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Relationship between endoplasmic and ectoplasmic oscillations during chemotaxis of *Physarum polycephalum*

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Summary. The plasmodium of *Physarum polycephalum* usually migrates coordinately as one whole body even in a complicated environment. By measuring oscillation phenomena in endoplasm and ectoplasm separately during chemotactic process, we studied the mechanism of information processing to achieve such a coordination. (1) The interaction between endoplasmic oscillators was long-range, competitive according to the length of period, and fast (18 cm/min). Ectoplasmic one was short-range. (2) After a partial stimulation of attractant to the organism, the period at the stimulated portion decreased first, and a global phase gradient appeared in endoplasm. Then ectoplasm at the non-stimulated portion was entrained to the endoplasmic pattern, and the migration direction at each part changed in accordance with the phase gradient as a whole body. (3) When the endoplasmic interaction was interrupted, the above coordinated response was not observed. These facts suggest that two-layer coupled oscillator system composed of endoplasm and ectoplasm play important roles for such an information integration.

Keywords: *Physarum polycephalum*; Information processing; Coordination; Chemotaxis; Oscillation; Endoplasm; Ectoplasm.

Introduction

The plasmodium of *Physarum polycephalum* is a large unicellular organism exhibiting chemotactic behavior (Coman 1940, Ueda et al. 1975, Knowles and Carlile 1978, Ueda and Kobatake 1982). Although the plasmodium has no specially differentiated organs for the information processing such as nervous system, it coordinately migrates as one whole body even in a complicated environment. It is very interesting how such large organism integrates the information from its surroundings in a highly coordinated manner.

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The plasmodium exhibits many kinds of oscillation phenomena, for instance, tension development (Kamiya 1970, Wohlfarth-Bottermann 1975), intra-cellular concentration of Ca^{2+} (Yoshimoto et al. 1981 a, Kuroda et al. 1988), ATP (Yoshimoto et al. 1981 b), H^+ (Nakamura et al. 1982), NADH (Mori et al. 1987). It has been suggested that these rhythms have close correlations with the information processing in chemotaxis. Attractants and repellents decrease and increase the periods, respectively (Durham and Ridgway 1976). A partial application of an attractant produces a spatial phase gradient from the applied site to the other regions (Hejnowicz and Wohlfarth-Bottermann 1980, Ueda et al. 1986, Matsumoto et al. 1986). The migration direction uniquely coincides with the spatial phase gradient (Matsumoto et al. 1988). We also confirmed the same relationship in the surface temperature oscillation (Tanaka et al. 1987). Recently, Ueda et al. (1990) reported that ATP wave propagates from the front region to the rear in the migrating plasmodium.

However, the self-organization mechanism of these spatio-temporal patterns for information processing still remains obscure. It has been reported that spatial synchronization between ectoplasmic oscillations is mediated by endoplasmic streaming (Yoshimoto and Kamiya 1978), and that the streaming is driven by ectoplasmic contraction rhythms (Kamiya and Kuroda 1958). These facts suggest that two kinds of oscillations in endoplasm and ectoplasm play important roles in generating the spatio-temporal coherence. Therefore, measuring these two rhythms separately in chemotactic process, we tried to clarify an information integration mechanism for the coordinated movement.

Materials and methods

Organism

The plasmodium of *Physarum polycephalum* was cultured by using the method of Camp (1936) and stored as sclerotia. Before use, the plasmodium was reactivated and allowed to migrate on a sheet of 1.5% agar gel plate without feeding in the dark at room temperature (20–23 °C). After 20 to 40 h from the start of the reactivation, a segment of smooth plasmodial strand, about 40 mm long and 0.3 to 0.5 mm in diameter, was excised carefully from the network portion and used for the experiments.

Experimental implementations

Measurement of transmitted light intensity

The plasmodial strand was transferred on a cellulose sheet (Union Carbite) placed on a 1.5% agar gel plate of 5 mm thick. After 1 to 2 h, a part of the strand was covered with another cellulose sheet of 5 mm wide. The thickness of the strand was defined as a spacing between the two sheets and adjusted to about 0.1 mm as shown in Fig. 1 a. The specimen was kept in humid air in a container at room temperature (20–23 °C). This treatment did not interrupt the migration of the plasmodium.

Microscopic observation (Olympus, BH-2, objective A10PL) was started more than 1 h after this treatment. Illuminating the entire strand by diffuse white light of about 300 lux from below, the observed image was projected on a TV monitor (Sony, KX-20HF1) through a high-sensitivity camera (Ikegami, CTC9000) as shown in the same figure. The transmitted light intensity on the image was transformed proportionally into an electrical potential by directly attaching a phototransistor (Toshiba, TPS-607) on a surface of the monitor. The distance between the two detectors for endoplasmic and ectoplasmic regions was adjusted to 200 μm in absolute size. The diameter of the measuring portion was about 25 μm. These potentials were digitized (Canopus, ADX-98E) and stored in a personal computer (NEC, PC-98XA). Data acquisition was achieved at 2 sec interval.

An example of the time courses of the transmitted light intensity measured in endoplasm and ectoplasm is represented in Fig. 1 b. They were analyzed by calculating a period and a phase difference as shown in the same figure. The phase difference sometimes fluctuated between in-phasic and anti-phasic states. Especially, the wave form in endoplasm was not superimposed over that of ectoplasm during anti-phasic state. These facts indicate that oscillation phenomena in endoplasm and ectoplasm are recorded exclusively.

The temporal change of the transmitted light intensity is thought to be influenced by the thickness, shuttle streaming and configuration of cytoplasmic components. The temporal change of the thickness at the observation point was measured at about 6 μm. Sandwiching the strand by two pieces of hard plastic sheets designed to be highly permeable for oxygen (Hoya, HARD58), its effect was estimated to be less than 8%. The rhythm obtained under cross nicol condition (Olympus, BH-POL) was always anti-phasic to that of normal condition both in endoplasm and ectoplasm (data not shown). By considering the report of Ishigami et al. (1987), it is suggested that the optical oscillation has close correlation with birefringent fibrils. If the rhythm in endoplasm was passively induced by spatial movement of endoplasm, it should be symmetrical with respect to a turning point of the shuttle streaming. However, since it was random (data not shown), the effect is thought to be sufficiently small. These facts suggest that the oscillation of the transmitted light intensity is mainly

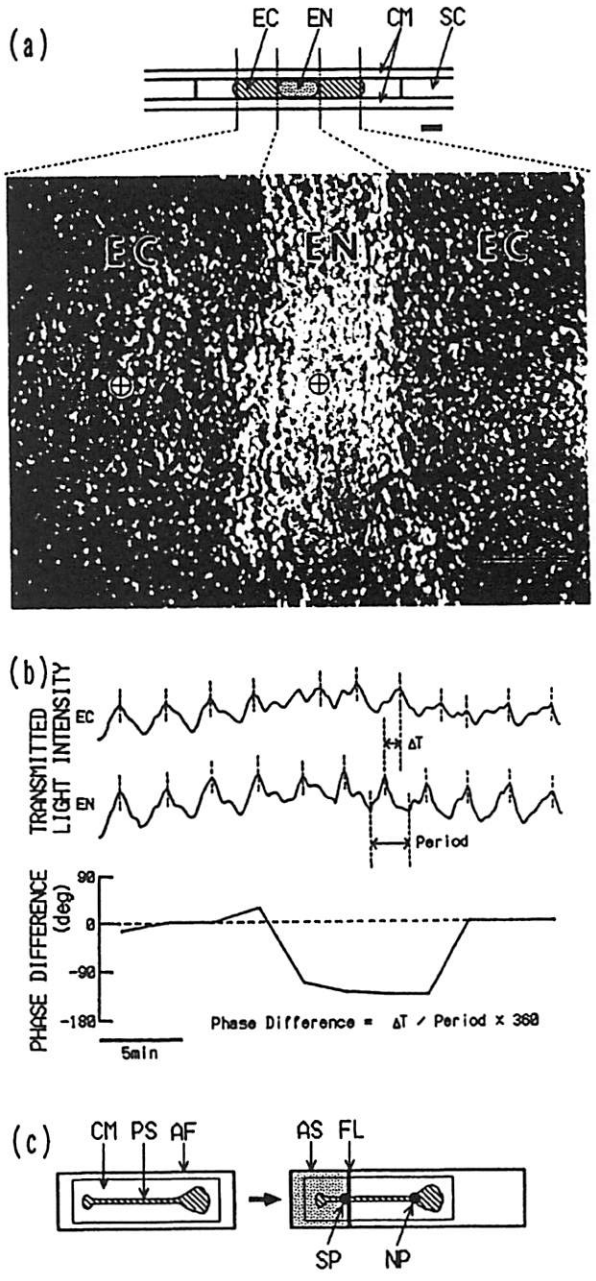

Fig. 1. Experimental system to measure the endoplasmic and ectoplasmic oscillations. **a** Schematic illustration of a cross section of the observation point (top) and its monitored image (bottom). ⊕ Measuring point. **b** Time course of the transmitted light intensity. A period was defined by the interval between the two successive minimum peaks. To obtain a phase difference, time interval of the two corresponding maximum peaks between the two oscillations was divided by one of the two periods and multiplied by 360°. **c** Stimulation to the plasmodium. *EN* Endoplasm; *EC* ectoplasm; *CM* cellulose membrane; *SC* spacer; *PS* plasmodium; *AF* agar gel plate; *AS* agar gel plate with stimulant; *FL* thin film; *SP* and *NP* observation point in stimulated and non-stimulated portions. Bars: 100 μm

attributable to the configuration change of fibrous components in cytoplasm.

Measurement of net flow time

To measure the migration velocity during chemotaxis, the temporal change of the shuttle streaming was analyzed from the monitored image. An approximated index of the streaming, net flow time, was defined as a summation of the time intervals between the two successive reversals of the streaming direction, and duration time to the migration direction observed before stimulation was given positive value. Since time average of diameter and streaming velocity in one shuttle did not exhibit significant difference between the two directions (data not shown), it is suggested that the index is approximately proportional to the net transported volume in shuttle streaming. Thus, the velocity of the endoplasmic transportation was defined as the net flow time in every 5 min interval.

Procedures for the observation of chemotactic response

When an attractant was applied to a part of the plasmodium, whole parts of the organism migrated toward the stimulus coordinately. On the other hand, when a repellent was applied in the same way, the repulsive response was observed in the neighborhood of the stimulus (data not shown). Accordingly, the reversal process of the migration direction as a whole body after application of an attractant was investigated as the most simple case exhibiting the coordinated migration in chemotaxis.

Within 1 to 2 h from the treatment for the optical observation, the plasmodium selected one of the directions and started to migrate toward the direction as a whole body. Then, an agar gel plate containing attractant (10 mM glucose or oatmeal extract) was placed in backward direction of the migration as shown in Fig. 1 c. Between the two plates, thin film (American Can Company, Parafilm) was inserted to prevent diffusion. Then, sliding the cellulose sheet on which the plasmodium was placed, we attached the end of the plasmodium to the stimulus in about 1 cm long. The transmitted light intensity and the net flow time were measured in the stimulated and non-stimulated portions, respectively.

Results

Relationship between oscillations and migration during chemotaxis

Temporal change of the oscillations

Figure 2 a shows an example of the time courses of the oscillatory pattern observed in endoplasm and ectoplasm during chemotactic process induced by 10 mM glucose.

These results were analyzed as the relative change of period as shown in Fig. 2 b. First, the period of ecto-

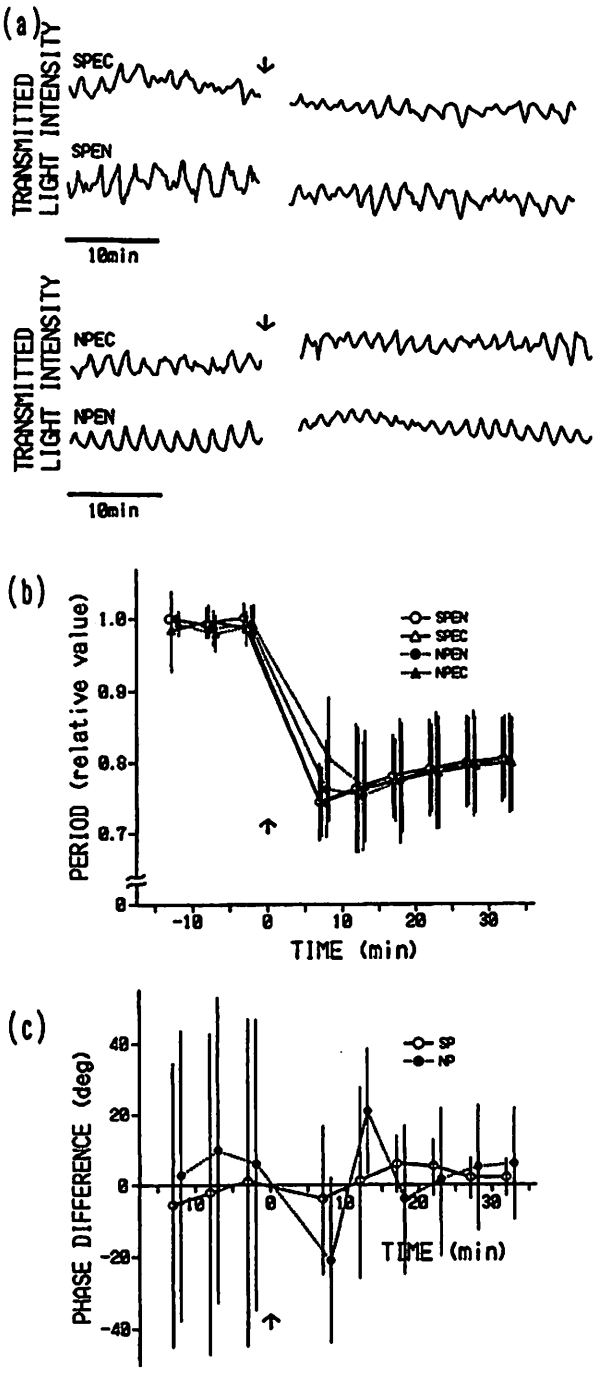

Fig. 2. Temporal change of the relationship between endoplasmic and ectoplasmic oscillations. a Time courses of the transmitted light intensity. Since the treatment for stimulation takes a few minutes, data were not obtained in the interval. b and c Period (relative value) and phase difference between endoplasm and ectoplasm. They were firstly calculated in each sample as a mean value in every 5 min interval, and they were re-averaged among many samples. The period was normalized by the mean value of endoplasmic rhythm obtained in the first interval. The phase advance of EC to EN was given positive value. Abscissa indicates the time from the stimulation (10 mM glc). Arrows stand for the onset of the stimulation. Similar results were also obtained in the chemotaxis to oatmeal. *SPEN* and *SPEC* EN and EC at observation point in stimulated portions (SP); *NPEN* and *NPEC* EN and EC at observation point in non-stimulated portions (NP). n = 7 and 11 for SP and NP, respectively. Bars indicate standard deviation

plasm coincided with that of endoplasm in each observation point. After stimulation, they decreased simultaneously by about 25% at the stimulated portion. However, at the non-stimulated portion, the response of ectoplasm delayed to endoplasm until 15 min later. This fact implies that the periods of endoplasm and ectoplasm become different in this interval, and that the decrease of period propagates spatially through endoplasm. After this transition process, they synchronized again and these periods gradually increased. The phase difference between endoplasm and ectoplasm was calculated from the same data as shown in Fig. 2c. Before stimulation, remarkable phase difference was not observed at each portion. After stimulation, the phase difference at the stimulated portion did not change while that of the non-stimulated portion largely fluctuated. Since the periods of endoplasm and ectoplasm did not coincide in this time interval (Fig. 2b), such response is thought to be observed. After this transition process, the synchronized state was recovered. On the other hand, the standard deviation of the phase difference decreased at both portions after stimulation. This fact implies that the intensity of mutual interaction between endoplasmic and ectoplasmic oscillations increased.

The spatial relationship of oscillatory pattern between stimulated and non-stimulated portion was measured in the same chemotactic process. Figure 3a represents an example of the time courses of endoplasmic oscillations recorded simultaneously at both observation points.

The spatial phase difference between them was calculated as shown in Fig. 3b. First, the oscillations at both observation points spatially synchronized, and

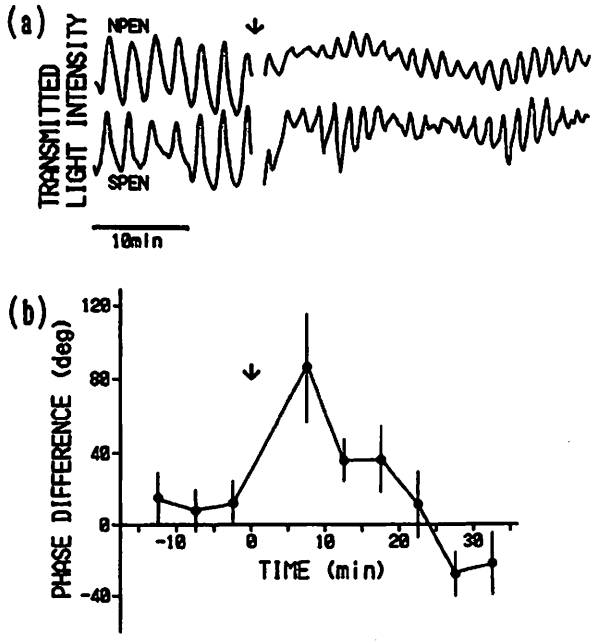

Fig. 3. Temporal change of the spatial relationship between endoplasmic oscillations. a Time course of the transmitted light intensity. b Spatial phase difference. The plasmodial strand was laid in the form of "U"-shape, and the two observation points were imaged in the same visual field simultaneously by using a low magnifying power objective (Olympus, Plan 2). The phase advance of SP to NP was given positive value. Other details are the same as in Fig. 2. n = 6. Bars indicate standard deviation

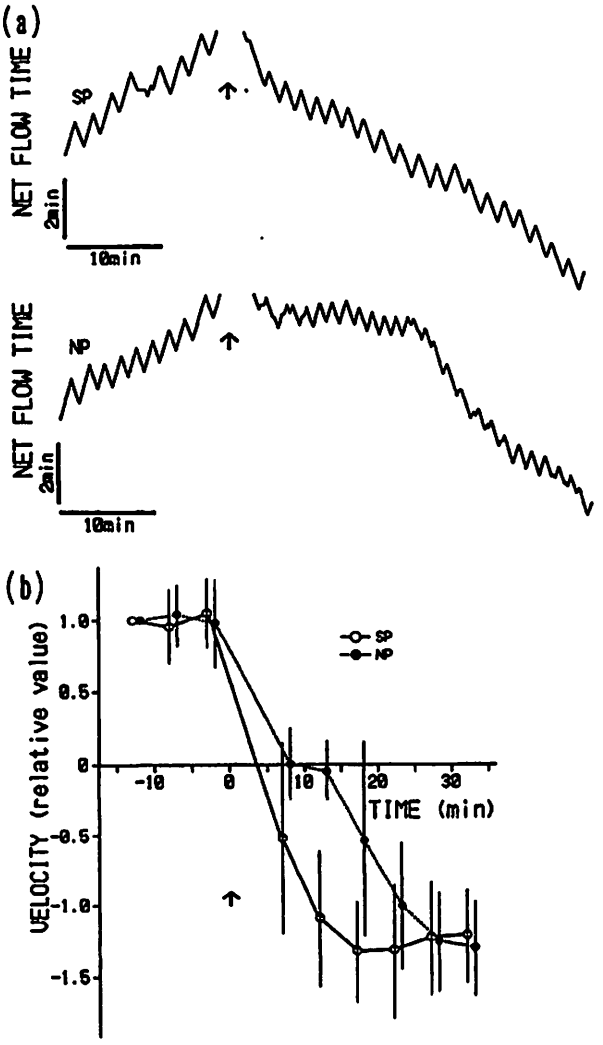

Fig. 4. Temporal change of the migration. a Time courses of the net flow time. b Velocity of endoplasmic transportation (relative value). It was normalized by the mean value obtained in the first time interval. The value in the first interval at SP and NP was 45.5 sec and 47.1 sec, respectively. Other details are the same as in Fig. 2. n = 7 and 11 for SP and NP, respectively. Bars indicate standard deviation

significant phase difference was not observed. After stimulation, the phase in the stimulated portion immediately advanced to the others. Then, the stable phase difference of about 40 degrees was observed until 20 min after stimulation. The direction of phase advance pointed to the stimulated portion in the plasmodium. This large phase difference disappeared after this time interval.

Temporal change of the migration

Figure 4a represents an example of the time courses of the net flow time during the same chemotactic process.

To investigate the migration pattern, the temporal change of the endoplasmic transportation velocity was calculated from the net flow time as shown in Fig. 4b. First, the velocity at the stimulated and non-stimulated portion exhibited little difference. After stimulation, the velocity at the stimulated portion rapidly reversed. This response is thought to be simultaneous with the decrease of period (Fig. 2b) and the emergence of the endoplasmic phase gradient (Fig. 3b). However, at the non-stimulated portion, the velocity decreased to zero until 15 min after stimulation, i.e., stop of migration. After the transition process, the velocity started to reverse. This timing is thought to coincide with the resynchronization between endoplasm and ectoplasm (Fig. 2b and c). Finally, the velocity at both portions coincided, and the coordinated migration as a whole body was recovered in the direction of endoplasmic global phase gradient (Fig. 3b).

These facts indicate that the transition process in the oscillation phenomena precedes the complete reversal of the migration direction as a whole body. Thus, it is suggested that the oscillations in endoplasm and ectoplasm play important roles in the information processing for the coordinated migration in chemotaxis.

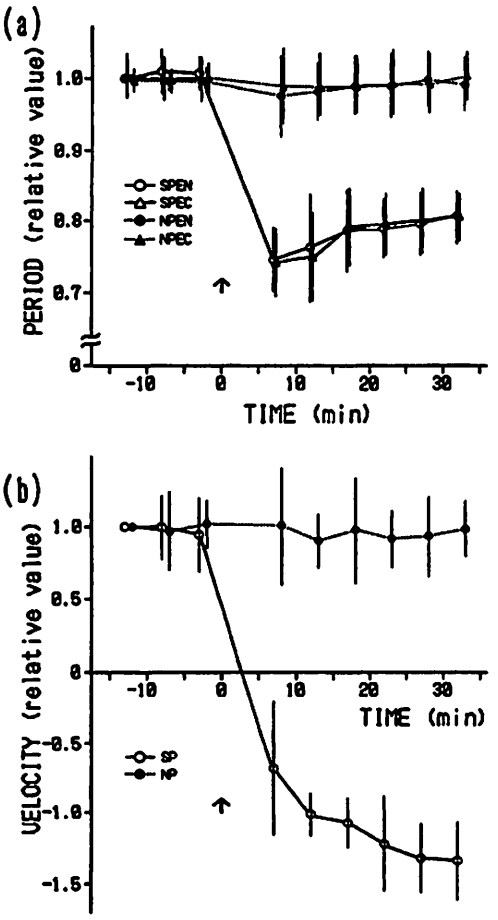

Fig. 5. Effects of inhibition of the endoplasmic interaction. a Period (relative value). b Velocity (relative value). An air bubble about 5 mm long was formed in endoplasm at the middle between the two observation points. The migration direction did not change and its velocity did not decrease more than 15% by this treatment. Then the ectoplasmic rhythms around the bubble were not interrupted. The stimulus was applied about 30 min after this treatment. Other details are the same as in Figs. 2b and 3b, respectively. n = 4 for each portion. Bars indicate standard deviation

Effects of inhibition of endoplasmic interaction

Figure 5a shows the temporal change of period under inhibition of the endoplasmic interaction during the same chemotactic process. After stimulation, the responses in endoplasm and ectoplasm were not observed at the non-stimulated portion. This fact indicates that the period variation spatially propagates through endoplasm. Thus, it is suggested that the interaction in endoplasmic oscillations is long-range while that of ectoplasmic ones is short-range.

Figure 5b shows the temporal change of the velocity of endoplasmic transportation under the same conditions. After stimulation, since the velocity at the non-stimulated portion did not change, the discrepancy of migration direction in a body was observed. This fact indicates that the endoplasmic interaction plays an important role in generating the coordinated movements in chemotaxis. Particularly, since the direction of the spatial phase gradient in endoplasm uniquely corresponded to the migration direction (Figs. 3b and 4b), it is suggested that the global phase gradient in endoplasm represents the integrated information which controls the migration as a whole body.

Spatial entrainment of oscillations in endoplasm

Effects of period variation

The relationship between the period variation and its spatial propagation in endoplasm was investigated as shown in Fig. 6 a. When the period decreased after stimulation, its variation propagated to the non-stimulated portion regardless of the stimulants. When the period increased, such response was hardly observed at the non-stimulated portion. Endoplasmic streaming was not inhibited under these conditions. These facts suggest that the endoplasmic interaction is asymmetrical according to the length of period. A rhythm with comparatively shorter period is thought to propagate in the longer distance.

On the other hand, when the environmental conditions for the plasmodium were relatively improved independent of whether the stimulant was an attractant or a repellent, the period decreased at the stimulated portion and vice versa. This fact suggests that the information from the surroundings are estimated in comparison with the internal state of the plasmodium and coded by the period change.

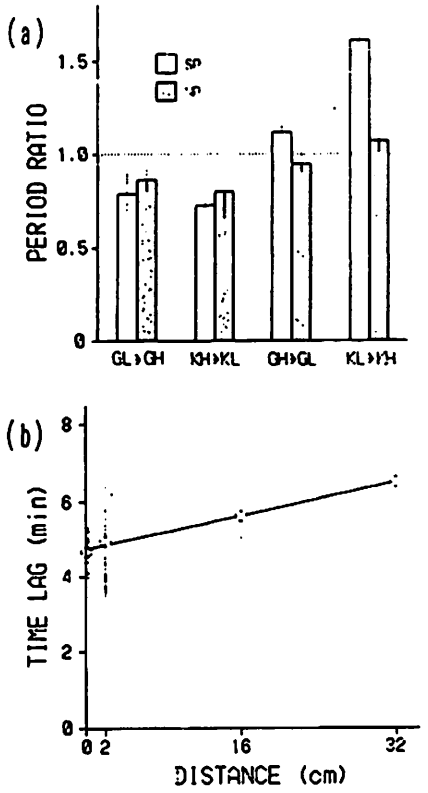

Fig. 6. Spatial entrainment of oscillations in endoplasm. **a** Relationship between period change and its spatial propagation. By using the similar stimulation method exhibited in Fig. 1 c, whole parts of the strand were first placed on the basic stimulant, then an end of it was attached to another stimulus. The response at NP was measured in endoplasm at 2 cm apart from SP. A mean period in 15 min interval from 5 min after stimulation was divided by that of just before stimulation. The bottom part of the figure shows the sequence of stimuli. To clarify a direct response at SP, a short plasmodial strand of 1 cm length was attached to the stimuli in the same sequence. *GH* and *GL* 10 and 1 mM glc; *KH* and *KL* 60 and 10 mM KCl. n = 5 for each column. **b** Relationship between distance from the stimulated portion and time-lag of period variation. Stimulus (10 mM glc) was induced at an end of the strand. The response was observed at 1 cm apart from the opposite end. Preparing various lengths of strands, the distance was regulated. The time-lag was defined as the time interval between the onset of stimulation and the timing of 10% decrease of period. n = 9, 16, 5, and 4 for distance 0, 2, 16, and 32 cm, respectively. Other details are the same as in Fig. 2. Bars indicate standard deviation

Propagation velocity

The relationship between the time-lag of period variation and the distance from the stimulated portion was measured as shown in Fig. 6 b. The time lag increased proportionately as the distance increased. The propagation velocity of spatial entrainment in endoplasmic oscillations was estimated to be about 18 cm/min. Since this value is much larger than the mean velocity of the shuttle streaming (2–3 cm/min), it is suggested that the endoplasmic interaction is mediated not only by the shuttle streaming but also by other dynamic mechanisms occurring in the streaming.

Discussion

We propose a model of the information processing system for the coordinated migration in chemotaxis of *Physarum* plasmodium as shown in Fig. 7. It is composed of two levels of internal subsystems, the higher level corresponds to the endoplasmic oscillator system and the lower one to the ectoplasm.

The function of the endoplasmic oscillator system is thought to integrate the environmental information

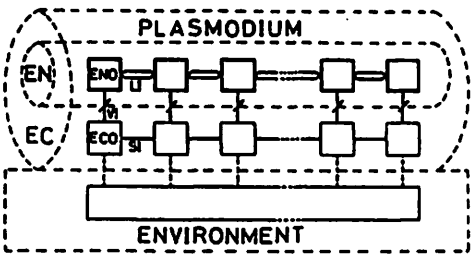

Fig. 7. Schematic representation of the information processing system in the plasmodium. *EN* Endoplasm; *EC* ectoplasm; *ENO* endoplasmic oscillator; *ECO* ectoplasmic oscillator; *LI* long-range and competitive interaction; *SI* short-range interaction; *VI* variable interaction

and to regulate the migration pattern coordinately as a whole body. Since the interaction between endoplasmic oscillations is long-range, competitive according to the length of period, and fast, it is very convenient to self-organize a single spatial phase gradient as an integrated information even when many periods representing the complicated external environment exist in a large plasmodium. Then it is suggested that the direction of the global phase gradient in endoplasm regulates the migration direction as a whole body.

The function of the ectoplasmic oscillator system is thought to represent the local environmental conditions around the plasmodium, because its interaction is short-range. Then it is suggested that the information from the environment is estimated in comparison with the internal state of the plasmodium, and the result is coded by the period variation.

An information integration dynamics is speculated as follows. First, environmental condition is received by the ectoplasmic oscillator system in each part as a period variation and transmitted to endoplasm. Even if many rhythms with different periods are generated according to the complicated circumstances, they are processed in parallel due to the competitive interaction in endoplasm. Then, the one with the shortest period indicating the best-conditioned portion slaves the other oscillations, and single global phase gradient around the part is self-organized in endoplasm as an integrated information. Finally, rhythms in ectoplasm are modulated to coincide with the endoplasmic ones owing to the increase of coupling intensity. Thus, the migration direction in each part is also regulated according to the endoplasmic phase gradient. Through these dynamic processes, global coherence in oscillations and migration as a whole body are thought to be established.

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