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Unified mechanism for relay and oscillation of cyclic AMP in *Dictyostelium discoideum*

(slime molds/cell differentiation/adenylate cyclase/allosteric model/dissipative structures)

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ABSTRACT **A modified version of an allosteric model for adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] previously analyzed for sustained oscillations of adenosine 3′:5′-cyclic monophosphate (cAMP) in *Dictyostelium discoideum* [Goldbeter, A. (1975) *Nature* 253, 540–542] is examined to see whether it can account for the relay of cAMP pulses. Oscillations occur around a nonequilibrium, unstable stationary state when system parameters are in a certain domain. It is found that relay can occur outside this domain, in a restricted set of parameter values for which the solution ultimately tends to a stable steady state. A suprathreshold level of extracellular cAMP is needed to elicit relay which consists in a pulsatory synthesis of intracellular cAMP. Theoretical predictions are compared with the results of experiments on cAMP relay and oscillation in aggregation-competent cells of *D. discoideum*. The model suggests an explanation for the emergence of aggregation centers and for a sequence of developmental events observed in interphase amoebae.**

The cellular slime mold *Dictyostelium discoideum* is a major model for the study of development (1, 2). When deprived of nutrients, amoebae of this species aggregate around centers by a chemotactic response to adenosine 3′:5′-cyclic monophosphate (cAMP), and further develop into multicellular fruiting bodies (3). The process of aggregation has a periodicity of several minutes. Successive "steps" of inward amoeboid movement appear to propagate outward from the center toward the periphery of the aggregation territory.

It has long been suggested that aggregation centers autonomously release the attractant in periodic fashion. The other cells respond to attractant stimulation by moving towards its source and by relaying the signal (4–8). In addition to their role as chemotactic signals, periodic pulses of cAMP stimulate amoeboid differentiation (9, 10).

Both the relay (11, 12) and the autonomous oscillation (13, 14) of cAMP have been demonstrated experimentally in suspensions of *D. discoideum* cells. We show here that oscillation and relay can be explained by a unified model based on regulatory mechanisms reported in the literature, the two different qualitative behaviors corresponding to two different parameter regimes. Our work can be regarded as extending Goldbeter's demonstration (15) that oscillation can be generated by the intracellular regulation (16) of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1]. Here, we consider a compartmental model in which control of intracellular cAMP synthesis by external cAMP is analyzed explicitly.

The unified mechanism for relay and oscillation of cAMP suggests an explanation for the emergence of aggregation centers and for a temporal sequence of developmental events observed during interphase in *D. discoideum*.

Abbreviation: cAMP, adenosine 3′:5′-cyclic monophosphate.

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RESULTS

In an attempt to capture the essential biochemical mechanisms responsible for oscillation and relay, let us consider the activating effect of extracellular cAMP on adenylate cyclase (17). This activation follows the binding of cAMP to a receptor that might be specific for relay (18). The simplest assumption one can make is that the cAMP receptor on the cell surface is a regulatory part of adenylate cyclase; such an assumption is consistent with the finding that the catalytic site of this membrane-bound enzyme faces the interior of the cell (19). We thus consider the following three concentration variables in a basic compartmental model (see Fig. 1):

$$\alpha, \text{ intracellular ATP;}$$

$$\beta, \text{ intracellular cAMP;}$$

$$\gamma, \text{ extracellular cAMP.} \qquad [1]$$

Here the actual concentrations are normalized by dividing them respectively by K_S, K_P, and K_P, where K_S and K_P are the Michaelis constant of adenylate cyclase for ATP and the dissociation constant of complexes formed by the enzyme with cAMP at the regulatory (receptor) site.

The time variation of the metabolite concentrations in the model is governed by the following equations:

$$d\alpha/dt = v - \sigma\phi$$

$$d\beta/dt = q\sigma\phi - k_t\beta$$

$$d\gamma/dt = (k_t\beta/h) - k\gamma$$

in which

$$\phi = \alpha(1+\alpha)(1+\gamma)^2/[L + (1+\alpha)^2(1+\gamma)^2]. \qquad [2]$$

Here v denotes a constant ATP input rate, divided by K_S; σ denotes the maximum adenylate cyclase activity, divided by K_S; $q = K_S/K_P$; k_t measures the assumed proportionality of intracellular cAMP transport rate to intracellular cAMP concentration; the dilution factor h is the ratio of extracellular fluid volume to cell volume in the experiments with cell suspensions; k measures the assumed linear rate at which extracellular cAMP is destroyed by the membrane-bound (20) and extracellular (21) forms of phosphodiesterase; L denotes the allosteric constant of adenylate cyclase.

It is assumed for definiteness that adenylate cyclase is an allosteric dimer obeying the concerted model of Monod *et al.* (22), with exclusive binding to the R state. A quasi-steady-state hypothesis for the enzymatic forms has been used in the derivation of Eqs. 2. We note that we have found no essential difference in behavior if transport is proportional to $(\beta - \gamma)$, or takes a Michaelian form.

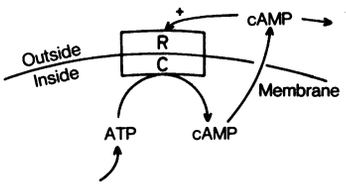

FIG. 1. Compartmental model for relay and oscillation of cAMP. Capitals R and C refer to the receptor and to adenylate cyclase, respectively. The + sign indicates that binding of cAMP to the receptor results in cyclase activation (see details in *text*).

We can view the situation somewhat differently, by concentrating our attention on the interior of the cell. It has been shown (15) that the activation of adenylate cyclase by 5′-AMP, which is one of the regulatory loops found experimentally by Rossomando and Sussman in *D. discoideum* (16), is sufficient to give rise to oscillatory cAMP synthesis. It is noteworthy that with the variable identifications,

$$\alpha, \text{ intracellular ATP;}$$

$$\beta, \text{ intracellular cAMP;}$$

$$\gamma, \text{ intracellular 5′AMP;} \qquad [3]$$

the governing equations remain those of Eqs. 2, with $h = 1$. Now, however, constants k_t and k relate, respectively, to the phosphodiesterase and 5′-nucleotidase reactions, while the dissociation constant K_P refers to complexes formed by adenylate cyclase with 5′-AMP.

Oscillations also occur if intracellular cAMP directly activates adenylate cyclase because the governing equations are then identical with those describing the phosphofructokinase reaction; the latter enzyme is responsible for glycolytic oscillations observed in yeast and muscle (23). Moreover, oscillations can occur when adenylate cyclase is simultaneously subjected to positive feedback by intracellular and extracellular cAMP. Thus, the biochemical oscillator can, in principle, be solely intracellular or extracellular; it can also be intra- and extracellular at the same time. As discussed by Gerisch *et al.* (24), the two latter mechanisms are the most likely on experimental grounds. We show below that only the externally controlled mechanisms are capable of relay.

Eqs. 2 admit a single stationary state $(\alpha_0,\beta_0,\gamma_0)$, with $\beta_0 = qv/k_t$, and $\gamma_0 = qv/hk$. The steady-state substrate concentration, α_0, is obtained by solving the second degree equation derived from the relation $v = \sigma\phi$. As in ref. 15, it can be shown that there is a range of parameters for which Eqs. 2 produce sustained oscillations of an amplitude and frequency that are uniquely determined by the particular parameter set chosen. One such oscillation is depicted in Fig. 2. In Fig. 3, we illustrate the presence of an oscillatory region in a parameter space composed of ATP injection rate (v), adenylate cyclase activity (σ), and phosphodiesterase activity (k). Outside the oscillatory domain, all solution trajectories generally lead to a unique, stable steady state. An exception occurs when the ATP injection rate exceeds the maximum cyclase activity, in which case substrate accumulates continually.

By numerical integration of the system of differential Eqs. 2, we attempted to find parameter triplets just outside the oscillatory domain that provide a sizable overshoot in adenylate cyclase activity and hence yield a pulse of cAMP as a response to an external cAMP signal. Many attempts failed: the system responded to an initial elevation of external cAMP with at most a slight increase in cAMP before reverting to its steady state.

Results of a successful attempt are shown in Fig. 4. Here the system is operating under a set of conditions that are identical

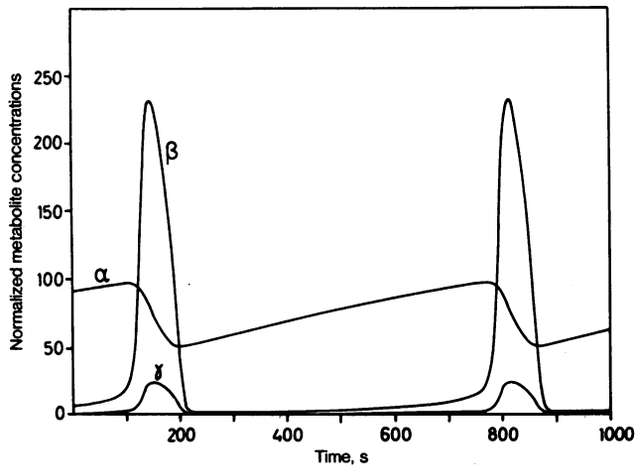

FIG. 2. Autonomous periodic oscillations of normalized concentrations of intracellular ATP (α), intracellular cAMP (β), and extracellular cAMP (γ). The curves are obtained by integration of Eqs. 2, for $v = 0.1\ s^{-1}$; other parameter values are given in the legend to Table 1.

to those of Fig. 2, except that the ATP injection rate v is decreased by a factor of 2.5, dropping the system outside the oscillatory domain near point A of Fig. 3. Just as in the cell suspension experiments (11, 12, 14, 25), there is a large amplification of the initial external cAMP signal, the peak in external cAMP lags behind the internal cAMP peak, and the waveform and amplitude of the relay are quite similar to those of the oscillation.

Both the time course and the amplitude of the relayed pulse depend on the amplitude of the external cAMP signal, as shown in Fig. 5. A noteworthy feature of the model is that relay takes place only above a critical value of the initial level of external cAMP (γ_i). The transition is extremely steep, and occurs for values of γ_i between 1.846 and 1.848 in the case considered. The amplitude of the internal pulse remains practically constant when γ_i exceeds the threshold concentration.

As to the time course of the relay response, the data in Fig. 5 show that the time to the maximum of the peak decreases exponentially as the amplitude of the initial signal increases.

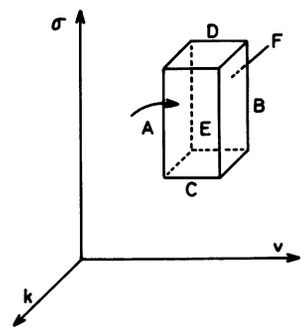

FIG. 3. The rectangular parallelepiped schematizes a domain in which autonomous oscillations can be found in a parameter space composed of ATP injection rate (v), adenylate cyclase activity (σ), and phosphodiesterase activity (k). The letters A through F represent regions exterior to the six faces of the parallelepiped in which relay capability was sought. The heavy arrow entering the parallelepiped through A represents a slow developmentally controlled parameter variation that could bring about the observed transitions between cells whose cAMP production is always small (inert cells), amplifies cAMP signals (relay cells), or spontaneously and periodically reaches significant levels (autonomous oscillators).

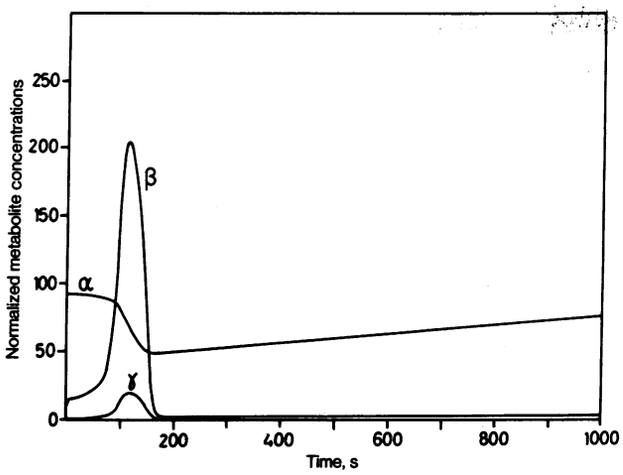

FIG. 4. Relay of a cAMP signal. The situation is that shown in Fig. 2 except that ATP injection rate v is decreased by a factor of 2.5. Initially, intracellular ATP (α) and intracellular cAMP (β) have their steady-state values. Exterior stimulation is simulated by setting extracellular cAMP at a value that is two times its steady-state level (the steady-state values are $\alpha = 92.366$, $\beta = 10$, and $\gamma = 1$).

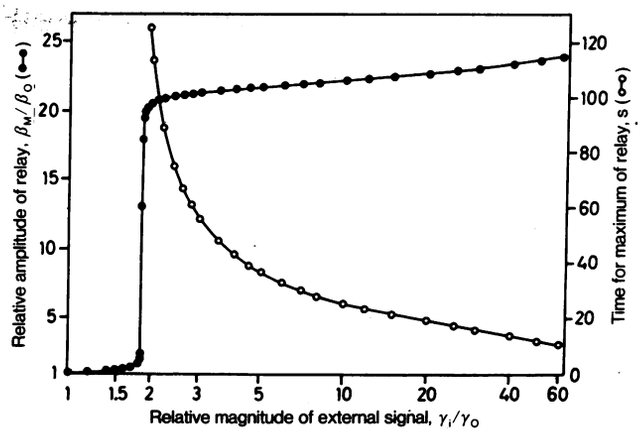

FIG. 5. Dependence of the relay response on the amplitude of the external cAMP signal. The curves give the amplitude and the time course of relay as a function of the ratio (γ_i/γ_0), where γ_i and γ_0 are the initial and steady-state values of the normalized concentration of external cAMP (in the case considered, $\gamma_0 = 1$), respectively. The amplitude of relay is given as the maximum of the intracellular cAMP peak, β_M, divided by the steady-state level β_0. The second curve shows the time at which the maximum of the peak, β_M, is reached after the initial stimulation. Parameter values are those of Fig. 4.

By contrast, the half-width of the relayed pulse is practically independent of γ_i; in the case of Fig. 5, it remains in the range 51–55 s as the ratio (γ_i/γ_0) passes from 1.85 to 60. As is the case for sustained oscillations (15), all characteristics of relay such as the amplitude, half-width and time course depend on the various parameters of the model, such as v, k, or σ.

In many biological systems, the qualitative agreement that we have mentioned would be the best matching one can expect between theory and experiment. The slime mold system is unusual in that there is a great deal of quantitative data. A number of points of quantitative comparison head the rows of Table 1. We see in Table 1 that the results of Figs. 4 and 5 provide semiquantitative agreement with experiment.

There exists an alternative mechanism for relay in the model. In region B of Fig. 3, a pulse in β can be obtained when the initial value of β and γ is assumed to be lower than the steady-state value. This makes no sense for system **1** wherein γ corresponds to extracellular cAMP, because relay is initiated by an increase in this chemical. However, for system **3** this assumption would be appropriate provided the external pulse caused a rapid decrease in internal cAMP and/or 5′-AMP. Nonetheless, several considerations indicate that such a mechanism is unlikely on experimental grounds. One reason is that it gives rise to pulses whose maximum is usually three times larger than the steady-state value; this amplification is too small to account for the observations listed in Table 1.

By contrast, amplification factors of the order of 20 or more are found in system **1**, as shown in Fig. 4. It should be stressed that we have found such a significant relay only for a restricted set of parameter values in region A of Fig. 3, in the immediate vicinity of the oscillatory domain. On the other hand, although we considered a few parameter sets in each of the neighborhoods A–F depicted in Fig. 3, our search of parameter space was hardly exhaustive. Such a search is probably not worthwhile until experiments have increased confidence that the main qualitative features of the system have been identified.

DISCUSSION

Experimental studies have shown (28) that several hours after the onset of interphase, full chemotactic response to cAMP appears first, then relaying of cAMP pulses begins, followed by autonomous generation of the periodic cAMP signal.

Table 1. Comparison of relay in the model and experiment*

Relay	Relative amplitude†	Time for maximal relay (s)	Half width (s)	Delay between extra- and intracellular cAMP (s)	Min[ATP]/ max[cAMP]$_i$	Max[cAMP]$_i$/ max[cAMP]$_e$	Steady-state ratio [ATP]/[cAMP]$_i$
Experiment	10–25	100–120	60	30–40	50	20–50	10^3
Model	20	113	53	3	25	10	9.24×10^2

* Experimental data are taken from Gerisch *et al.* (11, 14, 25); we have calculated mean orders of magnitude for the concentration ratios on the basis of the following figures: maximum intracellular cAMP concentration during relay (max[cAMP]$_i$): 10–25 μM; maximum extracellular cAMP concentration (max[cAMP]$_e$): 0.5 μM; basal (steady-state) level of ATP and of intracellular cAMP: 1–2 mM and 1–2 μM (upper limit), respectively. The theoretical data are obtained for the parameter values of Fig. 4: $v = 0.04\ s^{-1}$, $\sigma = 1.2\ s^{-1}$, $k = k_t = 0.4\ s^{-1}$, $L = 10^6$, $q = 100$, $h = 10$. Although arbitrary, these parameter values are in a physiological range; the value of h is of the order of the dilution factor in the experiments (11, 14). Agreement is reached with the observed nucleotide levels when constants K_S and K_P are taken as 10 μM and 0.1 μM. Then the steady-state concentrations of ATP, intracellular cAMP, and extracellular cAMP are: 0.924 mM, 1 μM, and 0.1 μM, respectively. The value $K_P = 0.1$ μM is of the order of that found for the dissociation constant of the cAMP receptor in *D. discoideum*. Experimental values for this constant are in the range 10 nM–0.1 μM (18, 26, 27).

† Relative amplitude of relay is given as the maximum of the intracellular cAMP peak divided by the steady-state level of intracellular cAMP.

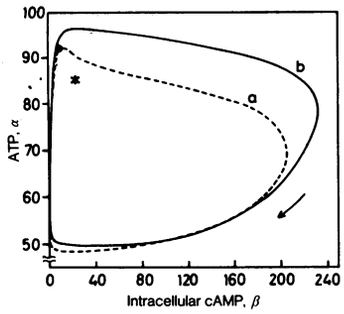

FIG. 6. A trajectory for relay and for oscillation in the (α,β) phase plane. In curve a, relay starts at the steady-state values of α and β (●), following a slight increase in the third variable γ. Curve b shows a limit cycle enclosing the unstable steady state (*). The arrow indicates the direction of movement. The situations described by curves a and b are those of Figs. 4 and 2, respectively.

With respect to the generation of cAMP signals, *D discoideum* cells, in the course of their development, thus can make two transitions; from inert cells to relay cells, and from relay cells to autonomous oscillators. The shift from inert cells to relay cells corresponds to a shift in the phase space from a situation in which solution trajectories head fairly directly for the final steady state, which is a stable node or focus, to one in which the trajectories only reach the final state after traversing an extensive loop (Fig. 6, curve a). Note that this shift does not correspond to a shift in the solutions' topological character.

The relay-oscillator transition occurs when parameters are such that one loop closes back upon itself to form a limit cycle around the singularity (Fig. 6, curve b). This is a topological change. From the point of view of irreversible thermodynamics, the oscillations represent a temporal dissipative structure because they occur around a nonequilibrium unstable steady state (29). The similarity of the two phase-plane trajectories shown in Fig. 6 conforms with the similarity in waveform and amplitude observed for relay and oscillation of cAMP in the experiments with cell suspensions (11, 14, 25). Moreover, the link predicted theoretically between the time for maximal relay and the period of oscillation is close to that observed in the experiments: for closely related values of the parameters, the two quantities in the model are of the order of 100 s and 600 s, respectively; these data compare with the experimental mean values of 100 s and 420 s (11–14).

Our unified mechanism for relay and oscillation suggests an explanation for the two transitions described above. The explanation rests on the supposition that some of the parameters of the system change slowly with time. Indeed, it has already been shown in *D. discoideum* that the activity of certain enzymes appearing in the model, such as phosphodiesterase (26, 30) and adenylate cyclase (31), is under developmental control and varies during interphase. The observed developmental sequence can be explained by any combination of parameter changes (symbolized by the heavy arrow in Fig. 3) that brings the system from well outside the oscillatory domain (inert cells) to the vicinity of this domain in one of the locations where relay is possible, and finally, for cells that become oscillators, to the interior of the domain.

A study of the development of the relaying competence in a population of *D. discoideum* cells has shown that there is a distribution of metabolic states among interphase amoebae (32). Given such a distribution, we can analyze the biochemical nature of aggregation centers in terms of the adenylate cyclase model. We identify center-founding cells in aggregation territories with those cells that are the first to reach the regime of sustained oscillations of cAMP. Note that an ability of amoebae to evolve into and out of the instability domain of the cyclase reaction also can explain how new aggregation centers may emerge in a population from which center-founding cells are continuously removed, and how the capacity for autonomous oscillation disappears after a few hours.

In favor of our hypothesized unified mechanism for relay and oscillation of cAMP is the observation that both phenomena have the same temperature dependence in *D. discoideum* (33). That association of relay capability with oscillation might be a widespread biological phenomenon is suggested by the generality of the mechanisms employed in our theoretical discussions. Furthermore, a similar association between pulsatory amplification of initial perturbations, sometimes referred to as excitability, and oscillation has been demonstrated for nonenzymatic chemical systems, both in a model (34) and in experiments (35, 36). Generation of a pulse as a result of initial displacement from steady-state conditions has also been noted in models for membrane excitation (37, 38). These models involve transitions between multiple stationary states, in contrast with the present situation where the relaying system admits a single steady state.

With respect to the experiments on relay in *D. discoideum*, the present analysis provides a qualitative explanation for three observations not listed in Table 1. First, the all-or-none response to the external cAMP signal, illustrated in Fig. 5, accounts for the phenomenon that aggregation-competent cells of *Dictyostelium* relay the external cAMP signal only when the latter exceeds a threshold (6). The model provides an explanation of this effect at the molecular level; the data for the external signal in Fig. 5 are of the magnitude of the pulses applied in the experiments (11, 17, 25) when the dissociation constant K_P is taken as 0.1 μM (see the legend to Table 1). Given this value for K_P, the data of Fig. 5 correspond to a threshold for relay close to 0.2 μM. Such a value is precisely that obtained for this threshold by H. Parnas and L. A. Segel (manuscript submitted for publication) in computer simulations of *D. discoideum* aggregation, when cAMP secretion by signaling cells is assumed to last one minute. The existence of a refractory period for relay (5–7) is also a property of the model. Indeed, the system which has produced a cAMP pulse cannot generate any further pulse until the substrate has been replenished to a critical level.

The third remark relates to the observation from cell suspension experiments that a small peak in intracellular cAMP usually follows the external stimulation by a few seconds (11, 25). This puzzling behavior could be explained by noticing that when a cAMP pulse is given to a suspension, some cells are likely to "see" this pulse as larger than the final concentration it will reach in the solution after complete homogenization of the latter by stirring. As shown in Fig. 5, the time course of the relay drops to about 10 s when the amplitude of the external signal is larger than the threshold by a factor of 30. It is thus conceivable that the few cells first hit by the pulse would relay after such a short time; their response would be limited by homogenization of the suspension and would appear as a small peak of intracellular cAMP following external stimulation. If this explanation is valid then this small initial peak of intracellular cAMP should diminish with increasing stirring of the suspension. The theoretical predictions on the time course, amplitude, and half width of relay as a function of the initial signal could also be checked in cell suspension experiments.

A last point of comparison with experiment concerns the time variation of ATP in the course of relay and oscillation. As indicated in Figs. 2 and 4, the theoretical amplitude of ATP variation in both cases is usually less than that of intra- and

extracellular cAMP by two orders of magnitude. For values of the parameters close to those of Fig. 2, the periodic variation of α can be restricted to a range of $\pm 10\%$ around the mean. This could account for the observation that the total intracellular ATP remains practically constant during relay and oscillation (25). Some kind of compartmentation of ATP could also explain why local oscillations would be masked in the assay of the total ATP pool.

It should be stressed that the behavior of the model qualitatively changes when the ATP level is maintained constant. Eqs. 2 then reduce to two differential equations which do not admit any periodic solution. Instead, the model may exhibit transitions between multiple steady states. For a given set of parameter values, the system may then function in either one of two stable states corresponding to different activities of adenylate cyclase. Evolution to one state or to the other depends on initial conditions. It thus appears that some variation of ATP is needed in the model to generate sustained periodicity in cAMP synthesis, when no additional feedback is considered.

In conclusion, we realize that our ideas will doubtlessly have to be modified to some extent as more is learned about transport, about the allosteric and regulatory properties of adenylate cyclase in *D. discoideum*, and about the process by which binding to the cAMP receptor results in adenylate cyclase activation. Nonetheless, our simulations indicate that major components of the signal system may well have been identified and that two successive developmental transitions may be brought about by a continuous change in one or two parameters.

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