Eccentric motions of spiral cores in aggregates of *Dictyostelium* cells

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Siegert and Weijer presented evidence indicating the existence of spiral waves of cell migration induced by cyclic adenosine monophosphate (cAMP) in hemispherical aggregates (mounds) of *Dictyostelium* cells [Current Biol. 5, 937 (1995)]. Here I report experimental evidence of the eccentric motion of the spiral core in single mounds of the wild-type strain NC-4. I observed two types of motion of the spiral core. One type is a core shift, in which a spiral core initially situated at the center of a circular mound moves toward the circular boundary of the mound. Another type is a continuous drift of a spiral core along the circular boundary. To obtain some insights into the mechanism of the motion of the spiral cores, I perform a numerical simulation of spiral waves in two-dimensional excitable media with a circular boundary. [S1063-651X(98)10004-1]

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INTRODUCTION

Spiral waves in two or three dimensions are one of the most remarkable spatiotemporal patterns in nonequilibrium excitable or oscillatory media, and have been the subject of extensive research. One active field of recent investigation of spiral waves concerns the transition from regular rotation to eccentric motion of the spiral core. Under some conditions, the trajectory of the tip and a spiral wave is not circular, but rather epicyclelike [1–3]. Spiral wave core motion induced by periodically modulated external force have been examined in a chemical reaction, the Belousov-Zhabotinsky system [4,5]. Furthermore, interactions between two spiral waves were also shown to result in core moving [6–10]. Very recently, it was reported that the rectilinear filament of a simple scroll wave (three-dimensional spiral wave) in the Belousov-Zhabotinsky reaction gel system may be induced to drift by applying temperature gradients parallel to the filament [11]. This treatment leads to a helical shape change of the filament. Here, I report experimental evidence of core motion of spiral waves in a biological system, that is, a hemispherical aggregate (mounds) of *Dictyostelium* cells.

Upon starvation, *Dictyostelium* amoebae begin a process which eventually induces them to aggregate. Aggregation itself is regulated by cell-to-cell communication through an intercellular chemoattractant, cAMP (cyclic adenosine monophosphate), which is secreted by the cells. During aggregation, spiral waves of optical density in thin cell layers may be observed [12], which are correlated with changes in cell shape. The spiral wave of optical density is a reaction to the self-organized cAMP spiral wave in the still two-dimensional cell layer. In fact, the cAMP spiral wave has been directly detected by using isotope dilution-fluorography by Tomchik and Devreotes [13]. Cell aggregation in two dimensions mediated by the cAMP spiral wave is followed by the formation of a hemispherical aggregate of cells, the mound. Before the mound stage, cells begin to differentiate into two cell types—prestalk (pst) and prespore (psp) cells—which have been observed to distribute to random positions in the early mound. The subsequent morphogenetic changes of the hemispherical mound lead to the formation of a fruiting body, often via the slug stage. Cell differentiation proceeds during the stages from the mound to the fruiting body, and the pst and psp cells finally differentiate into stalk and spore cells in the fruiting body, respectively.

Weijer and co-workers obtained evidence of spiral or circular waves of cAMP in the mound by observing the optical density waves and/or using cell tracking techniques [14,15]. Very recently, Vasiev, Siegert, and Weijer [16] presented a hydrodynamic model for *Dictyostelium* mound formation. Their simulations showed that the spiral wave in the mound tends to meander when the excitability of the mound decreases. They proposed that the mechanism of such a meandering process includes the following two points. In less excitable media, the size of a spiral core becomes larger and the spiral tip is situated close to the mound boundary. Subsequent interaction between the spiral tip and the mound boundary leads to spiral meandering. A similar behavior of spiral waves had been found in the complex Ginzburg-Landau equation and the experimental system of the Belousov-Zhabotinsky zone reaction with a circular boundary [17–19]. Furthermore, Vasiev, Siegert, and Weijer [16] proposed that the above mentioned mechanism was responsible for the meandering of the spiral wave and of the mound itself in the streamer *F* mutant NP368.

Here, by using the wild strain NC-4, I present experimental evidence that the spiral core, situated initially at the center of the mound, shifts toward the circular boundary of the mound. Based on a numerical simulation of two-dimensional spiral waves in an excitable medium with a circular boundary, I suggest another mechanism of the core shift from the center to a new asymmetric location, which the authors of Ref. [16] did not consider. The pst cells located in random positions in an early mound sort gradually to the center of the mound by chemotaxis to the cAMP spiral wave. This causes spontaneous formation of a gradient of excitability from the center to the boundary of the mound, and leads to the core shift.

METHODS

Experiment

Dictyostelium discoideum NC-4 cells were grown on agar plates with *Escherichia coli* as nutrient for 24 h at 22 °C in

the dark. After this incubation, a portion of the cells on an agar plate began to aggregate, because of the local scarcity of nutrient. The cells were washed three times by 20-mM potassium phosphate buffer (pH6.8), and washed by centrifugation to remove the bacteria. The harvested cells were then incubated in 0.0025% neutral red (Sigma) in potassium phosphate buffer for 2 min at 26 °C, and then washed three times with the buffer solution. The neutral red stained cells were deposited on 1.5% distilled water agar and incubated for 17 h at 8 °C. In some cases, one half of the starved cell population was stained by neutral red and mixed with unstained cells and put on 1.5% distilled water agar. Changes in the spatial distribution of neutral red strained cells in early mounds were recorded by time-lapse video recorder (SONY EVT-820) via a charge-coupled device (CCD) video camera (SONY CCD-IRIS) mounted on an inverted microscope (NIKON TMS-F). The measurements were performed at room temperature (20±2 °C). Illumination was minimized in order to avoid toxic reactions to neutral red induced by light. In some cases, the light exposure time for the mounds was manually set to about 5 s every 15 or 20 min.

Numerical simulation

In order to obtain some insight into the mechanism of the core shift of spiral waves in a mound, a hemispherical mound was modeled as a two-dimensional circular excitable media, and numerical simulations of the two-dimensional spiral waves were performed. In the numerical simulation, I used the Barkley model [20] as follows:

$$\partial u/\partial t = \varepsilon^{-1} u(1-u)(u-(v+b)/a) + D\Delta u, \qquad (1)$$

$$\partial v/\partial t = u - v. \qquad (2)$$

This model was used to simulate three-dimensional cAMP waves in a *Dictyostelium* slug by Steinbock *et al.* [21]. The value u represents the extracellular cAMP concentration, and v the fraction of the cAMP receptors in the active state [21]. The parameters a and ε are fixed at 0.4 and 0.0085, respectively. The parameter b represents a threshold and controls excitability. The diffusion constant D is 1.0. The time step per iteration was 0.001. Two-dimensional 100 $\times$100 cells were constructed, within which a spiral wave was created with the parameter $b=0.03$. Later, in order to mimic the mound after the pst cells had sorted to the center, a circular boundary with the radius $r=35$ cells was imposed and simultaneously the value of b for the cells within the circle of radius $r=35$ cells was changed as follows. According to Steinbock *et al.* [21], b is specified as 0.01 for the highly excitable region (corresponding to the pst cells), and left as 0.03 for the less excitable region (corresponding to the psp cells). Therefore, $b=0.01$ for the circular region of $0<r\leqslant 10$ and $b=0.03$ for the ring of $10<r\leqslant 35$. In the outer region of $r>35$, the reaction terms of Eqs. (1) and (2) were omitted.

RESULTS AND DISCUSSION

Mounds with various diameters could be induced to form by varying the cell density during starvation. It seemed that no rotational movements of cells occur in smaller mounds, although I have not yet clarified the relationship between the diameter of the mounds and rotational cell movement. Mounds below a critical size might not support a cAMP spiral wave. The influence of the circular domain size on the spiral wavelength were investigated in the NO+CO reaction system on a Pt(100) surface, and it was found that below a critical size the medium no longer supports spiral formation [22].

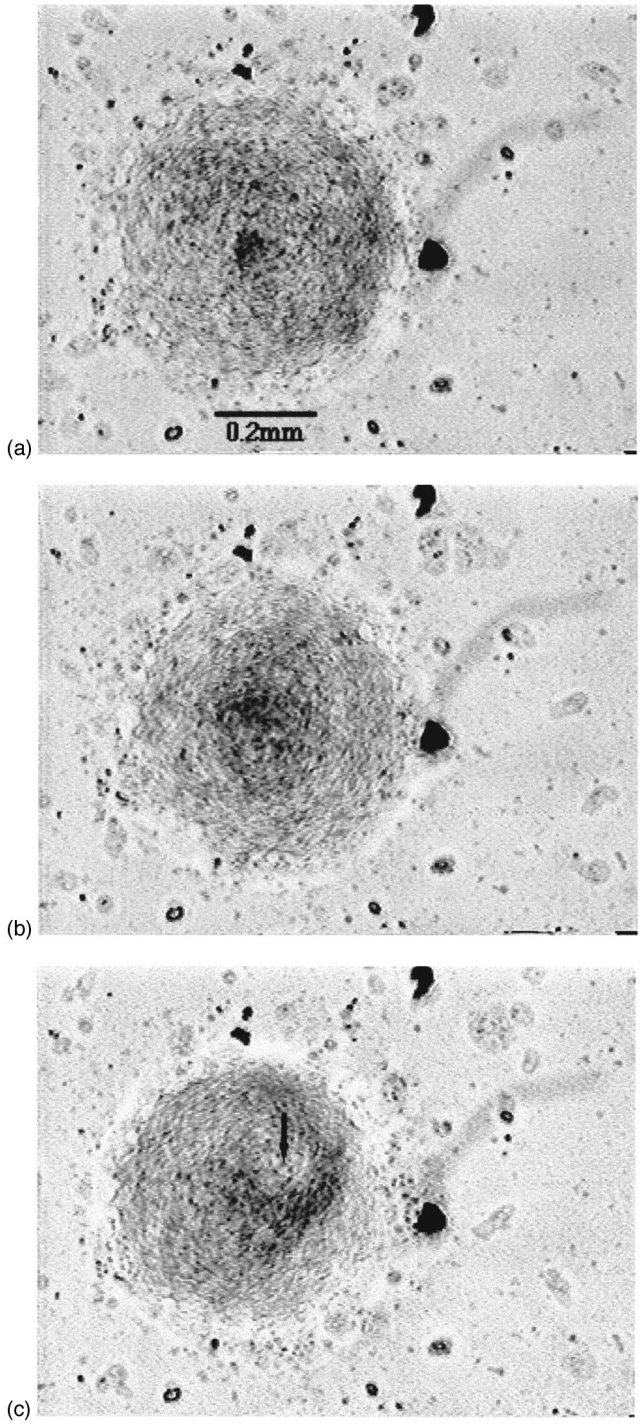


FIG. 1. Accumulation of neutral red stained pst cells in the center of a mound with rotational cell movement in the clockwise direction, and the subsequent dispersal of the cluster of the accumulated cells. (a) 0 min. (b) 40 min. (c) 50 min. The arrow in (c) shows a new spiral core.

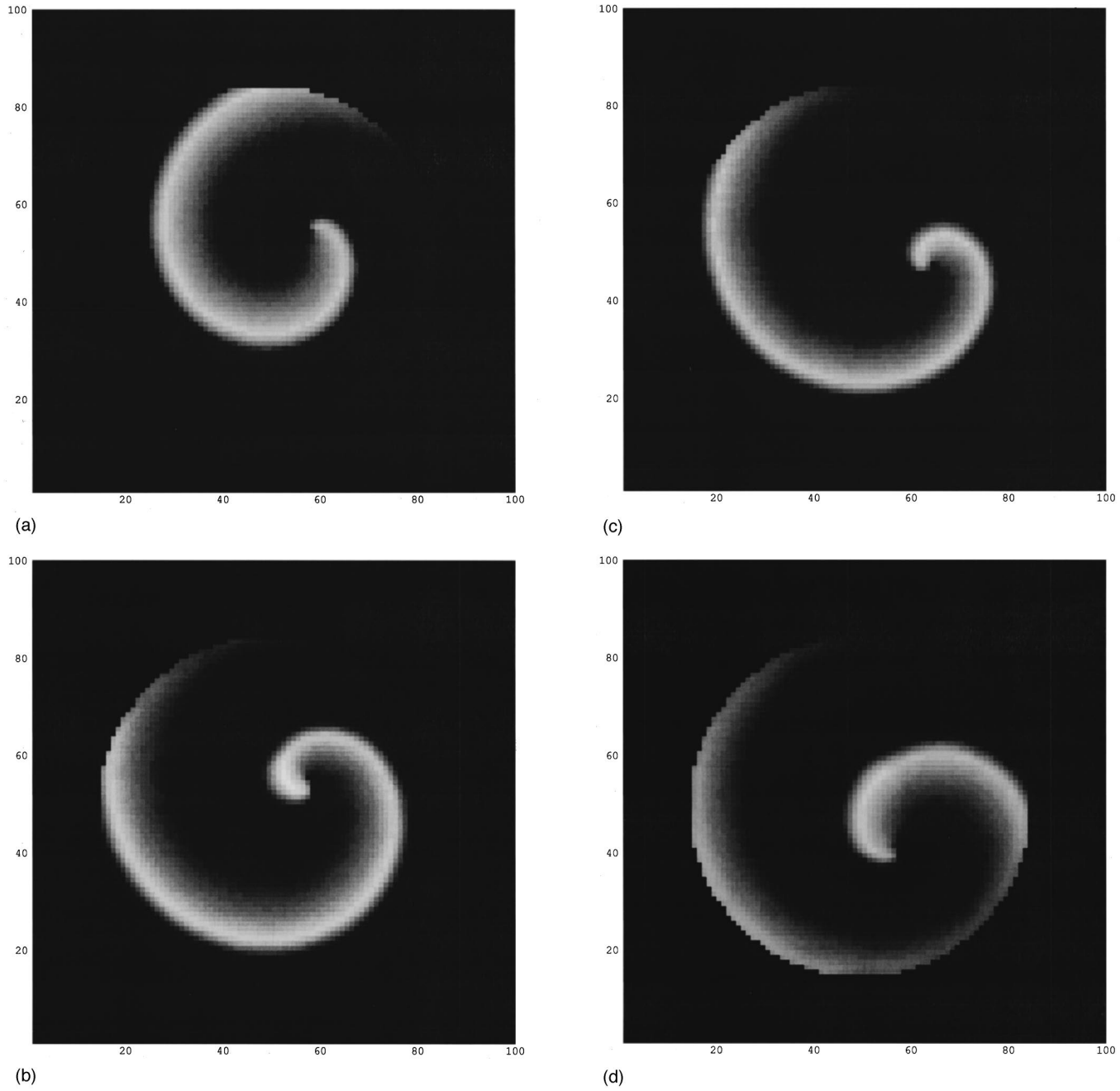
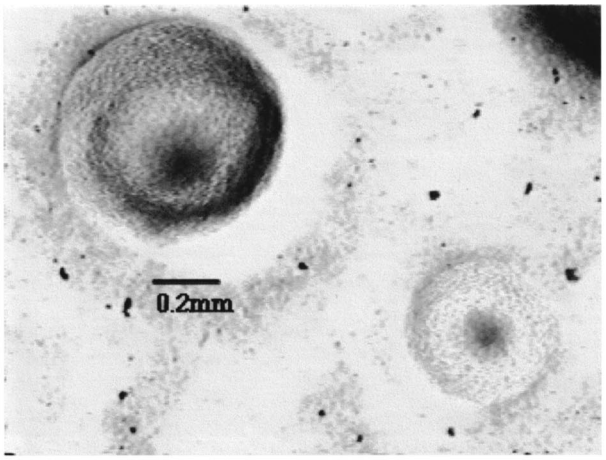


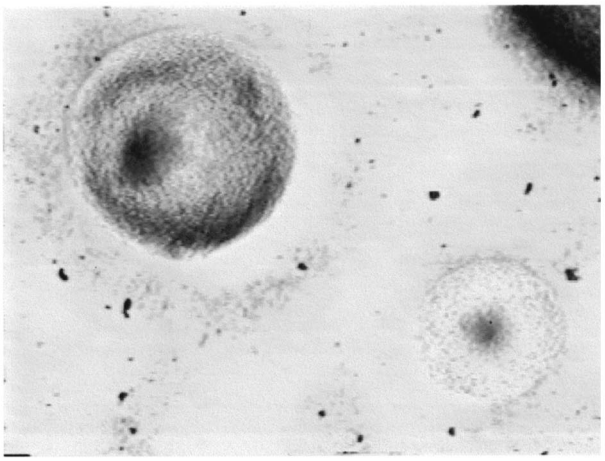
FIG. 2. Time series of the spiral wave of the variable ν (proportion of active cAMP receptors) after changing the threshold parameter b of the cells within the circle of radius $r=10$ cells and the center (50,50). (a) $t=12\,010$. (b) $t=13\,000$. (c) $t=16\,000$. (d) $t=20\,000$.

Figure 1 shows an example of a mound during rotational cell movement. This mound was formed from a mixture of neutral red stained and unstained cells. Neutral red strained pst cells gradually accumulated in the center of the mound [Figs. 1(a) and 1(b)]. The rotational direction of cells in the mound was clockwise. The movement of the cells indicates the propagation of a cAMP spiral wave in the mound. The accumulated pst cells in the center no longer show rotational movement, but only constrain movement in the center of the mound. This behavior provides evidence that the center of the mound corresponds to the core of the cAMP spiral wave. One explanation for the accumulation of neutral red stained pst cells at the center of the mound requires a greater chemotactic response to the cAMP wave of the pst cells than the psp cells. In fact, this difference was reported in Ref. [23]. During the rotational movements of the cells in the mound, a center [arrow in Fig. 1(c)] of cell rotation emerged and, subsequently, degradation of the cluster of accumulated pst cells was observed. This suggests that the spiral core, situated initially in the center, shifts toward the circular boundary of the mound. This core shift is not the case of meandering, because the location of the new center of cell rotation remains stationary.

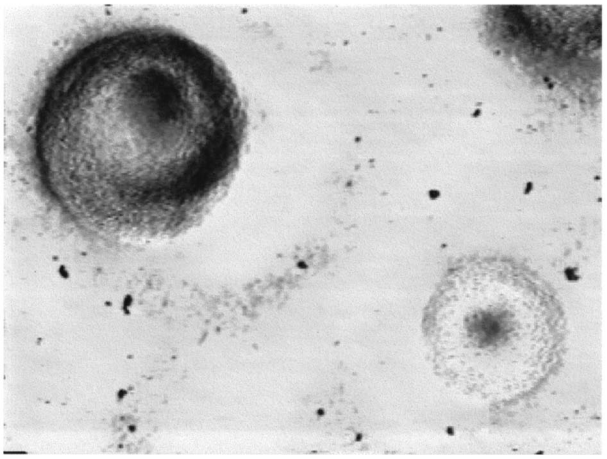
I performed a numerical simulation to obtain some insight into the mechanism of the core shift of the cAMP spiral wave in a single mound. My computation was substantially the same as that used for cAMP waves in the slug of *Dictyostelium* by Steinbock *et al.* [21]. First, a spiral wave was



(a)



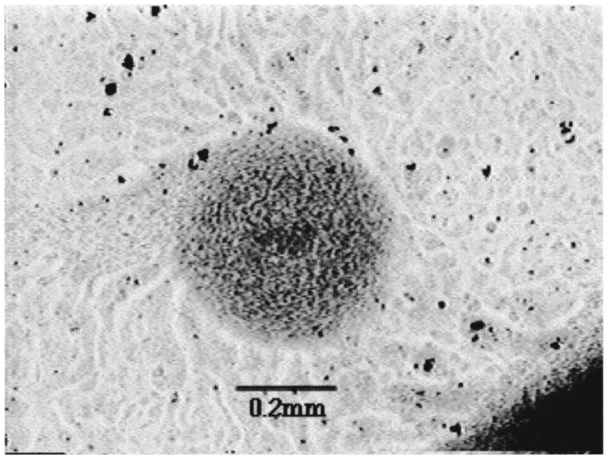
(b)



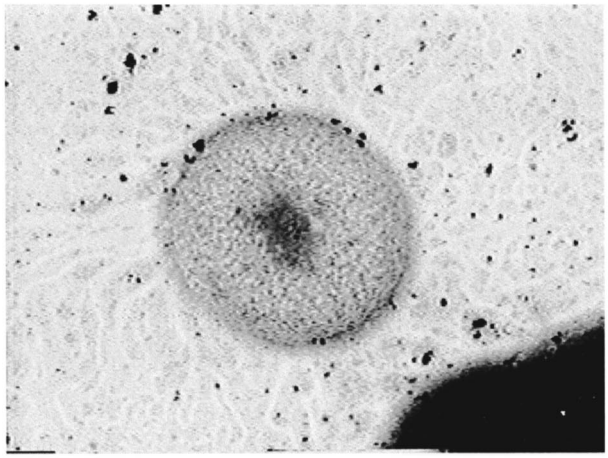
(c)

FIG. 3. The spiral core drifting continuously along the boundary of a mound. (a) 0 min. (b) 15 min. (c) 30 min. The direction of the rotation of the cells and the spiral core in the mound is clockwise.

created in two-dimensional 100×100 cells. At one time point ($t=12\,000$), a circular boundary of radius $r=35$ cells was imposed, and the value of the parameter b of the cells within the circle of $r=10$ was changed from 0.03 to 0.01. This procedure was performed so as to reflect the accumulation of the neutral red stained pst cells in the center of a mound. Figure 2 shows a time series of the spiral wave after changing b. A core shift from the center to an asymmetric location was induced. In contrast to Fig. 2, the location of the spiral tip remains stationary in the center if b remains globally fixed to 0.03. This computation is preliminary, but the result shown in Fig. 2 appears to correspond to the experimental observation demonstrated in Fig. 1. I propose that graded separation of pst and psp cells leads to the spontaneous formation of a radial gradient of excitability from the center to the boundary, and that this inhomogeneity induces core shifts of the cAMP spiral wave. The authors of Ref. [24], using the light-sensitive Belousov-Zhabotinsky reaction system, observed the drifting of spiral waves in an excitable medium with a gradient of refractoriness. Furthermore, they reported using a cellular automaton model to produce a simulation similar to that in the present work.



(a)



(b)

FIG. 4. The transition from a mound (a) to a ring structure (b) after 35 min. In (b), there are only a few stained cells in the center of the ring.

I not only observed the core shift shown in Fig. 1, but also that the core drifted continuously along the circular boundary of a mound. Figure 3 shows the core drifting along the boundary of the mound (the upper left mound). In this case, the mound was formed entirely of starved and neutral red stained cells. The rotational direction is clockwise. The circular region within the mound presumably consists of neutral red stained cells and neutral red itself on the surface of the agar plate. The latter accumulated in the spiral core during

rotational movement of the cells. This point is illustrated in Fig. 4, which shows a transition from a mound to a ring structure. Such a transition was observed in relatively small mounds which displayed rotational cell movement. It is apparent in Fig. 4(b) that residual neutral red accumulates in the center of the ring, which retains only a few cells. The core drift along the circular boundary of the mound has been also reported in mounds of a streamer *F* mutant NP368 by observing the wave of optical density [16]. Furthermore, the spiral core drift along the boundary of finite circular media has been reported in both experimental Belousov-Zhabotinsky reaction systems and in a numerical calculation [17–19]. In these studies, the interaction between the boundary and the spiral tip was proposed as the mechanism of core drift. In the present study, not only the interaction of the spiral wave with the circular boundary, but also the gradient of excitability in the mound caused by cell sorting process, might be responsible for the continuous core drift along the boundary, demonstrated in Fig. 3. To clarify these processes, long-termed numerical simulations, which include the movement of the pst and psp cells, will be needed and are now in progress.

In the present work, I obtained evidence for (1) the core shift of the cAMP spiral wave from the center to an asymmetric location, and (2) continuous core drift along the circular boundary in the mounds. The transition from regular rotation to movement of the spiral core is attributed to redistribution of pst cells at the center of the mound. Of special interest is the relationship between the movement of the pst cells and their final positions. Such information might be obtained by using cell tracking techniques during the late mound stage and during the transition to the slug.

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