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

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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 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 stimulus (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,
The course of the stimulation Pt. Phasmidum

The graph shows the time course of the rise in [Ca^{2+}]_i following electrical stimulation with 50 Hz, 5 V, 20 ms pulses. The x-axis represents time from the stimulus in minutes, and the y-axis represents the [Ca^{2+}]_i in mM. The graphs show a rapid increase in [Ca^{2+}]_i immediately after the stimulus, followed by a decrease to baseline levels.

RESULTS

Conductance at room temperature (24°C) in the dark, and effect of increased light on conductance. (A) Effect of increased light on conductance. (B) Effect of increased light on conductance

Figure 1. The intracellular spiking threshold after the stimulus. The graph shows the effect of increased light on conductance, with a light intensity of 500 lux. The x-axis represents time from the stimulus in minutes, and the y-axis represents the [Ca^{2+}]_i in mM. The graph shows a rapid increase in [Ca^{2+}]_i immediately after the stimulus, followed by a decrease to baseline levels.

Figure 2. The intracellular spiking threshold after the stimulus. The graph shows the effect of increased light on conductance, with a light intensity of 500 lux. The x-axis represents time from the stimulus in minutes, and the y-axis represents the [Ca^{2+}]_i in mM. The graph shows a rapid increase in [Ca^{2+}]_i immediately after the stimulus, followed by a decrease to baseline levels.

Figure 3. The intracellular spiking threshold after the stimulus. The graph shows the effect of increased light on conductance, with a light intensity of 500 lux. The x-axis represents time from the stimulus in minutes, and the y-axis represents the [Ca^{2+}]_i in mM. The graph shows a rapid increase in [Ca^{2+}]_i immediately after the stimulus, followed by a decrease to baseline levels.

Figure 4. The intracellular spiking threshold after the stimulus. The graph shows the effect of increased light on conductance, with a light intensity of 500 lux. The x-axis represents time from the stimulus in minutes, and the y-axis represents the [Ca^{2+}]_i in mM. The graph shows a rapid increase in [Ca^{2+}]_i immediately after the stimulus, followed by a decrease to baseline levels.

Figure 5. The intracellular spiking threshold after the stimulus. The graph shows the effect of increased light on conductance, with a light intensity of 500 lux. The x-axis represents time from the stimulus in minutes, and the y-axis represents the [Ca^{2+}]_i in mM. The graph shows a rapid increase in [Ca^{2+}]_i immediately after the stimulus, followed by a decrease to baseline levels.
The figure shows the migration velocity of cells in response to a chemical stimulus. The x-axis represents the time from stimulus onset (in minutes), and the y-axis represents the migration velocity in μm/min.

(a) Time from stimulus onset (min) vs. Migration velocity (μm/min): The graph shows a decrease in migration velocity over time, indicating that cells move less as time progresses.

(b) Time from stimulus onset (min) vs. Time from stimulus offset (min): The graph indicates that after a certain period, the cells stop migrating completely.

(c) Time from stimulus onset (min) vs. Period (s): The period of oscillation is shown to increase over time, indicating a change in cell behavior.

(d) Time from stimulus onset (min) vs. Concentration (μM): The concentration of the stimulus is shown to decrease over time, affecting cell migration.

(e) Time from stimulus onset (min) vs. Position (μm): The position of the cells is shown to move towards the stimulus source, indicating chemotaxis.

(f) Time from stimulus onset (min) vs. Cumulative migration distance (μm): The cumulative distance traveled by cells is shown to increase with time, indicating active movement.

(g) Time from stimulus onset (min) vs. Cell count: The graph shows a decrease in cell count over time, indicating cell death or migration.

(h) Time from stimulus onset (min) vs. Change in cell size: The cell size is shown to increase over time, indicating cell swelling or growth.

(i) Time from stimulus onset (min) vs. Secondary migration velocity (μm/min): The graph shows a decrease in secondary migration velocity over time, indicating a decrease in cell activity.

(j) Time from stimulus onset (min) vs. Secondary position (μm): The position of the cells is shown to move away from the stimulus source, indicating a change in direction.
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 decenerates on the way and cannot propagate to the whole plasmodium.

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**REFERENCES**

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