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Aggregation territory formation and cell streaming in a model of the cellular slime mould Dictyostelium discoideum

A reaction-diffusion-advection model of multicellular morphogenesis during in the aggregation phase of the microorganism Dictyostelium discoideum *is reviewed. Linear analysis identifies a novel chemotaxis-driven instability which appears to underlie the cell streaming phenomenon observed experimentally. Numerical simulations show that the model captures in considerable detail the establishment of aggregation territories and the formation of branching cell stream patterns which characterize aggregation* in situ.

1. Introduction

The establishment of pattern and form during the development of multicellular organisms remains one of the most challenging problems of biology (for an overview of theoretical work see e.g. [8]). Here we report on a theoretical study of the mechanisms of pattern formation in a model system of developmental biology, the cellular slime mould *Dictyostelium discoideum* (*Dd* hereafter). The life cycle of *Dd* exhibits a relatively simple transition from a free-living single cell stage to multicellularity, during which many of the cell-cell interactions characteristic of development in more complex organisms can be observed [2]. This transition is initiated by the aggregation of about 10^5 cells in distinct territories, and ca. 1–2 hours after the beginning of aggregation, a branching pattern of cell streams is formed within these aggregation territories in which *Dd* amoebae establish direct cell-cell contacts (c.f. [4]). Here we review briefly a recently proposed model of the aggregation process [4]. Its linear analysis provides a scenario for the onset of cell streaming. The patterning dynamics of the full model are studied in numerical simulations which extend previous work to the biologically relevant situation of more than a single aggregation centre.

2. The model

Aggregation necessitates a coordinated motile response of the amoebae in an area much larger than the typical cell dimensions. In *Dd* this is achieved by the coupling of chemotaxis (i.e., directed cell motion in a chemical gradient) to chemical cell-cell signalling. A ubiquitous biological signalling molecule, cAMP[1], serves as extracellular messenger, mediating both cell communication and orientation. On the basis of the experimentally characterized biochemical and motility dynamics of a single amoebae in response to cAMP, we derived a model of the aggregation process in the cellular ensemble [4]. Its three dynamic variables, the cell density distribution, $n(x,y,t)$, the extracellular cAMP concentration, $u(x,y,t)$, and the cellular sensitivity to cAMP (the fraction of cAMP receptors per cell in the active state), $v(x,y,t)$ are governed by the following reaction-diffusion-advection system:

$$\frac{\partial n}{\partial t} = \nabla \cdot (\mu \nabla n - \chi(v) n \nabla u), \tag{1}$$

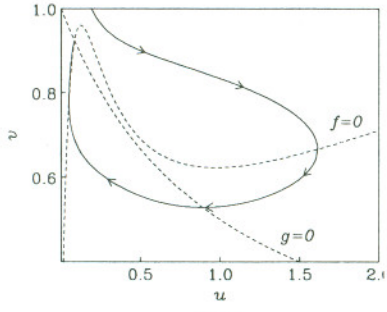
$$\frac{\partial u}{\partial t} = f(n,u,v) + \nabla^2 u, \tag{2}$$

$$\frac{\partial v}{\partial t} = -g_1(u)v + g_2(u)(1-v), \tag{3}$$

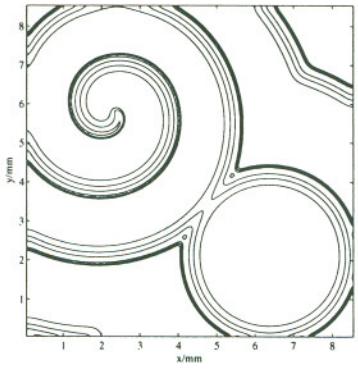
where $\nabla = (\partial/\partial x, \partial/\partial y)$. Zero-flux boundary conditions are employed. System (1)–(3) is derived by coupling a version of a successful cAMP signalling model [7,10] with a minimal model of the chemotactic response of *Dd* amoebae [3,5]. It is based on two modes of action of cAMP: the activation of both chemotaxis and its own synthesis on a short timescale, and the subsequent desensitization of the cellular machinery towards further stimulation on a somewhat longer timescale (primarily via a conversion of the cAMP cell surface receptors to an inactive form). We use the following expressions for the rates of desensitization and resensitization,

$$g_1(u) = k_1 u, \quad g_2(u) = k_2, \tag{4}$$

[1] cyclic adenosine 3',5'-monophosphate



(1a)



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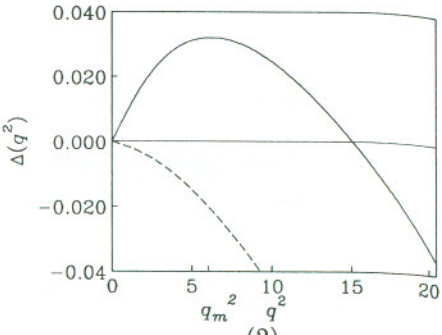

(2)

Figure 1: Behaviour of system (2)–(3) with cell density "clamped" at a constant value ($n = 1.0$); (a) phase plane with typical excitation trajectory, and (b) contour plot of spiral wave solution (upper left of domain) and concentric wave (lower right; the pacemaker region in the centre is silent at the time of the snapshot) on a two-dimensional spatial domain. Parameters: $r = 70.0$, $a = 0.014$, $b = 0.2$, $d_1 = 1.64$, $d_2 = 1.82$, $k_1 = k_2 = 2.5$; timescale 2.5 min, length scale 220 μm, cAMP scale 5×10^{-7} M.

Figure 2. Dispersion relation $\Delta(q^2) = \ln \rho^*(\lambda = 0, q^2)$ for cell patterning in the y-direction. Parameters as in Figure 1, and $\mu = 0.012$, $A = 0.72$, $m = 10.0$, $\kappa = 0.7$, $K = 0.8$, $\chi_0 = 0.5$ (solid line), $\chi_0 = 0.2$ (dashed line).

and for cAMP synthesis and degradation,

$$f(n,u,v) = \frac{rn(bv+v^2)(a+u^2)}{(1-\kappa n/(1+Kn))(1+u^2)} - \left(\frac{d_1 n}{1-\kappa n/(1+Kn)} + d_2\right) u, \tag{5}$$

which are somewhat simplified versions of the corresponding terms in [7]. Expression (5) incorporates the autocatalytic stimulation of cAMP synthesis coupled to the cellular sensitivity (v). Extending the previous models cAMP signalling models [10], the cell density dependence of cAMP synthesis and degradation is included. The chemotactic drift term in (1) also depends on the cellular sensitivity to cAMP, via

$$\chi(v) = \chi_0 v^m/(A^m + v^m), \tag{6}$$

with a "Hill coefficient" $m > 1$ and a suitably chosen response threshold $0 < A < 1$ [3]. The cell "diffusivity" μ will in general be a decreasing function of the cell density; for simplicity, we take it to be a positive constant. The remaining model parameters $(a, b, d_{1/2}, k_{1/2}, r, \kappa, K, \chi_0)$ are also positive; for numerical estimates of the parameters see [4,5].

3. Linear analysis

If the cell density is "clamped" at a constant value, (2)–(3) essentially reduces to a two-variable description of cAMP signalling. This is a valid approximation for the initial phase of aggregation as cells are practically homogeneously distributed. Equations (2)–(3) then constitute a particular realisation of a generic excitable medium, and admit the characteristic wave solutions observed experimentally (cf. [10] and references therein). Figure 1 shows the characteristic excitable phase portrait of the local kinetics of (2)–(3) with $n = 1$, and typical two-dimensional wave solutions of the reaction-diffusion system.

When the cell dynamics are included, the immediate effect of chemotaxis on cell density is determined by the ratio of advective velocity, $w = \chi(v)\nabla u$, to cAMP wavespeed, c. Considering plane waves (a reasonable approximation for the situation away from the aggregation centres formed by pacemakers and spiral wave cores), and neglecting the small diffusive term in (1), it is straightforward to derive the following expression for the cell density profile $N(z)$ in the travelling wave coordinate $z = x - ct$:

$$N(z) = \frac{n_0}{1 - w(z)/c}, \tag{7}$$

where n_0 is the initial homogeneous cell density. As *in situ* $\max w(z)/c \approx 0.1$, the homogeneous cell density remains practically undisturbed by the cAMP waves, and the wave solutions to the full model (1)–(2) are close to the ones obtained with "clamped" cell density. This is supported by numerical calculations and also corresponds well to

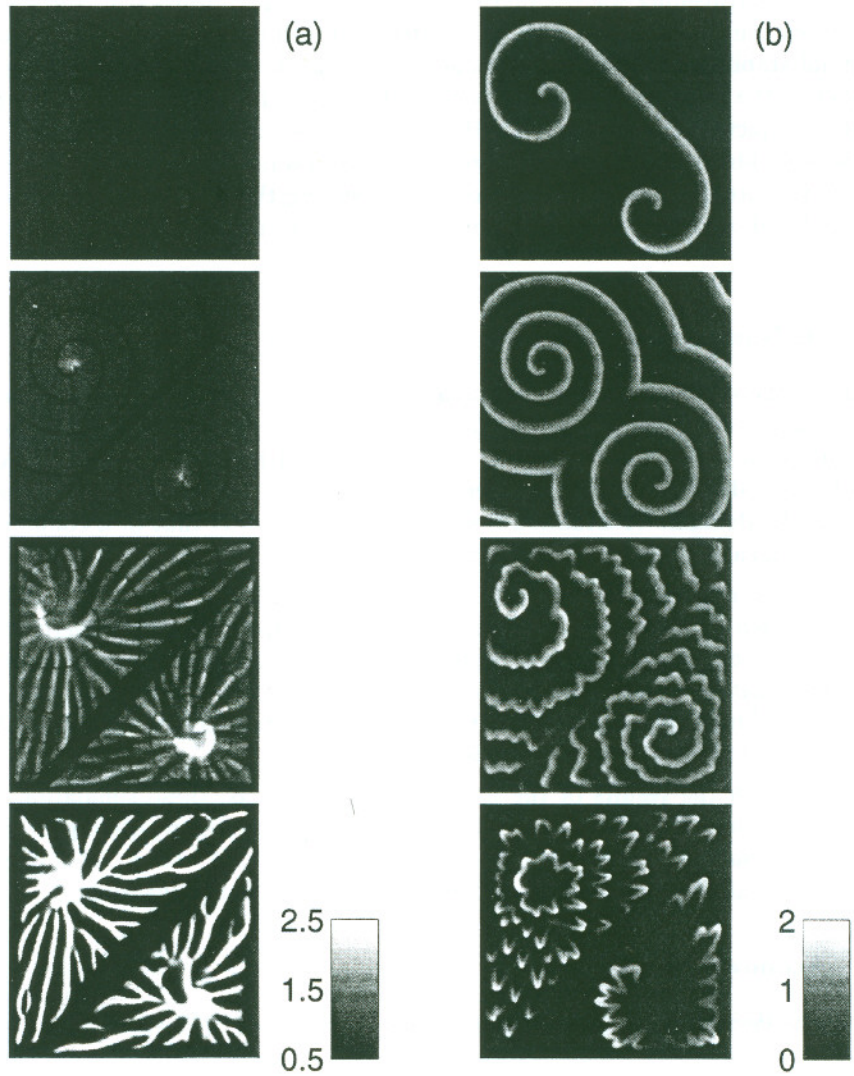

Figure 3: Spatio-temporal evolution of (a) cell density, and (b) cAMP concentration in a numerical simulation of system (1)–(3). The dimensional domain size is as in Figure 1 (b) (8.5×8.5 mm); snapshots are taken after 1 min, 10 min, 40 min and 80 min (top to bottom). Initially a small random perturbation between -0.075 and 0.075 was added to the homogeneous cell density (1.0) at every mesh point; the double spiral was initiated by a plane wavefront with two free ends. Parameters as in Figures 1 and 2, with $\chi_0 = 0.5$.

experimental observations [1]. However, it is not guaranteed that the wave solutions which are known to be stable for the "clamped" density case [9] remain stable in the full system. We proceed to show that they are in fact unstable on a two-dimensional spatial domain. The instability is intrinsically linked to chemotactic cell movement.

Let $(N(z), U(z), V(z)) = (N(z+Z), U(z+Z), V(z+Z))$, $z = x - ct$ be a plane periodic travelling wave solution to system (1)–(3), with period Z. The evolution of a small perturbation of this solution is governed by the linearization of (1)–(3) around (N, U, V), in (z, y, t)-coordinates. Separable solutions are sought in the form $\underline{a}(z)\exp\{\lambda t + iqy\}$, yielding the following ordinary differential equation system for $\underline{a}(z)$ with Z-periodic coefficients,

$$\begin{pmatrix} \tilde{a}(z) \\ a_2''(z) \\ 0 \end{pmatrix} - c\underline{a}'(z) + \begin{pmatrix} -\lambda - \mu q^2 & \chi(V(z))N(z)q^2 & 0 \\ f_n(N(z),U(z),V(z)) & f_u(N(z),U(z),V(z)) - \lambda - q^2 & f_v(N(z),U(z),V(z)) \\ 0 & g_u(U(z),V(z)) & g_v(U(z),V(z)) - \lambda \end{pmatrix} \underline{a}(z) = 0,$$

where

$$\tilde{a} = [\mu(z)a_1'(z) - \chi(V(z))Na_2'(z) - [\chi(V(z))a_1 + \chi_v(V(z))Na_2]U'(z)]';$$

this system describes the evolution of the amplitude of Fourier modes perpendicular to the direction of wave propagation. We can expect disturbances in the cell distribution, as the main source of small inhomogeneities, to evolve

essentially in the travelling wave frame, because they do not propagate with the cAMP waves. Therefore we numerically track the Floquet multiplier associated with cell conservation for the homogeneous perturbation ($q = 0$) and marginal stability with respect to time ($\lambda = 0$), $\rho^*(\lambda = 0, q^2)$ (i.e., we have $\rho^*(0,0) = 1$). We conjecture ρ^* to characterize the behaviour of initial perturbations in cell density under repetitive forcing by the cAMP waves. The result is depicted in Figure 2. For the parameter set characterizing normal aggregation, one finds a band of unstable wavenumbers, with a maximal growth rate at a wavelength of about 500 μm, that is, several cell diameters. Notice that the situation can be stabilized by decreasing the chemotactic cell motility. Thus we expect to find a chemotactic break-up of the initially homogeneous cell layer under the influence of the repetitive cAMP waves (cf. [5]).

4. Numerical solutions

To investigate the consequences of this instability, we solved system (1)–(3) numerically on a rectangular domain. An alternating direction implicit finite difference method is applied to the diffusion operator, and the reaction and advection terms are evaluated explicitly, using an upwind method for first spatial derivatives. This resulted in a robust and reasonably fast numerical scheme, with which we were able to follow the evolution of several aggregation territories in one domain. A situation frequently seen *in situ* is that of a counter-rotating double spiral of cAMP waves, and a typical simulation result is depicted in Figure 3.

Very clearly one observes the break-up of the initially homogeneous cell layer in two distinct aggregation territories in which a cell stream pattern is formed. Comparison with experimental results (e.g. Figure 1 in [4]) shows how closely our minimal model reproduces the natural aggregation sequence, with respect to the formation of distinct aggregation territories, cell streaming within each territory, and also the cAMP spiral wave dynamics (increase in signalling frequency). Furthermore, the characteristic wavelength of the stream pattern compares well with the analytical prediction of the previous section.

Together with the results of the linear stability analysis, these simulations provide good evidence that the model (1)–(3) captures the essential cellular mechanisms of the aggregation process. In particular, cell streaming is the result of a chemotactic instability forced by repetitive cAMP waves. This phenomenon provides an interesting example of a biological morphogenetic process based on a dynamic instability.

Acknowledgements

We would like to thank P.C. NEWELL *(Oxford) and* J.A. SHERRATT *(Warwick) for stimulating discussions.*

5. References

1 ALCANTARA, F. & MONK, M.: Signal propagation during aggregation in the slime mould *Dictyostelium discoideum*. J. Gen. Microbiol. **85** (1974), 321–334.

2 DEVREOTES, P.N.: *Dictyostelium discoideum*: a model system for cell-cell interactions in development. Science **245** (1989), 1054–1059.

3 HÖFER, T., MAINI, P.K., T., SHERRATT, J.A., CHAPLAIN, M.A.J., CHAUVET, P., METEVIER, D., MONTES, P.C., MURRAY, J.D.: A resolution of the chemotactic wave paradox. Appl. Math. Lett. **7** (1994), 1–5.

4 HÖFER, T., SHERRATT, J.A., MAINI, P.K.: Dictyostelium discoideum: cellular self-organization in an excitable biological medium. Proc. Roy. Soc. Lond. B **259** (1995), 249–257.

5 HÖFER, T., SHERRATT, J.A., MAINI, P.K.: Cellular pattern formation during Dictyostelium aggregation. Physica D **85** (1995) in press.

6 KELLER, E.F., SEGEL, L.E.: Initiation of slime mold aggregation viewed as an instability. J. Theoret. Biol. **26** (1970) 399–415.

7 MARTIEL J.-L., GOLDBETER, A.: A model based on receptor desensitization for cyclic AMP signaling in *Dictyostelium* cells. Biophys. J. **52** (1987), 807-828.

8 MURRAY, J.D.: Mathematical Biology (second edition). Springer-Verlag, Berlin 1993.

9 TYSON, J.J., KEENER, J.P.: Singular perturbation theory of traveling waves in excitable media (a review). Physica D **32** (1988), 327–361.

10 TYSON, J.J., MURRAY, J.D.: Cyclic AMP waves during aggregation of *Dictyostelium* amoebae. Development **106** (1989), 421–426.

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