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ORIGINAL ARTICLE

# Oscillations and Waves of Cyclic AMP in *Dictyostelium*: A Prototype for Spatio-Temporal Organization and Pulsatile Intercellular Communication

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**Abstract** The amoebae *Dictyostelium discoideum* aggregate after starvation in a wavelike manner in response to periodic pulses of cyclic AMP (cAMP) secreted by cells which behave as aggregation centers. In addition to autonomous oscillations, the cAMP signaling system that controls aggregation is also capable of excitable behavior, which consists in the transient amplification of suprathreshold pulses of extracellular cAMP. Since the first theoretical model for slime mold aggregation proposed by Keller and Segel in 1970, many theoretical studies have addressed various aspects of the mechanism and function of cAMP signaling in *Dictyostelium*. This paper presents a brief overview of these developments as well as some reminiscences of the author's collaboration with Lee Segel in modeling the dynamics of cAMP relay and oscillations. Considered in turn are models for cAMP signaling in *Dictyostelium*, the developmental path followed by the cAMP signaling system after starvation, the frequency encoding of cAMP signals, and the origin of concentric or spiral waves of cAMP.

## 1. Slime mold aggregation and theoretical biology

In 1970, Evelyn Keller and Lee Segel published a landmark paper in the *Journal of Theoretical Biology*, entitled: "Initiation of slime mold aggregation viewed as an instability" (Keller and Segel, 1970). This article marked the entry of *Dictyostelium* amoebae into the field of theoretical biology. Before turning to biology Lee had gained wide recognition in the field of Applied Mathematics, in which he wrote a classical textbook with C.C. Lin, his former teacher at MIT (Lin and Segel, 1988). Lee was working at the time in hydrodynamics, investigating the role of instabilities in the formation of Benard convection patterns in fluids heated from below. This phenomenon represents one of the most striking examples of nonequilibrium self-organization (Glansdorff and Prigogine, 1971). Lee became attracted

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to biology after seeing pictures of wavelike patterns of slime mold aggregation in the laboratory of John Ashworth, then at the University of Leicester, where Lee was visiting his brother in law P.D. Weitzman, a biochemist. Patterns of aggregating slime molds reminded him of Benard patterns seen in hydrodynamics. This is how slime mold aggregation became the topic of this paper.

A paper on slime mold aggregation based on a symmetry breaking instability was particularly timely because it coincided with the rapid development of studies of temporal and spatial self-organization in nonequilibrium chemical systems, as exemplified by oscillations and waves in the Belousov-Zhabotinsky reaction (Nicolis and Prigogine, 1977). To this date, the wavelike patterns observed during *Dictyostelium discoideum* aggregation represent one of the most beautiful and best understood examples of spatiotemporal self-organization at the cellular level. In *D. discoideum*, the wavelike nature of aggregation (Gerisch, 1968; Alcantara and Monk, 1974) results from the existence of a cellular rhythm in the production of cyclic AMP (cAMP), the molecule that controls chemotaxis in the course of aggregation. Oscillations of cAMP soon became a topic of choice for theoretical modeling. Although cAMP appears to be the primary oscillator underlying wavelike aggregation, some evidence suggests that a second oscillator, perhaps based on $Ca^{++}$, might also operate in *Dictyostelium* (Nanjundiah, 1998).

The purpose of this contribution to the volume dedicated to the memory of Lee Segel will be to present an account of theoretical studies of the cAMP signaling system controlling aggregation in *Dictyostelium*, the very phenomenon that attracted Lee to theoretical biology and to which he often returned thereafter (Parnas and Segel, 1978; Segel, 1984, 2001; Barchilon and Segel, 1988). I will also describe the circumstances in which I began to work with Lee on this question, and will briefly discuss current views of the mechanism and function of oscillations and waves in *Dictyostelium* cells. As discussed in detail elsewhere (Goldbeter, 1996), oscillations and waves of cAMP in the slime mold *D. discoideum* represent one of the most striking examples of spatiotemporal self-organization at the cellular level, and provide a prototype for pulsatile intercellular communication in higher organisms, for example, pulsatile hormone secretion. *Dictyostelium* also represents an organism of choice for studying development and differentiation (Devreotes, 1989; Loomis, 1996; Eichinger et al., 2005).

## 2. Oscillations and relay of cAMP in *Dictyostelium* cells

In contrast to aggregation in *Dictyostelium minutum*, which proceeds in a nonoscillatory manner (Gerisch, 1968), aggregation in *D. discoideum* possesses a wavelike nature (Fig. 1). In this species, starved amoebae start to collect in waves moving toward cells that behave as aggregation centers (Gerisch, 1968; Alcantara and Monk, 1974). The periodicity of the phenomenon is of the order of 5–10 min. Based on observations under the microscope, Shaffer (1962) suggested that centers periodically release a chemotactic substance called acrasin. Konijn and Bonner later established the chemical nature of this signal, which turned out to be cAMP (Konijn et al., 1967). The biochemical aspects of chemotaxis and morphogenesis in *Dictyostelium* have recently been reviewed (Manahan et al., 2004; Weijer, 2004).

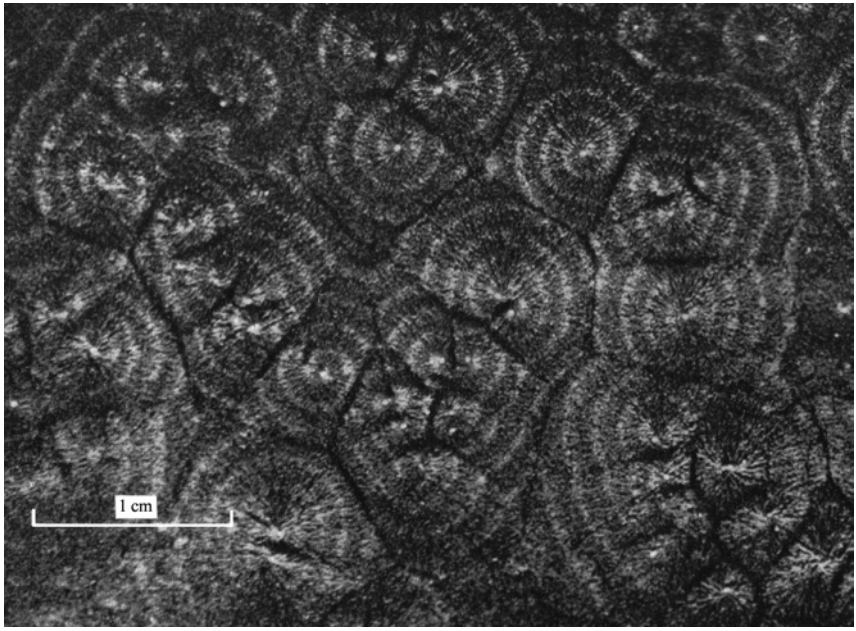



**Fig. 1** Wavelike aggregation of *Dictyostelium dioscoideum* amoebae after starvation (from Alcantara and Monk, 1974).

The first evidence for a biochemical clock controlled by cAMP came from studies in *D. discoideum* cell suspensions (Gerisch and Hess, 1974). These studies showed that periodic changes in light scattering can occur spontaneously in such suspensions, and that a pulse of extracellular cAMP can phase shift the oscillations. At the same time Rossomando and Sussman (1973) reported that cAMP synthesis in *D. discoideum* is positively controlled by 5′AMP, which is obtained through degradation of cAMP by phosphodiesterase. I heard of these results in a seminar given by Maurice Sussman shortly after I moved to the Weizmann Institute in September 1973 for a 2-year post-doctoral stay with Roy Caplan, with whom I prepared a review on oscillatory enzymes, and in which a section was devoted to cAMP oscillations in *Dictyostelium* (Goldbeter and Caplan, 1976). At the Weizmann Institute I soon met Lee Segel, who has just arrived from the US to become head of the Department of Applied Mathematics. I knew Lee from his papers published a few years earlier, but had never met him.

Making the link with the results of Gerisch and Hess on cAMP-controlled light scattering oscillations in cell suspensions, I built a first model (Goldbeter, 1975) that predicted the occurrence of sustained oscillations of cAMP, based on the regulatory scheme proposed by Rossomando and Sussman for adenylate cyclase, the cAMP-synthesizing enzyme. Shortly after this paper was published, Gerisch and co-workers reported their beautiful experimental studies showing that in continuously stirred suspensions of *D. discoideum* cells, the cAMP signaling system can display two distinct modes of dynamic behavior. They first demonstrated oscillatory synthesis of cAMP with a period close to 10 min (Gerisch and Wick, 1975),

and further showed that cells unable to oscillate autonomously were capable of relaying cAMP signals: when subjected to a suprathreshold, transient increase in extracellular cAMP, cells initially in a stable steady state synthetize a pulse of intracellular cAMP before returning to the stable state that prevailed before perturbation (Roos et al., 1975).

The next step in modeling the cAMP signaling system was carried out in collaboration with Lee (Goldbeter and Segel, 1977). We decided to address the link between relay and oscillations of cAMP. At the time, some experimentalists suggested that the two modes of dynamic behavior were due to distinct molecular mechanisms. We wanted to explore the possibility that a single mechanism operating with different parameter values could account both for suprathreshold relay of cAMP pulses and for autonomous oscillations of cAMP. We searched for a mechanism capable of producing a pulse of intracellular cAMP in response to the extracellular perturbation and arrived at a simple possibility (which later proved to be too simple). In the absence of oscillations, when the steady state is stable, it is possible to trigger a pulse of cAMP if the initial effect of the extracellular signal is to lower instantaneously the level of intracellular cAMP below the steady state level. Then, if the steady state is a stable focus, the system responds to a decrease of cAMP by making a loop in the phase plane before returning to steady state. This excursion corresponds to the synthesis of a cAMP pulse, and can be accompanied by a series of pulses of decreasing amplitude as the system returns to the stable steady state via damped oscillations. Lee called this the "window shade mechanism" because the pulsatile response was triggered by an initial decrease of intracellular cAMP.

However, no evidence existed for an initial decrease of intracellular cAMP in response to the extracellular cAMP pulse, nor for any triggering of damped oscillations. We were rescued from this dilemma by Günther Gerisch, who invited me to visit him at the Biozentrum in Basel where he generously showed me results of unpublished experiments that led us to abandon the idea of the window shade mechanism. Lee and I resumed our search for conditions that produced a pulse of intracellular cAMP in response to a suprathreshold increase in extracellular cAMP, and eventually found that such conditions did indeed exist.

Abandoning the earlier model (Goldbeter, 1975) based on regulation that could not be corroborated by further experimental studies, the new model we considered was based on the activation of adenylate cyclase upon binding of extracellular cAMP to a membrane receptor. The three variables in this model were the concentrations of intracellular ATP and cAMP—respectively the substrate and product of adenylate cyclase—, and the concentration of extracellular cAMP. Self-amplification, which is the hallmark of cAMP synthesis in *D. discoideum*, originates from the positive feedback exerted by extracellular cAMP: when the latter binds to the receptor, it triggers the synthesis of intracellular cAMP, which is transported into the extracellular medium, where it binds to the receptor and further enhances cAMP synthesis by adenylate cyclase. In this model, oscillations result from the coupling of the positive feedback loop to the depletion of ATP due to its transformation into cAMP.

By carrying out numerical simulations we found a range of initial conditions in the phase plane for which the system, initially in a stable steady state, was

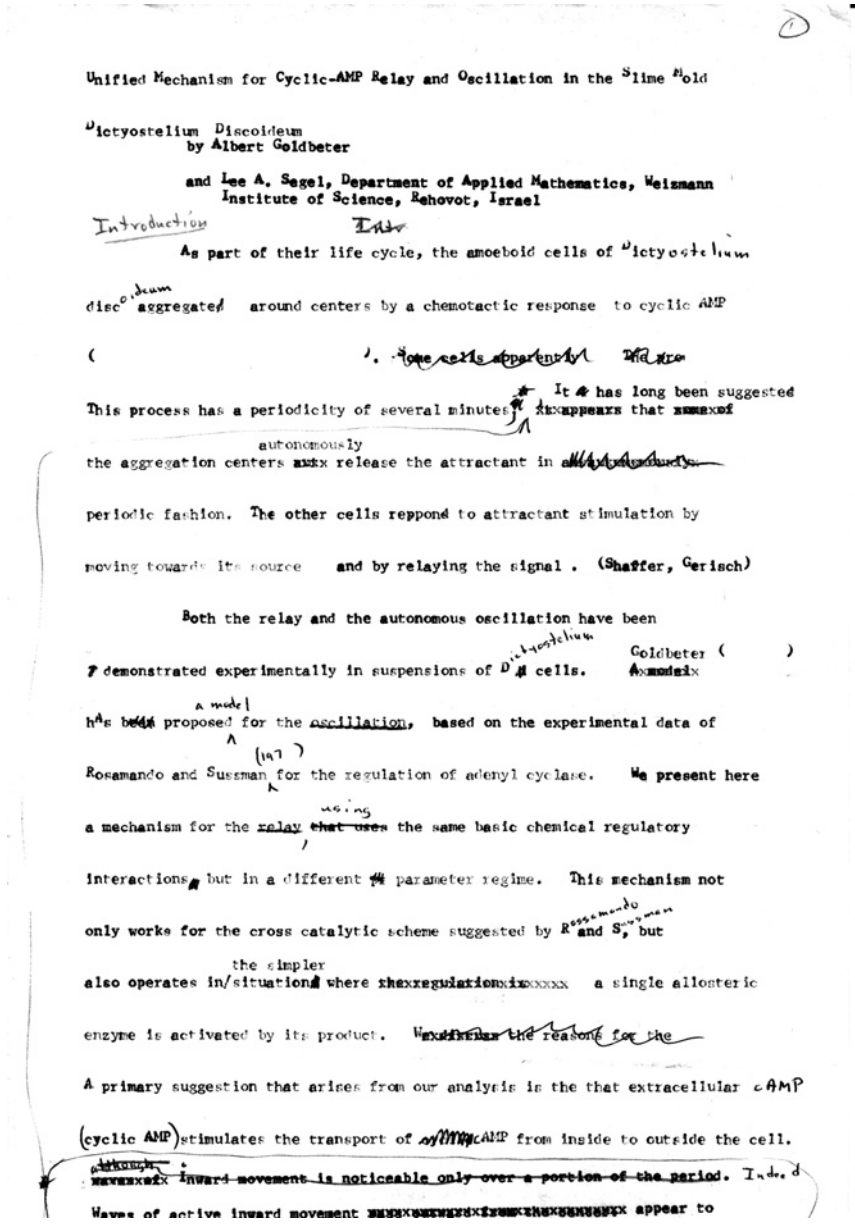
capable of amplifying in a pulsatile manner a small transient increase in extracellular cAMP. This behavior occurs when the steady state is located in the immediate vicinity of the domain where sustained oscillations occur. This led us to write a paper entitled "Unified mechanism for relay and oscillations of cyclic AMP in *D. discoideum*" (Goldbeter and Segel, 1977). I still remember vividly how we wrote this article on the terrace of Lee's apartment in Rehovot, overlooking the orange grove of the Weizmann Institute. Lee was sitting at a small desk, on which a compact electrical typewriter was poised. We discussed at length the wording of each sentence, and Lee would begin typing it, leaving a large space between lines, which would facilitate subsequent insertions or corrections (see Fig. 2 which reproduces the first page of the original draft of this paper which I kept, in contrast to the drafts of most of my other papers). I was bemused by the way the paper emerged from this exercise in controlled improvisation. The process gave us much fun, which was compounded by the endless flow of jokes that Lee loved to tell. I never experienced greater pleasure in writing a paper with a co-author.

The finding of a region in the phase space where the system was capable of relaying pulses of extracellular cAMP was later clarified with Thomas Erneux by means of phase plane analysis. The use of nullclines allowed us to show that relay reflects the excitability of the cAMP signaling system in *Dictyostelium*, and that this mode of dynamic behavior is necessarily linked to the occurrence of sustained oscillations for closely related parameter values (Goldbeter et al., 1978; Goldbeter, 1980).

## 3. Dynamical transitions in cAMP signaling and the developmental path in parameter space

Beyond its role in controlling cell aggregation after starvation, the cAMP signaling system in *Dictyostelium* provides a prototype for the onset of a biological rhythm in the course of development. Studies from the group of Gerisch indeed showed that the cAMP signaling system undergoes a series of sequential transitions in dynamic behavior during the hours that follow starvation (Gerisch et al., 1979). At first cells are not capable of relaying pulses of extracellular cAMP, then they become able to relay and, a few hours later, they begin to autonomously secrete periodic pulses of cAMP. They later lose the capability to oscillate and again become able to relay cAMP signals.

Since we had developed a unified model for relay and oscillations of cAMP in *Dictyostelium* Lee Segel and I decided to investigate the dynamical bases of this sequence of developmental transitions. We determined the dynamic behavior of the cAMP signaling system in the parameter space formed by adenylate cyclase and phosphodiesterase, the enzymes involved, respectively, in cAMP synthesis and degradation. To account for the transitions no relay-relay-oscillations-relay, we found that cells should follow a path in this parameter space that would lead them from a stable, non excitable steady state to a stable, excitable steady state. Then cells would enter the domain of sustained oscillations around an unstable steady state before quitting it to return to the domain of excitability. We proposed to call this trajectory in parameter space the "developmental path" (Goldbeter and



Unified Mechanism for Cyclic-AMP Relay and Oscillation in the Slime Mold

Dictyostelium Discoideum
by Albert Goldbeter

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Introduction

As part of their life cycle, the amoeboid cells of Dictyostelium discoideum aggregate around centers by a chemotactic response to cyclic AMP (

). Some cells apparently find

This process has a periodicity of several minutes. It has long been suggested that the aggregation centers release the attractant in an autonomously periodic fashion. The other cells reppond to attractant stimulation by moving towards its source and by relaying the signal. (Shaffer, Gerisch)

Both the relay and the autonomous oscillation have been demonstrated experimentally in suspensions of Dictyostelium cells. Goldbeter ( ) has proposed a model for the oscillation, based on the experimental data of Rosamando and Sussman (1971) for the regulation of adenyl cyclase. We present here a mechanism for the relay using the same basic chemical regulatory interactions, but in a different parameter regime. This mechanism not only works for the cross catalytic scheme suggested by Rossomando and Sussman, but also operates in the simpler situation where the regulation is a single allosteric enzyme is activated by its product. [illegible] the reason for the

A primary suggestion that arises from our analysis is the that extracellular cAMP (cyclic AMP) stimulates the transport of cAMP from inside to outside the cell. [illegible] Inward movement is noticeable only over a portion of the period. Indeed Waves of active inward movement [illegible] appear to

**Fig. 2** First page of the draft of the article published by Goldbeter and Segel (1977) on a unified mechanism for relay and oscillations of cAMP in *Dictyostelium* cells (the draft was typed and annotated by Lee Segel, as described in the text).


**Fig. 3** Picture taken around 1980 while the author (AG, *right*) was visiting Lee Segel (*left*) in Boston, when Lee was spending a sabbatical year at Harvard. The picture was taken by Ruth Segel (Lee's wife) in front of the hotel where AG was staying. The inscription "Welcome CAMP" refers to some convention held at the time in the hotel, on an unidentified theme . . . unrelated to cyclic AMP!

Segel, 1980). The notion of a developmental path suggests that aggregation centers are those cells that are the first to enter into the domain of sustained oscillations of cAMP. If these cells are removed, other cells, which follow them on the developmental path but are still in an excitable state, will eventually enter the domain of autonomous oscillations, thus behaving as new aggregation centers.

To account for the observed transitions, the developmental path predicted by the model corresponded to a progressive increase in the activity of adenylate cyclase and phosphodiesterase (Goldbeter and Segel, 1980). Such biochemical changes are indeed observed in *Dictyostelium* during the hours that follow starvation (Loomis, 1979). The continuous increase in the two enzyme activities in the phase leading to aggregation can therefore underlie the discontinuous transitions in dynamic behavior displayed by the cAMP signaling system. Similar conclusions were later reached in a more detailed model for cAMP signaling based on receptor desensitization (Lauzeral et al., 1997). This shows the robustness of the notion of developmental path in parameter space applied to the case of cAMP signaling in *Dictyostelium*.

Around the time when the developmental path paper was published, I visited Lee in Boston where he was spending a sabbatical at Harvard. The picture reproduced in Fig. 3 shows us standing in front of the hotel where I was staying. We liked the fortuitous association with cAMP!

## 4. Model for cAMP signaling based on receptor desensitization

In any model for oscillations the key to the mechanism of periodic behavior is in understanding the repetitive sequence of events that first brings an increase in a certain variable, then a decrease, before a new cycle begins with yet another increase. In the model for cAMP signaling, positive feedback by extracellular cAMP on adenylate cyclase underlies the rapid rise in intracellular cAMP. The nature of the process that brings this phase of explosive increase to an end and finally causes a decline in cAMP is less clear. Also unexplained is the process by which cAMP will resume its rise at the end of this phase of decrease.

In the model developed in collaboration with Lee, the process limiting self-amplification in cAMP synthesis was the drop in the ATP concentration, caused by its transformation by adenylate cyclase into cAMP (Goldbeter and Segel, 1977). However, ATP is in the mM range, while intracellular cAMP is in the $\mu$M range. It is thus doubtful that ATP would become a limiting factor. Measures of the ATP level in the course of cAMP oscillations revealed but a minor variation (Roos et al., 1977).

Studies by Devreotes and coworkers in the mid-eighties showed that when it binds to its receptor on the cell membrane, cAMP induces its covalent modification which takes the form of reversible phosphorylation (Devreotes and Sherring, 1985). Moreover, cAMP oscillations are accompanied by the periodic alternation of the receptor between its phosphorylated and nonphosphorylated states (Gundersen et al., 1989). Many studies indicated at the time that receptors often become desensitized through reversible phosphorylation or some other form of covalent modification.

Building on these results, Martiel and Goldbeter (1987) proposed a model for cAMP signaling based on receptor desensitization. This model incorporates both a positive and a negative feedback by extracellular cAMP. Binding of cAMP to its receptor triggers the activation of adenylate cyclase as well as the transition of the receptor into a desensitized (phosphorylated) state. In this three-variable model, the explosive rise in cAMP synthesis is limited by the drop in the fraction of active receptor. Desensitization begins as soon as the level of extracellular cAMP starts to increase. Once the level of cAMP has dropped to a minimum, the receptor resensitizes through dephosphorylation. A new cycle of oscillations begins upon binding of extracellular cAMP to the receptor in its active state. As in the previous model, relay of suprathreshold pulses of cAMP occurs in a parameter domain adjacent to the domain in which sustained oscillations occur (Martiel and Goldbeter, 1987; Goldbeter, 1996).

## 5. Exact adaptation

In many sensory systems the response triggered by an external stimulus decreases in the course of time and returns to basal levels even though the stimulus is maintained at a high value. This physiological process is called adaptation. Adaptation can be partial or exact. In the latter case, the system returns precisely to the same steady state regardless of the level of stimulation. Exact adaptation is observed

in bacterial chemotaxis (Macnab and Koshland, 1972), and in the cAMP relay response in *Dictyostelium* (Dinauer et al., 1980).

In the summer of 1985 Peter Devreotes and I joined Lee Segel at the Weizmann Institute to work on this problem. Building on previous work devoted to adaptation of the bacterial chemotactic response (Goldbeter and Koshland, 1982), we considered a two-state receptor model, with a ligand that binds with different affinities to these two states, which are in conformational equilibrium. We assumed that a certain activity is generated by a linear combination of the four liganded or free receptor states to which an activity coefficient is attributed. The mathematical problem was to determine whether, for a given set of parameter values, it is possible to find a set of activity coefficients that would ensure that the total activity generated upon ligand binding to the receptor returns to the same steady state, regardless of the magnitude of the stepwise increase in ligand concentration. We found, to our delight, that a unique set of activity coefficients satisfying this constraint indeed exists. Lee was instrumental in carrying out the mathematical derivations that led to this surprising result (Knox et al., 1986; Segel et al., 1986). Alternative molecular mechanisms for exact adaptation have since been proposed (Alon et al., 1999; Levchenko and Iglesias, 2002). A recent modeling study was specifically devoted to a detailed analysis of the intracellular biochemical events mediating the chemotactic response in *Dictyostelium* cells (Ma et al., 2004).

## 6. Frequency encoding of cAMP pulses in intercellular communication

Key experiments published independently in 1975 by two groups showed that cAMP signals in *Dictyostelium* are frequency encoded. In a mutant of *D. discoideum* unable to aggregate, cAMP pulses administered at the physiological frequency of one pulse every 5 min are capable of rescuing the mutant and lead to multicellular aggregation (Darmon et al., 1975). In contrast, continuous stimulation by cAMP does not lead to aggregation. In the wild type, the administration of cAMP pulses after starvation accelerates development in the phase leading to aggregation. This effect is obtained with cAMP pulses delivered every 5 min but not when the interval between pulses is reduced to 2 min, or when the cAMP signal becomes continuous (Gerisch et al., 1975). Pulses of cAMP administered at random intervals in wild type are likewise less effective (Nanjundiah, 1988). These observations demonstrate the importance of the frequency of pulsatile cAMP signals.

The model based on receptor desensitization provides a unified explanation for these experimental observations. It shows that when the pulse is given at regular intervals, the receptor has sufficient time to resensitize during a 5-min interval, but not during a 2-min interval. In the latter case, when the next pulse arrives the amount of active receptor will not be sufficient to produce a large-amplitude response. In these conditions the synthesis of cAMP elicited by repetitive pulses of extracellular cAMP will be reduced, while it is nearly maximum when the pulse is given every 5 min. For the same reason, continuous stimulation by cAMP causes receptor desensitization and permanent attenuation of the cAMP response (Martiel and Goldbeter, 1987).

The model further predicts the existence of an optimal pattern of pulsatile stimulation by cAMP, which maximizes cellular responsiveness, i.e. the amount of cAMP synthesized in a given time in response to cAMP pulses. For the experimentally determined values of the rate constants measuring receptor desensitization and resensitization, the model predicts that the optimal pulsatile signal of cAMP should have a duration of about 4 min, with an interpulse interval of about 3 min (Li and Goldbeter, 1990). Increasing or decreasing the values of these rate constants should change the characteristics of the optimal pattern of pulsatile stimulation, a subject that could be easily explored with mutants.

The cAMP signaling system in *Dictyostelium* provides a useful prototype for pulsatile intercellular communication in higher organisms. It can be viewed as a primitive hormonal communication system, in which cAMP plays the role of both the hormone and the intracellular second messenger—this duality underlies the self-amplification property that is the hallmark of cAMP synthesis in *Dictyostelium*. The link with hormonal signaling goes beyond this analogy. Most hormones are indeed released in a pulsatile manner, and the frequency of pulsatile secretion often governs the efficacy of the hormonal signal.

The study of a general model of a receptor undergoing reversible desensitization shows that when the hormone that binds to the receptor is applied in a pulsatile manner, there exists an optimal pattern of periodic stimulation that maximizes target cell responsiveness (Li and Goldbeter, 1989). The results account for the existence of such an optimal pattern of stimulation for the hormone GnRH, which is secreted by the hypothalamus in the form of a 5-min pulse every hour. The model further shows that the optimal periodic signal is more efficient than pulses delivered in a random or chaotic manner (Li and Goldbeter, 1992). This is very similar to *Dictyostelium.*

## 7. From oscillations to waves of cAMP

The wavelike aggregation of *D. discoideum* cells after starvation provides a striking example of spatiotemporal organization at the supracellular level. In their pioneering paper of 1970 Keller and Segel showed that the initial homogeneous cell spatial distribution becomes unstable when the rate of secretion of chemotactic factor by the cells exceeds a critical value. A key result of this analysis, which applies particularly well to *D. minutum*, was that no heterogeneity in cell function—i.e., no center—is needed to trigger aggregation. Not considered explicitly in this first study was the wavelike nature of aggregation in *D. discoideum* cells. The periodic nature of aggregation in this species is directly linked to the oscillatory synthesis of cAMP, which is released in the form of periodic pulses by cells behaving as aggregation centers. Experimental observations indicate that aggregating cells form either concentric or spiral waves (Gerisch, 1968; Alcantara and Monk, 1974; Dormann et al., 1998). The waves of cellular movement are superimposed on waves of the chemoattractant, cAMP (Tomchik and Devrotes, 1981).

Much work has been devoted to understanding the onset of spatio-temporal self-organization (Höfer et al., 1995) and the transition from concentric to spiral patterns of wavelike aggregation, both experimentally and theoretically (Levine et al., 1996; Palsson and Cox, 1996; Dallon and Othmer, 1997; Palsson et al., 1997). Building on the notion of developmental path which was initially proposed for the transitions in dynamic behavior observed for the cAMP signaling system, Lauzeral et al. (1997; see also Halloy et al., 1998) investigated the effect of desynchronization of cells following the developmental path in parameter space. The results indicate that concentric waves of cAMP form first when cells enter the domain of autonomous oscillations, while neighboring cells are in an excitable state and relay these signals. However, the heterogeneity in parameter values, due to the distribution of cells on the developmental path, creates defects that eventually lead to breaks in concentric waves. From these defects spiral patterns arise, which are maintained even when all cells have become excitable and no one is capable any more of sending out periodic pulses of cAMP. Besides desynchronization other factors may favor the transition from concentric to spiral waves. The transition is likely facilitated by the chemotactic movement of cells, which further amplifies heterogeneities within the aggregation field (Dallon and Othmer, 1997).

## 8. Intracellular versus extracellular feedback loops in the origin of cAMP oscillations in *Dictyostelium*

In recent years work by Loomis and coworkers has raised the possibility that cAMP oscillations in *D. discoideum* may originate from an intracellular regulatory network rather than from the mixed positive and negative feedback exerted by extracellular cAMP (Laub and Loomis, 1998; Maeda et al., 2004). These authors obtained evidence for an intracellular feedback loop involving MAP kinase and the cAMP-dependent protein kinase PKA, which would inactivate adenylate cyclase after a cAMP pulse. Numerical simulations of a model based on this intracellular negative feedback loop confirm that it can produce sustained oscillations of cAMP.

To establish which of the two feedback loops plays a prominent role in the origin of cAMP oscillations, Cox and coworkers recently examined the patterns of wavelike aggregation in a variety of mutants lacking components of the intracellular and extracellular regulatory loops. They reached the conclusion that the primary (but not necessarily sole) source of the oscillations resides in the regulation exerted by extracellular cAMP upon binding to its membrane receptor (Sawai et al., 2005).

Interestingly, the possibility of cAMP oscillations due to intracellular regulation of adenylate cyclase by PKA seems to exist not only in *Dictyostelium* but also in yeast. In this organism Jacquet et al. (2003) recently observed a stress-induced oscillatory shuttling of the transcription factor Msn2 between cytosol and nucleus. They since obtained evidence suggesting that this periodic phenomenon is controlled by intracellular cAMP oscillations, via the control of adenylate cyclase by PKA. Periodic activation of PKA by cAMP oscillations in yeast would thus

underlie the repetitive, coherent shuttling of the transcription factor Msn2 into and out of the nucleus.

## 9. Computing an organism

Beyond aggregation and the formation of cAMP waves, many other aspects of *Dictyostelium* morphogenesis have attracted the attention of theoreticians (Nanjundiah, 1997; Weijer, 2004). Thus, several modeling studies focused on cell movement within the slug (Bretschneider et al., 1995) and on the process of slug migration (Odell and Bonner, 1986; Vasiev and Weijer, 2003; Dallon and Othmer, 2004).

One of the most ambitious attempts is due to Marée and Hogeweg (2001, 2002) who used a hybrid cellular automata/partial differential equation approach to simulate the whole multicellular phase of the slime mold life cycle, from aggregation to fruiting body formation. To accompany the publication of this study, Lee wrote a comment (Segel, 2001), the title of which I borrowed for this brief section. In the words of Lee, the authors provided "a computer simulation of the frog-prince transition of slug into fruiting body." The comment by Lee summarizes well his views about *Dictyostelium* modeling and, more generally, about the art of building theoretical models in biology.

## 10. From theoretical biology to computational systems biology: onset of a propagating wave

The work of Lee Segel covers a wide range of topics in theoretical biology, extending from enzyme kinetics and pattern formation to developmental biology, neurobiology and immunology. Few workers in the field have been so active and so eclectic in selecting important biological problems and addressing them by means of theory. Lee was unanimously respected, not only for his influential work, but also for his exceptional human qualities. I had the good fortune to meet him during my post-doctoral stay in 1973–1975 at the Weizmann Institute, and had the privilege of working with him on several occasions over the years, from that period up to the 1990s, when we published our last joint paper (Segel and Goldbeter, 1994).

Slime mold aggregation was the main topic of my collaboration with Lee, and in concluding this article, I would like to use this phenomenon as a metaphor for the wave of interest in theoretical studies in biology (Goldbeter, 2004). One may wonder why this interest is increasing so rapidly. One reason is clearly the arrival of new workers in the field, reflected by the swift development of Systems Biology and Computational Biology. In *Dictyostelium*, cAMP waves propagate only when the signals sent autonomously by aggregation centers are relayed, once the number of responding cells exceeds a threshold. Such conditions are apparently met for the propagation of a wave of interest in the field of theoretical or mathematical biology, also referred to by a variety of new names such as computational cell biology or (computational) systems biology. Lee Segel played a pioneering role in triggering this wave, and the exemplary nature of his work ensures that it will be sustained.

**Acknowledgements**

I wish to dedicate this article to the memory of Lee Segel, whose disappearance is a deep loss to the field and to his numerous friends around the world. Many of the ideas presented in this paper were developed with Lee over the years. I thank Ted Cox and Vidya Nanjundiah for their comments on the manuscript. This work was supported by grant #3.4636.04 from the *Fonds de la Recherche Scientifique Médicale* (F.R.S.M., Belgium) and by the European Union through the Network of Excellence BioSim, Contract No. LSHB-CT-2004-005137. This article was prepared while A.G. held a "Chaire Internationale de Recherche Blaise Pascal de l'Etat et de la Région Ile-de-France, gérée par la Fondation de l'Ecole Normale Supérieure" at the University of Paris Sud-Orsay (France), in the Institute of Genetics and Microbiology directed by Professor Michel Jacquet, whose hospitality is gratefully acknowledged.

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