CELL STRUCTURE AND FUNCTION 18: 111–115 (1993)

Information Propagation by Spatio-Temporal Pattern Change of Ca^{2+} Concentration throughout *Physarum polycephalum* with Repulsive Stimulation

Kiyohisa Natsume*, Yoshihiro Miyake[1], Masafumi Yano[2] and Hiroshi Shimizu[2]

Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology, Iizuka, Fukuoka 820, [1]Department of Information and Computer Engineering, Kanazawa Institute of Technology, Nonoichi, Ishikawa 921, and [2]Faculty of Pharmaceutical Sciences, University of Tokyo, 7–3–1 Hongo, Bunkyo-ku, Tokyo 113, Japan

Key words: *Physarum polycephalum*/Pattern of Ca^{2+} concentration/Information processing/Repulsive response

ABSTRACT. **The development of a spatio-temporal pattern of Ca^{2+} concentration (Ca^{2+} pattern) in the plasmodium of *Physarum polycephalum* during repulsive response was studied using fura-2. In the migrating cell, the gradient of the Ca^{2+} concentration (Ca^{2+} gradient) immediately showed a decrease in local concentration in the area (S-site) stimulated by 50 mM KCl. The concentration rose and then decreased in a site neighboring the S-site. This transient increase of Ca^{2+} concentration, the duration of which was approx. 10 minutes, was propagated to the site most distant from the S-site. There, the Ca^{2+} concentration gradually rose and remained at a high level. Twenty-five minutes after stimulation, a new Ca^{2+} gradient was established throughout the plasmodium. The migratory direction of the cell as a whole then changed. In this process, although the period of Ca^{2+} oscillation changed at the S-site, this change was only local to the site. During the information processing of the local repulsive stimulus, the transient Ca^{2+} increase propagated the local information about the stimulus to the non-stimulation sites (NS-sites), leading to the generation of a new pattern and the start of coordinated migration of the plasmodium.**

The Ca^{2+} pattern, either Ca^{2+} oscillation or a Ca^{2+} gradient recently observed in several cells (1, 6, 16, 17) is thought to be related to cell function, and some models have been proposed for the generation of the pattern (5, 9). The plasmodium has both a Ca^{2+} oscillation and a Ca^{2+} gradient, which play important roles in the information processing of the cell.

The plasmodium of *Physarum polycephalum* is a giant unicellular organism that usually displays coordinated migration as a whole body. Motility is affected by chemotactic stimulation (3, 4, 7, 12, 13). 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. The Ca^{2+} pattern described above is related to this process.

The Ca^{2+} gradient, which is related to the migratory direction, exists in the plasmodium like those of other chemicals, ATP, ADP, cAMP, and cGMP (11, 14, 15). The concentration of Ca^{2+} also oscillates in the cell for a period of 1 to 3 minutes (11, 18). When attractive chemicals are stimulated to the retreating area of the cell, the Ca^{2+} concentration at the S-site increases with the decrease in the period of Ca^{2+} oscillation. The period change is propagated immediately to the whole area of the cell. The Ca^{2+} pattern throughout the cell is then regenerated and the plasmodium begins to migrate in the opposite direction as a whole (11).

When repulsion is stimulated locally to the plasmodium, the period of the oscillation of transmitted light intensity is lengthened at the S-site. However, this change is not propagated to the NS-sites, unlike the case of attractive stimulation (10). In the information processing for a repulsive stimulus, a Ca^{2+} pattern other than the pattern induced by the attractant may be established in the cell. Therefore, in the present study, the development of the pattern was measured when the front of the plasmodium had a local repulsive stimulation. A repulsion-induced Ca^{2+} pattern is discussed and compared with an attractant-induced pattern.

MATERIALS AND METHODS

Organism. The plasmodium of *Physarum polycephalum* was cultured by the method of Camp (2). It was allowed to migrate on a 1.5% agar gel in a trough overnight without feeding before use.

Measurement of the intracellular spatio-temporal pattern of Ca^{2+} concentration. A plasmodial strand, approx. 10 mm in length and 0.6–0.8 mm in diameter, was excised from a large plasmodium, placed on a cellulose sheet on plain agar,

* Address for correspondence

and incubated. A few hours later, a fura-2 solution, containing 1.2 mM fura-2, 25 mM KCl, 1 mM NaCl, and 10 mM HEPES at pH 7.4, was microinjected at a concentration of 10–15 μM fura-2 into the cell. Within 30 minutes, the fura-2 solution diffused uniformly throughout the cell. The intracellular Ca^{2+} concentration was then measured using a fluorescence microscope system equipped with a photomultiplier tube. At the measurement, the plasmodial strand, which reached 2 cm in length, had begun to migrate in one direction, generating a fan-like structure at both ends. The time course of Ca^{2+} concentration along the cell was measured at four sites 5-mm intervals apart, as shown in the Fig. 1 inset. The Ca^{2+} concentration at each site was measured every 10 seconds from each plasmodium. The numbers of sites used in the text and the figure legends correspond to those in the inset of Fig. 1. The intracellular Ca^{2+} concentration was quantitatively estimated by the method of Kudo and Ogura (8).

In the plasmodium, the cytoplasm flows to and fro every few minutes. The streaming transports the cytoplasm from the rear to the front when the cell is migrating. Under the conditions of the present study, the average net flow every 5 minutes, which is called the "migration velocity", was calculated from the flow time of the streaming of the plasmodium (10, 11).

Chemical stimulation was applied by exchanging part of the agar gel plate under the front part of the cell with one containing 50 mM KCl. The plasmodium always showed normal repulsion behavior at this concentration. All experiments were conducted at room temperature (24°C) in the dark.

RESULTS

Time course of the intracellular spatio-temporal pattern of Ca^{2+} concentration and migration velocity by repulsion. The plasmodium showed a Ca^{2+} gradient when the cell was migrating unidirectionally. With 50 mM KCl stimulation at the front, typical developments of Ca^{2+} concentration along the cell are shown in Fig. 1. The concentration at site 1, the S-site, decreased immediately upon exposure to the stimulant and reached a steady state 14.5 minutes after the stimulation. At site 2, the concentration increased with the stimulus, began to decrease after 9.5 minutes, and reached a steady state. At site 3, the concentration increased from 4.5 minutes and decreased 14.5 minutes after the stimulus, reaching a steady state level higher than that before the stimulation. At site 4, the concentration began to increase after 9.5 minutes, reached a steady state, and remained at that level even after 19.5 minutes.

To examine the above results statistically, the time

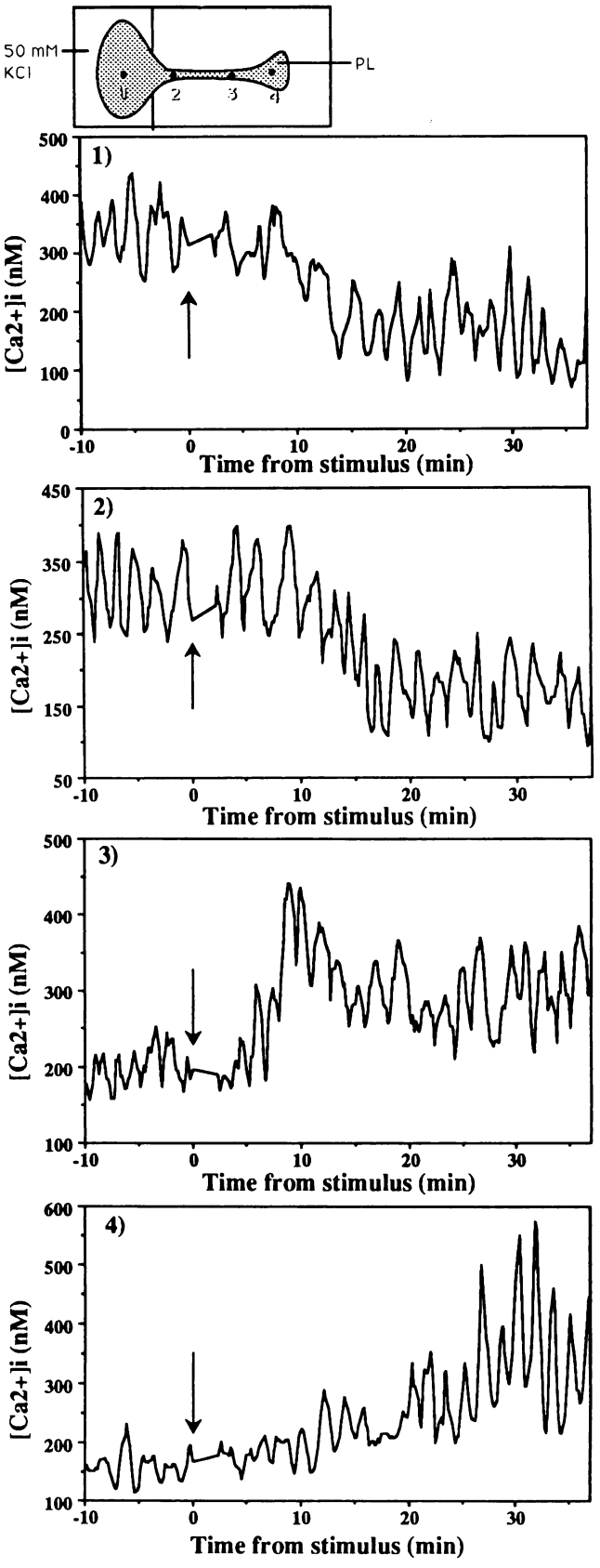

Fig. 1. An example of the typical temporal patterns along plasmodia stimulated with 50 mM KCl. The numbers in the figures indicate the measurement sites of the Ca^{2+} concentration in the inset. The repulsive stimulus to the cell is shown in the inset. The arrows indicate the onset of the stimulation. PL: plasmodium.

course of the averaged Ca^{2+} concentrations at the four sites was calculated (Fig. 2). The same tendencies were found. Also, with the repulsion, the Ca^{2+} gradient was found to reverse and reach a steady state after 20 minutes.

The development of the Ca^{2+} gradient along the cell is shown in Fig. 3. In this figure, the pattern change of Ca^{2+} concentration slower than the change of Ca^{2+} oscillation is shown. Local stimulation decreased the concentration at site 1, while the concentration at site 2 increased. Concentrations at the other sites showed no change. Thus, a mountain-like pattern of Ca^{2+} concentration, with a peak at site 2, was generated in the cell. The concentration at site 2 then began to decrease. The duration of this transient Ca^{2+} increase was approx. 10 minutes. This was much longer than the period of the Ca^{2+} oscillation (see below). The transient increase at site 2 was propagated to the other NS-sites, sites 3 and 4. Nine and a half minutes later, the Ca^{2+} concentration at site 3 increased and then decreased. Five minutes after that, the concentration at site 4 increased and re-

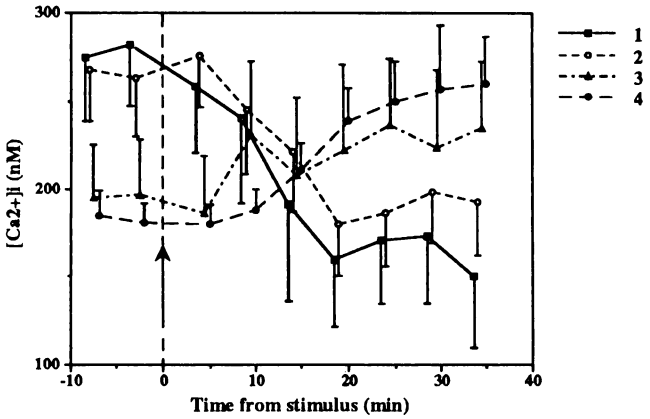

Fig. 2. Time courses of averaged Ca^{2+} concentrations along the plasmodium by 50 mM KCl stimulation. The average Ca^{2+} concentrations for 5 minutes were calculated for sites 1 to 4 in the inset of Fig. 1 (site 1: n=6, site 2: n=6, site 3: n=7, site 4: n=5). The number of each site is indicated in the Fig. 1 inset. The bars represent standard deviation (S.D.).

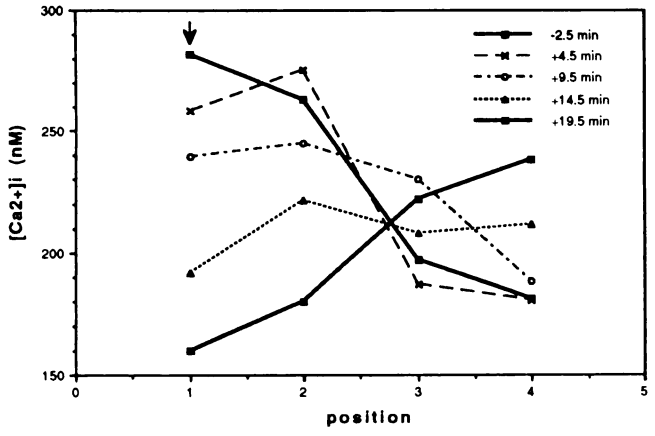

Fig. 3. Time courses of the spatial Ca^{2+} concentration pattern after 50 mM KCl stimulation. This spatial pattern was obtained from the values in Fig. 2. Before stimulation, the Ca^{2+} gradient, higher at site 1, gradually decreased at site 4 in the plasmodium (white squares). With KCl stimulation locally at site 1 (arrow), the gradient was reversed as a whole cell at 20 minutes after the stimulus (black squares). In the transient process, the rapid increase at site 2 with the stimulation decreased at 9.5 minutes. This transient Ca^{2+} increase was propagated to the other NS-sites, sites 3 and 4. At site 4, the Ca^{2+} concentration did not decrease and remained high. A new Ca^{2+} gradient was established.

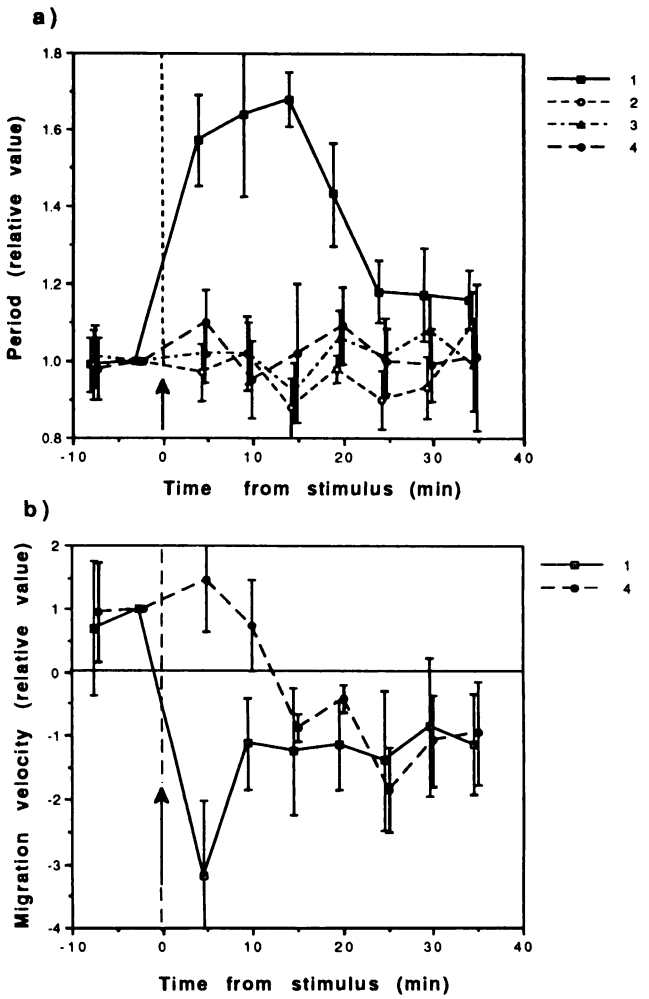

Fig. 4. Time courses of the period of Ca^{2+} oscillation (a) and the migration velocity of the plasmodium (b) after 50 mM KCl stimulation. Both values in the figures ((a) and (b)) were normalized by the value at minus 2.5 minutes. (a) The period changes at sites 1 to 4 are shown. (b) The migration velocities at sites 1 and 4 are shown. The sign of the migration velocity was positive when the cell moved towards site 1. The bars=S.D. ((a) site 1: n=6, site 2: n=6, site 3: n=7, site 4: n=5, (b) site 1: n=8, site 4: n=5)

mained at a new high level. Thus, a steady regenerated Ca^{2+} gradient was established.

The Ca^{2+} concentration in the plasmodium oscillated with a 1- to 2-min period (Fig. 1). The time course of the periods from sites 1 to 4 are shown in Fig. 4 (a). The period at site 1 became 1.4 to 1.7 times that before stimulation. Recovery began 20 minutes after the stimulus. At the sites other than site 1, the periods did not change significantly.

The time courses of the migration velocity are shown in Fig. 4 (b). Before stimulation, the velocities at sites 1 and 4 were the same and the plasmodium moved to site 1 as a whole body. On stimulation, only site 1 reversed the migratory direction immediately, while site 4 remained the same as before stimulation. After 15 minutes, the migratory direction at site 4 was also reversed. The velocities at both sites became the same at 25 minutes, when the plasmodium again began to migrate as a whole.

DISCUSSION

Giving the plasmodium a local repulsive stimulus at a frontal site caused the reversal of both the Ca^{2+} gradient and the migratory direction of the cell. The new gradient was established before the coordinated migration and was maintained during the migration (Figs. 2 and 4 (b)). Therefore, the Ca^{2+} gradient throughout the cell is related to the coordinated motility of the cell, as seen in the case of the attractive stimulation.

During information processing, the Ca^{2+} gradient was reversed throughout the plasmodium, although the cell was stimulated locally with chemicals. An attractant stimulus shortened the period of Ca^{2+} oscillation at the S-site. The change was immediately propagated to the whole cell and a new Ca^{2+} gradient was generated. Thus, the propagation of the period change of the oscillation may be necessary to the generation of the new gradient (11) when the plasmodium is stimulated with attraction. In the present study, the period of Ca^{2+} oscillation at the S-site was also immediately lengthened by the repulsive stimulus. This period change, however, was only local at the S-site (Fig. 4 (a)), and did not propagate to the whole cell. Instead of that, the transient Ca^{2+} increase with much longer duration than the period of the Ca^{2+} oscillation was immediately induced at the site neighboring the S-site, and propagated to the whole cell before the Ca^{2+} gradient was established (Figs. 2 and 3). Therefore, with the repulsive stimulation, the propagation of the transient Ca^{2+} increase may be necessary for the cell to establish a new gradient.

This is consistent with the change of the migration direction of the plasmodium stimulated with chemicals. In the case of an attractive stimulation, the NS-site immediately stopped the migration with the period change of the Ca^{2+} oscillation seen at the S-site (11). On the other hand, with the repulsion, the NS-site most distant from the S-site did not change the migration direction until the transient Ca^{2+} increase was propagated to the site (Figs. 3 and 4 (b)).

The attractant-induced Ca^{2+} pattern differs from the repulsion-induced one as described above. There is, however, a common character between them from the viewpoint of the information processing. With the cell stimulated locally with chemicals, the temporal pattern, that is, the frequency change of Ca^{2+} oscillation, or the transient Ca^{2+} increase, could propagate the local information to the whole cell in the early stage of processing the information. The spatial pattern, that is, the Ca^{2+} gradient throughout the cell, could then be generated and maintained for the coordinated migration as a whole in the following stage.

In the present study, by local repulsive stimulus to the cell, the plasmodium changed its migratory direction as a whole. However, by the same stimulation, Miyake *et al.* (10) found the cell not to change the direction. Since the plasmodial strand they used was four times as long as ours, it is suspected that the length of the plasmodium may affect the Ca^{2+} pattern. It is likely that when the cell is longer than limit of length, the transient Ca^{2+} increase induced near the S-site degenerates on the way and cannot propagate to the whole plasmodium.

Acknowledgements. The authors thank Dr. K. Kometani and Mrs. Y. Natsume for their helpful comments and encouragement.

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(Received for publication, September 18, 1992
and in revised form, April 17, 1993)