Organization of Community Behavior during Initiation of Morphogenetic Aggregation in a Population of *Dictyostelium discoideum* Amoebae

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ABSTRACT. Using microcomputer image processing, we analyzed the organization of community behavior during the initiation of aggregation in densely populated *Dictyostelium* amoebae. Just after starvation, cells behaved randomly in both space and time. The first spatial organization was the appearance of transitory regions in which the amoebae oscillated at the same frequency but with different phase. Next, waves began to propagate concentrically within small regions, but these domains were unstable, appearing and disappearing here and there. As time passed, waves propagated for longer distances, and eventually definite centers appeared. Spectral analysis showed that the center was characteristic in having highly ordered oscillation. Later, amoebae began aggregating at these wave propagation centers. Thus, populations of oscillating amoebae organize community behavior through competitive interaction among local oscillators, the most ordered enslaving the others.

When starved, populations of *Dictyostelium discoideum* amoebae migrating on an agar surface turn from a simple mixture of independent cells into a coordinated society: Cells aggregate and form a grex which later differentiates into spores and stalks (6, 7, 8). Aggregation is brought about by signaling through oscillatory cAMP excretion and by the chemotactic ability of cells to respond to this signal (5). The aggregation mechanism has been extensively studied after formation of a definite aggregation structure: Cells at the center periodically release pulses of cAMP that are relayed outward through the population by the other amoebae (2). The mode of wave propagation has been studied *in situ* from changes in optical density (3, 4); but, very little is known about the initiation process itself. It is a community response generated by a group of inter-communicating cells (8); i.e., a dynamic process, statistical and stochastic in nature. Therefore, we have used computer image processing to study how populations of *Dictyostelium* amoebae develop organized community behavior during the initiation of morphogenetic aggregation.

MATERIAL AND METHODS

Organism and Observation. The organism used was *Dictyostelium discoideum* (Ax-3) cultured at 22°C under shaking in medium containing (per liter) peptone 14.3 g, yeast extract 7.5 g, glucose 15.4 g, Na₂HPO₃ 12H₂O 1.28 g and KH₂PO₄ 0.486 g. 3-day cultures were washed with phosphate buffer 3 times by centrifugation (3000 g, 1 min), after which

Fig. 1. Typical time course of the light intensity at a given position. Time zero is the point at which the amoebae were washed with phosphate buffer. Oscillation becomes more and more regular as time passes. The aggregation center forms about 7 h after starvation at position (P_1).

a drop of the cell suspension was spread on hydrophobic agar (1). Cells were distributed uniformly and densely after the drop settled, and migration was confined within the wet region. The agar was kept moist in a plastic petri dish (O.D.=9 cm) on which part of the top-cover had been replaced by a cover glass. The cell density was 10^5 – 10^6 cells/mm², and a few layers of cells formed. The time course for morphogenesis under these conditions was similar to previous reports (8); aggregation began about 6 h after starvation, and the grex began to migrate at about 10–12 h.

Micro-computer image processing. Cells were mounted on a stage of a phase-contrast microscope (Nikon, model XB-NT). Their pictures were taken by a TV camera (Hitachi, model HV-141) and recorded by a time-lapse video recorder (National, AG-6011). The video images were digitized (Altek, model ALT-256), then transferred to a microcomputer and processed (Nippon Electric Co., model PC9801). To amplify changes in the light intensity produced by changes in the optical density, the image at time t was subtracted that at the previous time $t-t_1$. The value of t_1 was chosen as 150 sec, because it gave the most enhanced images without affecting the oscillation characteristics. A typical time course of oscillation at a point is shown in Fig. 1. Lastly, each image was displayed at 30-sec intervals as 32×32 squares with 17 shades. Coordination in the cell population appears as a spatial pattern or as propagating waves (Fig. 4).

The velocity of wave propagation was determined as follows: Regions of equal phase on the oscillation patterns were connected by dotted and solid lines as indicated in Fig. 2. By comparing two successive patterns at intervals of 90 sec, we could determine the magnitude and direction of wave propagation by taking vectors normal to this curve along the wave front (arrows, Fig. 2B).

Spectral characteristics of a time course at a given point also were studied by Fourier analysis of an FFT program for successive 30-min intervals. A time-series $f(t)$ (Fig. 1) was

Fig. 2. Determination of wave propagation vectors and their spatial distribution. (A) and (B) are oscillation patterns 90 sec apart. Wave propagation vectors are shown by arrows. See text for details.

Fig. 3. Frequency entrainment as the first spatial organization. (A) Oscillation pattern 4.2 h after starvation. (B) A region in which oscillations are entrained into the same frequency (by shaded area). These regions with this feature appear, but each entrains into different frequencies; therefore, they are marked differently with solid and broken lines. (C) Time courses of oscillation within the entrained region (P2, P3 and P4) and without it (P1).

expanded into a Fourier series:

$$f(t) = \sum_{n=0}^{\infty} \{a_n \cos(2\pi nt/T) + b_n \sin(2\pi nt/T)\} \quad (1)$$

in which the Fourier components a_n and b_n are

$$a_n = 2/T \cdot \int_{-T/2}^{T/2} f(t) \cos(2\pi nt) dt$$

and

$$b_n = 2/T \cdot \int_{-T/2}^{T/2} f(t) \sin(2\pi nt) dt \quad (2)$$

Here T denotes an interval. The power of the n -th component is defined as $a_n^2 + b_n^2$; hence, it is a measure of the extent to which oscillation with the frequency n contributes in $f(t)$. The power spectral density is a normalized distribution of $a_n^2 + b_n^2$ against n . Therefore, the sharpness in the distribution of the power spectral density is a measure of the time order at a given point (Fig. 5) and was determined for 32×32 points over the picture. With this measure, we could determine the point at which the most ordered oscillation was occurring in relation to the formation of an aggregation center.

RESULTS AND DISCUSSION

Development of spatial organization in the oscillation pattern. Dictyostelium amoebae oscillate at all stages of morphogenesis, but the mode of coordination in

Fig. 4. Successive patterns of propagating waves toward formation of an aggregation center. Times after starvation are indicated in the figure. Initially, the waves are unstable, appearing and disappearing here and there (A-C). Eventually, a definite center appears (D). The aggregation center is indicated by the symbol ($\times$) in (D). Points indicated by ($\times$) and ($\odot$) respectively correspond to P_1 and P_2 in Fig. 3.

community behavior differs from one stage to another. A typical time course of the transmitted light intensity at a given point, which happens to develop into an aggregation center, is shown in Fig. 1. Qualitatively, irregular oscillations gradually organize themselves into regular ones as morphogenesis proceeds. Note, however, that no visible aggregation is present before 7 h of starvation. What follows is the quantitative analysis of this early organization of spatial and temporal structures in populations of *Dictyostelium* amoebae.

The first distinguishable organization that appeared about 4 h after starvation is shown in Fig. 3. Before this, the amoebae behaved randomly, showing no correlation among oscillations (data not shown). At this point, regions appeared in which the oscillation frequencies were the same although the phases differed, as shown by the shaded area in Fig. 3B. Fig. 3, A and C, shows that even in the same region, the phases of oscillation differ.

The next step in the organization is the onset of wave propagation. Successive stages of spatial organization toward the formation of an aggregation center are shown in Fig. 4. Initially, concentric waves begin to propagate here and there, which soon fade away and the random pattern resumes. Other transitory patterns appear at different positions. These processes continue for a time, which indicates that the amoebae communicate locally, attempting to form an aggregation center. Probably, because oscillation itself is not ordered in long terms at this stage (Figs. 1 and 5), this spatial

Fig. 5. Successive organization of the temporal order. Power spectra are compared at two characteristic points, the soon-to-be center (P_1) and the periphery (P_2). Spectral distribution at P_1 becomes steep much earlier than at the other points.

pattern can not develop into a definite aggregation center. As time passes, concentric waves propagate for longer distances; but, the position of the local center still fluctuates (compare A, B and C). Eventually, 5-7 h after starvation, a certain local center no longer moves about, and concentric waves become longer and longer (D). Soon afterward, aggregation of amoebae at this oscillation center becomes visible; an aggregation center has been established.

Temporal organization of the oscillation. Development of the temporal order at two points is compared in Fig. 5; one (P_1) later developed into an aggregation center, the other (P_2) was located at the periphery of the concentric wave propagation (Fig. 2). Before the 5th h after starvation had passed, spectral distributions were similarly broad in both cases, evidence that the oscillation was random in the frequency domain as it was in space (Fig. 2). About the 6th h after starvation, oscillation at the soon-to-be center of aggregation became highly ordered, mainly because of the decrease in low frequency components; whereas, that at the soon-to-be periphery remained diffuse. Formation of ordered oscillation seems to trigger the formation of an aggregation center. Therefore, every organism in the population of *Dictyostelium* amoebae potentially has the ability to acquire highly ordered oscillation, hence to become an aggregation center. But, where and how aggregation is initiated is governed stochastically through competition among many local oscillators.

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