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MODELS OF AGGREGATION IN *DICTYOSTELIUM DISCOIDEUM*: ON THE TRACK OF SPIRAL WAVES

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Abstract. This work is concerned with some aspects of the social life of the amoebae *Dictyostelium discoideum* (Dd). In particular, we shall focus on the early stages of the starvation-induced aggregation of Dd cells. Under such circumstances, amoebae are known to exchange a chemical messenger (cAMP) which acts as a signal to mediate their individual behaviour. This molecule is released from aggregation centres and advances through aggregation fields, first as circular waves and later on as spiral patterns. We shall recall below some of the basic features of this process, paying attention to the mathematical models that have been derived to account for experimental observations.

1. **Introduction.** Amoebae of the species *Dictyostelium discoideum* (Dd for short) are nucleated cells that live in forest soils, feeding on bacteria. These cells proliferate by cell fission as long as there is enough food supply, a life style not very different from that of many other microorganisms sharing this habitat. What is more peculiar to Dd is its response when food is exhausted. Indeed, under starvation, up to 10^5 Dd cells aggregate together to form a multicellular pseudo-organism. This last enters into a developmental programme to form a fruiting body where a ball of cells is supported on top of a thin stalk. Cells inside the ball differentiate into resistance forms, called spores, which can remain in a dormant state for several weeks. Spores are disseminated by the wind or by small animals, and can be reactivated to originate new amoebae if deposited in a rich environment.

The formation of the Dd fruiting body has many aspects in common with animal embryonic development. In both cases a large number of cells are able to organize themselves to form complex structures of characteristic shape and size. These cells are originated by the proliferation of a single precursor cell in the case of animal embryos. Instead, in Dd many individual cells aggregate together to form a multicellular body. In the case of animals, these cell structures differentiate in a coordinated manner, following specific pathways to yield tissues and organs. Cell

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differentiation proceeds along with morphogenesis, so that each specific cell type, tissue and organ is eventually located at the appropriate place of the final structure.

Making a complex structure, such as an animal or a fruiting body, is in the first place an architectural problem. To start with, it is necessary to define the axes of the object: top and bottom, anterior and posterior, right and left. Once the spatial frame is defined, cell growth, migration and differentiation have to be coordinated, so that each cell ends up at its right position. These processes are often mediated through the establishment of organizing centres at given positions of the structure. These centres regulate growth migration and cell differentiation in their competence fields, through the emission of signals that diffuse in the surrounding medium. This represents an exceedingly complex process in animals, where millions of cells differentiate into a few hundreds of cell types, organized in many tissues and organs.

Dictyostelium discoideum offers the opportunity to study a developmental process at a smaller scale: two main cell types, stalk cells and spores, are organized in a small and relatively simple structure. However, the same basic processes mediate development in Dd and in more complex organisms such as ourselves: generation of shape (morphogenesis) and size, establishment of organizing centres, coordination of cell proliferation, migration and differentiation. The relative simplicity of Dd developmental plan has made possible the mathematical modelling of some of the processes involved, a fact which will be discussed in some detail below.

Dd development is initiated by the aggregation of individual cells into mounds. In this process, cells migrate towards aggregation centres by chemotaxis, that is, attracted by a chemical compound that diffuses from the aggregation centres. In the case of Dd, the chemoattractant was identified as adenosine $3', 5'$–cyclic monophosphate (cAMP), cf. [16]. This substance is produced, and secreted in a pulsatile way, in the centre of each aggregation field, so that cAMP concentration increases in the extracellular media for a short time to decrease thereafter. Concentration waves are transmitted through the aggregation field, giving raise to circular and spiral waves that are maintained through aggregation. Cells respond to these waves by migrating in the direction of increasing cAMP concentration, so that they eventually converge towards the aggregation centres.

The role of cAMP in Dd development is not restricted to aggregation. Once the mound is formed, cells continue to move towards increasing cAMP concentrations and, at the same time, initiate their differentiation into prestalk or prespore cells, a process which is also dependent on cAMP signalling. Aggregating cells express different proteins that allow them to adhere to neighbour cells and to the extracellular matrix. Cell adhesion is necessary to maintain the integrity of the aggregate. Moreover, differential adhesion between prestalk and prespore cells, together with differences in chemotaxis towards cAMP, are important determinants in subsequent developmental steps.

In this note we shall focus on describing the main aspects of the early aggregation process, prior to the formation of the mounds where Dd cells will eventually condensate. Subsequent features of the Dd developmental programme will only be quickly mentioned herein, and will be discussed in more detail elsewhere. Particular attention will be paid to the mathematical models (and the need of further refinements thereof) that have been suggested to account for some of the key features observed, both at a quantitative and a qualitative level.

We conclude this Introduction by describing the plan of the article. In Section 2, an overview of the main facts known about aggregation of Dd amoebae, triggered by starvation, is provided. Section 3 is concerned with reviewing the main elements of cAMP-induced signal transduction operating at any single Dd cell. In Section 4 we shall deal with the stage at which organizing centres are formed. Efforts made to explain the transition from circular to spiral optical patterns will be discussed therein. Some recent experimental facts linking individual firing of cAMP (in wild-type strains as well as in mutants) with the nature of the microscopic patterns observed, will then be described in Section 5. Section 6 contains some concluding remarks. Finally, a short description of the role played by cAMP signalling in the developmental processes following aggregation, and some mathematical models proposed to explain them, are provided in an Appendix A, while a brief review of the biochemistry of cAMP oscillations is gathered in Appendix B at the end of the paper.

2. **Starvation-induced aggregation in Dd: an overview.** The process of aggregation in wild type (WT) Dd cultures which is triggered by food exhaustion, lasts for about 8 hours. During the first $4-5$ hours, changes in the gene expression programme occur, which result in the induction of genes necessary for aggregation and the repression of genes required for proliferation. At the end of this initial period, Dd cells are aggregation-competent. In particular, they express sufficiently high levels of proteins required for cAMP-based signalling (cAMP receptors, cyclases, phosphodiesterases, ...). Proteins required for chemotaxis are also expressed in this time lapse.

For aggregation to occur, a minimum cell density is required, of the order of 2.5×10^4 cells/cm^2 [10]. Laboratory experiments, most of which are concerned with Dd growth in monolayers (that can be considered to be bidimensional media), are usually performed at higher densities, of about $4-65\times 10^4$ cells/cm^2. Under such conditions, and taking 11 μm (1 $\mu m = 10^{-6}$ meters) as the average diameter for Dd cells, the average distance among cells is $50-80$ μm for the first figure above, and $2-8$ μm for the second one.

In general, a given colony of Dd cells which is about to start aggregation is composed of individuals at different stages of their own cell cycle, whose length is about 8 hours [5]. For this reason, Dd colonies cannot be considered as consisting of identical units, since different cells have reached different levels of maturity. In some cases, however, a suitable manipulation allows to perform experiments on age-homogeneous populations [24].

At the beginning of aggregation, a few cells (which are thought to be at a comparatively advanced stage of their cell cycle), start emitting pulses in a periodic manner. Pulses are usually produced every $5-6$ minutes, although periods of $7-8$ minutes have also been observed [25]. They propagate with a speed of about $300\mu m$/min [35], a wavelength of $2-3$ mm, and an amplitude of about 1 mm [42]. In particular, under laboratory conditions any cell in the plate receives one of these pulses every $6-10$ minutes, and remain under its influence for about 3 minutes.

Upon receiving a cAMP wave, cells are stimulated to internally produce cAMP, part of which is secreted outside to keep the signalling process on. Intracellular cAMP concentrations of 20 μM (1 $\mu M = 10^{-6}$mol) have been described, and extracellular concentrations up to 7 μM have been reported [9]. Each cell takes 15 seconds on average to react to cAMP arrival. It then produces such substance for

about 1.5 minutes, after which it ceases to respond to external cAMP, and enter into a refractory period which lasts for about 5 minutes ([6]).

When Dd is cultured in monolayers, cAMP propagation produces target and spiral wave patterns that can be observed by means of darkfield microscopy (Figure 1), and are similar to those displayed by wave solutions of reaction-diffusion systems of an excitable type [31, 30]. The diffusion coefficient of extracellular cAMP has been estimated as $D = 1.5 \times 10^{-4}$ cm^2/sg [8].

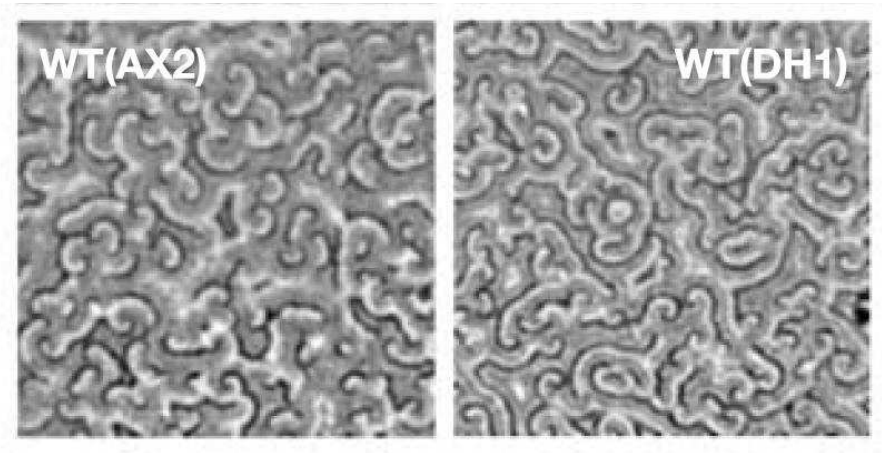

FIGURE 1. WT Dd cells showing spiral patterns (Reproduced with permission from Macmillan Publishers Ltd : Nature (Sawal et al. 433, 323-326), copyright 2005).

Arrival of cAMP signals is followed by a migration process towards the cAMP source. This type of cell activity is observed as long as cAMP concentration increases (which occurs over the wavefront) and then ceases. Cell migration velocity has been estimated as 20 μm/min [5]. In a typical aggregation process, as many as 50 cAMP waves are produced before cells eventually join each other. At that juncture, streams of Dd cells are formed that converge towards aggregates. This part of the process usually takes $2-3$ hours after signalling starts.

The formation of amoebae aggregates reveals some characteristic features. One of these is size-regulation: aggregates are repeatedly observed to achieve a characteristic size (of up to 10^5 cells), depending on the number of cells initially available and the dimensions of the surrounding medium [36]. Another important aspect is the transition from a bidimensional setting (typical of monolayer experiments) to a three-dimensional one, as cells in the condensate pile up each other to eventually yield solid mounds.

After such mounds have been formed, cells located therein begin to differentiate into two alternative cell types: prespore and prestalk cells. These two cell types are initially interspersed in the mound, but they soon segregate [41]. We remark on pass that a mathematical model for the prestalk/prespore patterning has been proposed in [29], which is based on the mathematical theory of activator-inhibitor systems described in [28]. Prestalk cells migrate to the upper part of the mound, where they form a small protuberance called the tip. Prespore cells stay in the

lower part of the mound. This structure then elongates vertically to form a finger-like structure, called first finger. Posterior development can follow two alternative pathways, as shown in Figure 2. Under favourable environmental conditions, the first finger continues development on the spot where it was formed. In this case, prestalk cells that were located at the top of the structure migrate through the prespore cells to reach the substratum. At the same time, these cells differentiate into stalk cells, vacuolize, elongate and secrete a rigid cellulose wall, dying at the end of the process. Prestalk cells are continuously added on top of the forming stalk, contributing to its elongation. The growth of the stalk makes possible the upward movement of prespore cells, that rise from the substratum to form a ball of cells on top of the stalk, the sorus. Prespore cells in the sorus differentiate into spores that become surrounded by a hard shell, consisting of cellulose and proteins, undergo dehydratation and adquire their characteristic ellipsoidal shape. Spores show very low metabolic activity and are viable for weeks. These resistance forms can be dispersed and therefore reach more favourable environments. Then spores germinate, their hard shell is broken, and a new amoeba emerges.

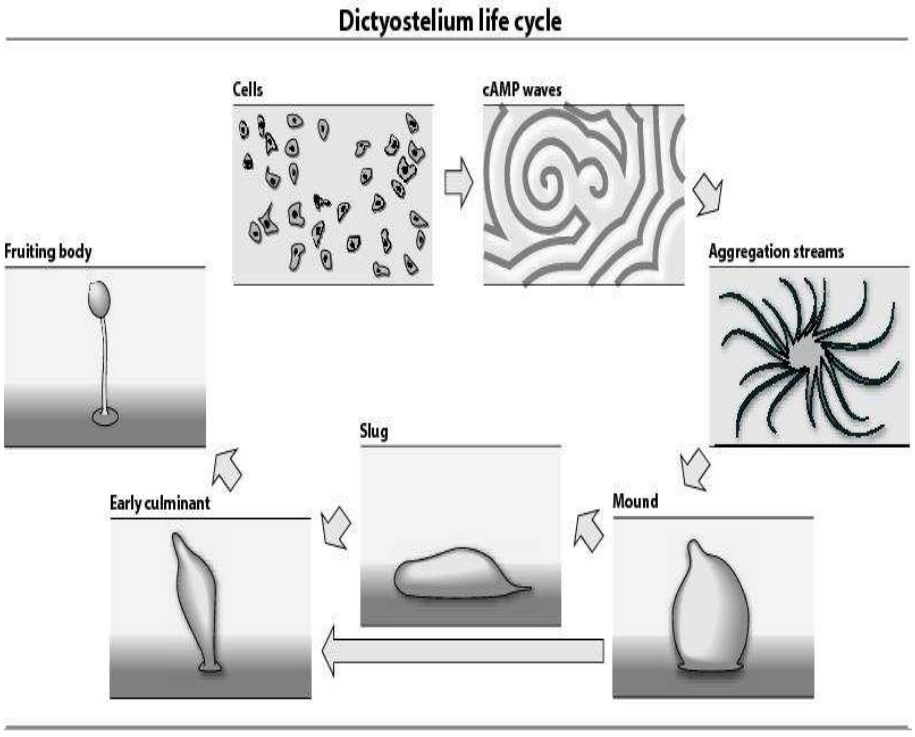

FIGURE 2. Key steps in the evolution of Dd cultures after starvation.

Under some circumstances, a longer developmental programme can take place. Dd structures at the first finger stage can sense environmental conditions and determine if they are adequate for spore dispersion. If this is not the case, the finger structure falls on the substrate and a migratory structure is formed, called slug [32]. The organization of the slug is similar to that of the first finger, with the prestalk

cells placed in the front of the structure and prespore cells in the back. Slugs are phototrophic and thermotrophic structures that move towards places that would provide better conditions to complete development. In nature, this migration can represent moving from the middle of a mass of leaves being degraded on the flour to its surface, where spores are easier to disperse. Slug structures, while looking like small animals, are just aggregates of individual cells, and their movement is the result of the coordinated migration of the constituent cells. Eventually, the slug arrives at a place more favourable to complete development. In this case, the slug stops, rounds up, prestalk cells migrate to the top of the structure and evaginate therein, thus forming a new finger. Prestalk cells then migrate through the prespore cells, and culmination proceeds as previously described. The overall sequence of facts is sketched in Figure 2.

3. **Individual cell behaviour: the internal signalling network.** We now focus into the biochemical cascade corresponding to the internal production of cAMP (to be denoted by cAMPi) induced by the action of external cAMP (henceforth represented by cAMPe). While some aspects of the corresponding regulatory pathways have yet to be elucidated, much is already known about its basic ingredients. For instance, following [25] (cf. also [17]) the key elements of this network can be represented in the diagram of Figure 3 (see also Appendix B for a more detailed description).

Following [25], supplementary material, the interaction scheme can be coached in mathematical terms as follows:

$$\begin{cases} \dfrac{\mathrm{d}u_1}{\mathrm{d}t} = k_1 u_7 - k_2 u_1 u_2, & \dfrac{\mathrm{d}u_5}{\mathrm{d}t} = k_9 u_1 - k_{10} u_4 u_5, \\ \dfrac{\mathrm{d}u_2}{\mathrm{d}t} = k_3 u_5 - k_4 u_2, & \dfrac{\mathrm{d}u_6}{\mathrm{d}t} = k_{11} u_1 - k_{12} u_6, \\ \dfrac{\mathrm{d}u_3}{\mathrm{d}t} = k_5 u_7 - k_6 u_2 u_3, & \dfrac{\mathrm{d}u_7}{\mathrm{d}t} = k_{13} u_6 - k_{14} u_7, \\ \dfrac{\mathrm{d}u_4}{\mathrm{d}t} = k_7 - k_8 u_3 u_4, & \end{cases} \tag{1}$$

where $u_1, u_2, \ldots, u_7$ denote respectively the concentrations of (in the case of enzymes, the active forms of) AcA, PKAR, Erk2, RegA, cAMPi, cAMPe and Car1. The positive parameters $k_1, \ldots, k_{14}$ stand for the kinetic constants correspondng to activation ($k_1, k_3, k_5, k_9, k_{11}, k_{13}$), inhibition ($k_2, k_6, k_8, k_{10}$) and decay ($k_4, k_{12}, k_{14}$). Constant k_7 represents production of RegA from a generic substrate at a constant rate. Note that no space dependence of $u_i (1 \leq i \leq 7)$ is considered in equations (1), which amounts to assume reactions to occur in a well-stirred medium.

As observed for instance in [17], a direct measurement of all constants k_i ($1 \leq i \leq 14$) in equations 1 is not possible in general. One reason is that some of the proposed interactions in Figure 3 need not be direct. In some cases, estimates on some of the k_i's can be derived by means of actual experiments, but in general one is led to select values for which simulations reproduce observed behaviours. In particular, a suitable choice of the k_i (cf. [25], supplementary material) gives raise to oscillations in some of the variables in (1), which closely resemble those experimentally seen within suitable time ranges. In particular, oscillations in cAMPi entrained by oscillations in cAMPe appear (cf. Figure 2 in [25]). Moreover, bursting oscillations in AcA are obtained under suitable upregulation of AcA basal levels, which are shown to proceed in accordance with oscillations in Erk2 and other components of

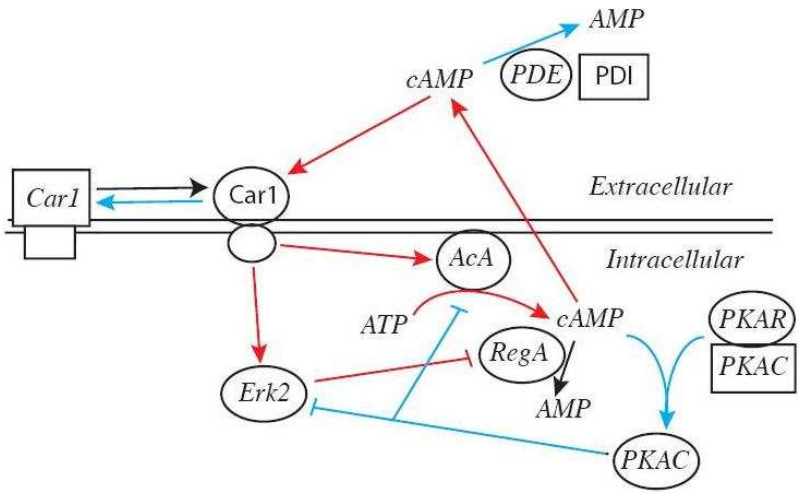

FIGURE 3. (Adapted from [25]): In this picture arrows represent activation or conversion of substrates, and bars denote inhibition. PDE is a secreted phosphodiesterase that hydrolizes cAMPe and PDI stands for an inhibitor of PDE. Car1 is the cAMP receptor in the cell membrane. The active form is represented as surrounded by a circle, and the inactive form (corresponding in this case to a refractory period) is written within a box, a convention to be retained henceforth. AcA is aggregation adenylyl cyclase. PKAC is the catalytic subunit of a cAMP-dependent protein kinase that is inactivated by the regulatory subunit PKAR. The PKAR-PKAC dimer is dissociated upon cAMP binding, thus activating PKAC. This last in turn downregulates Erk2, a protein kinase activated by cAMP binding to Car1, and that phosphorilates RegA, inhibiting its enzymatic activity. RegA is a phosphodiesterase that hydrolizes cAMPi. This last is produced by the action on AcA on a ATP substratum, and is in turn secreted outside the cell, thus contributing to a cAMP feedback loop. Regulatory circuits that activate cAMP synthesis (relay) are indicated in red, and those contributing to subsequent decrease in cAMP concentration are depicted in blue.

the circuit described in Figure 3. Interestingly enough, such oscillatory behaviours are restricted to a particular stage of the developmental process (see Figure 4 and Figure 4 in [25]).

4. **A macroscopic picture: the generation of signalling waves.** When a cell expresses large enough amounts of the enzymes required for cAMP synthesis, it starts secreting it to the surroundng media. In this way, such cell becomes a possible attracting centre for neighbouring cells. The cAMP signal is not merely transmitted by passive diffusion. Instead, an active relay process appears, whereby each cell detects extracellular cAMP coming from a preceding cell, and secretes cAMP as a response to that signal, which is in turn detected by the following cells. cAMP waves are emitted outwardly from aggregation centres, since each cell enters a refractory period to the cAMP signal soon after receiving it. Therefore, when a

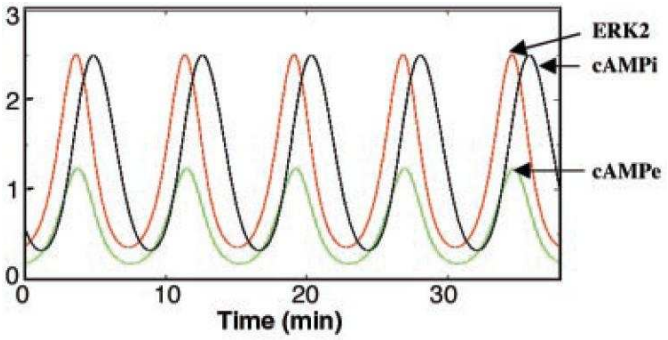

FIGURE 4. Output of the circuit simulation of cells at 4 hours of development. Periodic changes in ERK2 activity and the internal and external levels of cAMP are indicated (arbitrary units) (Reprinted with permission from M. Maeda et al. Science 304, 875-878. Copyright (2004). AAAS).

cell relays cAMP, preceding cells do not respond to it, and only cells that are further away from the initial signalling centre receive and transmit the signal. Adaptation lasts for several minutes, after which internal cells are able to join the signalling process again.

The mechanism thus described corresponds to an oscillatory system, where cAMP concentration raises and decreases cyclically around each pulsating cell. The frequency of the oscillations has been estimated in $5-7$ minutes, and cAMP concentration may raise above background values for about 3 minutes in each cycle. It is customary to call pacemakers to these early signalling cells (or groups of neighbouring cells that may become entrained by synchronization), whose presence characterizes the beginning of aggregation. Different pacemakers may exhibit different oscillation periods (see for instance Figure 1 in [21]).

If unchecked, the behaviour just sketched would lead to a pattern consisting of expanding circular waves (the so-called target patterns, cf. [31]) around each signalling centre. However, what darkfield microscopic observation reveals is that wild-type (WT) strains of Dd soon show a coexistence of circular and spiral patterns, and by 5 hours after starvation spiral wave territories (with a diameter of ~ 4 mm, corresponding to a few wavelengths) dominate and persist for the following $1-2$ hours ([13, 18, 21, 22, 33, 38], see figure 2). The question then naturally arises of explaining the transition from a target-dominated towards a spiral-prevalent stage as aggregation proceeds, a problem that remains largely open to this day.

From an experimental point of view, a number of control parameters have been identified that lead to the prevalence of either type of pattern. For instance, in [19] observations are reported that hint at a transition between targets and spirals depending on cell density. More precisely, at low values of the cell density σ (say, for $\sigma \equiv \sigma_L = 7.3 \times 10^5$ cells/cm^2) the asymptotics is dominated by circular waves spreading out of pacemakers. In this situation, firing centres entrain each other, and the dynamics proceeds from random firings to periodic events. Moreover, firing centres survive until late aggregation stages (~ 11 hours after starvation). On the other hand, in the case of high densities ($\sigma \equiv \sigma_H = 21.9 \times 10^5$ cells/cm^2), spiral

waves dominate at late times, and they eventually suppress the firing events after a transient period of competition with targets.

Further light on the interplay between both types of patterns was shed by the discussion made in [20] (cf. also [21]) on wave resetting. These authors observed that addition of a spatially uniform pulse of cAMP dramatically affects the signalling process. More precisely, if applied early enough after starvation, spirals are temporally suppressed, to eventually reappear (and take over) later on. However, if cAMP is supplied at later times, spirals are effectively wiped out, and only targets will emerge – and remain – for the rest of the process. As observed in the aforementioned works, such behaviour bears considerable analogies with that occurring in cardiac defibrillation (cf. [46] and references therein).

4.1. **Desynchronization of cells along their developmental path.** In this paragraph we shortly recall the approach followed in [18] (cf. also [13]) to understand the target-spiral transition just mentioned before. Its starting point is a model for cAMP oscillations, based on the reversible desensitization of its membrane receptor, which exists in two states: active and inactive. Only the complex formed by cAMP with the active receptor can activate AcA, the enzyme required to produce cAMPi, whose external secretion closes the cAMP loop (cf. [11, 27]). In particular, the following three equations are proposed for the total fraction of active receptors (ρ), and the normalized concentrations of cAMPi (β) and cAMPe (γ):

$$\frac{\mathrm{d}\rho}{\mathrm{d}t} = -f_1(\gamma)\rho + (1-\rho)f_2(\gamma), \tag{2}$$

$$\frac{\mathrm{d}\beta}{\mathrm{d}t} = q\sigma\Phi(\rho,\gamma,\alpha) - (k_i + k_t)\beta, \tag{3}$$

$$\frac{\mathrm{d}\gamma}{\mathrm{d}t} = \frac{k_t}{h}\beta - k_e\gamma + D\triangle\gamma, \tag{4}$$

where D is the diffusion coefficient of cAMPe,

$$f_1(\gamma) = \frac{k_1 + k_2\gamma}{1+\gamma}, \quad f_2(\gamma) = \frac{k_1L_1 + k_2L_2c\gamma}{1+c\gamma},$$

$$\Phi(\rho,\gamma,\alpha) = \frac{\alpha(\lambda\theta + \epsilon Y^2)}{1+\alpha\theta+\epsilon Y^2(1+\alpha)}, \quad Y = \frac{\rho y}{1+\gamma},$$

and $k_1, k_2, L_1, L_2, c, \lambda, \alpha, \theta, h, \epsilon, \sigma, k_e$ are suitable biochemical parameters (cf. [11], Chapter 5). We point out that, if the diffusion term is dropped in equation 4, which amounts to consider a stirred suspension of amoebae, the resulting ODE system is known to allow for sustained oscillations of cAMP [27]. In [18] the authors explored in detail the role of parameters σ and k_e in equations (2)–(4), which represent the kinetic rates of AcA and phosphodiesterase respectively. In particular, a study was made of the influence of the variation in time of σ and k_e on the resulting signalling patterns. To this end, several developmental paths (each corresponding to a given form of $\sigma(t)$ and $k_e(t)$ as time proceeds) where considered, and the resulting solutions of equations (2)–(4) were compared. A striking conclusion is that only a class of developmental paths is able to yield the target-spiral transition; see Figure 5 below.

4.2. **Regulation by phosphodiesterase inhibition.** In [33], a modification of the previous scenario which explores the influence of space inhomogeneities was considered. More precisely, to the regulatory mechanisms being postulated in equations (2)–(4), a fourth equation was added, namely:

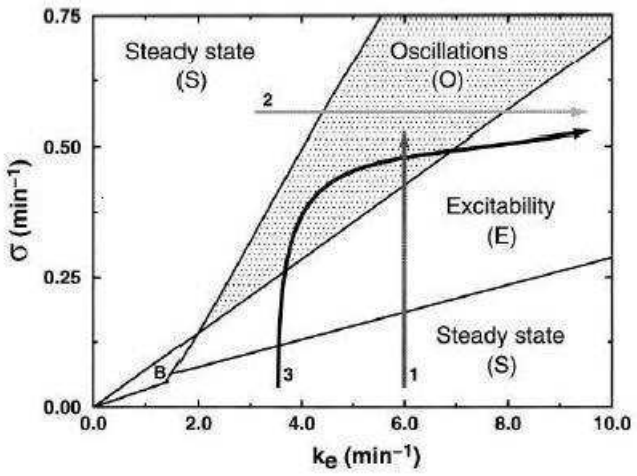

FIGURE 5. Stability diagram showing the different modes of dynamic behavior of the cAMP signalling system in the adenylate cyclase (σ)-phosphodiesterase (k_e) parameter space. The different regions are those of a stable, nonexcitable steady state (S), excitability (E) (defined here as the capability of sustaining without attenuation the propagation of a wave of cAMP upon a suprathreshold elevation of extracellular cAMP), and sustained autonomous oscillations of cAMP (O). The arrows refer to possible developmental paths followed by the signalling system in this parameter space as a result of variation in enzyme activies during the hours that follow starvation. Only path 3 gives rise to the formation of stable, fully developed spirals of cAMP. Reproduced with permission from [18]. Copyright (1997), National Academy of Sciences, USA.

$$k_e = \frac{k_{e,max}}{1+w}; \qquad k_{e,max} > 0. \tag{5}$$

This corresponds to a situation where cAMPe is degraded by a external phosphodiesterase (PDE), which is in turn negatively regulated by a PDE inhibitor (PDI), whose concentration in normalized units is given by $w(x, y, t)$. Numerical simulations of (2)–(5) were performed, and the results compared with actually observed patterns. In particular, it was noticed that if inhomogeneous values of $w(x, y, t)$ are randomly selected, and then kept constant during subsequent times, then when randomly distributed pulses of cAMP are assigned as initial values, such pulses give raise to circular waves. When interacting with new pulses of cAMP, broken circles result which eventually evolve into double spirals. The way in which spirals arise out of circular waves is explained in [33] as follows: when cAMP waves collide, they annihilate, since cells on the rear of impinging waves are in a refractory state to cAMP stimulation. However, when a new pulse is emitted shortly after a (circular) wave has passed, it breaks up to produce a half-circle which eventually becomes a double spiral; cf. Figure 6 below.

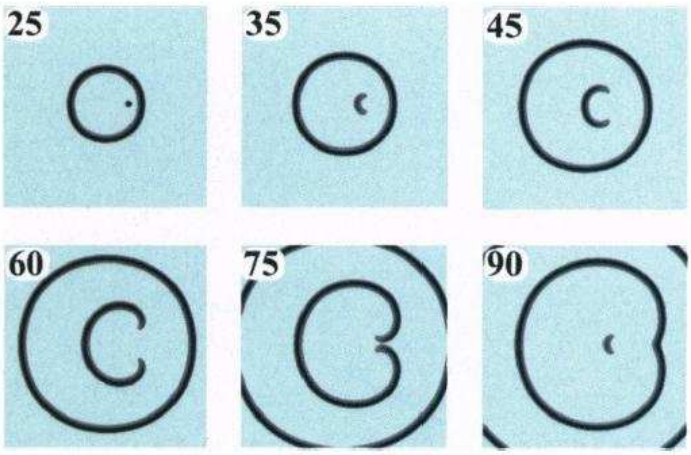

FIGURE 6. Formation of double spirals by interaction of a new pulse with a recently passed wave. Numbers indicate frame (there are 25 computational steps between each frame number). A cAMP pulse is initiated at frame 25 and gives rise to a double spiral (frame 45). Double spiral regenerates and forms the beginning of a new double spiral (frame 90). $k_e = 3.58$ uniformly. Reproduced with permission from [33]. Copyright (1996), National Academy of Sciences, USA.

It was further observed in [33] that, if levels of $w(x, y, t)$ are uniformly increased in time, the evolution already described is still observed, although significant changes in the wavelengths and rotational periods of the unfolding spirals are noticed as w increases.

4.3. **An effective-medium approach to excitability.** As it is apparent from our previous discussion, the simulations performed on equations (2)–(5) by tuning k_e (respectively, on (2)–(4) by regulating σ, k_e) share a common feature: both require that the corresponding parameters be externally driven. As a consequence, such equations cannot be considered closed in mathematical terms. An alternative approach consists in deriving the observed patterns from a mathematical model for the evolution of some effective (that is, averaged) quantities of the medium under consideration. In principle, this can be done in a heuristic way without making precise the manner in which these effective, macroscopic quantities are related to the underlying biochemical, microscopic precesses.

For instance, in [15, 19] the following hybrid model has been proposed to account for the target-spiral transition in Dd cultures:

$$\frac{\partial C}{\partial t} = D\triangle C - \Gamma C + H(C - C_T)C_r, \tag{6}$$

where $H(s) = 1$ if $s > 0$, $H(s) = 0$ otherwise,

$$C_T = \left(C_{max} - \frac{At}{t+T} \right) (1 - E), \tag{7}$$

$$\frac{\partial E}{\partial t} = -\alpha E + \beta C. \tag{8}$$

More precisely, amoebae in this model are treated as cellular automata on a two-dimensional lattice, and may assume three possible states: excitable, excited and refractory. When excitable cells at a given point (x, y) and a time t experience a cAMP concentration exceeding $C_T = C_T(x, y, t)$, they become excited and release a cAMP pulse C_r. Following cAMP relay, cells enter into a refractory state where no cAMP secretion occurs. During that period, C_T decreases from a value C_{max} to a value $C_{min} < C_{max}$ after a time $t = \tau > 0$ (cf. (7)) when they enter again into the excitable state. Excitability, denoted by E in (7)–(8), also modulates the value of C_T, while at the same time increases with cAMP concentration, see (8). It also undergoes a Arrhenius-type decay, as represented by the first term on the left of (8). Once again, D, T, A, Γ, α and β are parameters to play with. For detailed information on cellular automata techniques, the reader is referred to [4].

The results reported in [19] form numerical simulation of (6)–(8) can be summarized as follows. When E is kept constant at a value $E_{max} = 0.93$ (and therefore (8) is dropped), concentric waves (targets) emanating from these firing sites are obtained, and no coherent spiral patterns develop in time. The same situation occurs upon addition of small inhomogeneities in cell density or excitability. On the other hand, when the full system (6)–(8) (referred to in [22] as the genetically coupled model) is considered, long-range spiral structures unfold, independently of the initial state taken to start the simulation.

5. **From individual to collective behaviour.** We have shortly recalled in our previous section some of the models proposed to observe the macroscopic transition from target to spiral patterns in Dd monolayer cultures. We have also reviewed in Section 3 the basic ingredients of the cAMP-induced signal transduction process occurring at each individual cell. A question that naturally arises is that of deriving the observed macroscopic behaviour from the interaction of a large number of individual cells, each of them acting according to its own biochemical programme. This is a typical multi-scale modelling problem, on which only partial results seem to have been obtained so far.

Related to this, a remarkable fact has been reported in [38]. These authors observe that mutants defective in the production of some of the ingredients of the network described in Figure 3 are still able to display optical density oscillations, but their ability to produce self-organizing spiral patterns is seriously impaired. More precisely, while WT cells begin to produce oscillations at $3 - 4$ hours after plating, with a usual periodicity of $5 - 7$ minutes, and a total duration of $3 - 4$ hours, Erk2 (respectively RegA) mutants start later, at about 6 hours (respectively earlier, at $2 - 3$ hours). Periodic signalling in Erk2 mutants begins with a $5 - 7$ minutes periodicity, that gradually slows down to $10 - 11$ minutes and oscillations are maintained for up to 8 hours without aggregating. On the other hand, RegA mutants oscillate with a periodicity of ~ 7 mn. which lasts for about 2 hours, after which they aggregate to form small, unviable structures: see Figure 7 below.

In [38] it is stressed that the kinetics of the oscillations appeared to be quite similar in all strains. Given the scale used in Figure 1,B in [38] that is reproduced

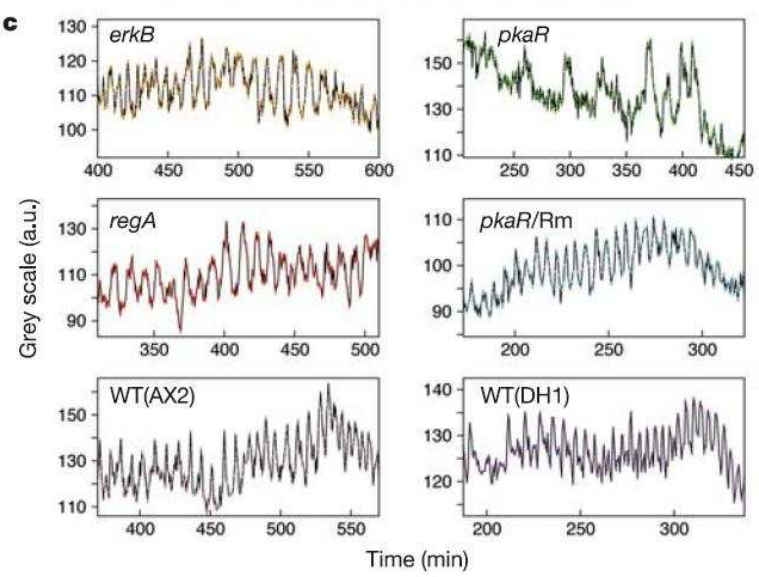

FIGURE 7. Signalling in mutants and WT strains of Dd (Reproduced with permission from Macmillan Publishers Ltd : Nature (Sawal et al. 433, 323-326), copyright 2005).

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above, each peak therein could correspond to the (average) behaviour of groups of about 10 cells each. The macroscopic behaviour of the RegA and Erk2 mutants, is characterized by an excess of spiral cores with respect to the WT cases. It is suggested in [38] that the failure to establish ordinary spiral fields may be due to the mutual anhilation of the many spirals developping in the mutant strains. A note of caution should be considered when analysing these results, since the complete absence of phosphodiesterase and protein kinase activiy has not been demonstrated. Residual activity in the mutant strains, coming either from other enzymes with similar activies or else from truncated proteins could remain in some of the mutants.

A discussion on the way in which these experimental facts could be related to a quantitative, mathematical model is provided in [38] (supplementary material). In particular, it is therein observed that when one considers equations (6)–(7) and:

$$\frac{\partial E}{\partial t} = \eta + \beta C \quad , \tag{9}$$

for some fixed constants η and β, it is possible to keep track of the transition from low excitability ($\beta < 0.001$, characterized by the presence of multiple firing centres) to high excitability ($\beta > 0.01$, for which persistence of spiral waves is observed). A number of technical assumptions are required (for instance, the use of extremely low values in the effective cAMP diffusion coefficient D, about 20 times lower that those customarily assumed).

6. **Concluding remarks.** Last years have behold a considerable advance in the elaboration of models to explain the oscillatory cAMP signalling that mediates Dd

aggregation. To this end, both intracellular and extracellular regulatory mechanisms have been considered. Most of the proposed models have tried to identify the minimal set of components that can produce oscillatory signalling. The advent of molecular genetics makes available a number of mutant strains where the activity of the components proposed in these models is compromised or altered. As a consequence, the analysis of cAMP signalling in these mutants is being used to check the validity of the proposed models, and will presumably continue to be a very useful tool in future research.

The continuous generation of new mutants will also allow for the identification of the other genes involved in cAMP signalling during aggregation and later development. New gene products will have to be incorporated in the proposed models or could even be the basis for new models. Subsequent development in that direction is expected to shed light on some aspects of cAMP regulation that are still not completely understood. One of these is the generation of signalling centres and the mechanisms that regulate their number and distribution in the aggregation fields. Current hypotheses assume that cells that initiate cAMP secretion were at specific points of their cell cycle at the moment of starvation. The use of synchronized cell populations could be of great utility to test these ideas.

One of the more interesting aspects in cAMP signalling in Dd is the transition from target to spiral cAMP waves. To explain the origin of the latter, several ideas have been put forward, for instance the existence of heterogeneities in the media or the different excitability of the cells. Further experimental studies may be necessary to define new regulatory mechanisms that, when incorporated into more complex models, would allow to understand the transition from target to spiral patterns in terms of measurable parameters of the Dd cell colonies.

Appendix A: cAMP signalling in Dd morphogenesis. Once formed, aggregates of Dd cells continue a developmental programme that involves cell differentiation and morphogenesis. cAMP signalling also plays a critical role in these two processes [7, 45]. As recalled before, Dd cells differentiate into two main cell types: prespore cells, that will form the spores, and prestalk cells that will form supporting structures such as stalk and basal disk. Prespore and prestalk cells initiate their differentiation programmes soon after aggregation. At the beginning, both cell types are interspersed in the aggregate. However, they soon segregate and prestalk cells concentrate in the central, upper part of the aggregate (tip). Cell type segregation is dependent on cAMP signalling coming from spiral waves that continue to be produced from the tip of the aggregate. Prespore and prestalk cells have different gene expression programmes at this stage of development, and differ in the way they react to the cAMP waves, as well as in the manner they transmit this signal. They also differ in their respective cell-cell adhesion properties. A mathematical model for cell type segregation based on differential adhesion between prestalk and prespore cells has been proposed in [26].

The structure described above, with prestalk cells in the upper part and prespore cells in the lower part, is kept until culmination, when the fruiting body is formed. Even during the migratory phase, the slug, a similar structure with prestalk cells in the front and prespore cells in the rear, is maintained. This layout is very important, since the tip of the aggregate (and the frontal part of the slug) have the capacity to organize the rest of the structure, holding some functional similarity to the organizer region of amphibian embryos [3]. This is probably due to the

fact that this region continues to be the source of cAMP signalling through all the developmental process. For instance, in the slug, cAMP waves are produced from the anterior end of the structure that are spiral in the prestalk region, and planar in the prespore region [45].

Cell migration towards the cAMP signalling centre is supposed to be important for the maintenance of the slug structure. Several mathematical models have been developed that account for slug structure and movement based on the principles described above: cAMP signalling and differential cell migration and adhesion of prestalk and prespore cells. Some of these models are based on the properties of viscous flows [44], others consider the cells as deformable ellipsoids [34], as groups of connected automata [26] or as moving particles [43].

The last step of Dd development is culmination. Prestalk cells, which stayed at the top of the structure, move downwards towards the substrate and progressively differentiate into stalk cells. Simultaneously, the prespore mass is raised from the substrate, attached to the upper part of the structure. cAMP continues to play a relevant role in this process, and is essential for spore differentiation. A theoretical model has been proposed that accounts for fruiting body morphogenesis [26].

Appendix B: the biochemistry of cAMP oscillatory waves. cAMP is synthesized inside the cells by adenyl cyclase enzymes, which use ATP as substrate. There are four adenyl cyclases in Dd, although only that encoded by the gene acA is expressed during aggregation. A fraction of the cAMP synthesized inside the cell is secreted to the extracellular media (through mechanisms not well established), and acts as a signalling molecule.

cAMP is degraded by phosphodiesterases to adenosine monophosphate. One intracellular phosphodiesterase, RegA, is expressed in aggregating Dd cells [39]. Similarly, there is also an extracellular phosphodiesterase, PDE [40]. Therefore the intracellular concentration of cAMP depends on the relative activities of the adenyl cyclase, AcA, and the phosphodiesterase RegA. The extracellular concentration depends on the rate of cAMP secretion from the cells and on the activity of PDE (that is modulated by the inhibitor PDI).

On its turn, the activity of AcA is regulated by the concentration of extracellular cAMP, cAMPe. Dd aggregating cells express some membrane proteins, the cAMP receptors, that bind cAMPe generating activation signals inside the cells. The cAMP receptor expressed at this stage is Car1 [23]. Binding of cAMP to Car1 activates a number of intracellular pathways that mediate the cell response to this signalling molecule. One of the pathways involves the activation of heterotrimeric G proteins that increase the activity of AcA, and therefore intracellular cAMP (cAMPi) concentration [14].

Binding of cAMP to Car1 also activates a second regulatory pathway that induces phosphorylation of the protein kinase Erk2. This last in turn phosphorylates RegA, inhibiting its phosphodiesterase activity, which contributes to increase the intracellular concentration of cAMP [39]. These activating loops are responsible for cAMP relay, since the cells produce cAMP in response to cAMPe. When a cell detects an increase in cAMPe, it induces internal synthesis of that substance within 15 seconds as the result of the activation of these two pathways.

cAMP synthesis and intracellular signalling are transient phenomena in aggregating cells, which is determinant for the oscillatory pattern of the signalling system [37]. One of the biochemical basis for this behaviour is receptor adaptation. After

a short period of activity, cAMP receptors become unable to respond to cAMPe. The molecular mechanism of Car1 adaptation is not completely understood.

cAMP binding induces phosphorylation of the receptor, and it was thought that this modification could produce adaptation. However, mutation of the phosphorylated residues did not alter adaptation, a refutation to that hypothesis [35].

Aggregating cells cease cAMP synthesis about 1.5 minutes after stimulation, and as a consequence, intracellular and extracellular cAMP concentrations decrease to basal levels 3 minutes after stimulation. The decrease in cAMPe concentration is most probably due to the activity of the phosphodiesterase PDE [2]. The drop in cAMPi concentration is due both to the inhibition of the adenyl cyclase AcA and to the activation of RegA. cAMP has long been known to be an intracellular second messenger that is able to activate several signalling pathways. One of them involves activation of the cAMP-dependent protein kinase PKA. This enzyme is usually found in the cytoplasm of the cells as a heterodimer composed by a catalytic subunit (PKA) and a regulatory subunit (PKR) that inhibits catalytic activity. cAMPi binds to the regulatory subunit producing its dissociation from the catalytic subunit, which thus becomes activated [12]. In aggregating cells, activated PKA has been proposed to mediate inactivation of AcA [25]. This system forms a feedback loop where high levels of cAMP inhibit its own synthesis. The protein kinase ErkB also becomes inactivated after PKA activation [1]. As mentioned before, ErkB is activated upon cAMP binding to its receptor, inhibiting the phosphodiesterase RegA. Phosphorylation of ErkB by PKA has the opposite effect, avoiding RegA inhibition and increasing cAMP degradation [25]. In this second feedback loop, and increase in cAMP concentration induces its own degradation through PKA activation and ErkB phosphorylation.

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