

Development of spatio-temporal pattern of Ca^{2+} on the chemotactic behaviour of *Physarum* plasmodium

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Summary. The development of a spatio-temporal pattern of Ca^{2+} concentration by a plasmodium of *Physarum polycephalum* during chemotaxis was studied using fura-2. Whenever the cell displayed coordinated migration in one direction as a whole body, a spatio-temporal pattern was established with a characteristic feature along the longitudinal axis. Calcium concentration oscillated with a period of a few minutes within the cell; the mean concentration at the front was higher than that at the rear. When the cell was given an attractant only at the rear end, the mean concentration rose at the site of application with an immediate increase in the frequency of oscillation. First, the change of the frequency is propagated toward the other end and then the mean level of the Ca^{2+} concentration at the non-stimulated site decreases. As a result, the Ca^{2+} gradient is reversed along the cell, which then begins to migrate in a coordinated manner in the reverse direction. This study showed that the spatio-temporal pattern of Ca^{2+} concentration is closely related to information processing for coordinated migration in chemotaxis. The role of the pattern in that process is discussed.

Keywords: *Physarum polycephalum*; Spatio-temporal pattern of Ca^{2+} concentration; Information processing; Intracellular Ca^{2+} ; Chemotaxis

Introduction

The plasmodium of *Physarum polycephalum* is a giant unicellular organism that usually displays coordinated migration as a whole body. The motility behaviour is affected by chemicals (Coman 1940, Carlile 1970, Ueda et al. 1975, Knowles and Carlile 1978, Ueda and Kobatake 1982). Even when a cell receives a local chemical

stimulus, it can properly process the information for the entire cell to behave in a well coordinated manner as a whole body. However, there is no specially differentiated organ for the processing. Therefore, the plasmodium seems to require a coherent and self-organized spatio-temporal pattern throughout it, which agrees with the migratory direction, in the information processing.

This can be seen from the rhythmic phenomena. When a chemical is applied to a portion of the plasmodium, a phase difference, which agrees with the migration direction, of the oscillation of thickness change is formed in the cell (Matsumoto et al. 1986, 1988). Thus, the phase gradient may be one candidate for the processor of the information. If this is true, when the plasmodium is stimulated, a phase gradient should be generated before the plasmodium displays a coordinated change in migratory direction. The relationship between the gradient and the migratory change is, however, not yet clear. Recently, Miyake et al. (1991) reported that the generation of a phase gradient precedes the start of the coordinated behaviour. Thus, the phase gradient can process the information for the coordinated behaviour of the plasmodium. Nevertheless, it has not been clarified how a spatial phase gradient can cause the motility behaviour. In addition, the phase gradient of temperature oscillation disappears after the cell begins to migrate (Tanaka et al. 1987) coordinately. Thus, some pattern other than phase gradient should also be related to the information processing for the coordinated behaviour of the plasmodium.

A gradient of chemicals, ATP, ADP, cAMP and cGMP, which agrees with the migratory direction, ex-

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ists in the plasmodium (Ueda et al. 1986). This gradient changes in response to local chemical stimuli (Ueda et al. 1987, 1988 c), the change being in agreement with the cytoskeletal organization, which is responsible for cell migration (Ueda et al. 1990). Thus, the self-organized pattern of chemical concentration may be another candidate for the processor of cell information. To clarify this, it is necessary to observe not only the pattern but also the behavioural change during the processing of the plasmodium under stimulation.

In this study, we observed both the spatio-temporal pattern of Ca^{2+} concentration and the change of migratory behaviour during the information processing of the plasmodium. Calcium ion is known to regulate the cytoskeleton of the plasmodium, which is related to the migration of the cell (Hasegawa et al. 1980, Furuhashi and Hatano 1989). Also, the Ca^{2+} concentration in the plasmodium displays oscillation (Yoshimoto et al. 1981). Our present work showed that a steady spatio-temporal pattern of Ca^{2+} concentration always exists in the plasmodium when the cell displays coordinated migration. By attractant stimulation, another steady and coherent pattern was generated and the cell began to migrate in a coordinated manner.

Materials and methods

Organism

The plasmodium of *Physarum polycephalum* was cultured by the method of Camp (1936). It was allowed to migrate on 1.5% agar gel in a trough overnight without feeding before use. A plasmodial strand about 10 mm in length and 0.6–0.8 mm in diameter was used.

Experimental apparatus

The system used to measure Ca^{2+} concentration is schematically shown in Fig. 1A. It is composed of a fluorescence microscope (Olympus, BH-2-RFK), a photomultiplier tube (Hamamatsu Photonics Co., R-268) and a recording instrument. A plasmodium subjected to microinjection as mentioned below is placed on the stage of the fluorescence microscope. Ultraviolet (UV) light from an ultra high pressure mercury lamp (Osram, HBO 100W/2) is conducted to the stage through a dichroic mirror and an epifluorescence objective lens (Olympus UVFL $\times 20$). The UV light is filtered by band-pass filters of 340 nm and 360 nm (Nihon Shinku Kougaku Co., BPF and Hard-BPF, respectively). The illumination area of the cell is only about 150 to 200 μm in diameter. Of this area, the area of ectoplasm in the plasmodium usually accounts for about 30% and that of flowing endoplasm for 70%. The fluorescence is detected through an interference filter of 498 nm (Nihon Shinku Kougaku Co.). Its intensity is recorded with a pen-recorder after amplification by a photomultiplier tube.

Measurement of Ca^{2+} concentration

Figure 1B shows the procedure of the present experiment. A plasmodial strand is excised from the middle part of a large plasmodium

and put on a piece of cellulose sheet on plain agar that is 2 mm thick. In both sides, vinyl sheet is put for the plasmodium not to form fan-like structure in the middle region (Fig. 1B, a). One to two hours later, fan-like structures appear at both ends of the strand (Fig. 1B, b). Next, a fura-2 solution, containing 1.2 mM fura-2, 25 mM KCl, 1 mM NaCl and 10 mM HEPES at pH 7.4, is micro-injected into the plasmodium for a concentration of 10–15 μM of fura-2 in the cell. In relative high concentration, fura-2 is sequestered in lysosomes in a cell. In the present experiment, however, that was little happened. Within half an hour, the solution diffuses uniformly throughout the cell. The sample is then covered with two plastic sheets (Hoya, Hard58), specially designed for good oxygen permeation, to reduce the thickness change of the cell. Under these conditions, the migratory and chemotactic behaviours of the cell did not differ from the

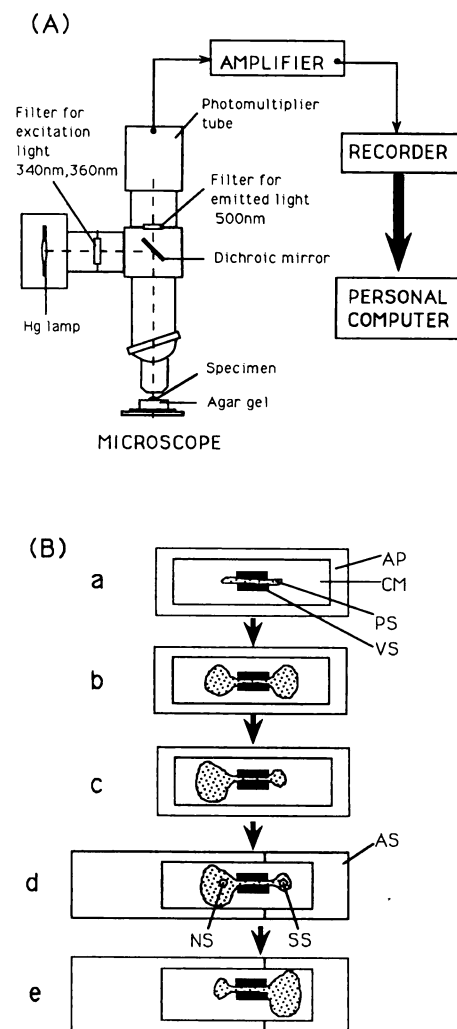


Fig. 1. A Schematic illustration of the apparatus for measuring of Ca^{2+} concentration in *Physarum* plasmodium using Ca^{2+} indicator, fura-2. B Procedure for stimulus application and observation of the chemotactic response. Each circle in the plasmodium (d) represents the illumination area, which was about 150 to 200 μm in diameter. AP Agar gel plate; CM cellulose membrane, PS plasmodial strand, VS vinyl sheet, AS agar gel plate containing the stimulant, SS stimulation site, NS non-stimulation site

normal ones. In an hour, one of the two fans becomes larger than the other (Fig. 1 B, c), indicating that the cell has begun to migrate towards the bigger fan. This cell, that is 1.5 to 2 cm long, is then transferred to the above system and stimulated.

Chemical stimulation is applied by exchanging part of the agar gel plate under the rear fan with one containing chemicals such as 10 mM glucose, 10 mM galactose, 10 mM KH_2PO_4 (Fig. 1 B, d).

The Ca^{2+} concentration in the plasmodium can be quantitatively estimated from the ratio of the intensity of fluorescence at 340 nm to that of 360 nm (Gryniewicz et al. 1985, Tsien et al. 1985); it was calibrated by the method of Kudo and Ogura (1986). At then, since the plasmodium has the autofluorescence at 340, 360 nm, the ratio was calculated from the values subtracted the autofluorescence from the raw data at each wave length. Ca^{2+} concentration was measured in the fans formed at the two ends of the strand, if not mentioned otherwise. The stimulated rear is referred to as the "stimulation site (S-site)" and the non-stimulated front fan as the "non-stimulation site (NS-site)". The NS-site was about 1 cm far from the S-site (Fig. 1 B, d). The Ca^{2+} concentration of each site was measured for 15 min before stimulation. After the exchange of agar which usually took about 2 min, the concentration was measured again for 35 min. The plasmodium was illuminated with UV light with a cycle of 1 s on at each excitation wavelength and 8 s off. Ueda et al. (1988 a, b) have reported that UV light lengthens the period of oscillation and induces the avoidance reaction of the cell. Hence, the duration of illumination has to be as short as possible. Under the present conditions, UV light altered neither the rhythmic phenomena nor the chemotactic behaviour of the cell (data not shown).

All experiments were performed at room temperature (24 °C) under the dark condition.

Estimation of migration velocity

The cytoplasm of a plasmodium flows to and fro every few minutes, termed "shuttle streaming". When the cell is migrating, the streaming transports cytoplasm from the rear to the front. Hence, the amount of the cytoplasm transported by the streaming can be regarded as a good index of cell migration. Under the present conditions, we can estimate the amount by separately integrating the period of flow in each direction, from the S-site to the NS-site and in the opposite direction. The averaged net flow every 5 min is calculated and called the "migration velocity". Further details are described by Miyake et al. (1991).

Results

Spatio-temporal pattern of Ca^{2+} concentration before and after stimulation by attractant

The plasmodium already had a gradient of Ca^{2+} concentration, when it migrated in one direction. One typical example is shown in Fig. 2 a. Each point represents the averaged Ca^{2+} concentration for 5 min in a cell. The concentration at the front was about 140 nM higher than that at the rear. When the rear part of the cell was stimulated by 10 mM glucose, the Ca^{2+} gradient was reversed as shown in Fig. 2 b. Then the migration direction also was reversed. Thus, this gradient of Ca^{2+} in the cell agreed with the migratory direction. Similar gradients were found for the other three cells.

Typical temporal patterns in the reversal process are shown in Fig. 3. The observation points are at the front (S-site, Fig. 3 a) and at the rear (NS-site, Fig. 3 b) of the plasmodium. At both sites, the Ca^{2+} concentration constantly oscillated with periods of a few minutes before the stimulation. Their oscillating levels were in a steady state and differed from each other. The mean level of the front (NS-site) was about 244 nM and that of the rear (S-site) was about 189 nM for 15 min before the stimulation. This absolute Ca^{2+} concentration was about in the same range as that in the microplasmodia reported by Kuroda et al. (1988).

The stimulation caused a marked change in the Ca^{2+} concentrations at the two sites. At the S-site, the concentration rapidly increased and then gradually rose for 10 min to reach a steady state. The plateau con-

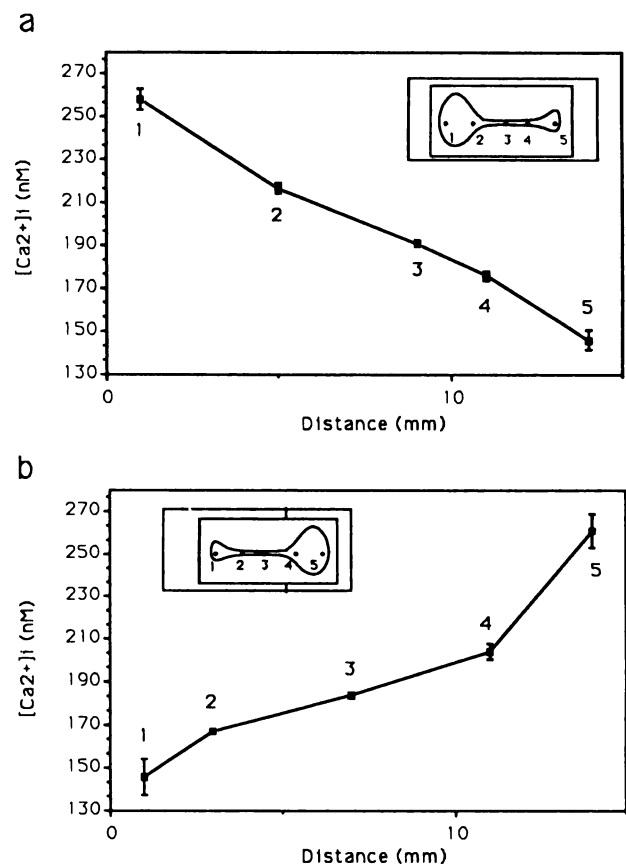


Fig. 2. Typical spatial pattern of Ca^{2+} concentration along the unipolar plasmodium **a** before and **b** 35 min after stimulation with 10 mM glucose. We measured Ca^{2+} concentration for 5 min at every five points along the longitudinal axis. Numbers in each figure correspond to the positions in each inset. The points represent the averaged concentrations for 5 min of Ca^{2+} change in one plasmodium. S.D. value is so small in each position, since the change of Ca^{2+} concentration for 5 min there was small in this plasmodium. Bars = S.D. (standard deviation)

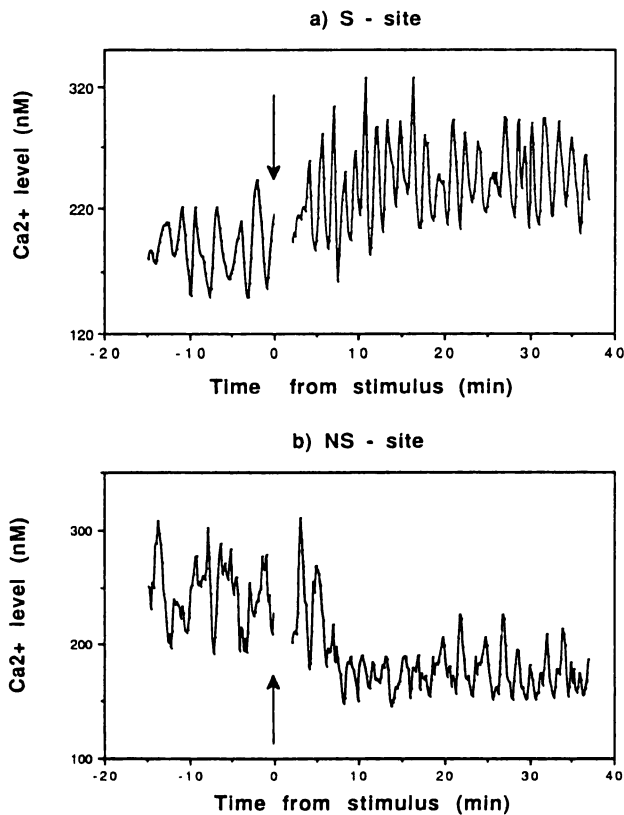


Fig. 3. Examples of the developing temporal pattern of Ca^{2+} concentration at S- and NS-sites when a plasmodium was stimulated with 10 mM glucose. **a** S-site (stimulation site), **b** NS-site (non-stimulation site). In this and the following figures, arrow indicates glucose application

centration of 246 nM averaged from 10 to 35 min (Fig. 3 a). At the NS-site the Ca^{2+} concentration gradually decreased and also reached a steady state at 175 nM, averaged, which lasted about the same time as the S-site (Fig. 3 b).

These time courses were statistically averaged ($n = 7$ and $n = 11$ for the S- and the NS-site) and the same tendency was found (Fig. 4). Before the stimulation, the averaged concentration at S-site, 170 ± 12 nM (mean concentration \pm S.D.), was higher than that at NS-site, 224 ± 16 nM (mean concentration \pm S.D.), which was averaged for -15 to 0 min. On the other hand, after the stimulation, the values were reversed at 5 min and reached a steady state. The plateau concentration after the stimulation was 223 ± 31 nM (mean \pm S.D.) and 173 ± 28 nM (mean \pm S.D.), which were averaged for 15 to 35 min. In addition, Ca^{2+} concentrations at the two sites were reversed 5 min after the stimulation and reached a steady state at about 15 min.

The onset of local stimulation changed the periods of Ca^{2+} oscillation at both sites. They became shorter

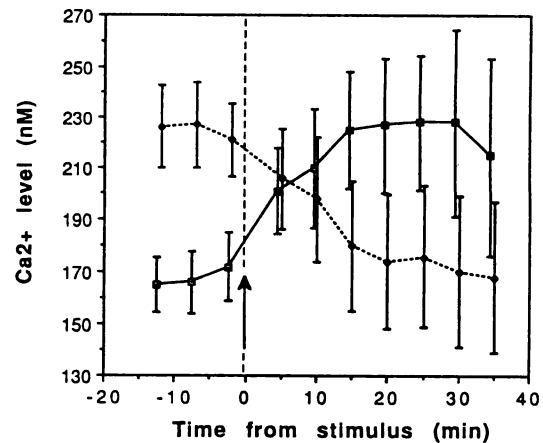


Fig. 4. Time courses of averaged Ca^{2+} concentrations at S- and NS-sites. Solid and broken lines stand for Ca^{2+} concentrations at the S- and the NS-site, respectively. The averaged values for every 5 min interval were calculated and then averaged for each site as shown (S-site: $n = 7$, NS-site: $n = 11$). Bars = S.D.

(Fig. 3 a and b); about 70–80% on the average. As shown in Fig. 5 a, the changes at both sites occurred as soon as the plasmodium was stimulated, with recovery starting at 20 min.

Similar responses were observed with the other kinds of attractants, such as 10 mM galactose and KH_2PO_4 (data not shown).

Correlation between the Ca^{2+} pattern and migratory direction of the plasmodium

The velocities at both S- and NS-sites of the plasmodium were almost the same as a unit before stimulation, as shown in Fig. 5 b. Hence, the cell showed coordinated migration to the NS-site as a whole body. When it was stimulated at the rear part (S-site) with 10 mM glucose, the velocities at the two sites became different. At the S-site, it immediately decreased to a negative level while at the NS-site it remained about zero, indicating that the cell had reversed its migratory direction only at the S-site. About 10 min later, the NS-site value also decreased below zero. The velocities at both sites were small and significantly different, which indicates that the cell was migrating in separate parts and not as a coordinated whole. The speed of both sites gradually increased in the reversed directions. About 20 min after the stimulation, they reached a steady state with the same velocity throughout the entire cell as that before the stimulation. Finally, the plasmodium began to migrate in a coordinated manner toward the attractant.

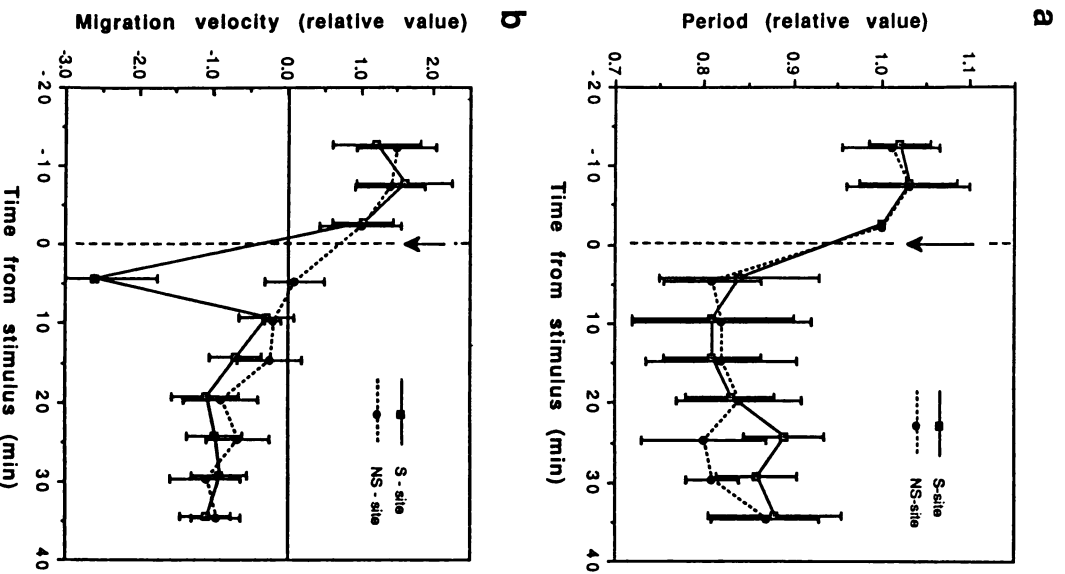


Fig. 5. **a** Time courses of the periods of Ca^{2+} oscillation at S- and NS-sites with glucose stimulation. The period is defined as the interval between the successive minimum peaks. The averaged periods were calculated for 5 min intervals and normalized by that at -2.5 min in each datum (S-site: $n = 6$, NS-site: $n = 10$); then they were all averaged for each site. With the stimulation, the periods at both sites immediately became short; they began to recover 20 min after the stimulation. Bars = S.D. **b** Time courses of migration velocities of the plasmodium with attractant stimulation. The velocities averaged for 5 min intervals at S- and NS-sites are shown. All values were normalized using that for the NS-site at -2.5 min: S-site ($n = 9$) and NS-site ($n = 7$). Bars = S.D.

Discussion

From the information processing viewpoint, the plasmodium requires a coherent and steady spatio-temporal pattern of Ca^{2+} concentration to begin migration as a whole body.

How does the gradient of Ca^{2+} make the plasmodium show coordinated migration? In the cell, migration is

caused by biased shuttle streaming, which is driven by the contraction difference between its ends (Kamiya 1950). The tension development is decreased by a Ca^{2+} concentration higher than 100 nM (Yoshimoto and Kamiya 1984). These findings indicate that a steady gradient of Ca^{2+} concentration through the cell causes a contraction difference between the rear and the front, from which the biased streaming results, and the plasmodium starts coordinated migration.

Stimulation causes changes in the frequency of the Ca^{2+} oscillation at the NS-site as well as the S-site (Fig. 5 a); a change in the S-site propagates throughout the plasmodium. After that, a steady gradient of Ca^{2+} is generated (Fig. 5 b). In this period, a spatial phase gradient throughout the cell is also observed (Matsumoto et al. 1986, Tanaka et al. 1987, Miyake et al. 1990); the phase gradient disappears after the cell begins to migrate (Tanaka et al. 1987). Nevertheless, the Ca^{2+} gradient remains. Therefore, in the information processing for the coordinated behaviour, rhythmic phenomena could relate to the early stage, during which the plasmodium selects the best direction in which to migrate (Miyake et al. 1991). In the following stage, the spatial Ca^{2+} gradient can generate the coordinated behaviour and maintain the migratory direction of the cell.

In the present study on the information processing for the coordinated behaviour, we stimulated the plasmodium with attractants. Repellents also change the spatio-temporal pattern (Ueda et al. 1987, 1988 c). Our next study will be on the pattern of Ca^{2+} concentration after repellent stimulation to further clarify the information processing of the plasmodium.

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