Final Problem Set ***Assigned:*** ***11.29.04***
Due in class ***Due:*** ***12.09.04***

Dictyostelium amoebae are free living cells with a remarkable twist: under the stress of starvation, large numbers of amoebae are able to collect together to form a single multi-cellular organism (Fig. 1). The entire process begins when starving amoebae emit pulses of the chemoattractant cAMP, inducing the surrounding cells to move in their direction and to secrete cAMP themselves. This process generates outgoing spiral waves of cAMP which direct the entire population towards the original source (Fig. 2). We will try to understand the origin of and response to these cAMP waves.

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1. Chemical kinetics of cAMP signaling

Two species of cAMP receptors exist in the *Dictyostelium* cell membrane: the 'activator' A, and the 'inhibitor' I, both of which act on a third protein R. When bound to cAMP, a pair of A molecules catalyzes the conversion of R to an active form R^*, and a pair of I molecules catalyzes the reverse reaction (Fig. 3).

(5) *a.* The initial binding of cAMP (C) to its receptors is described by:

$$A + C \underset{k_A^-}{\overset{k_A^+}{\rightleftharpoons}} AC \qquad\qquad I + C \underset{k_I^-}{\overset{k_I^+}{\rightleftharpoons}} IC\,.$$

Write down equations giving the time evolution of $[AC]$ and $[IC]$. Now assume that $[AC] << [A_{tot}]$, and $[IC] << [I_{tot}]$; let $a \equiv [AC]$, $i \equiv [IC]$, and $c \equiv [C]$. By making a convenient choice of units, show that these equations can be written in the form

$$\frac{da}{dt} = k_A^-(c-a) \qquad\qquad \frac{di}{dt} = k_I^-(c-i)\,. \qquad (1)$$

(5) *b.* The reactions involving activation and inactivation of *R* reach a rapid equilibrium:

$$R \underset{k_R^- \cdot i^2}{\overset{k_R^+ \cdot a^2}{\rightleftharpoons}} R^* .$$

The a^2 and i^2 terms arise because it takes two molecules of *AC* or *IC* to catalyze these conversion reactions. Setting $\beta = k_R^- / k_R^+$, find an expression for the rapid equilibrium value of $r \equiv [R^*]$ in terms of *a*, *i*, and $[R_{tot}]$.

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2. Positive feedback and oscillations

The molecule *R* is an enzyme known as adenylate cyclase, which in its active form catalyzes the conversion of ATP into cAMP in the cytoplasm. The presence of extracellular cAMP thus stimulates the synthesis of intracellular cAMP, which in turn is secreted into the environment, creating a positive feedback loop (Fig. 3). Let $c_1 \equiv [\mathrm{cAMP_{in}}]$; let the rate of cytoplasmic cAMP synthesis be $k_1 r$; and let the rate constant for its secretion be k_0. cAMP is continuously degraded by phosphodiesterase enzymes both inside and outside the cell, with rate constants γ_1 and γ_0, respectively. The entire network is described by the following equations:

$$\frac{dc_1}{dt} = k_1 r - (\gamma_1 + k_0)c_1 \qquad \frac{dc}{dt} = k_0 c_1 - \gamma_0 c \, . \tag{2}$$

(5) *a.* Assuming that the concentrations c_1 and *a* reach rapid equilibrium, reduce the four equations in (1) and (2) to two equations for the slow variables *c* and *i*. Let $k \equiv k_I^-$. Show explicitly the choice of units required to produce the following simplified form:

$$\frac{dc}{dt} = \frac{c^2}{\beta \cdot i^2 + c^2} - c \qquad \frac{di}{dt} = k(c - i) \quad . \tag{3}$$

(10) *b.* When *Dictyostelium* is grown in liquid medium, extracellular cAMP is well stirred, making its concentration uniform over space. However, under such conditions, the cAMP concentration is known to oscillate over time. Find the conditions on *k* and β so that the system is oscillatory.

(5) *c.* On a graph of *i* vs. *c*, plot the nullclines and simulate the time evolution of the system for an oscillatory case.

(5) *d.* Suppose it only required a single molecule of *AC* or *IC* in order to catalyze the *R* conversion reactions. Comment on the stability of the system in this case.

3. Diffusion and cAMP waves

When cells are grown on a plate, cAMP diffusion is slow, and the extracellular cAMP concentration is no longer uniform. Consider a plate on which there exists a uniformly distributed population of cells. Each cell senses cAMP in its environment, and secretes fresh cAMP in response. This new batch of cAMP is able to reach neighboring cells, stimulating them to synthesize more cAMP, and so on. The situation is similar to one in which a number of radio transmitter towers (cells) are used to detect, amplify, and re-broadcast a weak radio signal (cAMP). The cAMP concentration now varies over space as well as time. For simplicity, we will analyze a 1-dimensional case, with cells uniformly distributed along a line. We can assume that the cells have fixed positions over the timescales considered, because their chemotaxis is relatively slow. This system obeys the equations

$$\frac{\partial c}{\partial t} = \frac{c^2}{\beta \cdot i^2 + c^2} - c + D\frac{\partial^2 c}{\partial x^2} \qquad \frac{\partial i}{\partial t} = k(c - i)\,.$$

We have provided MATLAB code which simulates the time evolution of a reaction-diffusion system. Modify the code to implement these equations. The system is assumed to extend from x = -1 to x = +1, with no flow at the boundaries. Use the following initial conditions:

$$c(x, t = 0) = \exp(-x^2 / 2\sigma^2) \qquad i(x, t = 0) = i_0\,.$$

That is, provide an initial pulse of cAMP centered at the origin, and some non-zero amount of inhibitor activity in all cells.

(10) *a.* Use the following parameters: $\beta = 4$; $k = 0.5$; $D = 10^{-7}$; $\sigma = 0.1$; $i_0 = 0.1$. Run the simulation to see the emergence of cAMP waves emanating from the origin. Plot out a typical cAMP profile, indicating the direction of motion of the waves.

(5) *b.* Run the simulation again, this time with a k value which produces a non-oscillating system. Describe the typical cAMP profile once the transients have died out. Can cells find the initial source of cAMP based on this type of profile?

(5) *c.* A simple concentration gradient would allow cells to find the cAMP source. Why do you think *Dictyostelium* uses waves of cAMP rather than a gradient in order to trigger cell aggregation?

4. Receptor clustering and signal amplification

The cells must now sense and move towards the source of the cAMP waves. It is unknown precisely how the amoebae are able convert the small front-to-back difference in cAMP concentration into the large output signal necessary to drive cell motion. However, a similar amplification of small *temporal* changes in ligand concentration into large changes in receptor activity has been observed in bacterial chemotaxis networks. We will now discuss a receptor clustering model that has been proposed in order to explain this amplification. Consider a receptor molecule R that is activated by the binding of some ligand L. Let $\alpha = 0,1$ represent the activity of the receptor. Thus,

$$\underset{(\alpha=0)}{R + L} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \underset{(\alpha=1)}{RL}$$

(5) *a*. The probability that the system will be found in a state with energy E is proportional to $e^{-E/kT}$. Show that the energy of the receptor molecule may be written in the form

$$-E/kT = A \cdot \alpha .$$

By calculating the mean activity $\overline{\alpha}$ and equating this to the value known from chemical kinetics, find an expression for A in terms of L, k_+ and k_-.

(5) *b*. Now consider a lattice of receptors R_i, each with activity α_i. Suppose that an active receptor is able to activate nearby receptors even if they are not ligand-bound. The energy of R_i can then be written as

$$-E_i/kT = A \cdot \alpha_i + B \cdot \Sigma_{j=1}^{n}(\alpha_i - ½)(\alpha_j - ½)$$

where the sum over j is a sum over the n nearest neighbors of R_i. How does the state of the neighboring receptors influence the energy of R_i?

(5) *c*. Assume that the activity of each neighboring receptor may be approximated by its mean value. That is, $\Sigma_{j=1}^{n}\alpha_j \cong n\overline{\alpha}$. By substituting this expression into the above equation, find an expression for the mean activity $\overline{\alpha}_i$ of receptor R_i.

(5) *d*. There is nothing special about the particular receptor R_i: our calculations could equally have been applied to any other receptor in the system. Therefore, the mean activity $\overline{\alpha}_i$ must be equal to the mean activity $\overline{\alpha}$ of the neighboring receptors. Apply this consistency condition to find an equation for $\overline{\alpha}$, and show how this equation may be solved graphically. (Hint: it is easier to work with the variable $s = \overline{\alpha}$ -1/2.)

(10) *e*. Explore the possible system responses as a function of the parameters A and B. For low values of B, the equation has a single solution for all A values. For high values of B, the system goes from having one, to three, then back to one solution as A is swept from -∞ to +∞. Find the critical value B_c which separates these two behaviors. Explain why the system response changes as this critical value is crossed. Which regime is more relevant for understanding signaling?

(5) *f.* Assuming $B < B_c$, calculate the logarithmic amplification

$$G = \left.\frac{\partial \ln(\alpha)}{\partial \ln(L)}\right|_{L_0} = \left.\frac{L}{\alpha}\frac{\partial \alpha}{\partial L}\right|_{L_0},$$

where $L_0 = k_- / k_+$ is the dissociation constant for ligand-receptor binding. Plot G as a function of B, and verify that it approaches the correct limit as B approaches zero. We see that the amplification can be made arbitrarily large by appropriately tuning B. What is the possible disadvantage of doing this?

(10) *g*. Based on published experimental data, estimate the logarithmic amplification at each stage of the *Escherichia coli* chemotaxis network. Is there any evidence that receptor clustering contributes to this amplification?