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Interaction between cell shape and contraction pattern in the *Physarum* plasmodium

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Abstract

The relationship between cell shape and rhythmic contractile activity in the large amoeboid organism *Physarum polycephalum* was studied. The organism develops intricate networks of veins in which protoplasmic sol moved to and fro very regularly. When migrating on plain agar, the plasmodium extends like a sheet and develops dendritic veins toward the rear. After a particular stimulation, the vein organization changes into veinless or vein-network structures. In both structures, the mixing rate of the protoplasm, which is related to communication among contraction oscillators, decreased compared with that of the dendritic one. Accompanying these changes in vein structure, the spatio-temporal pattern of the rhythmic contraction changed into a small-structured pattern from a synchronized one. In the above process, cell shape affects the contraction pattern, but, conversely, the contraction pattern effects the cell shape. To demonstrate this, a phase difference in the rhythmic contraction was induced artificially by entraining the intrinsic rhythm to external temperature oscillations. New veins then formed along the direction parallel to the phase difference of the rhythm. Consequently, the vein organization of the cell interacts with the contractile activity to form a feedback loop in a mechanism of contraction pattern formation.

Keywords: *Physarum*; Pattern formation; Network morphology; Contraction; Oscillation

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1. Introduction

The plasmodium of *Physarum polycephalum* is a large multinuclear amoeboid organism that shows rhythmic contraction everywhere within the cell. The spatial patterns of the rhythmic contraction are closely related to the behaviors of this organism [1–4]. In this study, the mechanism of the pattern formation was experimentally studied, especially from the viewpoint of cell shape.

The rhythmic contraction is almost synchronous in the organism, but the synchrony of the contraction has some variation in space and time. Hydrostatic pressure is generated among different parts in the organism and accordingly produces streaming of the protoplasm [5]. In this model, the streaming is regulated by the contraction pattern. On the other hand, there is evidence that the streaming itself affects the contraction rhythm: streaming is necessary to synchronize the contraction [6], and insufficient streaming causes an anti-phase relationship between the two parts [7]. In this way, the contraction pattern and the protoplasmic streaming are coupled [8,9].

So far, changes of the cell shape have not been taken into account in spite of the fact that plasmodial veins are the channels of the protoplasmic flow and exchange in the plasmodium. Here, we focus on the effects of the cell shape, especially the vein organization, on the rhythmic contraction. First, three very different types of vein structure are described, and the mixing rate of the protoplasm and the contraction pattern in these structures are observed. Next, we show a reverse case in which new veins are formed artificially by controlling the oscillation patterns. The physiological mechanism of pattern formation of the rhythmic contraction is discussed.

2. Methods

2.1. Organism

The true slime mold, the plasmodium of *Physarum polycephalum* (strain HU554 x HU560), was cultured with oat flakes on a 1% agar gel at 25°C in the dark. The extending tip (4 mm × 4 mm) was cut from a large plasmodium in a culture trough (25 cm × 35 cm) and placed on a new agar gel in a Petri dish (9 cm in diameter). After a few hours in the dark, the organism derived from the cut portion had extended concentrically to a diameter of 3 cm, and this was used in the experiments.

2.2. Stimulation of the organism for inducing various cell shapes of the plasmodium

Changes in the cell shape were induced by three methods. (1) The organism was allowed to move on agar gel containing either 10 mM phenylalanin, 2 mg/ml casamino acid or 40 mg/ml ground oats flakes. (2) The organism was irradiated with an ultraviolet light (253 nm, 15 W, Type #GL-15, National Electric Co., Tokyo, Japan) from above at a distance of 20 cm for 15 min. (3) The organism was exposed to temperature elevation to 33°C from 25°C.

2.3. Measurements of the spatio-temporal patterns in the rhythmic contraction

The spatio-temporal dynamics of the rhythmic contraction were monitored as previously described [10,11]. The organism was illuminated either from below with an infrared emission diode (approx. 950 nm), or from above at an angle, and observed from above with a video camera that fed into a microcomputer. From the brightness level $I(x,t)$ at position x and at time t, the oscillatory component $\Delta I(x,t)$ was calculated by subtracting the drifting component [10,11].

2.4. Estimation of mixing rate of the protoplasmic sol in the plasmodium

The mixing rate of the protoplasm was measured by incorporating marked particles made of oat flakes ground in an earthenware motor with blue ink (type #4001, Pelican, Germany) into the plasmodium. The particles were fed to the plasmodium and were incorporated in approximately half a day, turning the plasmodium greenish. A 1-mm^3 portion of the stained plasmodium was amputated and placed on the periphery of an

approximately 3 cm diameter unstained plasmodium. The two plasmodia were allowed to coalesce into a single plasmodium with stained particles that were transported to various parts of the organism. A single particle being moved away from the site of the coalescence could be followed under a microscope, and its destination was determined after half a period. The distribution of the destination gave a qualitative measure of the mixing rate of the protoplasm.

2.5. Measurements of cell shape under the control of the contraction pattern due to the periodic change of temperature

Fig. 1 shows the experimental setup for varying the temperature periodically and observing the changing oscillation patterns and cell shape. A Peltier-effect device (PD) was sandwiched by two copper plates (CP), and a heat irradiator (IR) was attached to the lower part. Two similar modules were set 1 mm apart as shown in the figure. On the upper plates, the 2–3-mm-thick agar gel on which the plasmodium extended was placed in the middle. The temperature of the agar plate was monitored by thermo-sensors (TS) on both sides, and readings were transmitted to a personal computer which controlled the PDs. The temperatures on both sides, T_1 and T_2 (°C), were varied as $T_1 = A\sin(2\pi\omega t) + T_0$ and $T_2 = A\sin(2\pi\omega t + \psi) + T_0$, where $T_0 = 25$°C, $A = 1$°C, $\omega = 0.95\omega_i$ and t is time (second). The quantity ω_i is the reciprocal of the contraction period (peak-to-peak time in a unit of second), which was averaged over several changes just before the temperature oscillations were applied. The quantity ψ was π or $(2/3)\pi$ to make the phase difference between two parts as large as possible. Because the contraction oscillation synchronized with the temperature oscillation, the contraction pattern could be controlled [2,12]. The contraction pattern and the cell shape were simultaneously observed.

2.6. Quantification of vein orientation and the degree of synchrony in the rhythmic contraction

The veins are channels for the protoplasmic flow, and the vein-arrangement index (VAI) indicates the direction of the flow itself. A plasmodium changes its vein organization, so the orientation of the veins is a quantitative function of time. VAI was determined as shown in Fig. 2. The thick veins in picture a were extracted and approximated by straight lines in b. Each straight line **V** (a vector quantity) was decomposed into two components $\mathbf{V}^x$ and $\mathbf{V}^y$, where the y-axis is parallel to the gap (indicated by the white dashed line in Fig. 2a) separating the two thermo-modules (Fig. 2c). The parameter VAI was calculated by adding all the veins,

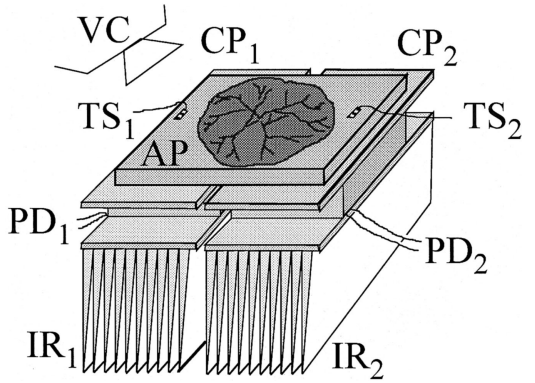

Fig. 1. Experimental set-up for varying the temperature and observing the oscillation pattern and cell shape. The temperature on each side was varied sinusoidally with the same period, but phases (ψ) differed by $(2/3)\pi$ or π. VC, video camera; TS, thermo-sensor; PD, Peltier device; AP, agar plate; IR, heat irradiator; CP, copper plate. See Section 2 for details.

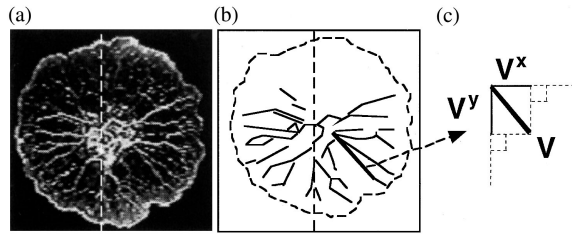

Fig. 2. Quantification of the vein orientation. (a) The circular plasmodium. Dashed line indicates the position of a narrow gap between two PDs in Fig. 1. (b) Main veins approximated by connected straight lines. (c) Projection of a line **V** in two directions, parallel ($\mathbf{V}^y$) and perpendicular ($\mathbf{V}^x$), to the narrow gap between the PDs. $\mathbf{V}^y$ and $\mathbf{V}^x$ were added up, and the ratio of the two sums was defined as the vein-arrangement index (VAI).

$$\mathrm{VAI} = (\Sigma_i|\mathbf{V}_i^x|)/(\Sigma_i|\mathbf{V}_i^y|),$$

where $\mathbf{V}_i = \mathbf{V}_i^x + \mathbf{V}_i^y$, and $|\mathbf{V}|$ indicates a length of $\mathbf{V}$.

The contraction rhythm sometimes fluctuated when temperature oscillations were applied [13]. The degree of the synchronization was quantified by averaging the difference between the periods of rhythmic contraction of the organism τ_i and those of the external temperature oscillation τ_f. The synchronization index (SI) was calculated as

$$\mathrm{SI} = -\left(\Sigma_i|\tau_i - \tau_f|\right)/\Sigma_i.$$

3. Results

3.1. Three patterns of vein organization and intracellular mixing of protoplasm after stimulation

Fig. 3 shows three typical patterns of vein organization in the plasmodium after various stimulation was applied. Fig. 3a,b show the vein structure in the plasmodium crawling freely in the culture conditions. The dendritic structure of the vein has developed, and a sheet-like structure was formed in the periphery. In this dendritic vein and sheet structure, marked particles from a peripheral site were transported to almost all sites within the plasmodium during a half period of the contraction cycle. Destinations of the particles seemed to be determined by chance, because the particles were shuffled while flowing in a vein and then distributed at many bifurcation points of the veins. As above, the protoplasmic sol was mixed throughout the plasmodium in one period of the contraction oscillation.

Fig. 3c shows the second type of vein organization, the veinless structure. The veins have disappeared, and the cell is shaped like a sheet. This structure was transiently observed when the temperature was changed from 25°C to 33°C. The disappearance of the veins was extreme when the plasmodium was on the phenylalanin-containing agar (10 mM). In the veinless structure, the mixing of protoplasm was found to be slow, as the marked particles in the cell were mixed no longer

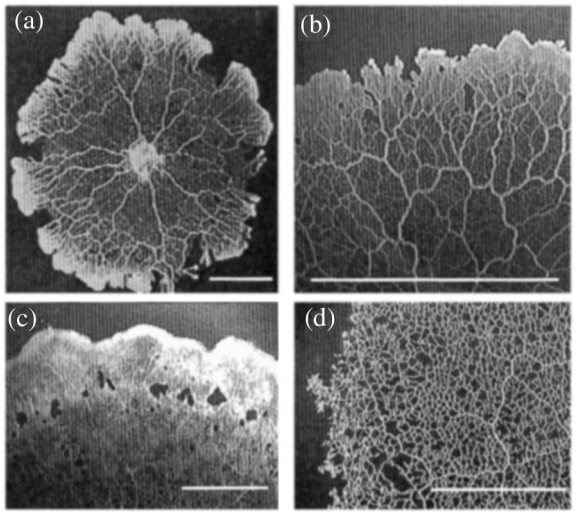

Fig. 3. Plasmodia with three typical patterns of vein organization: (a,b) dendritic vein and sheet structure in the culture conditions; (c) veinless structure; and (d) vein-net structure. Scale bar: 1 cm.

over the plasmodium in one period of the rhythmic contraction. Fig. 3d shows the third type of vein organization, the vein-net structure. The thickness of all the veins was similar, and their configuration resembled a net. This structure was observed: (1) when the plasmodium extended on the oat flake-containing agar or on the casamino acid-containing agar; or (2) at half a day after 15 min UV light irradiation (see Section 2). In this vein-net structure, protoplasmic mixing did not occur all over the organism in one cycle of contraction. In the latter two-vein patterns, the protoplasmic mixing was not global, as in the dendritic vein and sheet structure, but was local in one oscillation.

3.2. Different patterns of contraction in the plasmodium with the veinless and the vein-net structures

Fig. 4 shows a typical oscillation pattern in a plasmodium with the veinless structure. The black and white pattern is detailed (Fig. 4b) in comparison with the pattern in the dendritic vein and sheet structure (Fig. 4a). For example, a curved zone (the black part indicated by the arrow in Fig.

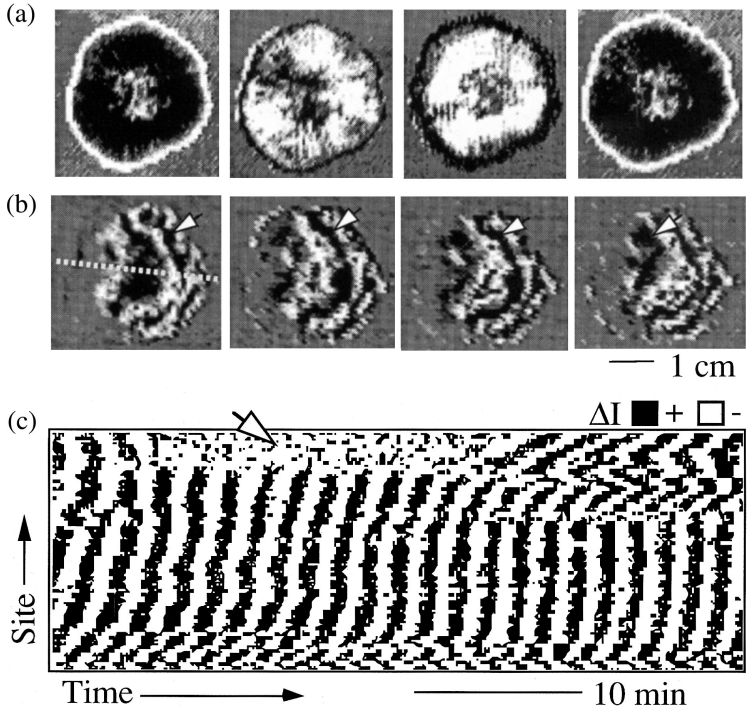

Fig. 4. The rhythmic contraction (ΔI) in the vein organization of Fig. 3a,c. (a) Synchronous pattern of the rhythmic contraction except in the peripheral part in the dendritic vein and sheet structure of Fig. 3a. Two-dimensional snapshots were taken every 24 s. ΔI is higher as the graded shades are darker. (b) Successive snapshots of the contraction in the veinless structure like Fig. 3c (every 40 s). The curving zone indicated by the arrows moved leftward. (c) Time variations of the contraction observed from the right to the left along the diameter indicated by the white dotted line in Fig. 4b. The arrow indicates the part that ceased the contraction oscillation.

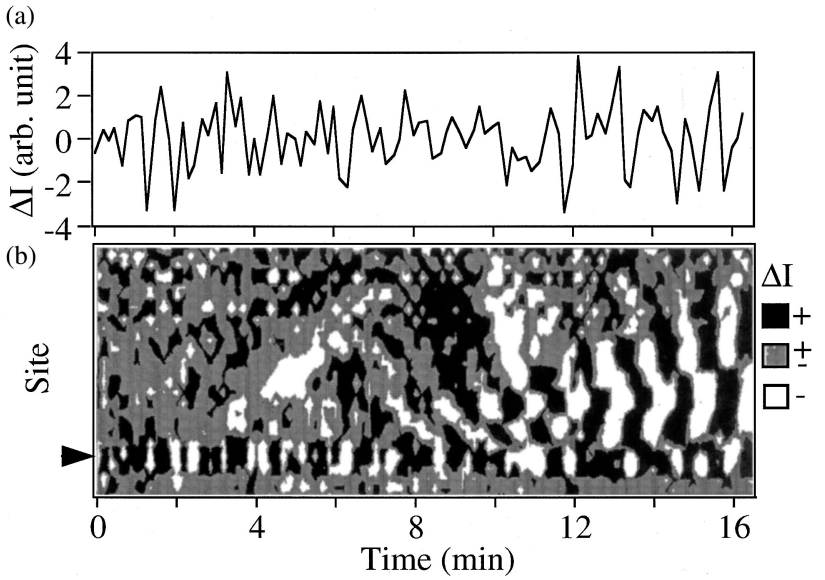

Fig. 5. The contraction pattern in the extremely veinless structure induced by phenylalanine treatment. (a) Time course of ΔI at a fixed site (indicated by the arrow in Fig. 5b) of the plasmodium. The variations are irregular. (b) One-dimensional variations of ΔI. ΔI is higher as the graded shades are darker.

4b) was formed and moved leftward. Fig. 4c shows the time course of spatially one-dimensional variations from the right to the left along the white dotted line indicated in Fig. 4b. Black and white stripes run between bottom and top in Fig. 4c, and the slope of the stripes varies in space and time. Specifically, although contraction waves were propagated in the cell, the propagation speed varied. And in some parts of the organism, the oscillation was suspended (indicated by the arrow). Synchrony of the contraction decreased, and the contraction pattern was complicated.

Fig. 5b shows a typical pattern of oscillation in a plasmodium with an extremely veinless structure. There are many black and white domains that are isolated from each other in Fig. 5b, which means that a contraction wave is no longer propagated throughout the cell. Fig. 5a showed the contraction oscillation at the site indicated by the arrow in Fig. 5b. Periods and amplitudes of the oscillations were also no longer constant, so the oscillations were disordered in space and time.

Fig. 6 shows oscillation patterns in a plasmodium with the vein-net structure. As indicated by

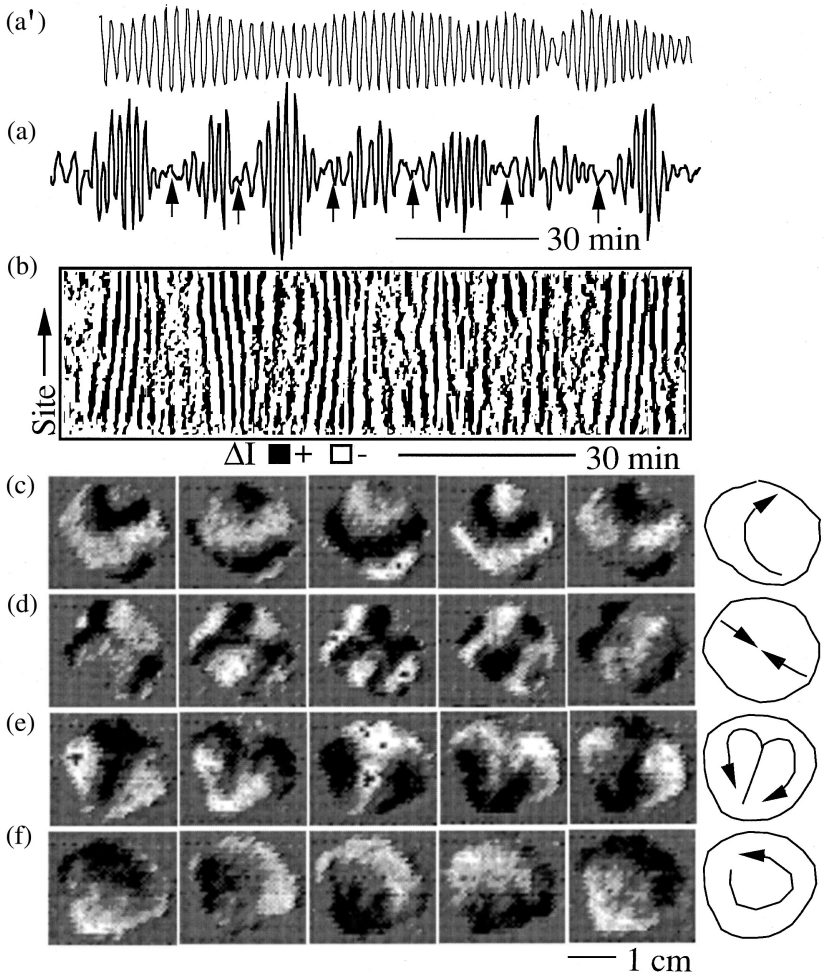

Fig. 6. The contraction patterns in the vein-net structure. (a) Time course of ΔI at a fixed site in the plasmodium. For the sake of comparison, the time course which was observed in the dendritic vein and sheet structure is shown in a. (b) Time course of one-dimensional variations. (c–f) Successive two-dimensional snapshots of the contraction pattern every 32 s. The line drawings indicate the propagation directions of the contraction waves. ΔI is higher as the graded shades are darker.

the arrows in Fig. 6a, the oscillations were damped transiently and repeatedly. When the oscillations were not damped, contraction waves propagated throughout the cell (Fig. 6b), but the two-dimensional patterns were different. Examples of these patterns are shown in Fig. 6c–f. Two zonal waves moved upward (c) or from opposite sides (upper left and lower right corners in Fig. 5d) and collided. A phase domain moved upward toward the periphery, split to two (left and right) at the top, and moved downward along the periphery to the bottom, like a double spiral pattern (e). Two semicircular domains rotated around the periphery like a single spiral pattern (f). None of the patterns continued for long.

The changes in contraction pattern that accompanied the different vein structures were also quite different.

3.3. Alteration of vein structure by artificial control of the contraction pattern

Fig. 7 shows modulation of the vein orientation under artificial control of the contraction pattern. As time progressed under conditions where the left half of the plasmodium oscillated with the phase difference of $2/3\pi$ $(=\psi)$ earlier than the right half, the veins remained horizontally oriented as before, but the vertical veins became unclear (Fig. 7A). The ratio of VAI compared to that just before the temperature oscillation [VAI(t_0)] increased from 1 to approximately 1.5 (Fig. 7B). Fig. 7C shows the relationship of SI and VAI at 20 min after the beginning of temperature oscillation. VAI increased together with SI; therefore, the changes in vein orientation depend on the actually realized degree of contraction control. As above, the veins were reinforced along the direction of the phase difference of the contraction, and weakened perpendicular to that direction.

Next, in order to test the effects of the contraction pattern on the generation of a new vein, a plasmodium with the veinless structure was prepared according to the results shown in Fig. 3c. Fig. 8Ba shows a rectangular piece of the veinless

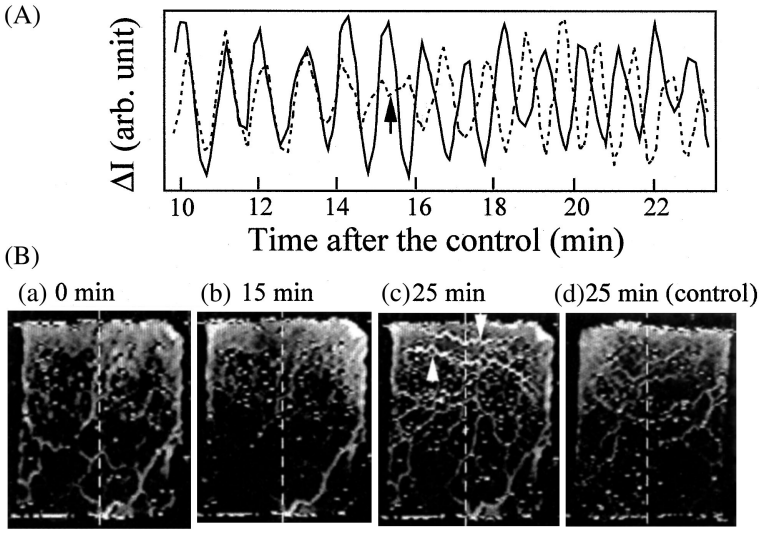

Fig. 7. Modulation of vein arrangement by artificial control of the contraction pattern. (A) Plasmodium at 0 (a), 6 (b) and 27 (c) min from the beginning of the oscillatory variations of temperature. Dashed white line indicates the position of the narrow gap between two PDs in Fig. 1. (B) Time course of VAI(t) normalized by that just before the temperature oscillations, VAI(t_0). (C) Relationship of VAI to SI.

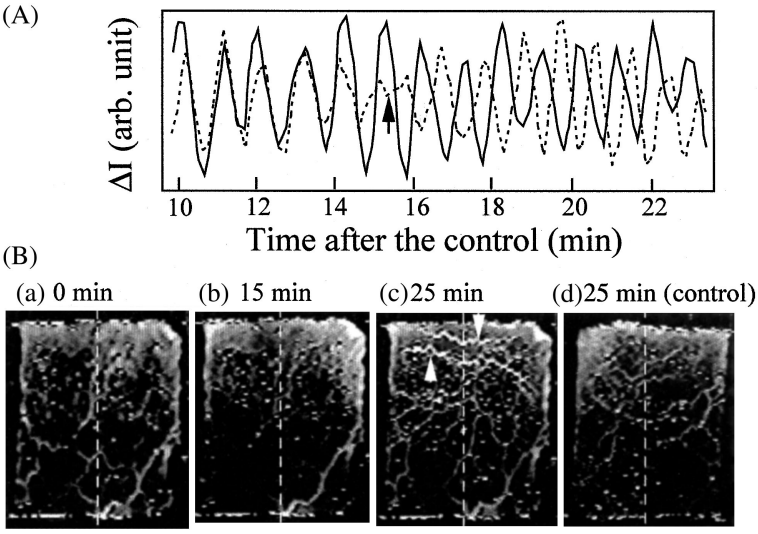

Fig. 8. Vein generation by artificial control of the contraction pattern. (A) Time course of ΔI in the right and the left parts separated at the dashed line in B. Two oscillations were inphase in the early stage, but became anti-phase 15 min from the beginning of the artificial control (indicated by the arrow). (B) Rectangular plasmodia at 0 (a), 15 (b), and 25 (c) min from the beginning of artificial control, and a control specimen under artificial control without a phase difference (d). Dashed white line indicates the position of the narrow gap between two PDs in Fig. 1.

plasmodium (approx. 1 cm $\times$ 1 cm) 20 min after being moistened with 10 mM phenylalanin solution. In these conditions, the contraction rhythm seemed irregular in space and time as described earlier so that the phase locking was unlikely to occur. The vein morphology was observed only in the cases in which phase locking occurred.

Fig. 8 shows the generation of the vein structure along the direction of the phase difference ($\psi = \pi$) of the contraction. The contraction phase became an anti-phase after 15 min of the temperature oscillation (indicated by the arrow in Fig. 8A). Just before the anti-phase contraction, there were no new thick veins in comparison to the appearance at 0 min (Fig. 8Ba,b). However, two thick veins were newly developed perpendicular to the direction of the phase difference of the contraction several minutes after the anti-phase contraction occurred (indicated by the arrows in Fig. 8Bc). Contrary to this, such veins were not observed when the contraction phases were synchronous (Fig. 8Bd). Consequently, when there were two regions that contracted in different phases for several minutes, a different structure developed in each of them.

4. Discussion

We considered morphogenesis of the three kinds of vein organization shown in Fig. 3 in relation to their contraction patterns. Veins developed along the phase difference of the contraction pattern, as described earlier in Fig. 8. First, when contraction timings were constantly different between the peripheral and the inner parts (Fig. 4a), thick veins were formed roughly along the radii (Fig. 3a). Second, when the contraction pattern was not constant, as shown in Fig. 6, isotropy of the vein network appeared. Third, when the contraction pattern seemed disordered in space and time, as shown in Fig. 5, a vein structure no longer appeared (Fig. 3b). These relationships are consistent with the results shown in Figs. 7 and 8. Thus, the correspondence of the vein structure and the contraction pattern is consistent with the very different types of cell shapes which naturally developed.

A possible mechanism of the vein generation due to the phase difference of the rhythmic contraction is described. The phase difference moti-

vates the flow of protoplasm [5]. Actin fibers are regularly arranged in the gel layer of the vein and regarded as the basic structure of the vein [14–18]. The arrangement can also be induced by an externally loaded tension. Let us imagine the situation in which viscous sol vigorously flows in the narrow cavity in a plasmodium. The flowing sol draws the immobilized gel layer. This drawing effect may be able to affect the actin fiber arrangement. Therefore, the drawing effect induced by the protoplasmic flow may result in the formation of a vein.

A possible mechanism of the pattern formation of the rhythmic contraction is based on the previous proposals [8,19,20]. A molecular system of the contraction is an actin–myosin system, which is immobilized in the outer layer of the cell [21,22]. The force-generating system interacts with oscillating chemical reactions of metabolites [23–28]. The chemical oscillations may be the primary oscillations, since they occur in the absence of mechanical oscillations [8,29]. Therefore, let us consider the spatial interaction of the chemical oscillations. Chemical substances diffuse in the outer layer and are transported to and from the inner layer. The streaming of the inner layer brings about a fast interaction among the contraction oscillators located in the outer layer. On the other hand, the results of this report indicated that the streaming modifies the vein structure. Because the vein is a channel of protoplasmic streaming, the streaming determines the manner of spatial interactions among the intracellular oscillators. Consequently, the protoplasmic streaming plays a key role in the pattern dynamics of contraction. The architecture of such a self-constrained system may be related to development of complex behaviors of the plasmodium.

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