INSTITUTE OF PHYSICS PUBLISHING NONLINEARITY

Nonlinearity **15** (2002) C1–C5 PII: S0951-7715(02)31067-3

COVER ILLUSTRATION

cAMP waves in *Dictyostelium* territories

Kyoung J Lee[1], Raymond E Goldstein[2] and Edward C Cox[3]

[1] National Creative Research Initiative Center for Neurodynamics and Department of Physics, Korea University, Seoul 136-701, Korea
[2] Department of Physics and Program in Applied Mathematics, University of Arizona, Tucson, AZ 85721, USA
[3] Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA

Received 21 November 2001
Published 12 December 2001
Online at stacks.iop.org/Non/15/C1

Abstract

The cover illustration is a stylized image, with a field of view of several millimetres, of cyclic AMP waves in populations of the single-cell micro-organism *Dictyostelium discoideum* on the surface of agar. These waves are 'target patterns' driven by pacemaker cells at their centres. Targets compete with rotating spirals during the stage of the life cycle of *Dictyostelium* in which travelling chemical waves induce chemotaxis towards wave centres and result in multicellular structures. We discuss recent progress in the study of this competition.

Under conditions of nutrient deprivation, populations of the single-cell organism *Dictyostelium discoideum* undergo a transition from independent cell behaviour to multicellularity [1]. This involves the generation of travelling chemical waves in the form of targets and/or spirals that then induce chemotaxis toward their centres. The resulting aggregate of differentiating cells then develops into a stalk structure topped by spores that can exist in a dormant state until more favourable times.

The *Dictyostelium* system is in many ways the 'hydrogen atom' of the morphogenesis problem, in which detailed connections between genetic factors and macroscopic behaviour can be worked out [2]. From the point of view of nonlinear dynamics and pattern formation, it displays the classical properties of an excitable medium [3]. The standard model of the signalling process, described by Martiel and Goldbeter [4], involves the basic step of sensing with membrane-bound receptors the chemical messenger 3′5′-cyclic adenosine monophosphate (cAMP) that has diffused from nearby cells, the subsequent internal production of cAMP and its release into the extracellular medium, and its degradation by the enzyme phosphodiesterase, thereby freeing the receptors.

The biochemistry summarized above is now well understood, but how it translates into spatio-temporal pattern selection is a matter of great current interest [5–11]. As in typical reaction–diffusion systems, the cAMP production of individual *Dictyostelium* cells may spontaneously oscillate, yielding a pacemaker for concentric circular waves, or simply be excitable, relaying those waves as expanding circles or rotating spirals. A central issue in the

0951-7715/02/010001+05$30.00

formation of large-scale coherent wave patterns in spatially extended populations is, thus, that of selection between targets and spirals.

Direct visualization of the chemical waves is not possible because of the extremely low concentrations of signalling chemicals, but classic experiments with radioactive labelling [12] have shown that dark-field imaging, which is sensitive to the differential scattering of light by cells that have polarized in response to locally high cAMP levels, accurately maps the waves. The cover illustration is a false-colour dark-field image, processed to highlight the wavefronts.

Spiral waves do not form from small-amplitude fluctuations in chemical concentrations about a homogeneous state, but rather via a roll-up phenomenon at the free ends of wave segments [13, 14]. When they appear, there is a population-density-dependent competition with targets, with low population density favouring circular waves, and high density favouring spirals [5]. Moreover, the spirals that eventually dominate emerge from broken wave segments created early during signalling, and ultimately entrain the pacemakers responsible for circular waves by virtue of their generally higher oscillation frequency.

In non-living reaction–diffusion systems such as the Belouzov–Zhabotinski reaction, oscillators often form around inhomogeneities [13, 14], and with modern continuously fed gel reactors it is possible to maintain the overall chemistry in a nearly constant state over time [15]. Thus, the overall chemical kinetics do not vary during the course of an experiment, and they are quite homogeneous within the reaction chamber. In contrast, living systems such as populations of *Dictyostelium* are intrinsically heterogeneous and evolve in time. This is especially true for the kinetic properties of cAMP oscillations. For example, figure 1 shows time series of waves emanating from three separate pacemakers, obtained by measuring the pixel intensity in time-lapse images during the signalling stage. There is a noticeable variation in the oscillation period.

The variation in kinetic properties during the course of the signalling stage is now understood to have a profound impact on waveform competition. In particular, there is an evolution from excitable to oscillatory and back [5, 6, 10, 11]. The initiation of circular waves

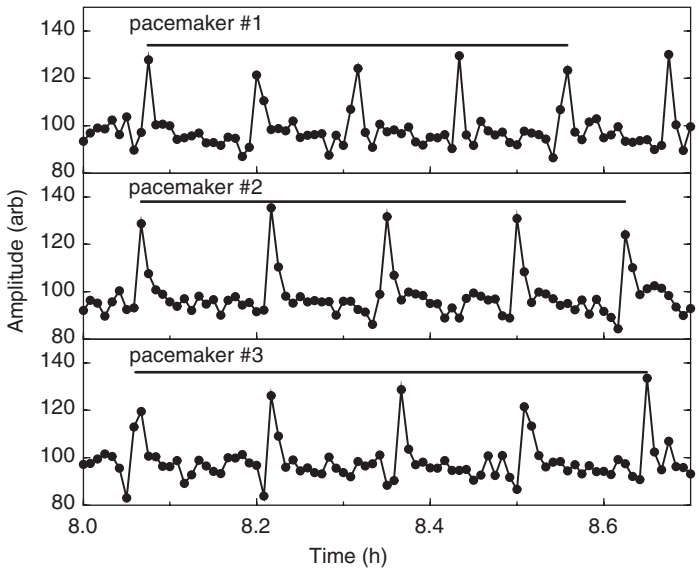

Figure 1. Time series of pixel intensities near three pacemakers, illustrating the variability in periods among them.

in the early signalling stage of *Dictyostelium* arises because members of the population are not in complete synchrony following starvation, and consequently some cells make the excitable-oscillatory transition before others. If fully developed spirals form, then they entrain the pacemakers and the pattern is entirely controlled by spirals.

A key test of the hypothesis that pacemakers observed early in the signalling stage are entrained was reported recently [16]. If, in the presence of spiral waves, the suppressed pacemakers never reappear to generate circular waves, it stands to reason that extinguishing those spirals might allow the pacemakers to regain control. By analogy with cardiac defibrillation, in which a large external voltage resets the waves of electrical activity, we found that application of a fine mist of dilute cAMP extinguishes the cAMP waveforms, after which signalling recovers within several wave periods as the applied cAMP diffuses away.

The ability to reset the waveforms depends upon when, during the signalling stage, the cAMP mist is applied. If done early, then spirals re-form and dominate the targets as usual. The results of cAMP application later in signalling are shown in figure 2 [16]. Panels (*a*)–(*c*) show

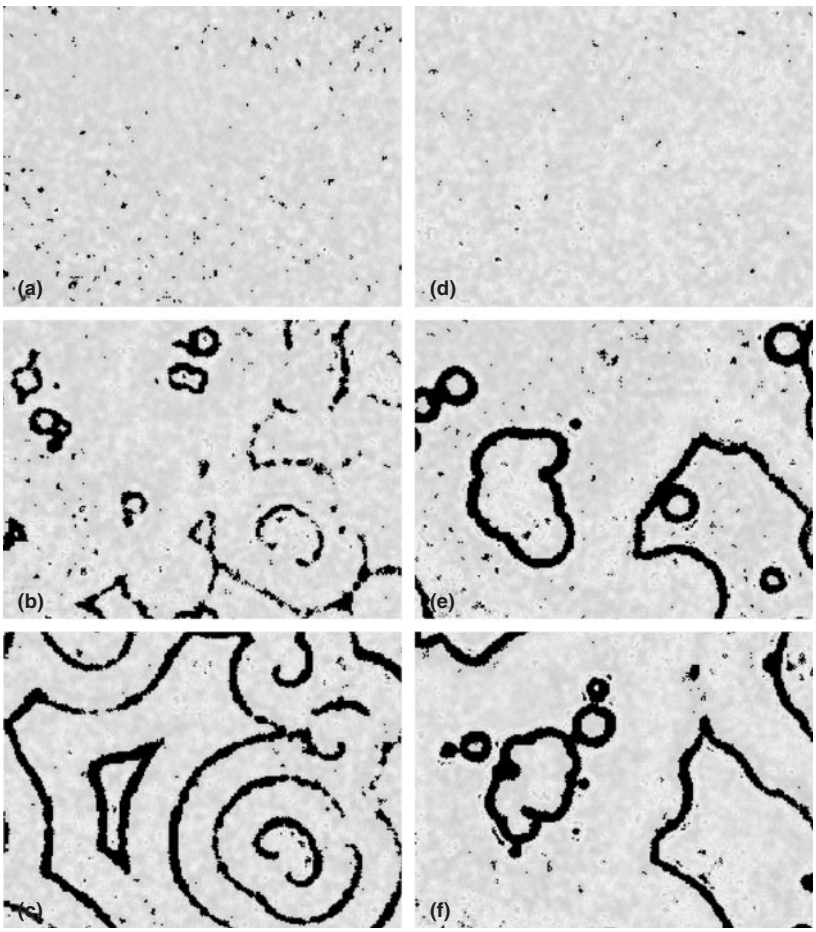

Figure 2. Images showing signalling activity before and after cAMP perturbation: (*a*) the homogeneous state after nutrient deprivation; (*b*) targets and spirals coexist several hours later; (*c*) spiral waves dominate even later; (*d*) several minutes after a cAMP mist is applied, the signalling has stopped; (*e*) targets driven by pacemakers are soon re-established and are persistent (*f*).

the typical free-running development of a spiral-dominated state from the initial non-signalling pattern. Panel (*d*), taken shortly after the cAMP application, indicates that signalling has been completely shut down, only to reappear in (*e*) as circular waves emanating from pacemakers. Remarkably, under these conditions these targets persist (*f*) and spirals are never formed: the precise analogue of cardiac defibrillation.

We may thus conclude that during the signalling stage of *Dictyostelium discoideum* there are two dynamical fixed points (figure 3): patterns with only targets and patterns with only spirals. The spiral state is stable to the introduction of targets by virtue of entrainment, but the converse is not true.

The basic outline of a possible biochemical explanation for these observations is now emerging. The starting point [8] is the issue of how targets created early in signalling produce spirals, and a key point is that the phosphodiesterase that degrades external cAMP is itself inhibited by a secreted protein inhibitor, PDE. Local random pulses of this PDE can cause premature wave initiation by increasing the local cAMP level, and thus premature firing behind a travelling circular wave. This in turn causes wave break-up and thus spiral initiation. Genetic studies [9] in which the inhibitor is not expressed show patterns without spirals, consistent with this theory. Moreover, it is known that the PDE is produced and secreted early during starvation,

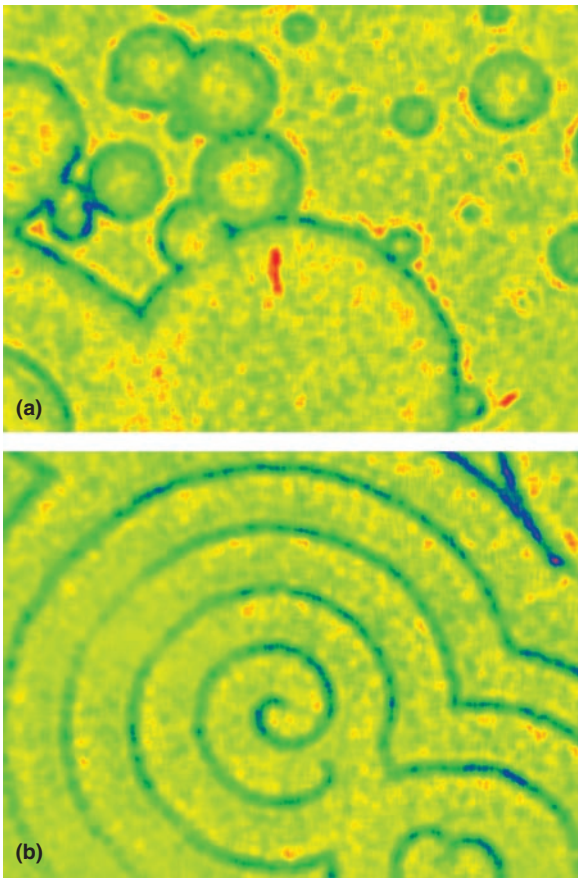

Figure 3. Spiral pattern (*a*) selected naturally by a population of *Dictyostelium* and the target pattern (*b*) controlled by autonomous pacemakers, following resetting with a mist of cAMP.

as the cells become excitable, but synthesis stops hours later. This process is a prime candidate for the regulator of slow evolution of the excitability of the medium. It illustrates how subtle and remarkable living systems are in self-regulation along a developmental path [17, 18].

References

[1] Bonner J J 1967 *The Cellular Slime Molds* (Princeton, NJ: Princeton University Press)
[2] Loomis W F 1975 *Dictyostelium Discoideum, A Developmental System* (New York: Academic)
[3] Meron E 1992 *Phys. Rep.* **218** 1
[4] Martiel J and Goldbeter A 1987 *Biophys. J.* **52** 807
[5] Lee K J, Cox E C and Goldstein R E 1996 *Phys. Rev. Lett.* **76** 1174
[6] Aranson I, Levine H and Tsimring L 1996 *Phys. Rev. Lett.* **76** 1170
[7] Levine H, Aranson I, Tsimring L and Truong T V 1996 *Proc. Natl Acad. Sci. USA* **93** 6382
[8] Pálsson E and Cox E C 1996 *Proc. Natl Acad. Sci. USA* **93** 1151
[9] Pálsson E, Lee K J, Goldstein R E, Franke J, Kessin R H and Cox E C 1997 *Proc. Natl Acad. Sci. USA* **94** 13719
[10] Falcke M and Levine H 1998 *Phys. Rev. Lett.* **80** 3875
[11] Lauzeral J, Halloy J and Goldbeter A 1997 *Proc. Natl Acad. Sci. USA* **94** 9153
[12] Tomchik K J and Devreotes P N 1981 *Science* **212** 443
[13] Agladze K, Keener J P, Müller S C and Panfilov A 1994 *Science* **264** 1746
[14] Tyson J J and Murray J D 1989 *Development* **106** 421
[15] Belmonte A and Flesselles J M 1996 *Phys. Rev. Lett.* **77** 1174
[16] Lee K J, Goldstein R E and Cox E C 2001 *Phys. Rev. Lett.* **87** 068101
[17] Goldbeter A and Segel L A 1980 *Differentiation* **17** 127
[18] Halloy J, Lauzeral J and Goldbeter A 1998 *Biophys. Chem.* **72** 9