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Rotating Cell Motion and Wave Propagation During the Developmental Cycle of Dictyostelium

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Abstract

During all stages of the complex development of the slime mould Dictyostelium discoideum single cells follow well defined patterns of motion. This chemotactic behavior is controlled by rotating waves of excitation during the early two-dimensional aggregation and during the subsequent migration stage of the three-dimensional slug. We present quantitative mutual-correlation analysis of chemotaxis in the center of spiral waves, revealing a well organized rotation of amoebas in this area. Furthermore, the control of recently observed rotational motion in slugs is simulated with a simple three-dimensional reaction-diffusion model.

Introduction

During the developmental cycle of the slime mould Dictyostelium discoideum single amoebas, dispersed over a substratum, aggregate towards an aggregation center. The cells collect in multicellular aggregates, which transform into a conditional motile stage, the slug. Subsequently, they differentiate to form a fruiting body bearing spores (Loomis, 1982). This process is coordinated and selforganized by travelling waves of cyclic adenosine monophosphate (cAMP) (Tomchik and Devreotes, 1981).

It is well known, that during the early two-dimensional stage of aggregation the system behaves as an excitable or oscillatory medium (Siegert and Weijer, 1989; Foerster et al., 1990). Hence, from a physicist's point of view it is comparable to other excitable systems, like the chemical Belousov-Zhabotinsky (BZ) reaction (Field and Burger, 1985) or the CO-oxidation on platinum (Jakubith et al., 1990), which show similar spatio-temporal patterns. The characteristic excitation patterns of these two-dimensional systems are expanding target patterns or rotating spiral waves. Nonlinear differential equations coupling local reactions with diffusive transport reproduce experimental observations qualitatively and quantitatively (Keener and Tyson, 1986; Tyson and Murray, 1989).

An interesting particularity of the Dictyostelium system is the existence of an additional transport process which is caused by chemotaxis. This motion is controlled by the local dynamics of the cAMP-concentration and occurs in a direction opposite to the direction of cAMP wave propagation (Devreotes, 1989). In a recent investigation (Steinbock et al. 1991) the cell velocity was found to vary periodically between nearly zero and 20–30 μm/min, with periods of 6–9 min.

This paper analyzes, in the first part, the chemotactic cell motion in the central region of two-dimensional spirals in quantitative detail and presents, in the second part, numerical simulations which elucidate the dynamics of three-dimensional wave propagation in Dictyostelium slugs. Furthermore, the connection between observed cell motion and controlling waves of excitation is discussed.

Two-dimensional stage of aggregation

Cells of Dictyostelium discoideum, axenic strain AX-2, were spread uniformly on an agar surface, containing only buffer and 2 mM caffeine. Due to the depletion of food sources the amoebas start to aggregate and form the described excitation patterns. Figure 1 gives an example of a rotating spiral wave observed by darkfield photography. To study the chemotactic cell motion in these patterns on a microscopic level, we used an inverse microscope (ZEISS, IM 35). The observed images (bright field illumination)

were stored as quick-motion movies on a time lapse video recorder. Single sequences of 30 frames were digitized by an image acquisition card and analyzed on a computer.

The velocity analysis is based on a mutual-correlation method (Hashimoto et al., 1993). A moving cell having a characteristic contour (e.g. the cell boundary) causes temporal intensity changes at the traversed sites of recorded picture elements (pixels). If the contour is nearly constant in time, the intensity functions of neighboring pixels are shifted due to the motion by a retardation time τ_0. This time shift is calculated by mutual-correlation functions $C_0^k(\tau)$ between the temporal brightness changes of a central pixel 0 and that of its neighboring pixels k. The fraction of pixel distance and retardation time τ_0, when $C_0^k(\tau)$ reaches its maximum, yields a velocity v in the direction towards the neighboring pixel k. The maximum value of $C_0^k(\tau)$ is a measure for the probability that the cell was moving towards the site k. Due to necessary restrictions to be made, a considerable number of pixels is omitted in the calculations (Hashimoto, 1993). Therefore we commonly use spatial averages in appropriately selected areas.

Figure 2 shows a typical microscopic view into the center of a spiral wave (A) and the corresponding velocity field of cell motion (B). Each of the velocity vectors in Fig. 2B was calculated by averaging the available information of the 51×51 surrounding pixels. The vortex-like velocity field indicates a rotating motion of amoebas in the central region of spiral waves. In the upper segment of this vortex the cells are migrating with the highest velocities ($\approx$ 15 μm/min), while the slowest motion is found in the central part ($\approx$ 1 μm/min). Further analyses revealed, that the high-velocity segment moves around the center with a period of approximately 8 min. The sense of rotation of this excited high-velocity segment is always opposite to that of amoebas. This observation is again caused by the opposite directions of cAMP-wave propagation and chemotactic cell motion.

The properties of the observed vortex-like cell motion can be explained by the geometry and dynamics of the spiral-shaped cAMP-wave. The spiral tip spinning around the core determines the velocity field and its position controls the location of the excited high-velocity segment. Thus, we found a new tool to observe the dynamics of cAMP-waves by analyzing the chemotactic cell motion.

Three-dimensional waves in migrating slugs

We have recently obtained evidence that three-dimensional rotating scroll waves appear to organize the motion of cells in Dictyostelium slugs (Siegert and Weijer, 1992). The anterior part of this multicellular body (20% of all amoebae) consists of prestalk cells, which ultimately build the stalk of the fruiting body. The remainder is formed by

prespore cells which differentiate to spores in the fruiting body.

Analysis of cell motion in slugs revealed that amoebae in the prespore zone move straight forward in the direction of slug migration, while cells in the prestalk zone move perpendicularly to the direction of slug migration, that is they rotate around the slug axis. In Fig. 3 tracks of cell movement are presented, which illustrate this behavior. This analysis suggested that the chemotactic signal spreads as a scroll wave in the front of the slug and converts into planar waves in the rear part. We proposed that this complex mode of wave propagation was caused by a change in excitability along the long axis of the slug (Siegert and Weijer, 1992). This is based on the finding that during aggregation the cells that will become prestalk show high frequency oscillations in optical density when isolated, while cells that will become prespore show slow oscillations (Weijer et al., 1984).

Martiel and Goldbeter (1987) proposed nonlinear kinetic rate laws for oscillations and signal relaying by Dictyostelium amoebas in well-stirred cell suspensions. These were extended by Tyson et al. (1989) to a reaction-diffusion model, which is able to describe spatio-temporal pattern formation (e.g. rotation of spiral waves). To investigate whether an three-dimensional excitable system exhibit a behavior that supports our hypothesis of wave propagation in slugs, we performed numerical simulations. Based on the previous simulations of wave propagation during the two-dimensional aggregation phase (Tyson et al., 1989), we calculated numerical solutions of an excitable reaction-diffusion system (Barkley, 1991):

$$\frac{\partial u}{\partial t} = \vec{\nabla}^2 u + \frac{1}{\epsilon} u(1-u)\left(u - \frac{v+b}{a}\right) \quad ,$$

$$\frac{\partial v}{\partial t} = u - v \quad .$$

In this system the excitability is controlled by the parameters a=0.4, ϵ=1/150 and b (specified below). The time step per iteration is dt=0.0103. The variable u (propagator) obeys a nonlinear reaction kinetics and qualitatively models the extracellular cAMP concentration, while v (controller) represents the fraction of the cAMP-receptor in its active state. The shape of the Dictyostelium slug is approximated by a cylinder. The total number of grid points in this cylinder (90800) is of the same magnitude as the number of amoebas in a typical slug. The cylinder is embedded in a rectangular box, surrounded by grid points obeying unexcitable kinetics (b=0.3). The difference in excitability between the prestalk and prespore region is modelled by a step function of parameter b along the symmetry axis of the cylinder (b_{pst}=0.01, b_{psp}=0.023).

The initial condition is a scroll wave along the long axis of the slug having constant excitability. After a certain time (t=880 iterations) a change in excitability along the long axis of the slug is introduced, as described above. In response to this change the scroll wave undergoes a complex transformation into a new pattern, as shown in Fig. 4. While the wave rotation in the region of high excitability (prestalk region) remains stable during the entire calculation, the scroll wave in the region of low excitability (prespore region) increases its wave length and rotation period. Subsequently, the whole structure becomes twisted in middle segments of the cylinder. The process of twisting and the higher frequency in the prestalk region causes a dramatic change of the pattern in the less excitable prespore zone: Planar wave fronts appear that are oriented perpendicular to the long axis of the cylinder. This spatial arrangement remains stable over more than 30 periods of scroll wave rotation. The interface between the region of scroll wave rotation and planar wave propagation displays more complex dynamics and alternating phases of weak and strong twisting.

The calculations demonstrate that the observed chemotactic cell motion in Dictyostelium slugs (Siegert and Weijer, 1992) can be readily explained by a partially decomposed scroll wave of chemical signals. They show that waves of excitation play a crucial role not only during cell aggregation but also during later stages in the developmental cycle of this biological system.

Conclusions

We have shown experimentally that the analysis of cell motion is a powerful tool to obtain informations about the controlling waves of excitation. Quantitative results on chemotactic movement of amoebas reveal not only temporal aspects (e.g. periodicity in aggregation), but also provide insight in geometrical properties of the occurring structures. Furthermore, it is possible to use this correlation between chemical waves and chemotaxis to model observed cell motion in three-dimensional Dictyostelium slugs by reaction-diffusion equations and thus, to obtain a more precise understanding of these fascinating and complex modes of wave propagation.

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Figure Captions

1. Darkfield photography of a rotating spiral wave (wavelength $\approx$ 3 mm). The dynamics of this selforganized pattern control the aggregation of Dictyostelium cells towards the center of the spiral.

2. (A) Microscopic image of the central region of a spiral wave (0.39×0.32 mm^2). (B) Corresponding vector-field of cell velocities obtained by mutual-correlation analysis. The amoebas of (A) perform a vortex-like rotation around the center of the spiral core.

3. (A,C) Tracks of cell motion in neutral red-stained amoebas in Dictyostelium slugs. Cells in the prespore zone move in the direction of slug motion, and cells in the prestalk zone move at $\approx 45°$ angles to the direction of slug migration. In all slugs recorded we observed cells rotating in the tip (prestalk zone). (B,D) Same tracks as in (A) and (C), but corrected for the slug speed (from Siegert and Weijer, 1992).

4. Three-dimensional representation of the numerical solution of reaction-diffusion equations, modelling modes of wave propagation in Dictyostelium slugs (see text). The highly excitable (prestalk) zone is located on the left side. Grid points with $v < 0.27$ are shown transparently, while the others are solid. The spatio-temporal dynamics of this stable structure can explain the observed chemotactic cell motion.