

Information Processing for the Organization of Chemotactic Behavior of *Physarum polycephalum* Studied by Micro-Thermography

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Summary

The spatial and temporal pattern of oscillating temperatures on the cell surface of a plasmodial strand of *Physarum polycephalum* was measured with a sensitive thermal image camera. The longitudinal tension of the strand was studied simultaneously. In the absence of chemical stimulation, the phases of the temperature oscillation observed at various portions of the strand were entrained with almost coincidental phase. The temperature and tension oscillation were synchronized, although the phase difference between them was occasionally changed. With local chemical stimulation, the phase of the temperature oscillation advanced in the portion to which the plasmodium would be induced to migrate. The phases between temperature and tension oscillations then became constant. The mechanism by which the plasmodium processes local information of chemical stimulus to global information for the migration is discussed.

Keywords: Chemotaxis; Information processing; Oscillation; *Physarum polycephalum*; Thermal image camera.

1. Introduction

The plasmodium of *Physarum polycephalum* is a large unified cell with a size in the order of a few hundred square cm's. This organism is well known to exhibit positive and negative chemotaxis; when chemicals are applied to any part of the cell, the whole organism migrates towards or away from the stimuli (COMAN 1940, CARLILE 1970, UEDA *et al.* 1975, KNOWLES and CARLILE 1978, UEDA and KOBATAKE 1982).

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The biased shuttle flow of the protoplasm, which looks like propagating waves (HEJNOWICZ and WOHLFARTH-BOTTERMANN 1980, UEDA *et al.* 1986, MATSUMOTO *et al.* 1986), causes the migration. This suggests that appropriate phase difference should be organized among the oscillations of the local contractile elements throughout the whole cell depending on the types of stimuli, since the streaming is driven by the intracellular pressure difference caused by the contractility (KAMIYA 1950 a). It has been reported that the contractile element of the stimulated portion contracts and relaxes by applying repellents and attractants, respectively (UEDA *et al.* 1976), which is related to the concentration change of intracellular chemicals, such as ATP, H⁺, cAMP, and cGMP (UEDA and GOTZ VON OLENHUSEN 1978, HIROSE *et al.* 1980, HIROSE *et al.* 1982, AKITAYA *et al.* 1984, MORI *et al.* 1986). However, the mechanism of the intracellular information processing to organize the appropriate phases among the local contractile elements over the entire cell has not been clarified.

The plasmodium has various kinds of synchronous cellular oscillations, such as local contractility (KAMIYA 1970, WOHLFARTH-BOTTERMANN 1975, SAMANS *et al.* 1978), protoplasmic streaming (KAMIYA 1950 b), intracellular Ca²⁺ concentration (YOSHIMOTO *et al.* 1981 a), intracellular ATP concentration (YOSHIMOTO *et al.* 1981 b), surface pH (NAKAMURA *et al.* 1982) and intracellular cAMP and cGMP (AKITAYA *et al.* 1984, UEDA *et al.* 1986). The plasmodium behaves as an integrated system of loosely coupled nonlinear oscillators which have respective inherent frequency (YOSHIMOTO and KAMIYA 1978). DURHAM and RIDGWAY (1976) examined

the effects of chemical stimuli on a small piece of the plasmodium and found that attractants increase and repellents decrease its oscillating frequency. In loosely coupled nonlinear oscillators, a change of local inherent frequency affects other oscillators; increase (decrease) of the frequency advances (delays) the phase of intimate oscillators. Thus, we suppose that spatial phase gradients formed among the intracellular chemical oscillations play an important role in information processing for the organization of the appropriate phase difference among the locally oscillating contractility.

In this report, we developed a sensitive thermal image camera and applied it to study the spatio-temporal phase differences of surface temperature oscillations on the plasmodial strand which was found to be related to the exothermic reaction of it. We measured the oscillations of the longitudinal tension of the strand simultaneously; and the effect of attractants and repellents was examined.

2. Materials and Methods

2.1. Organism

The plasmodium of *Physarum polycephalum* was cultured by the method of CAMP (1936). The plasmodium was allowed to migrate on 1.5% agar gel overnight without feeding before use. A plasmodial strand about 15 mm in length and 0.6–0.8 mm in diameter was used.

2.2. Experimental Setups

The plasmodial strand was horizontally connected between the extensions of two glass capillary tubes placed 5 mm apart as schematically shown in Fig. 1. Isometric tension was measured by a tension transducer (Akers, Horten, Norway, AE-801) which had a sensitivity of about 0.1 mg. Cylindrical filter paper was plugged into the tip of the extension, to which the end of the strand was attached. For chemical stimulations, solution of attractant or repellent was fed to the filter paper through the extension capillary. This construction was sealed in a lucite chamber with a water jacket. Temperature of the inside of the chamber was controlled by a bath-circulator (HAAKE, F 3-K) at $20\text{--}22 \pm 0.02^\circ\text{C}$. The window of the chamber was sealed with polyethylene film which passed infrared light from the object.

2.3. Micro-Thermography

For micro-thermography, a thermal image camera (JEOL, JTG-3300) was equipped with a lens of germanium ($\times 9$) and an infrared detector of a HgCdTe element (at 77 K), which was most sensitive to $10\text{ }\mu\text{m}$ infrared radiation. A two-dimensional thermal image was obtained by scanning a vibrating mirror. The time and spatial resolutions were 8 msec and $60\text{ }\mu\text{m} \times 60\text{ }\mu\text{m}$, respectively. The temperature was calibrated with an internal standard black-body radiation; thus the slow drift was suppressed within $0.01^\circ\text{C}/20\text{ min}$. The peak amplitude of the noise was less than 0.01°C . The attempt to measure the temperature of the plasmodium by thermistors has been reported by ALLEN *et al.* (1963). However we could observe for the

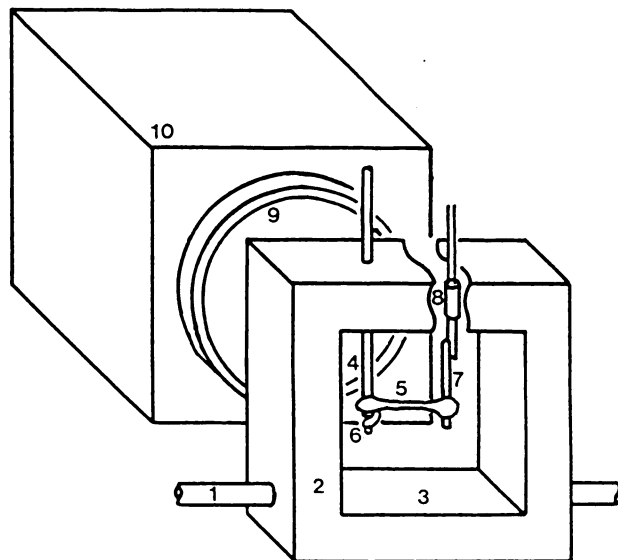


Fig. 1. A schematic illustration of the experimental apparatus. 1 water jacket, 2 lucite chamber, 3 window of the chamber, 4 extension of the stimulation end, 5 plasmodial strand, 6 cylindrical filter paper to which the stimulant is applied, 7 extension with the tension transducer, 8 tension transducer, 9 lens of germanium, 10 thermal image camera (JEOL, JTG-3300)

first time the spatial and temporal pattern of oscillating temperature on the cell surface owing to these high spatial and temperature resolutions.

Thermographic images of the strand were measured at five second intervals. To avoid the effect of the lateral slow movement of the strand, the highest temperature points along the strand were picked up and plotted as an one-dimensional pattern (Fig. 2) using a personal computer (PC 9801 E, NEC). The diameter of the strand undergoes oscillatory change with an amplitude of up to $50\text{ }\mu\text{m}$. However the heat capacity of the strand did not change significantly, because the temperature oscillation was found not to be correlated to the morphological changes.

The observed temperature is the function of heat production and its dissipation. In control experiments the dissipation constant was found to be less than 0.02 s^{-1} (data not shown). Thus the phase difference between the oscillation in the temperature and heat production is less than 20° when the period of the oscillation is longer than 120 seconds.

2.4. Data Analysis

The surface of the strand was divided into eight sections (1 to 8, as is shown in Fig. 2) and the temperature of each section was averaged. 128 data points of the temperature and tension fluctuations sampled at 5-second intervals were used for the fast Fourier transformation (FFT) analysis to determine the amplitudes and phases of their frequency components. Not only the power spectra of the temperature and tension fluctuations but also the phase differences in respect to the fundamental frequency of the oscillations in the local temperature and between the local temperature and tension were calculated. The latter was determined by the cross-spectra calculated from the real and imaginal components of two oscillations (HINO 1977).

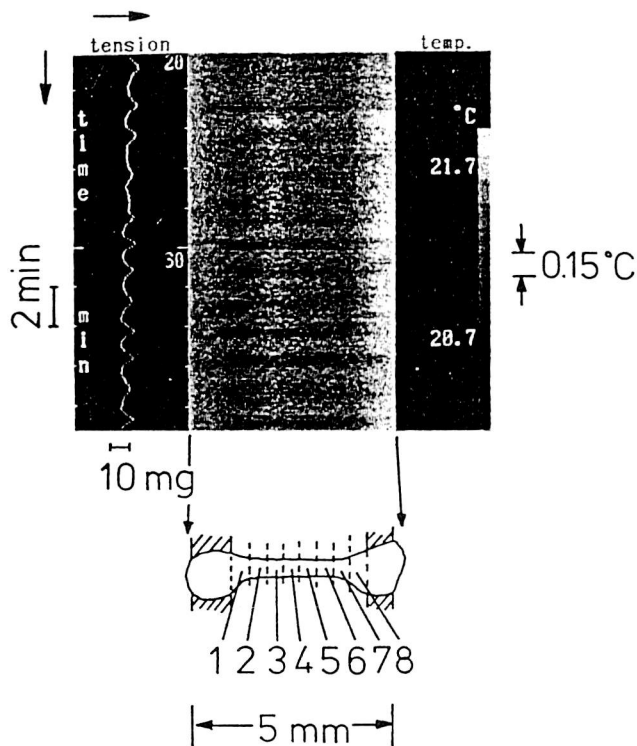


Fig. 2. A spatio-temporal pattern of the surface temperature oscillation together with longitudinal tension of the plasmodial strand in the absence of chemical stimulation. The temperature of the strand oscillates synchronously throughout an entire strand. The lowest part of the figure shows the correspondence to the real image of the strand. The surface of the strand was divided into eight sections (1-8). The temperature of each section was spatially averaged

3. Results

3.1. Absence of the Stimulation

Figs. 3 and 4 show time courses of the oscillations of local temperatures in 8 sections and longitudinal tension of the strand under the absence of stimulation. The phases of the temperature oscillation observed in various sections are almost coincident, except for the local fluctuation (denoted as ▼ in Figs. 3 and 4). FFT analysis (Figs. 8a and b) confirms that the phase differences between the oscillations are less than 60° . The tension oscillation shows a simple waveform while the temperature oscillation occasionally includes a lot of minor peaks (Figs. 3 and 4). Figs. 7a and b show the power spectrum of the longitudinal tension and temperature oscillation of the middle part of the strand. It is apparent that when the waveform is simple it is composed of a single frequency component, while the complicated waveform of the temperature oscillations includes minor frequency components.

In addition, the phase difference between the surface

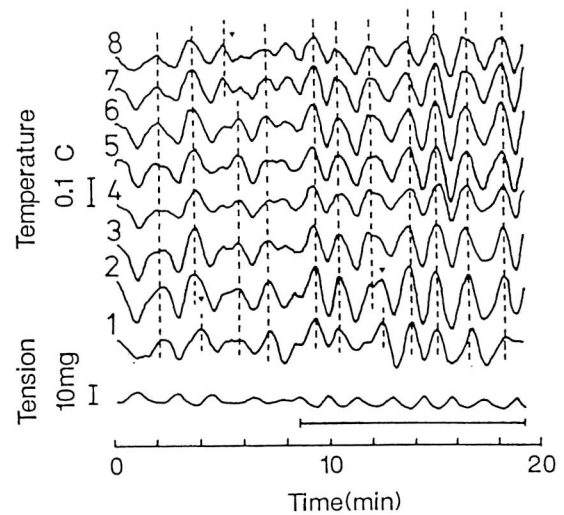


Fig. 3. Time courses of the surface temperature in the plasmodial strand together with longitudinal tension. The result of Fig. 2 (absence of stimulation) is plotted. 1-8 the averaged temperature of each section. — indicates the data to which FFT analysis is applied. ▼ shows the point where the local fluctuation is observed

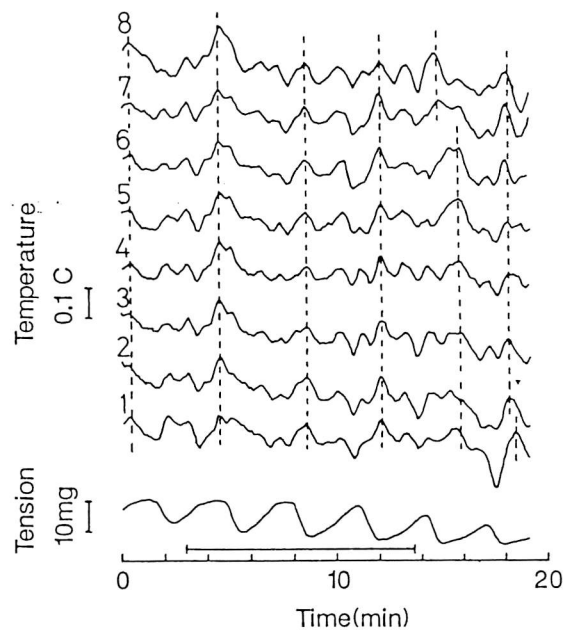


Fig. 4. Time courses of the surface temperature together with the longitudinal tension occasionally observed in the absence of stimulation. A lot of minor peaks are observed in the temperature fluctuation. Other details are the same as in Fig. 3

temperature and tension oscillation is constant for some time (Fig. 3), but varies occasionally (Fig. 4). Considering several different strands, the phase difference between the temperature oscillation of the middle part of the strand and tension oscillation has a wide range (Fig. 9a).

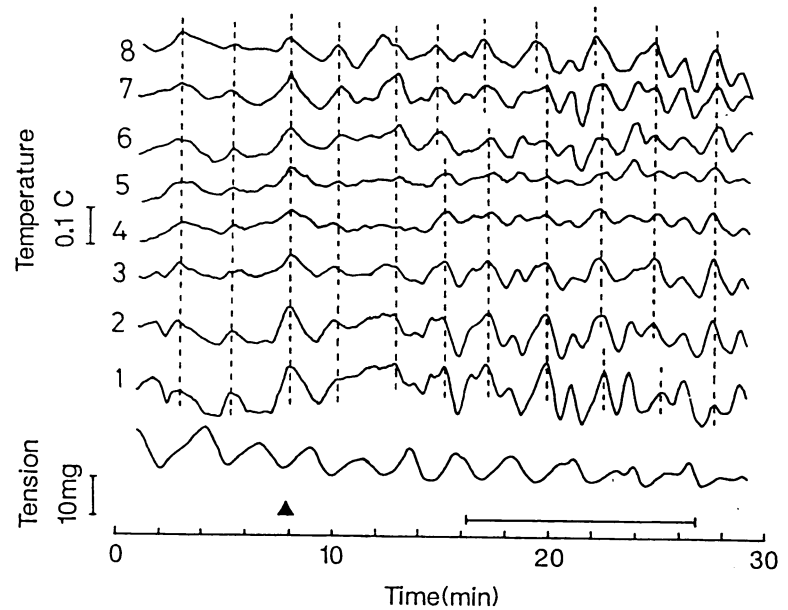


Fig. 5. Time courses of the surface temperature together with the longitudinal tension with the stimulation of 1 M KCl. The stimulation was applied at (▲) to the left end of the strand. Other details are the same as in Fig. 4

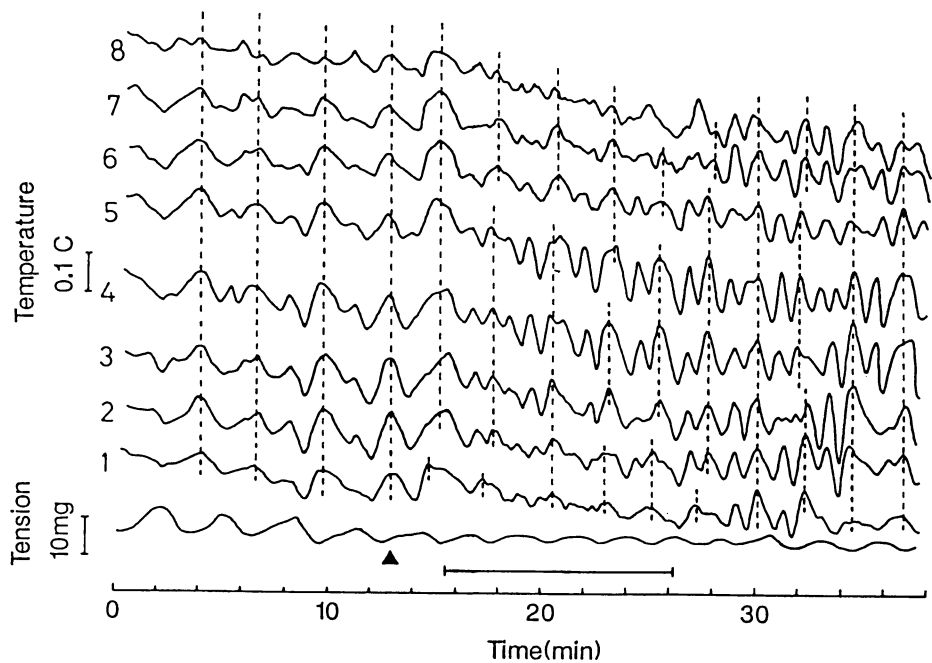


Fig. 6. Time courses of the surface temperature together with the longitudinal tension with the stimulation of 10 mM galactose. Other details are the same as in Fig. 5

3.2. Effect of the Repellent

Fig. 5 is the record when the avoiding behavior of the strand was induced with 1 M KCl (repellent). The phases of the temperature oscillations of the left part of the strand are delayed after the stimulation; thus, the spatio-temporal phase gradient is formed over the strand. As is shown in Fig. 8 *c*, the phase difference between the two ends is as large as 90° . The gradient lasts only for 20 minutes and then becomes flat (at the last part of Fig. 5). The avoiding behavior of the

plasmodium, however, continues, because an appreciable increase was observed in the size of the knot of the right end, suggesting the increase of the amount of the protoplasm.

The amplitude of the tension oscillation decreases with a small shoulder in the waveform. The temperature oscillations include minor peaks of significant amplitudes. The power spectrum (Fig. 7 *c*) shows the increase of second super-harmonic component in the oscillation, especially in the case of temperature oscillation. But no case could we observe the waveform

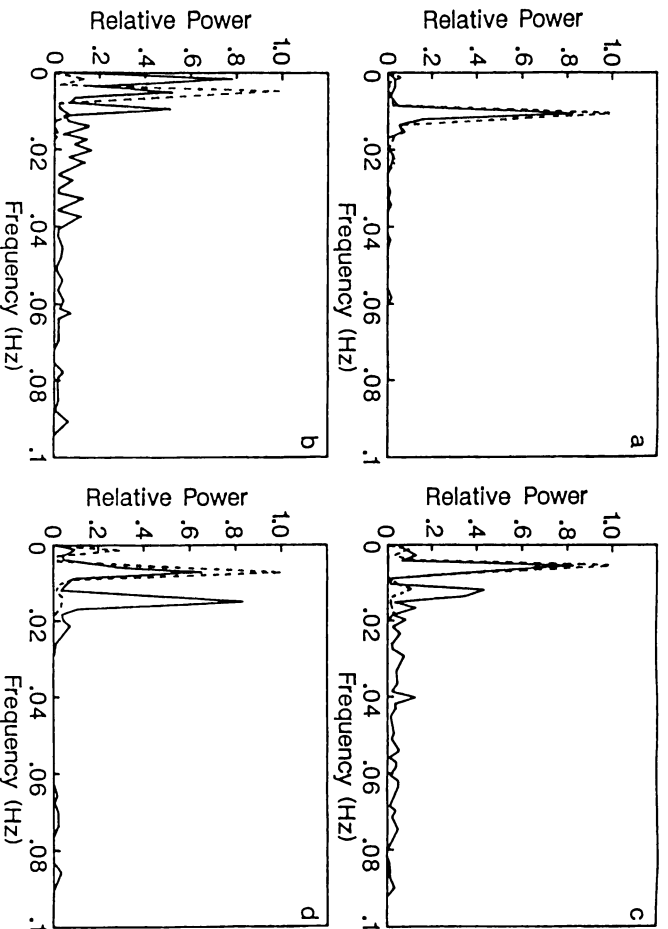


Fig. 7. Power spectra of the temperature and tension fluctuation calculated by FFT. Real line: the spectrum of the temperature of section 5. Broken line: the spectrum of the tension. (a) to (d) correspond to the results of Fig. 3 to 6, respectively.

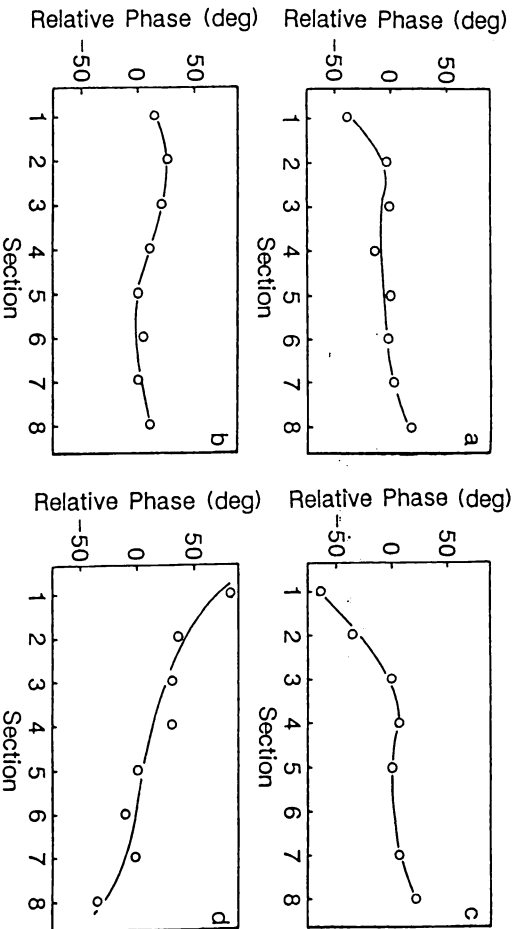


Fig. 8. Relative phase difference between the local temperature oscillations in each section (1-8) calculated by cross-spectra. (a) to (d) correspond to the results of Fig. 3 to 6, respectively.

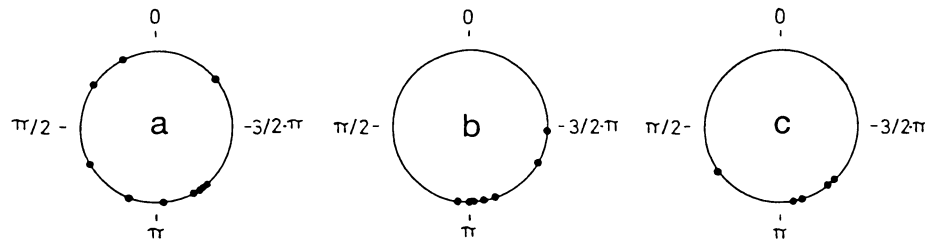
containing minor frequency components in the temperature oscillation as is seen in Fig. 4.

The phase difference between the surface temperature and tension oscillation becomes constant. Considering several different strands, the phase difference between the temperature and tension oscillation ranged from π to $3\pi/2$ (Fig. 9b). Similar reactions were induced in response to other repellents, such as 500 mM methanol, 500 mM ethanol or 1 M glucose (KNOWLES and CARLILE 1978, UEDA and KOBATAKE 1982).

3.3. Effect of the Attractant

Reaction to 10 mM galactose (attractant) is shown in Fig. 6. The phases of the temperature oscillations of the left part of the strand are advanced after the stimulation to form the gradient. As is shown in Fig. 8d, the phase difference between the two ends is about 120° . The gradient lasts only for 20 minutes and then becomes flat (at the last part of Fig. 6), while the approaching behavior continues, because increase of the size of the left end is observed.

Fig. 9. The phase relationship between the temperature oscillation (section 5) and longitudinal tension. (a) The absence of stimulation, (b) after the stimulation of 1 M KCl, (c) after the stimulation of 10 mM galactose. The results of 5–10 different strands are plotted



The amplitude of the tension oscillation decreases with a small shoulder in the waveform. The temperature oscillations include minor peaks of significant amplitudes. The power spectrum (Fig. 7d) shows the increase of second super-harmonic component in the temperature oscillation. But we did not observe the waveform containing minor frequency components in the temperature oscillation.

The phase difference between the surface temperature and tension oscillation becomes constant, and ranges from $3\pi/4$ to $5\pi/4$ (Fig. 9c). Similar reactions were induced by attractants such as 10 mM glucose, 10 mM KH_2PO_4 and an extract of oat flake.

4. Discussion

Without chemical stimulation, the phase relation between the oscillations of the exothermic reaction and contraction tended to be constant for some time and to fluctuate only occasionally (Figs. 4 and 9a). This indicates that the origin of each oscillation is different and they are loosely coupled.

The extent of the coupling increases with stimulation, because the phase relation between the oscillations of the temperature and tension did not vary after the stimulation (Figs. 9b and c). This suggests the induction of a definite relation between the exothermic reaction and motile system in the cell after the stimulation. The observed exothermic reactions probably occur in mitochondria, because in our preliminary experiment the inhibition of respiratory reactions suppressed the temperature oscillation but the tension continued oscillating.

MITO *et al.* (1980) reported that positive chemotaxis is caused by mitochondria while the negative chemotaxis is related to the other mechanism, since inhibition of mitochondria does not inhibit the repulsive motive force while inhibit the attractive one in chemotaxis. On the contrary to these, we observed that both positive and negative tactic behavior of the plasmodial strand examined by the fan formation and thickness of the protoplasm was inhibited under the anaerobic con-

dition, although local repulsive behavior was observed at the stimulated portion with the repellent (data not shown). These suggest that organization of the tactic behavior of the whole cell is related to the function of the mitochondria.

Without stimulation, the oscillations of the local temperatures were almost coincident (Figs. 8a and b) with occasional fluctuation. The phase relation between the local temperature oscillation was within a few tens of degrees. Thus, the exothermic reactions in the local areas were almost synchronized. The waveform of the tension oscillation was rather simple with a large amplitude, suggesting that the oscillations of the local contractile elements were almost synchronized coincidentally (YOSHIMOTO and KAMIYA 1978), although a little difference should be remained for the driving of the shuttle streaming.

The spatio-temporal pattern of the oscillating reaction was influenced by chemical stimuli applied to one end of the strand. The repellent and attractant, respectively, induced phase delay and advance at the stimulated end (Figs. 8c and d) to give a significant phase gradient throughout the strand. The polarity of the spatial phase gradient was consistent with the direction of the subsequent migration of the plasmodium: it moved to the side where the phase had advanced.

Since the small shoulders were observed in the tension oscillations after the stimulation, we can conclude that significant phase differences were produced between the oscillations of the local contractile elements. This suggests that for the driving of the biased shuttle streaming of the protoplasm the local contractile elements organize the phase difference between them. The phase gradient in the temperature was formed only for a few tens of minutes, while the migration continued for a long period. Thus, the formation of the gradient may be related to the process of organization of a consistent direction for the migration throughout the whole cell.

The local information on the type of stimuli may be encoded in the increase or decrease of the fundamental frequency of the local oscillation at the stimulated end

(DURHAM and RIDGWAY 1976). This change can induce a phase gradient between the local oscillations throughout the whole cell because of the mutual entrainment among them. We speculate that the global information on the migration direction of the entire cell is encoded in the polarity of the phase gradient and that the local part of the cell selects its migration direction, according to this polarity.

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