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Feedback induced biphasic response in the chemotaxis pathway of *Dictyostelium*

Liu Yang and Pablo A. Iglesias

Abstract— **Upon uniform stimulation by the chemoattractant cAMP, *Dictyostelium* cells exhibit a biphasic response. Initially, a rapid rise in the localized accumulation of PI(3,4,5)P_3 is accompanied by an increase in actin polymerization. This response peaks approximately 5–10 seconds after stimulation. It is then followed by a slower, smaller phase peaking approximately two minutes after the stimulus. Until recently, the nature of this biphasic response has been poorly understood. Moreover, the origin for the secondary phase is unknown. In this paper we conjecture the existence of a feedback path between the response and stimulus. Using a mathematical model of the chemoattractant-induced response in cells, and standard tools from control engineering, we show that positive feedback may elicit this second peak. Finally, we discuss some of the literature that suggests the possible existence of this loop.**

I. INTRODUCTION

Chemotaxis, the directional migration in response to a spatial gradient of chemical stimuli, is a central process found in many cells. Single-cell organisms, such as bacteria and amoebae rely on chemotaxis to search for nutrients [1], [2]. Multicellular organisms also depend on chemotaxis at several stages of development. For example, cells of the immune system, including neutrophils and fibroblasts, can use chemotaxis to search for pathogens [3].

Many cells that carry out chemotaxis exhibit adaptation to uniform chemoattractant stimuli. An adapting cell, when stimulated by a constant dose of chemoattractant, exhibits a large transient response. In time, however, the cell's response returns to its prestimulus levels. This "perfect" adaptation amounts to a step disturbance rejection, together with a signal detection. To do so, requires integral control feedback [4]–[6].

In this paper we study the adaptation property of the cAMP-mediated response in the social amoebae *Dictyostelium* [7], [8]. Individual *Dictyostelium* are usually found in soil feeding on bacteria. However, when faced by harsh environmental conditions, such as the loss of all nutrients, they undergo a fascinating developmental process that results in the development of cellular machinery that allows them to synthesize, secrete, and detect adenosine 3',5'-monophosphate (cAMP). In about six hours, and relying on chemotaxis, up to 100,000 cells will congregate and form a multicellular mound. Subsequent morphogenesis allows a fraction of the cells to survive by sporulation.

L. Yang and P.A. Iglesias are with the Department of Electrical and Computer Engineering, The Johns Hopkins University, 3400 N. Charles Street, Baltimore, MD 21218

The binding of extracellular cAMP to the cAMP-receptors (CAR1) leads to the activation of several downstream processes [9]; see Fig. 1. The receptor is coupled to a heterotrimeric G-protein consisting of three subunits. Upon stimulation, the α subunit dissociates from the β and γ subunits. This dissociation allows the β and γ subunits to activate a series of events involving a cascade of phosphoinositides. In particular, phosphorylation of the lipid PI(4,5)P_2 (phosphatidylinositol 4,5-bisphosphate) by the kinase PI3K (phosphoinositide-3-kinase) to form PI(3,4,5)P_3 (phosphatidylinositol 3,4,5-trisphosphate) and its dephosphorylation by the phosphatase PTEN (phosphatase and tensin homolog) are known to be crucial to the response of *Dictyostelium* cells to chemoattractant. Together, these enzymes elicit a localized accumulation of PI(3,4,5)P_3 at the leading edge. This elevated concentration of PI(3,4,5)P_3 induces a concomitant increase in actin polymerization, which is associated with pseudopod formation and motility.

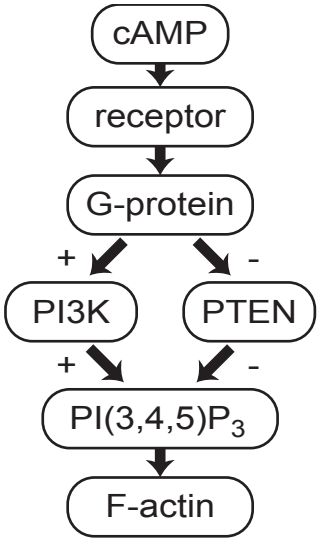

Fig. 1. **Signaling pathway regulating chemotaxis in *Dictyostelium*.** cAR1, the cAMP receptor, is a G-protein coupled receptor. Upon stimulation, the subunits of the G-protein dissociate and are able to signal downstream effectors. In *Dictyostelium*, this includes the two enzymes PI3K and PTEN. They serve to synthesise PI(3,4,5)P_3 at the cell membrane and the latter is associated with F-actin polymerization. Biochemical details of this pathway are described in [9]. A detailed mathematical model is found in [18].

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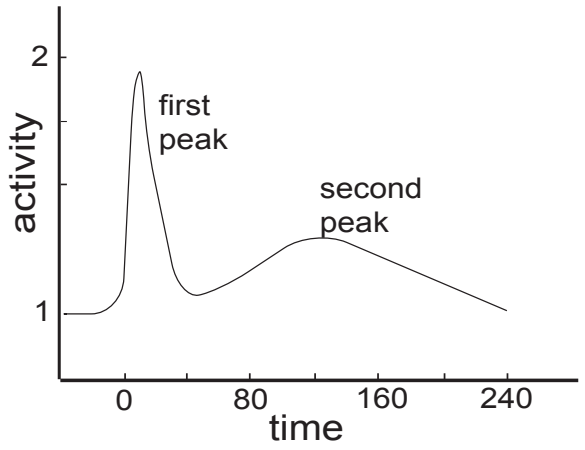

Fig. 2. **Existence of a second peak.** This graph shows the typical biphasic response observed in both $PI(3,4,5)P_3$ accumulation and actin polymerization in *Dictyostelium* after uniform chemoattratant stimulation. The data here is representative of experimental data from [14].

A. Can feedback explain the second peaks in the cAMP-mediated response?

Experimental studies of actin polymerization have shown that uniform cAMP stimulus generates a biphasic response [11]–[13] and that this also closely parallels a similar biphasic response in the accumulation of $PI(3,4,5)P_3$ at the plasma membrane [14]. As seen in Fig. 2, the uniform stimulus first induces a large response in actin/$PI(3,4,5)P_3$, peaking at levels approximately 2 fold higher than prestimulus values within 5 seconds. This first response disappears between 25–30 seconds. A slower, much smaller ($\approx 25\%$ higher than the prestimulus levels) second peak appears, peaking somewhere between 60–180 s after the original stimulus [14].

Currently, the origin of the second peak is unknown. It has been observed that the second peak correlates with the capacity of cells to extend lateral pseudopods in response to changes in the direction of a chemotactic gradient [14].

The existence of this secondary peak has led some biologists even to suggest that *Dictyostelium* cells "does not fully adapt to constant cAMP levels" [13].

In this paper we use tools from control engineering to study these second peaks. We conjecture the existence of a feedback connection between the response and stimulus. We then show that this feedback path could account for the biphasic response seen in actin/$PI(3,4,5)P_3$.

The rest of the paper is organized as follows. We begin by presenting a general model that has been used to explain the adaptation property observed in *Dictyostelium*. We also present a series of variations that differ slightly in the way that their components are interconnected, but which maintain the perfect adaptation property. We then present our main results in which linearized versions of these models are analyzed using root locus techniques. Finally, we discuss the implications of these models and present some concluding remarks.

II. MATHEMATICAL MODELS

Here we describe a model that has been suggested to explain the adaptation mechanism found in *Dictyostelium* [15], [16], [18]. We begin by postulating the existence of a *response regulator*, that can exist in both active (R) and inactive forms, and whose total concentration is given by R_T. This response could represent, for example, the binding sites for PI3K and PTEN that exist on the cell membrane.

We assume that the activation and inactivation are regulated by a pair of processes. The excitation (E) process induces an increase in the level of the response, whereas the inhibition (I) process lowers the response. Using mass action dynamics, an equation for the system is

$$\frac{dR(t)}{dt} = -k_{-r}I(t)R(t) + k_r[R_T - R(t)]E(t) \tag{1}$$

The steady-state level of the response is given by

$$\lim_{t\to\infty} R(t) = \frac{\frac{E(\infty)}{I(\infty)}}{K_R + \frac{E(\infty)}{I(\infty)}} R_T \tag{2}$$

where $K_R = k_{-r}/k_r$. Note that it is the *ratio* of excitation over inhibition that determines the steady-state concentration of active receptors. Now, assume that these enzymes are, in turn, regulated by the external signal C which is proportional to chemoattractant concentration:

$$\frac{dE(t)}{dt} = -k_{-e}E(t) + k_e C(t) \tag{3}$$

$$\frac{dI(t)}{dt} = -k_{-i}I(t) + k_i C(t) \tag{4}$$

Suppose that the cell is initially at steady-state with cAMP concentration $C_0 \neq 0$ and that, at time $t = 0$, this is changed to $C_1 \neq 0$. The enzyme concentrations obey

$$E(t) = E_1 + e^{-k_{-e}t}(E_0 - E_1)$$
$$I(t) = I_1 + e^{-k_{-i}t}(I_0 - I_1)$$

where $E_j = C_j K_E$, $I_j = C_j K_I$, for $j = 0, 1$ and

$$K_E = k_e/k_{-e}, \quad \text{and} \quad K_I = k_i/k_{-i}.$$

These expressions can then be replaced in (2) to show that the steady-state concentration of active response regulators is independent of cAMP concentration and equals the prestimulus level. It follows that the system is rejecting the step changes in cAMP perfectly.

Some observations about this system can be made. First, in analyzing the steady state behavior of the system, we have assumed stability. It is possible to show that if the input satisfies $C(t) \geq \varepsilon > 0$, then the system is uniformly bounded-input, bounded-output stable [19]. If we assume that the input is constant $C > 0$, then the system is uniformly exponentially stable.

Second, since perfect adaptation amounts to the rejection of step disturbances, we expect that this system can be

expressed with an integrator in feedback; this is indeed the case [8], [19].

Third, though it is not relevant for the analysis of this paper, this system can be made to exhibit static spatial sensing if we allow for a diffusive inhibitor. That is, whenever a spatially inhomogeneous C is applied, the response will also exhibit this property [15], [16].

Finally, note that to generate step increases in response to the chemotactic source — as is seen with the membrane-bound concentration of PI3K — we require that transiently, the increase in $E(t)$ be larger than that of $I(t)$. If this is the case, $C(t)$ will also increase transiently causing an increase in the concentration of $R(t)$. This is guaranteed, provided that $k_{-e} > k_{-i}$. Alternatively, a step decrease in the response — as is seen with the membrane-bound concentration of PTEN — is obtained when $k_{-e} < k_{-i}$.

The model presented here has been tested experimentally by measuring the response of *Dictyostelium* cells to varying combinations of temporal and spatial stimuli [17]. Strong agreement with the model has been observed [18].

A. Other models

Typically we assume that $R_T \gg R(t)$ so that we can replace

$$k_r[R_T - R(t)] \approx k_r R_T$$

The combination of (1), (3) and (4) can now be written as:

$$\Sigma_1 = \begin{cases} \dfrac{dE(t)}{dt} & = -k_{-e}E(t) + k_e C(t) \\ \dfrac{dI(t)}{dt} & = -k_{-i}I(t) + k_i C(t) \\ \dfrac{dR(t)}{dt} & = -k_{-r}I(t)R(t) + k_r R_T(t) \end{cases}$$

provides a simple mechanism for achieving perfect adaptation. Several modifications are possible. For example, the following two systems

$$\Sigma_2 = \begin{cases} \dfrac{dE(t)}{dt} & = -k_{-e}E(t) + k_e C(t) \\ \dfrac{dI(t)}{dt} & = -k_{-i}I(t) + k_i E(t) \\ \dfrac{dR(t)}{dt} & = -k_{-r}I(t)R(t) + k_r R_T C(t) \end{cases}$$

and

$$\Sigma_3 = \begin{cases} \dfrac{dE(t)}{dt} & = -k_{-e}E(t) + k_e C(t) \\ \dfrac{dI(t)}{dt} & = -k_{-i}I(t) + k_i E(t) \\ \dfrac{dR(t)}{dt} & = -k_{-r}I(t)R(t) + k_r R_T E(t) \end{cases}$$

both achieve perfect adaptation. They differ from Σ_1 only in the roles that the excitation and source take as the "inputs" to either the inhibitor or response equations. For example, in Σ_2, the inhibitor is stimulated by E rather than C. In Σ_3, the response is stimulated by E rather than C. Since, at steady-state, both these signals are proportional to each other, there is no change in the fact that the level of C does not show up in the steady-state concentration of R. This ability to replace E and C interchangeably suggests that the excitation subprocess can be eliminated completely, leading to the following second order system [20].

$$\Sigma_4 = \begin{cases} \dfrac{dI(t)}{dt} & = -k_{-i}I(t) + k_i C(t) \\ \dfrac{dR(t)}{dt} & = -k_{-r}I(t)R(t) + k_r R_T C(t) \end{cases}$$

III. RESULTS

We consider the possibility that the observed second peak may arise from a feedback between the response in these systems and the input. We can test this hypothesis through a root locus analysis on the transfer functions of the linearized systems. Specifically, we look for dominant complex-valued poles.

Before analyzing the response of these systems under the effects of feedback, we note that through a change of variables, we can reduce the system Σ_1 to a unitless system [15]. In particular, denoting $r = R/R_T$ as the fraction of active response regulators, $\tau = k_{-e}t$ as the dimensionless time, and $e = (k_r/k_{-e})E$, $c = (k_e k_r/k_{-e}^2)C$ as dimensionless concentrations, the system reduces to:

$$\begin{aligned} \frac{de}{d\tau} &= -(e-c) \\ \frac{di}{d\tau} &= -\alpha(i-c) \\ \frac{dr}{d\tau} &= -\beta i r + e \end{aligned}$$

where $\alpha = k_{-i}/k_{-e}$ and

$$\beta = [(k_{-r}/k_r)(k_{-e}/k_e)]/(k_{-i}/k_i).$$

With this formulation we note that, for a given fixed input s_0, the equilibrium satisfies

$$e_{ss} = i_{ss} = c_0, \qquad r_{ss} = \frac{1}{\beta}$$

Linearizing this system, with input c and output $y = r$, about this equilibrium generates the transfer function

$$G_1(s) = (1-\alpha)\frac{s}{(s+\alpha)(s+1)(s+\gamma)}$$

where $\gamma = c_0\beta$.

From this transfer function we observe the presence of a zero at $s = 0$, which guarantees the asymptotic rejection of steps. We also see that, if $\alpha = 1$, changes in the external signal are not detected because the excitation and inhibition processes are identical and their effects on the equation for r cancel each other. Thus, the external signal has no effect on the response. If $\alpha > 1$ the inhibitor is faster than the excitation leading to a negative response from a positive input.

A. *Effects of feedback*

We now consider the effects of feedback on the response of this system. Supppose that the stimulus s consists of both a contribution from the external source l and feedback from r:

$$s = l + kr$$

where k is the feedback gain.

From a simple root locus analysis (Fig. 3) we observe that, for both positive and negative feedbacks, a range of gains will guarantee complex-valued closed-loop poles. When negative feedback is used, the two fastest poles join to form a complex pair while the slower pole remains real and migrates towards the zero. This results in slower responses as the (absolute value) of the feedback gain is increased. Thus, we might expect that the contribution of the complex poles will be overshadowed by the slower poles and that no biphasic response would be observed. We found this to be the case.

Under positive feedback, it is the two slower poles that join and form a complex conjugate pair, before migrating towards the right-hand plane, while the fast (real) pole moves towards $-\infty$. We now expect that the complex poles will dominate and that they may elicit a biphasic response.

B. *Effect of feedback on* Σ_2–Σ_4

We now proceed to analyze systems Σ_2–Σ_4 as in Σ_1. First we non-dimensionalize, and then obtain the corresponding equilibria. Finally, we linearize these systems about these equilibria. The corresponding transfer functions are:

$$G_2(s) = \frac{s(s+\alpha+1)}{(s+\alpha)(s+1)(s+\gamma)}$$
$$G_3(s) = \frac{s}{(s+\alpha)(s+1)(s+\gamma)}$$
$$G_4(s) = \frac{s}{(s+\alpha)(s+\gamma)}$$

As expected, all transfer functions have a zero at $s = 0$ that guarantees step disturbance rejection. However, these transfer functions differ from $G_1(s)$ in two respects. We observe that no value of α will generate an identically-zero output as was seen in $G_1(s)$. Moreover, in contrast to $G_1(s)$, it is impossible to generate a negative response to a positive step.

By comparing the location of the open-loop poles and zeros of this transfer function with those of $G_1(s)$ we can predict the behavior under feedback. Note that $G_1(s)$ and $G_3(s)$ differ only with respect to the transfer function gain. Thus, by properly scaling the feedback gain the two transfer functions will generate the same root locus diagram and hence the same closed-loop behavior. $G_4(s)$ is straightforward to analyze since it has only two open-loop poles. When negative feedback is used, these poles will always be real, and hence no oscillatory behavior is to be expected. However, when $k > 0$ the root locus for this system behaves similarly to that of G_1. The only difference is the presence of a fast real pole in $G_1(s)$ that should not affect the response significantly.

Thus, the only transfer function which gives rise to a significantly different root locus than $G_1(s)$ is $G_2(s)$ because of the presence of the extra, stable, real zero at $s = -(1+\alpha)$. Note that this zero lies to the left of the two poles at α and 1, which are typically the two slower poles. If it lies completely to the left of all three poles, then complex-valued poles may arise with negative feedback, but these would be fast and the system performance would be dominated by the slow real pole that approaches the zero at $s = 0$. In contrast, if it lies to the right of the pole at γ (but still to the left of the poles at -1 and $-\alpha$) then negative feedback will never give rise to complex-valued poles.

In all cases, however, positive feedback may generate oscillatory behavior since it leads to a pair of dominant complex-conjugate poles, as in $G_1(s)$. Taking into consideration that the first peak should be approximately two times the steady state value, the best case response comes from system Σ_2, shown here in Fig. 4.

IV. DISCUSSION

Until recently, the nature of the second phase of PI(3,4,5)P_3 accumulation and actin polymerization has not been fully appreciated, possibly because it is difficult to observe unless conditions are carefully controlled [14]. Moreover, its origin is completely unknown.

In this paper we have shown, using straightforward tools from control engineering that a possible source for the biphasic response is the existence of a positive feedback loop. To do so we have used a simple model that explains the adaptive chemoattractant-induced response observed in *Dictyostelium* cells. While other models have been proposed to account for this adaptation, experiments have demonstrated that the cellular response is consistent with the models used in this paper.

Using linearizations of these models and root-locus analysis we showed that negative feedback can be used to obtain closed-loop systems with complex poles, but that these complex poles are relatively fast compared to the dominant real pole. Hence, any oscillatory contribution to the response from these poles is insignificant.

In contrast, by postulating a positive feedback connection between the response and stimulus, complex poles also arise, but these are dominant. Thus, biphasic responses can be obtained that are more reminiscent of the actin polymerization curves observed experimentally.

It should be noted here that in analyzing nonlinear systems, the order in which to apply linearization and feedback is not exchangeable. An analysis in which feedback is applied before linearization can also be carried out [21].

Having suggested the existence of a positive feedback loop, it is worth considering whether any evidence of such a loop has been reported experimentally.

In human neutrophils, it has been demonstrated that localization of PI(3,4,5)P_3 induces actin polymerization

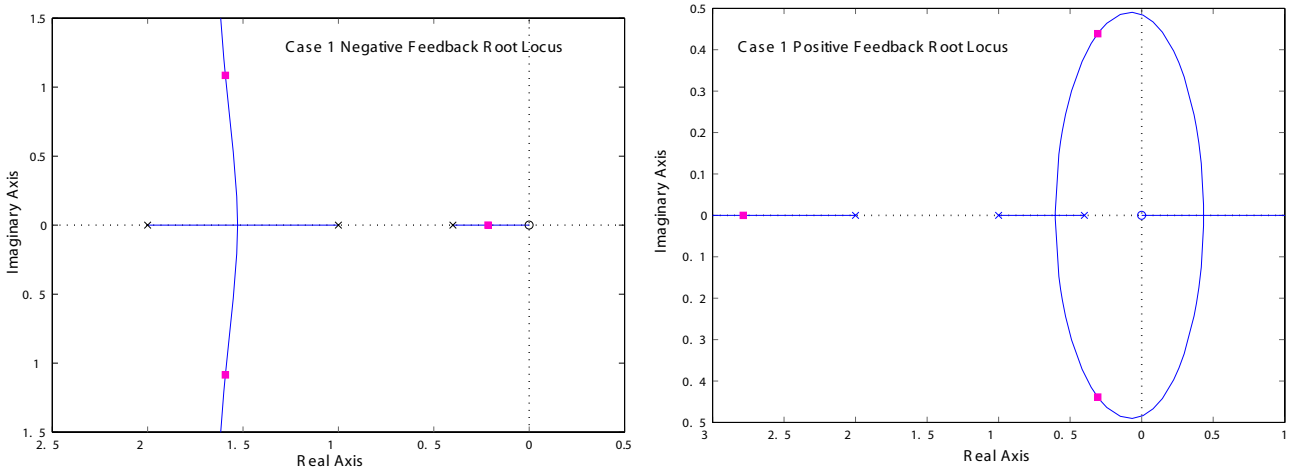

Fig. 3. **Root locus analysis.** The plots show the root locus analyses for the open loop transfer function $G_1(s)$ assuming negative and positive feedback loops. For these plots we have assumed that $\alpha = 0.4$, $\beta = 2$, $l = 1$. When negative feedback is used, the dominant pole is real. Two complex poles appear but they do not influence the step response greatly. With positive feedback, the complex poles are now the closest to the imaginary axis. This elicits an underdamped response and may explain the appearance of a second peak.

localized to the leading pseudopods. This, in turn, stimulated PI(3,4,5)P_3 synthesis, thereby completing a positive feedback loop [22]–[24]. However, experiments in *Dictyostelium* have not provided evidence for the existence of this loop [13]. In particular, in cells whenever actin polymerization has been inhibited, the second peak is still observed [13]. Nevertheless, it is still possible that the feedback is effected by PI(3,4,5)P_3 through a different intermediary.

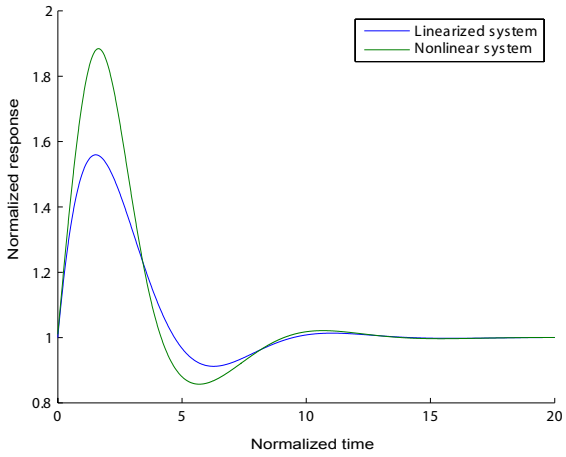

Fig. 4. **Step response.** We simulated the effect of positive feedback on systems Σ_1–Σ_4. We did this for both the original nonlinear and the linearized system. Shown is the response for Σ_2, though all four systems had approximately the same response. Parameters are $\alpha = 0.5$, $\beta = 0.6$, $k = 1$ and $l = 1.5$.

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