Spatio-Temporal Organization in Nonequilibrium Systems

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Wave propagation and cell movement control morphogenesis of the cellular slime mould *Dictyostelium discoideum*

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The cellular slime mould *Dictyostelium discoideum* is well suited to study cellular communication and the role of biological oscillations in spatio-temporal pattern formation. *Dictyostelium* cells live as solitary amoebae in the soil where they feed on bacteria. Upon starvation up to 10^5 cells aggregate over large distances into a multicellular mass, the slug (see fig. **1A**). The slug transforms during a complicated morphogenesis into a fruiting body consisting of a spore head supported by a stalk composed of dead vacuolated stalk cells. The spores disperse and germinate to single amoeba again. The aggregation and coordinated cell movement during slug morphogenesis require extensive cell-cell communication.

The aggregation of single cells into multicellular structures is directed by periodic cyclic-AMP signals and chemotaxis. Cells in the aggregation center periodically produce the chemoattractant cyclic Adenosine-Monophosphate (cAMP). cAMP is secreted into the extracellular medium, where it diffuses away. Neighbouring cells detect cAMP via cell surface receptors. These stimulated cells now produce themselves huge amounts of cAMP, which they in turn secrete. This feed-back process results in a wave-like propagation of the cAMP signal from cell to cell and from the center outwards. Extracellular phosphodiesterases degrades cAMP. After stimulation the cells are refractory to further stimulation. This property ensures unidirectional outward propagation of the signal. Stimulated cells respond with periodic chemotactic movement. Theys move in the direction of increasing cAMP concentrations. This leads to periodic waves of inward directed chemotactic movement. As a result the cells collect in the aggregation center. The cAMP wave propagation can be seen as outward propagating concentric or spiral optical density waves (see fig. **1B**). These waves are only visible in monolayers of cells ,i.e during the early stages of aggregation (1). In later aggregates these waves are no longer visible. After aggregation the cells form a multicellular "slug" which contains up to 10^5 cells. The slug has a defined polarity and is composed of at least three different cell types. The front quarter (tip) consists of two types of prestalk cells (pstA, pstB), the back three quarters consists of prespore cells. The prestalk cells will form the stalk of the fruiting body and the prespore cells the spores.

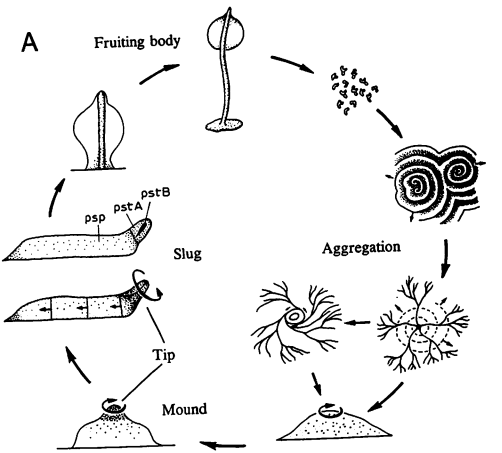
We investigated whether the same principles that govern aggregation, i.e wave propagation and chemotactic cell movement, are responsible for the complex slug morphogenesis. Since optical density waves are no longer visible we investigated cell behaviour and cell movement in late aggregates and slugs. Cell movement of labeled single cells in *Dictyostelium* slugs were

analysed by high resolution digital image processing. We found that all cells in a slug move in a periodic and chemotactic fashion as juged by their periodic shape and velocity changes (2). This implies that the cells respond to periodic signals. In order to deduce the spatial geometry of these signals we determined the movement trajectories of many cells in various parts of the slug. Cells in the front and the back of a slug move along completely different trajectories (see fig. **1C**). Prestalk cells move around the long axis of the slug, while prespore cells move forward parallel to the long axis. The cell movement data show that the chemotactic signal in the slug propagates as a three dimensional scroll wave in the front (prestalk zone), which transforms into planar waves in the back (prespore zone). This behavior is postulated to result from a difference in excitability of the cells along the long axis of the slug (2,3). The cells in the prestalk zone are more excitable than cells in the prespore zone. The biological significance of this particular type of wave propagation is that the core of the scroll wave will be a region of low continuous cAMP, conditions that favor the expression of stalk cell specific genes. In summary our experiments show that the morphogenesis of this relative simple biological system is controlled in two as well as in three dimensions largely by the dynamics of the underlying excitable system.

1. F.Siegert and C.J.Weijer, (1989) *J.Cell.Sci.* **93**, 325
2. F.Siegert and C.J.Weijer, (1991) *Physica D* **49**, 224
3. F Siegert and C.J.Weijer, (1992) *Proc.Natl.Acad.Sci.* **89**, 6433

Figure: The life cycle of *Dictyostelium discoideum*

The scheme (**A**) starts with single cells that aggregate in spirals as seen in darkfield optics. Moving cells are elongated and are seen as white bands due to increased light scattering (**B**). Cells which are not moving directionally are rounded and detected as dark bands. After stream formation waves emanating from the center propagate through the aggregation streams, while the cells move inward. The aggregation center can either be a mass of cells or temporarly form a loop. After tight aggregates (mounds) have formed the cells differentiate. Due to a higher oscillation frequency the prestalk cells form the tip in which the signals propagates as a (twisted) scroll wave as derived from the analysis of cell movement tracks (**C, white lines**). The wave rotates along its long axis as indicated by the black arrow. The mound now extends up in the air and forms a slug, which falls over and migrates away. The prestalk region stays a twisted scroll wave which decomposes upon arrival in the prespore zone in planar waves, due to the lower excitability of the prespore cells. The core of the slug is a region of low cAMP and this favors pstB expression, while the perifery of the tip with high cAMP favours pstA expression. The slug then converts by a series of not yet understood morphogenetic changes into a fruiting body, during which process the differentiation in the final cell types spore and stalk cells takes place.


A
Fruiting body
pstB
pstA
psp
Slug
Aggregation
Tip
Mound


B


C