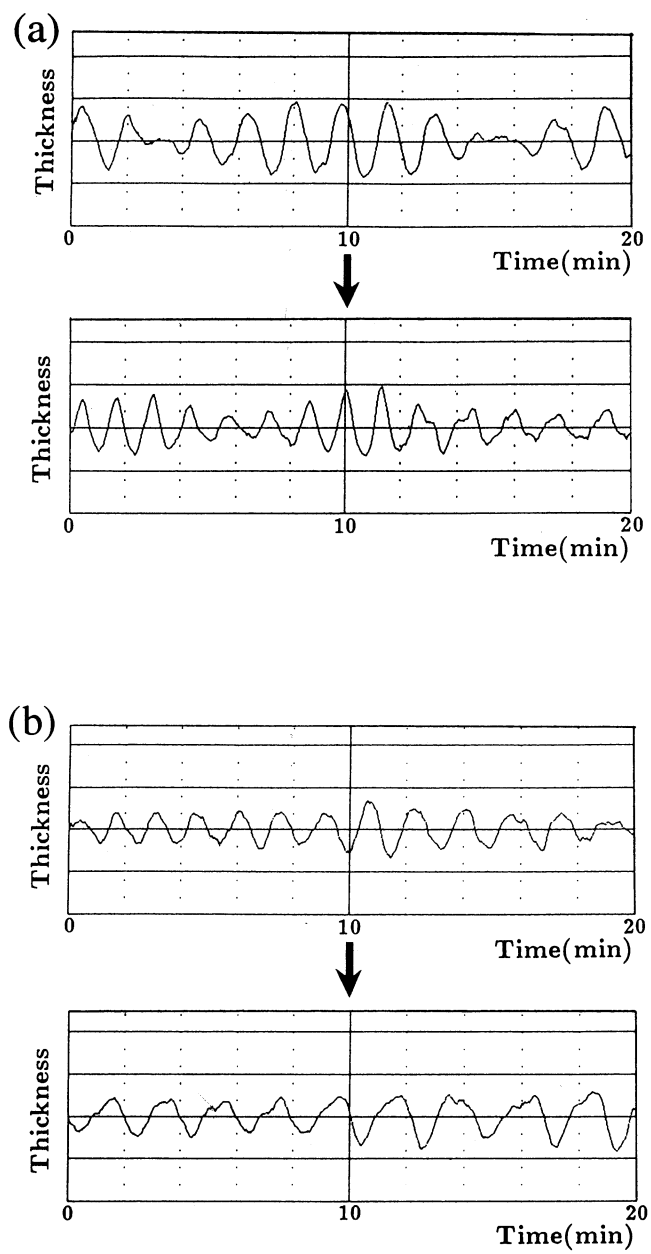


**Fig. 2.** Temporal development of the thickness oscillation observed under concentration increase from 0 mM to 30 mM. (a) Galactose. (b) KCl.

tal change, and after that they gradually recovered as shown in Fig. 5b. Under this experimental condition, it was observed that the larger concentration decrease induced the larger period decrease. These properties are similar to the cases in Fig. 3a.

*Period change and peak time.* These temporal developments were further analyzed with respect to the period change and the peak time. Figures 6a and b show the relationship between the concentration change of environmental condition and the intracellular period modulation in the above processes. In these figures, the period change was normalized by the period which was observed before the concentration change, and the data were averaged over 100 minutes among many samples (n=5). In each figure, the ordinate shows this normalized period change and the abscissa indicates the concentration change plotted in logarithmic scale.

Figure 6a shows the relationship between the concentration increase and the period change. In the case of galactose, the period change decreased monotonously when the concentration change was larger than the threshold value of 0.3 mM, and no period change occurred below the threshold value. In the case of KCl, the period change increased monotonously when the concen-

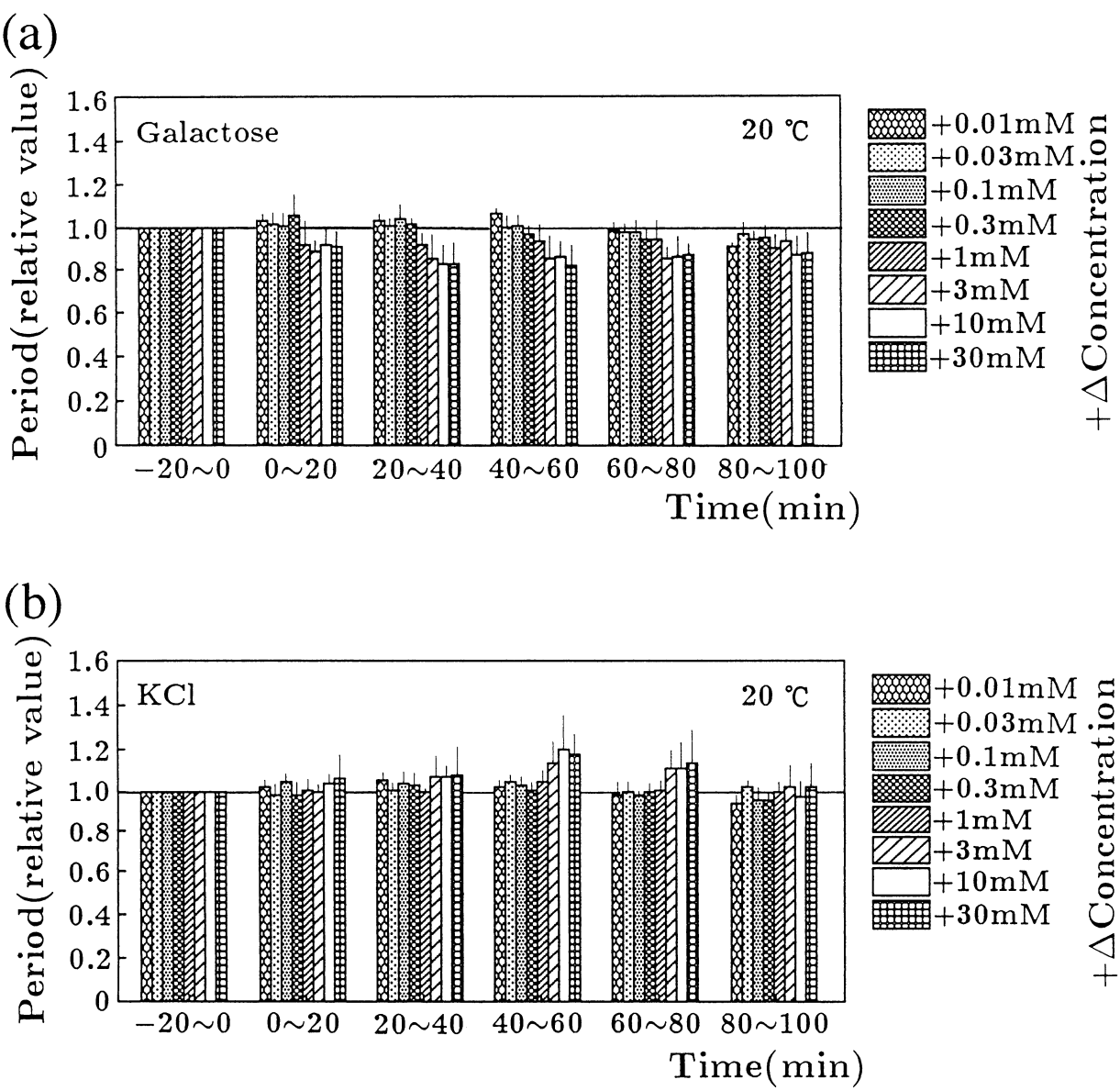


**Fig. 3.** Temporal development of the averaged period of the thickness oscillation observed under various concentration increases. (a) Galactose. (b) KCl.

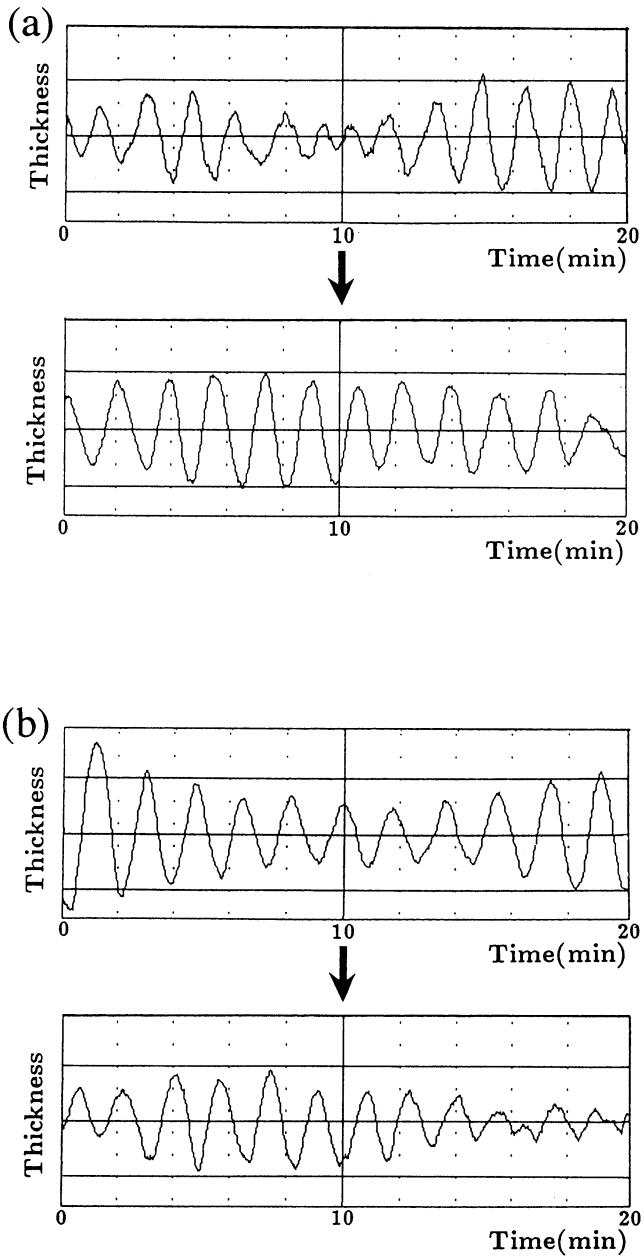


**Fig. 4.** Temporal development of the thickness oscillation observed under concentration decrease from 30 mM to 0 mM. (a) Galactose. (b) KCl.

tration change was larger than the threshold of 1.0 mM. Under these conditions, the period change was approximately proportional to the concentration change in logarithmic scale.

Figure 6b shows the relationship between the concentration decrease and the period change, and the abscissa indicates the negative concentration change in logarithmic scale. In the case of galactose, the period change increased monotonously when the concentration change was larger than the threshold of 1.0 mM. This is similar to the case of KCl shown in Fig. 6a. In the case of KCl, the period change decreased monotonously when the concentration change was larger than the threshold of 0.3 mM, similar to the case of galactose shown in Fig. 6a. These results strongly suggest that the relative change in environmental condition is encoded on the intracellular period modulation independent of the stimulant substances.

On the other hand, Figs. 7a and b show the relationship between the concentration change and the peak time in the same processes. Here, the peak time was defined and calculated as the time interval between the time of concentration change and the time of maximal period change. In each figure, the ordinate shows the peak time and the abscissa indicates the concentration change.

Figure 7a shows the relationship between the concentration increase and the peak time, and Fig. 7b shows the relationship between the concentration decrease and the peak time. In both cases, the peak times were almost constant independent of the concentration change, and their values were about 40 to 50 minutes. These results suggest that the change of environmental condition is not encoded on the peak time in the temporal development of intracellular oscillation.

Since similar results were obtained using glucose and NaCl as stimulants, it is thought that the above characteristics are not restricted to the responses to galactose and KCl.

## DISCUSSION

In this paper, we elucidated the relationship between the intracellular period modulation and the change of external environmental condition, from the viewpoint of intracellular information coding of the external condition in the *Physarum* plasmodium.

As for the information expression mechanism of external environment, it was clarified that the relative change in environmental condition is encoded on the relative change in intracellular period. The most important point is that the period change does not directly depend on the environment itself but depends on the change of the environment. Thus, attractive change of the environmental condition, such as concentration increase of attractant or concentration decrease of repellent, induces period decrease. In a similar manner, repulsive change, such as concentration increase of repellent or concentration decrease of attractant, induces period increase. These results strongly support our preliminary observations (6). This is essentially different from the proposed mechanism that the attractant and repellent induce period decrease and increase, respectively (2). We believe that the effect of the environment is

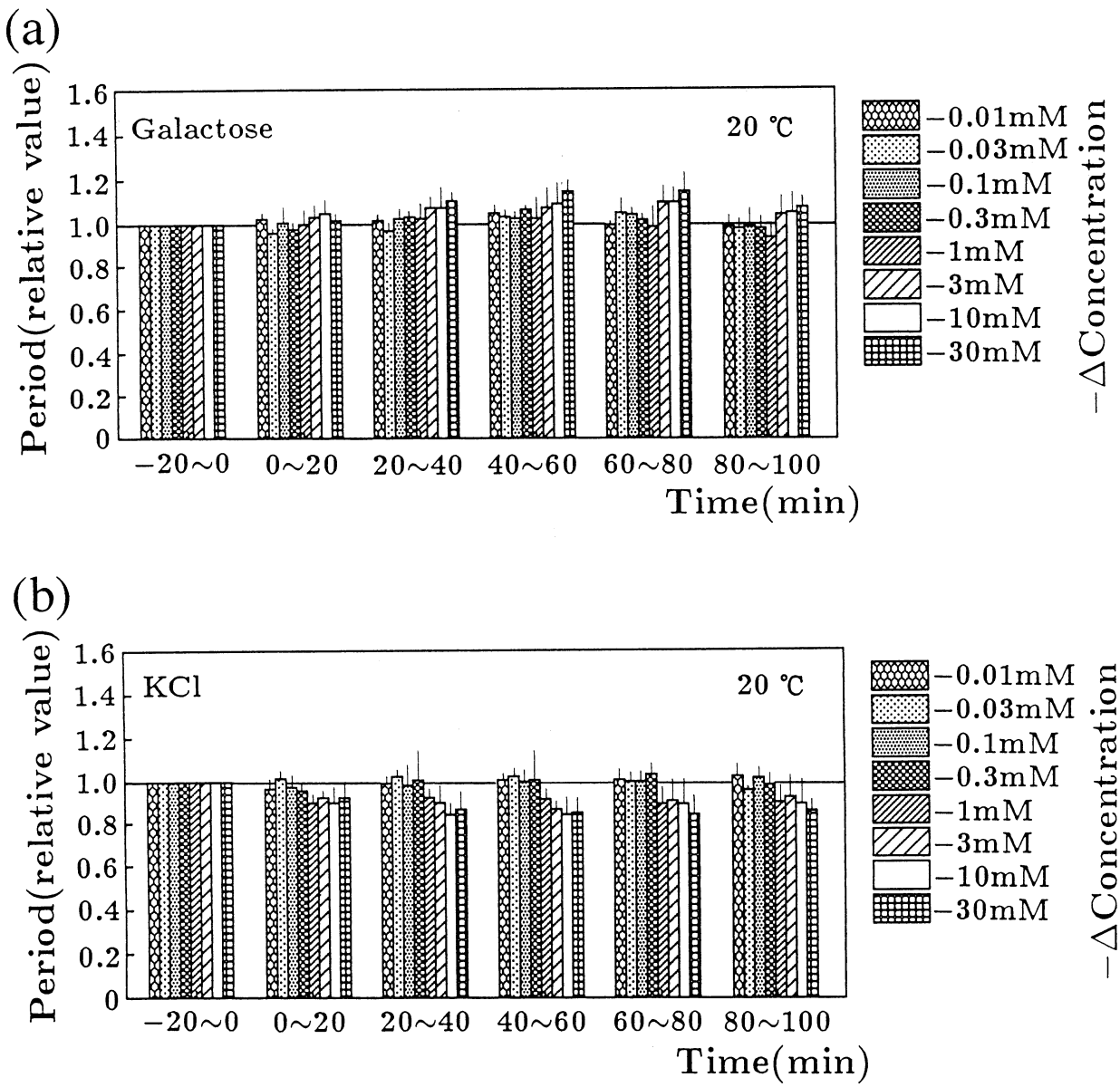


**Fig. 5.** Temporal development of the averaged period of the thickness oscillation observed under various concentration decreases. (a) Galactose. (b) KCl.

determined based on the relationship between the internal state of the organism and the external environment. Therefore, the concept of attractant and repellent should be generalized based on these experimental results.

In this information coding mechanism, two useful characteristics were found. One is that the better change in environmental condition induces the shorter period in intracellular oscillation. Since the oscillation with shorter period entrains the wider spatial region in intracellular oscillator system (6, 9, 10), this coding mechanism is thought to be very convenient for comparing local environmental conditions in the information processing process of the plasmodium. Thus, from a theoretical point of view (8), it is thought that information on local environmental conditions could be integrated into the global information which leads to the migration of the entire organism toward the area with the more favorable environmental conditions. The next is that this coding mechanism has threshold values, and there is a no-response region between period increase and decrease. Based on this property, this internal coding would be robust to small external perturbations. In addition, since similar threshold values were found in the

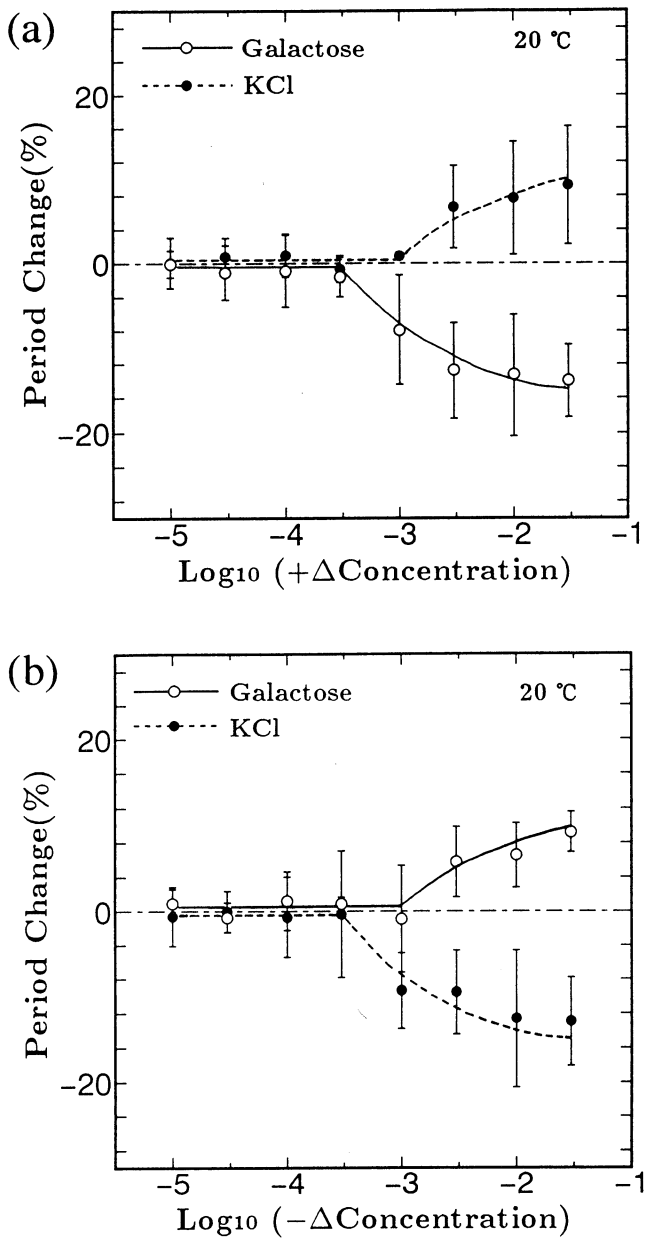


**Fig. 6.** Relationship between concentration change and period change. (a) Concentration increase. (b) Concentration decrease.

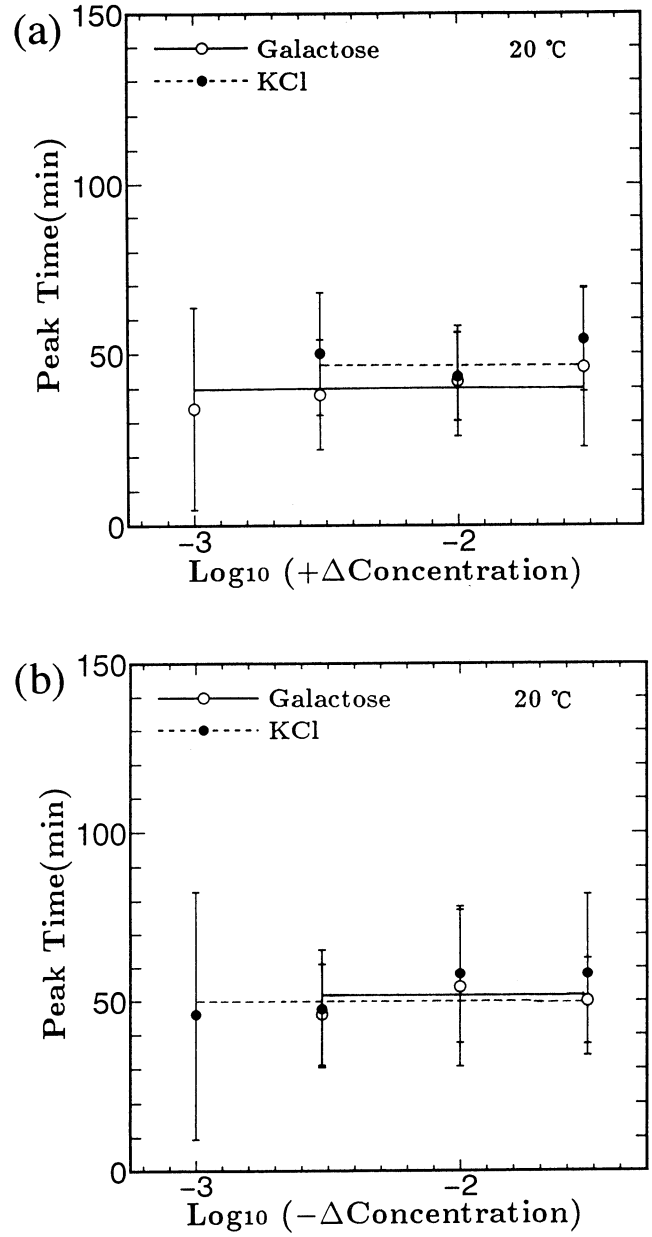


**Fig. 7.** Relationship between concentration change and peak time. (a) Concentration increase. (b) Concentration decrease.

relationship between the membrane potential and the concentration increase of stimulants in the chemoreception of the plasmodium (15), the period modulation might have close correlation with the regulation of the membrane potential.

From these experimental results, it is suggested that the sensory system in the plasmodium evaluates the environmental condition based on the relative relatinship between the internal memory state of the previous environment and the present external one. This relationship between internal and external states might provide a kind of framework for processing the meaning of information for the organism. This sensing mechanism is more intelligent than the conventional ones. It should be further investigated experimentally and theoretically.

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