Spatiotemporal dynamics of eucaryotic gradient sensing

Atul Narang and K.K. Subramanian

Department of Chemical Engineering, University of Florida, Gainesville, FL 32611-6005*

1 BACKGROUND

Figure 1: The four phases of a chemotactic cycle.

Cell migration plays a crucial role in our birth, survival and death. We are conceived as amorphous fertilized eggs. It is cell migration, among other processes, that sculpts a richly structured embryo from a fertilized egg. While we live, cell migration heals our wounds (Martin, 1997) and protects us from surrounding pathogens (Jones, 2000). But when we age, cell migration can accelerate death. Cancer metastasis is caused by directed migration of tumor cells from their primary site to their preferential sites of metastasis (Moore, 2001). A better understanding of the mechanism of cell migration will have profound biomedical consequences.

Most eucaryotic cells move by crawling on a surface. The crawling movement occurs in response to an external stimulus, which is frequently a chemical concentration gradient. The resultant motion propels the cells forward along the direction of highest increase in concentration. The chemical that induces the movement is called chemoattractant and the movement itself is called chemotaxis. Eucaryotic chemotaxis is cyclic and each cycle consists of 4 phases (Figure 1): (1) Extension of a protrusion (2) adhesion of the protrusion to the surface (3) contraction of the cell body and (4) retraction of the tail. Each phase of the cycle is a complex process involving the coordinated action of a large constellation of molecules (Lauffenburger and Horwitz, 1996). In this work, we confine our attention to gradient sensing, the mechanism that enables the cell to read the external gradient and extend a protrusion precisely at the leading edge, the region exposed to the highest chemoattractant concentration.

The extension of the protrusion involves localized actin polymerization at the leading edge. Soon after the cells are exposed to a chemoattractant gradient, the leading edge develops fingerlike actin-based structures called filopodia. The space between the filopods then fills up with an actin mesh to form a wide, sheetlike lamellipod. The localized polymerization of actin at the leading edge implies that the gradient sensing machinery amplifies the external signal. Indeed, the chemoattractant gradients imposed in the extracellular space are often quite small (1-2% concentration change over the length of the cell) (Tranquillo et al., 1988), but actin polymers synthesized in response to the gradient are found exclusively at the leading edge (Coates et al., 1992). A key problem of gradient sensing is the elucidation of the mechanism that mediates the formation of a highly polarized distribution of actin polymers in response to a relatively mild chemoattractant gradient.

The chemoattractant gradient is transmitted to the actin polymerization machinery by a signal transduction pathway that starts with receptors on the cell surface and terminates in proteins that catalyze actin polymerization. It is, therefore, conceivable that actin polymers inherit their polarized distribution from some molecule that is upstream of the polymers in the pathway. Recent experiments have

* Electronic address: narang@che.ufl.edu
• Identified certain membrane-resident phosphoinositides, namely, phosphatidylinositol 3,4,5-phosphopate (PIP$_3$) and phosphatidylinositol 4,5-phosphate (PIP$_2$), are among the earliest polarized components of the pathway.

• Studied the spatiotemporal dynamics of these phospholipids in response to various chemoattractant perturbations.

Here, we present a mathematical model that is based on known signaling pathways and captures most of the experimentally observed spatiotemporal dynamics. We begin by summarizing the signal transduction pathway and the spatiotemporal dynamics. In Section 2, we describe our mathematical model and show that the simulations agree qualitatively with the observed dynamics. In Section 3, we elaborate on the future direction of this research.

1.1 Signaling pathways

Figure 2: (a) The phosphoinositide cycle (b) Polarization of PIP$_3$ in response to a steady chemoattractant gradient (from (Servant et al., 2000)). The curve with a shallow gradient represents the chemoattractant distribution. The curve with a sharp gradient represents the distribution of the marker for PIP$_3$. (c) The principle of fluorescent imaging experiments.

The signaling steps that follow receptor activation are the subjects of ongoing research. The model systems studied most intensively are Dictyostelium discoideum and neutrophils. In these systems, receptor-ligand binding activates heterotrimeric G-proteins. The polarization of PIP$_2$ and PIP$_3$ suggest that activated G-proteins stimulate PI5K and PI3K, the enzymes that catalyze the synthesis of PIP$_2$ and PIP$_3$ (Figure 2a). Although the precise mechanism of PI5K and PI3K activation is unknown, there is growing evidence that it is mediated by certain small GTPases of the Rho family. It has been proposed, for instance, that PIP$_3$ recruits small GTPases which in turn activates PI5K (Tolias et al., 2000) and/or PI3K (Weiner et al., 2002). This creates a positive feed loop in which synthesis of PIP$_3$ stimulates the synthesis of even more PIP$_2$ and PIP$_3$. There is yet another positive feedback loop because activation of PI5K increases the rate of synthesis of PIP$_2$ and its downstream product, phosphatidic acid (PA), which is a potent activator of PI5K (Ishihara et al., 1998). Consequently, upon receptor activation, the synthesis rate of PIP$_2$ and PIP$_3$ can rapidly accelerate to high levels. Such high synthesis rates can be sustained for no more than a second because the concentration of phosphatidylinositol (PI) in the plasma membrane is quite small (Willars et al., 1998). Depletion of PI in the plasma membrane is prevented by the cytosolic PI transport protein (PITP), which transfers readily available PI from the endoplasmic reticulum to the plasma membrane (Cockcroft et al., 1999). The PIP$_2$ formed by successive phosphorylation of PI is hydrolyzed by PLC to diacylglycerol (DG) and cytosolic inositol 1,4,5-triphosphate (IP$_3$). Diacylglycerol is converted to PA and transferred to the endoplasmic reticulum for regeneration of PI. Inositol produced by rapid dephosphorylation of IP$_3$ via multiple pathways (Berridge and Irvine, 1989), also participates in PI regeneration.

Recent experiments have established a strong link between localized PIP$_2$ formation and lamellipod extension. Specifically, it has been shown that
1. PIP₂, in conjunction with GTP-bound Cdc42, is a strong activator of N-WASP, which in turn activates Arp2/3 (Zigmond, 2000).
2. Activated Arp2/3 mediates actin polymerization by nucleating the sides of pre-existing actin filaments. This promotes the formation of the branched filament network found in lamellipods (Mullins, 2000).
3. Actin polymerization by Arp2/3 can drive lamellipod protrusion (Borisy and Svitkina, 2000).
4. Ruffles form at the very same time and locations as PIP₂ localization (Tall et al., 2000).

Taken together, these facts suggest that localization of PIP₂ and PIP₃ at the leading edge plays a crucial role in lamellipod extension.

1.2 Dynamics

The principle of the experiments used to study the spatiotemporal dynamics in response to various chemoattractant gradients is illustrated in Figure 2c. Motile cells are transfected with chimeric proteins made by fusing a fluorescent protein either to the molecule of interest, or to a “marker” molecule that binds specifically to the molecule of interest and thus “reports” on it. The marker molecules commonly used for reporting on PIP₂ and PIP₃ are the pleckstrin homology (PH) domains derived from various proteins (Balla et al., 2000). The transfected cells are then exposed to various chemoattractant concentration profiles and the movement of the fluorescent chimeric proteins is visualized using confocal microscopy. The chemoattractant profiles imposed include steady or time varying gradients, obtained by appropriate manipulation of the chemoattractant flow rate through a micropipette and steady uniform profiles, obtained by immersing the cell in chemoattractant. Each chemoattractant profile reveals particular aspects of the dynamics associated with gradient sensing.

**Steady and time varying chemoattractant gradients** show the existence of amplification and help identify the first amplified component. In response to such chemoattractant profiles, it has been observed that

- When G-proteins are absent (Lilly and Devreotes, 1995) or inactive (Servant et al., 1999), there is no polarization. Evidently, the chemoattractant profile is transmitted to the cell through the receptors and G-proteins.
- The receptors remain uniformly distributed in both Dictyostelium (Xiao et al., 1997) and neutrophils (Servant et al., 1999). Furthermore, receptor occupancy (Ueda et al., 2001) and G-protein activity (Janetopoulos et al., 2001; Jin et al., 2000) are not significantly polarized. It follows that receptors and G-proteins are required for transmitting the extracellular signal, but they are not the source of the amplification.
- Membrane-resident phosphoinositides, PIP₃ (Servant et al., 2000; Meili, et al., 1999; Parent et al., 1998; Haugh et al., 2000) and PIP₂ (Honda et al., 1999; Tall et al. 2000), are strongly polarized. In neutrophils, the gradient of the marker for PIP₃ is six times the chemoattractant gradient (Servant et al., 2000) (Figure 2b).

At first sight, the strong polarization of phosphoinositides seems explicable in terms of a simple amplification model. It suffices to postulate that phosphoinositide synthesis responds to receptor activation in a highly cooperative manner (Hill-type kinetics). In this case, the phosphoinositide distribution will be similar in shape, but steeper in slope, when compared to the chemoattractant concentration profile. The following observation precludes this simple model. If a cell that is already polarized in a certain direction is exposed to a modest chemoattractant gradient along a different direction, a new pseudopod does not develop at the point with the highest chemoattractant concentration. Instead, the existing pseudopod turns and reorients itself along the new gradient (Jin et al., 2000; Zigmond et al., 1981). It is as if the pre-existing leading edge is more sensitive to chemotactic signals than all other regions of the cell. This phenomenon, called *polarized sensitivity*, suggests that during the course of pseudopod formation at the leading edge, a diffusible substance is formed that rapidly migrates away from the leading edge and somehow inhibits the generation of a new pseudopod in other regions of the cell. Interestingly, if the new chemoattractant gradient is relatively large and localized, the existing pseudopod retracts and a new one
grows along the direction of the new gradient (Chung et al., 2001; Firtel and Chung, 2000; Parent et al., 1998).

**Steady uniform chemoattractant concentrations** reveal two properties of motile cells, namely, spontaneous polarization and adaptation. When cells are exposed to such chemoattractant concentration profiles, phosphoinositides accumulate uniformly along the entire plasma membrane within 5-10 seconds. However, this uniform pattern does not persist for long. Within a few minutes, the phosphoinositide distribution polarizes at a random location. This phenomenon is called spontaneous polarization (Wang et al., 2002, Wedlich-Soldner and Li, 2003) to emphasize the fact that the cells polarize even though the chemoattractant concentration is macroscopically uniform. The random location of the polarization suggests that this phenomenon is stochastic in nature and results from the relatively small number density of molecules in single cells.

In Dictyostelium, the phosphoinositides return to their pre-stimulus uniform distribution within 100-200 seconds (Parent et al., 1998) but eventually the cells polarize. It is significant that phosphoinositides return to pre-stimulus levels even though the chemoattractant concentration is several orders of magnitude higher than the pre-stimulus levels (Parent et al., 1998). This remarkable phenomenon is a manifestation of adaptation (Othmer and Schaap, 1998). It follows that in Dictyostelium, the time scale of activation is 5-10 seconds, and the time scale of adaptation is 100-200 seconds. In neutrophils, the cells polarization spontaneously before there is any perceptible reduction in phosphoinositide levels at the plasma membrane (Wang et al., 2002). This suggests that in neutrophils, the time scale of adaptation is so slow that the cells polarize spontaneously before there is significant adaptation.

![Figure 3](image)

**Figure 3**: (a) The model scheme is an abstraction of the PI cycle shown in Figure 2. (b) Initial dynamics of the membrane phosphoinositides at any point of the cell membrane. The initial concentrations of active receptors, $\rho$, and inositol phosphates, $\iota$, are treated as control parameters. When $\rho$ is small or $\iota$ is large, there is a threshold (defined as the distance between the lower and intermediate steady states). As $\rho$ increases or $\iota$ decreases, the threshold becomes progressively smaller. At sufficiently large values of $\rho$ or sufficiently small values of $\iota$, the threshold disappears completely.

In short, the distributions of PIP$_2$ and PIP$_3$ display four distinct types of spatiotemporal dynamics in response to various chemoattractant gradients: (1) Amplification and threshold (2) polarized sensitivity (3) spontaneous polarization and (4) adaptation. The desire to capture these dynamics has spurred the development of several mathematical models. Three of these models contain a short-range activator that is synthesized autocatalytically, and a long-range inhibitor that inhibits the synthesis of the activator (Meinhardt, 1999; Narang et al., 2001; Postma and Van Haastert, 2001). They differ only with respect to the postulated mechanisms of the activation and the inhibition. The remaining two models contain a long-range inhibitor but there is no autocatalytic synthesis (Levchenko and Iglesias, 2002; Rappel et al., 2002). In the next section, we present the model and show that it captures the dynamics of amplification, threshold, polarized sensitivity and spontaneous polarization. The discussion of adaptation is deferred to Section 3.
2 MODEL AND SIMULATION

2.1 Model

The model is an abstraction of the phosphoinositide cycle (Figure 2a). It contains three variables corresponding to three “lumped” pools (Figure 3a), namely, membrane phosphoinositides ($P$), cytosolic and its phosphates ($I$), and phosphoinositides in the endoplasmic reticulum ($P_s$). The concentrations of these variables are denoted by $p$, $i$ and $p_s$, respectively. It is assumed that

1. The cell is two-dimensional and disk-shaped. Thus, $p$ and $p_s$ are based on the length of the plasma membrane and $i$ is based on the area of the cytosol.

2. Radial gradients of the cytosolic inositol phosphate pool are negligibly small. This is reasonable because inositol phosphates diffuse rapidly. However, angular gradients of this pool are considered because membrane phosphoinositides, being slow diffusers, develop steep angular gradients. These sharp gradients could induce mild angular gradients of inositol phosphates.

It follows from assumptions 1 and 2 that the angle, $\theta$, is the only spatial variable.

3. In the absence of receptor activation, there is basal synthesis and degradation of $P$ and $I$. Basal synthesis rates of $P$ and $I$, denoted $c_p$ and $c_i$, follow zero-order kinetics. Basal degradation rates, denoted $r_{p,d}$ and $r_{i,d}$, obey first-order kinetics with rate constants $k_p$ and $k_i$, respectively, i.e.,

$$
r_{p,d} \equiv k_p p, \quad r_{i,d} \equiv k_i i
$$

4. The receptors instantly inherit the chemoattractant profile imposed on the cells, and the receptor-mediated rate of formation of membrane phosphoinositides per unit length of membrane is given by

$$
r_{p,f} \equiv k_f r(t, \theta) p^2 p_s.
$$

Here, $k_f$ denotes the rate constant and $r(t, \theta)$ denotes the concentration of active receptors. The dependence on $p^2$ represents the autocatalytic and cooperative kinetics with respect to membrane phosphoinositides. This is an idealization of the two positive feedback loops shown in Figure 3a. The membrane phosphoinositides will play the role of a local activator in the model.

5. The inositol phosphate pool ($I$) stimulates transfer of phosphoinositides from the plasma membrane to the endoplasmic reticulum. Thus, the rate of removal of membrane phosphoinositides per unit length of the membrane is

$$
r_{p,r} \equiv k_r p i
$$

where $k_r$ denotes the rate constant. The rationale for this assumption is as follows. Inositol reacts with CDP.DG to regenerate PI in the endoplasmic reticulum (Figure 3a). An increase in inositol concentration will, therefore, drive the transport of PA from the plasma membrane to the endoplasmic reticulum and its subsequent conversion to PI. The inositol phosphates will act as a global inhibitor.

To simulate the experiments, it is assumed that before the cell is subjected to a chemoattractant perturbation ($t<0$), it is a homogeneous steady state, $(p^-, p_s^-, i^-)$, corresponding to a uniform chemoattractant concentration so that $r = r^- = constant$. At time $t=0$, the cell is perturbed by exposing it to a chemoattractant profile which is instantly mirrored by the active receptor profile, $r(t, \theta)$. The dynamics of $P$, $P_s$, and $I$ are then governed by the equations
\begin{align}
\frac{\partial p}{\partial t} &= k_f r_p^2 p_s - k_p pi + c_p - k_p p + \frac{D_p \partial^2 p}{R^2 \partial \theta^2}, \tag{1} \\
\frac{\partial p_s}{\partial t} &= -\left(k_f r_p^2 p_s - k_r p + c_p - k_p p\right) + \frac{D_p \partial^2 p_s}{R^2 \partial \theta^2}, \tag{2} \\
\frac{\partial i}{\partial t} &= s\left(k_f r_p^2 p_s - k_p pi\right) + c_i - k_s i + \frac{D_i \partial^2 i}{R^2 \partial \theta^2}. \tag{3}
\end{align}

Here, $D_p, D_i, D_s$ denote the lateral diffusivities of $P, Ps, I$, respectively, and $R$ denotes the cell radius.

The factor $s$, denoting membrane length per unit cell area, is required since synthesis and removal rates of $P$ are based on the length of the plasma membrane. Since the cell is circular, concentrations and fluxes must be equal at $\theta = 0$ and $\theta = 2\pi$. Thus, we require the periodic boundary conditions

\begin{equation}
x(0,t) = x(2\pi,t), \quad \frac{\partial x(0,t)}{\partial \theta} = \frac{\partial x(2\pi,t)}{\partial \theta}, t > 0 \tag{4}
\end{equation}

where $x \equiv p, p_s, i$. The initial conditions for the reduced equations are

\begin{equation}
p(0,\theta) = p^-, p_s(0,\theta) = p_i - p^-, i(0,\theta) = i^-, 0 \leq \theta < 2\pi \tag{5}
\end{equation}

The initial condition for $p_s$ reflects the assumption that the total amount of phosphoinositide in the membrane and the endoplasmic reticulum is conserved, so that the average phosphoinositide concentration, denoted $p_{ps}$, is constant (Narang et al., 2001).

It is convenient to define the dimensionless variables

\begin{align*}
\pi &\equiv \frac{p}{p_i}, \pi_s \equiv \frac{p_s}{p_i}, i \equiv \frac{i}{s p_i}, \xi \equiv \frac{\theta}{2 \times 3.1416}, \tau \equiv \frac{t}{1/(k_{sp} p_i)}
\end{align*}

and dimensionless parameters

\begin{align*}
\rho &\equiv \frac{r}{r_i}, \kappa_f \equiv \frac{k_f r_i p_i^2}{k_{sp} p_i}, \psi_p \equiv \frac{c_p / p_i}{k_{sp} p_i}, \kappa_p \equiv \frac{k_p}{k_{sp} p_i}, \delta_p \equiv \frac{D_p / C^2}{k_{sp} p_i}, \\
\kappa_i &\equiv \frac{k_i}{k_{sp} p_i}, \delta_i \equiv \frac{D_i / C^2}{k_{sp} p_i}, \rho^- \equiv \frac{r^-}{r_i}, \pi^- &\equiv \frac{p^-}{p_i}, \pi_s^- \equiv \frac{p_s^-}{p_i}, i^- \equiv \frac{i^-}{s p_i}
\end{align*}

where $C$ denotes the circumference of the cell and $r_i$ denotes the total number of receptors. Thus, we arrive at the dimensionless equations

\begin{equation}
\frac{\partial \pi}{\partial \tau} = \kappa_f \rho \pi^2 \pi_s - \pi t + \psi_p \kappa_p \pi + \delta_p \frac{\partial^2 \pi}{\partial \xi^2} \tag{6}
\end{equation}
\[
\frac{\partial \pi}{\partial \tau} = -\left( \kappa_f \rho \pi^2 - \pi \tau + \psi_{\rho} - \kappa_p \pi \right) + \delta_p \frac{\partial^2 \pi}{\partial \xi^2} \tag{7}
\]

\[
\frac{\partial \tau}{\partial \tau} = \kappa_f \rho \pi^2 - \pi \tau + \psi_{\xi} - \kappa_f \tau + \delta \frac{\partial^2 \tau}{\partial \xi^2} \tag{8}
\]

with initial conditions

\[
\pi(0, \xi) = \pi^- \cdot \pi^+ (0, \xi) = 1 - \pi^-, \tau(0, \xi) = \tau^-, 0 \leq \xi < 1 \tag{9}
\]

and periodic boundary conditions

\[
x(0, \tau) = x(1, \tau), \quad \frac{\partial x(0, \tau)}{\partial \xi} = \frac{\partial x(2\pi, \tau)}{\partial \xi}, \tau > 0 \tag{10}
\]

where \( x = \pi, \pi_x, \tau \).

2.2 Simulations

Equations (6-10), along with various choices of \( \rho(\tau, \xi) \) described below, were simulated using the NAG subroutine D03PHF (NAG, 1999). The parameter values used in the simulations are shown in Table I. The rationale for the choice of parameter values can be found in (Narang et al., 2001). To facilitate the comparison of the simulations with experimentally observed dynamics, it is useful to note that \( k_{sp} \sim 1 \) 1/sec (Narang et al., 2001). Hence, each unit of the dimensionless time, \( \tau \), corresponds to roughly 1 sec.

2.2.1 Amplification and threshold

In the following simulations, the cell is assumed to be at a uniform steady state \( (\pi^-, \pi^+, \tau^-) \) corresponding to the uniform active receptor profile, \( \rho = \rho^- \). At time \( \tau \geq 0 \), the cell is exposed to a steady chemoattractant gradient and the active receptor distribution (Figure 4a) instantly inherits the chemoattractant concentration profile. Despite the mild gradient of \( \rho \), a pronounced phosphoinositide peak ultimately develops at the leading edge, \( \xi = \frac{1}{2} \) (Figure 4b). Compared to the polarized distribution of membrane phosphoinositides, the concentration profile of inositol phosphates is virtually flat (Figure 4c).

In terms of the model, the formation of the phosphoinositide peak can be explained as follows. Because of their autocatalytic and cooperative kinetics, membrane phosphoinositides (P) are strongly amplified beyond a certain threshold. To see this, observe that immediately after receptor activation, \( \pi^-, \pi^+, \tau^- = 1 - \pi, \tau \), \( t \not\subset t^- \) and diffusion is negligibly small compared to reaction. Hence, the initial dynamics of the membrane phosphoinositides at any point of the plasma membrane is approximated by the equation

\[
\frac{\partial \pi}{\partial \tau} = \kappa_f \rho \pi^2 \left( 1 - \pi \right) - \pi \tau + \psi_{\rho} - \kappa_p \pi \tag{11}
\]

Figure 3b shows that if \( \rho \) is small at a point, the membrane phosphoinositides display bistable dynamics at that point, i.e., there are two stable steady states separated by an unstable steady state which acts as a threshold because \( \pi \) moves to the upper steady state only if it crosses the unstable steady state. If \( \rho \) is large at a point, the threshold disappears at that point, and every \( \pi \), no matter how small, tends to move toward
the upper steady state. Now, at time $t < 0$, $\rho = \rho^-$ everywhere and there is a threshold at every point. Upon activation of receptors at $t = 0$, $\rho$ becomes significantly large in a neighborhood of the leading edge. In this region, the threshold vanishes and the concentration of $P$ starts growing. Although $P$ diffuses slowly, it does have a tendency to spread throughout the cell membrane. This tendency is contained by the formation of cytosolic inositol phosphates ($I$) which rapidly diffuse away from the leading edge, and acquire a relatively flat profile. Rapid diffusion of $I$ has a two-fold effect. In the neighborhood of the leading edge, the inhibitory effect of $I$ is diminished, so that localized growth of the phosphoinositide peak increases. Outside this neighborhood, the higher concentration of $I$ promotes transfer of membrane phosphoinositides from the plasma membrane to the endoplasmic reticulum. The net effect of this transfer is to deplete the plasma membrane of its phosphoinositides, thus preventing the peak from spreading beyond the leading edge. Hence, within the leading edge, the steady state concentration of $P$ is higher than the initial basal level. Outside the leading edge, it is lower than the initial basal level.

Table I: Parameter values used in the simulations. (Narang et al., 2001; Zigmond et al., 1982)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>$\kappa_f$</td>
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<tr>
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<tr>
<td>$\kappa_p$</td>
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</tr>
<tr>
<td>$\delta_{\rho_0}$</td>
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</table>

Figure 4: Amplification of a small chemoattractant gradient: (a) Distribution of active receptors at $t \geq 0$ (b) Development of a pronounced membrane phosphoinositide peak at the leading edge of the cell at $\xi = \frac{1}{2}$ (c) Growth of the uniformly distributed inositol phosphate pool.

2.2.2 Polarized sensitivity

The model has also been used to explore the phenomenon of polarized sensitivity. To this end, the model cell is exposed to a chemoattractant gradient and allowed to form a steady state phosphoinositide peak consistent with this gradient. After the steady state has been reached, the polarized cell is subjected to a new chemoattractant gradient, different from the previous gradient. The simulations show that if the new chemoattractant gradient declines gradually from its maximum in such a way that the receptors in the neighborhood of the pre-existing phosphoinositide peak sense the influence of the new gradient, the pre-existing peak moves like a traveling wave to the point at which the new chemogradient gradient has a maximum (Figure 5b). On the other hand, if the new chemoattractant gradient is highly localized some distance away from the pre-existing phosphoinositide peak such that the receptors in the neighborhood of the pre-existing peak do not sense the influence of the new chemoattractant gradient, there is no wave motion. The pre-existing peak retracts, and a new peak grows at the maximum of the new gradient (Figure 5c). These results are consistent with the phosphoinositide dynamics observed in response to chemoattractant gradients.

To explain the wave-like motion of the peak (Figure 5b), it is useful to observe that the steady state membrane phosphoinositide peak formed in response to the first gradient is “inert” everywhere except in
the two thin “transition layers” surrounding the peak within which there is a sharp change in the gradient of membrane phosphoinositides. By “inert,” we mean that outside these transition layers, nothing is happening at steady state—there is neither diffusion nor synthesis of membrane phosphoinositides. The transition layers, on the other hand, are sites of intense activity even at steady state. In the upper half of a transition layer, there is rapid synthesis of membrane phosphoinositides which then diffuse into the lower half of the transition layer, from where they are promptly removed. The steady state is maintained by this precarious balance between synthesis of membrane phosphoinositides in the upper half of the transition layers and their removal in the lower half of the transition layers. If the balance is disturbed by imposing a shallow chemoattractant gradient that increases the rate of phosphoinositide synthesis relative to its rate of diffusion, the transition layer moves in a wave-like fashion at a velocity that is proportional to the net rate of phosphoinositide accumulation within the transition layer.

The response to sharp chemoattractant gradients (Figure 5c) can be explained as follows. After the steady state has developed, the concentration of the inhibitor, $I$, is high throughout the cell. This tends to increase the threshold at all points of the plasma membrane. When the gradient is switched, the active receptor concentration decreases at the previous “front” and increases at the current “front.” It follows from the earlier discussion regarding thresholds (see Figure 3b) that at the current “front,” the tendency of the threshold to increase due to elevated inhibitor concentrations is mitigated by the higher active receptor concentration. However, at the “previous” front, the tendency of the threshold to increase due to elevated inhibitor concentrations is further exacerbated by the lower active receptor concentration. The thresholds at the “previous” front become so large that despite the large concentrations of membrane phosphoinositides, they fall short of the threshold, and the pre-existing peak collapses.

![Figure 5: Response of a pre-existing phosphoinositide peak to a new chemoattractant gradient (Polarized sensitivity)](image)

- (a) Active Receptors
- (b) Shallow Gradient
- (c) Steep Gradient

2.2.3 Spontaneous polarization

The random location of the polarity in spontaneous polarization suggests that some variable that is upstream of the phosphoinositides in the signal transduction pathway undergoes stochastic fluctuations. The most upstream source of the stochastic fluctuations is receptor-ligand binding (Lauffenburger and Linderman, 1993). Indeed, fluctuations of the chemoattractant concentration are too small to cross the threshold for the formation of a phosphoinositide peak. These fluctuations are of the order $10^{-5}$%-1% with the higher end being attained only under rather special conditions. On the other hand, the number of active receptors frequently undergoes fluctuations that deviate from the mean value by up to ~1%, which are large enough to trigger spontaneous polarization of the cell.

Following Tranquillo et al. (1988), we construct a stochastic model of receptor-ligand binding. To this end, we partition a cell containing $r$ receptors into $n$ equal sections. We assume that this cell is exposed to some uniform chemoattractant concentration $l$. If ligand binds instantaneously to the receptors, the mean number of active receptors in each section, denoted $r_m$, is $\langle r_m \rangle l/(k^-/k^+ + l)$, where $k^-$ and $k^+$ are the rate
constants for receptor-ligand association and dissociation, respectively. The number of active receptors in each section, denoted, \( r_i \), is given by the stochastic differential equation

\[
d r_i = \left[ (k^+ r_i + k^- (r_m - r_i)) \right] dt + \sqrt{k^+ r_i (r_i/n - r_m)} + k^- r_m dW_i, \quad i = 1, 2 \ldots n
\]  

(12)

where the first term on the right denotes the deterministic part of receptor ligand binding, which has the effect bringing \( r_i \) back to its mean value, \( r_m \). The second term on the right denotes the stochastic part of the binding process. Here, \( dW_i \) denotes the Wiener process, which is a Gaussian random number generator with zero mean and standard deviation \( (dt)^{1/2} \). It should be noted that the standard deviation of the random process, \( \delta r_i \), is proportional to \( (r_i)^{1/2} \), but the relative spread, defined as the ratio, \( \delta r_i/r_m \), is inversely proportional to \( (r_i)^{1/2} \). Thus, the smaller the number of total receptors in a cell, the more pronounced the stochastic effects. If we define the dimensionless variables

\[
\tau \equiv \frac{t}{1/(k_r s_p)}, \quad \rho_i = \frac{r_i}{r_i/n}, \quad \rho_m = \frac{r_m}{r_i/n}, \quad \omega_i = \left( \frac{k_r s_p}{r_i/n} \right) \tilde{W}_i,
\]

and the dimensionless parameters,

\[
\lambda = \frac{l}{k^+ / k^-}, \quad \kappa = \frac{k^-}{k_r s_p},
\]

equation (12) becomes

\[
d \rho_i = \left[ \kappa^- (\lambda + 1) (\rho_m - \rho_i) \right] d\tau + \sqrt{\kappa^- \left( \lambda (1-\rho_m) + \rho_m \right)} d\omega_i, \quad i = 1, 2 \ldots n
\]  

(13)

To simulate the experiments showing spontaneous polarization, it is assumed that at \( \tau = 0 \), the cell is exposed to a small and uniform chemoattractant concentration, \( \lambda = \lambda^- \). At \( \tau \geq 0 \), the cell is immersed in a uniform concentration of chemoattractant \( \lambda^+ > \lambda^- \). Euler integration of (13) yields a noisy active receptor distribution, a typical snapshot of which is shown in Figure 6a. The response to stochastic receptor-ligand binding is then simulated by choosing this noisy active receptor distribution as the function \( \rho(\tau, \xi) \) in the model equations (6-8). The simulation shows that the sudden increase from \( \lambda^- \) to \( \lambda^+ \) causes an initial accumulation of membrane phosphoinositides, but this is followed by the formation of a phosphoinositide
peak at a random location (Figure 6b). The peak “wobbles” slightly even after it is fully developed because the stochastic fluctuations constantly perturb the system.

2.2.4 Variation of the phosphoinositide peak with respect to kinetic parameters and external signal

Figure 7 shows the variation of the steady state phosphoinositide peak with respect to three different distributions of the function $\kappa \rho$. We assume that these distributions are achieved by fixing the active receptor distribution, $\rho$, and varying the parameter, $\kappa_f$, i.e., cells with progressively higher levels of PI3K or PI5K are subjected to the same chemoattractant gradient. The simulations show that the development of the phosphoinositide peak occurs only within a certain range of $\kappa_f$. Even within this range of existence

![Figure 7](image.png)

Figure 7: Variation of the steady state phosphoinositide peaks with respect to different distributions of $\kappa_f \rho$: (a) Distributions of $\kappa_f \rho$ (b) The corresponding phosphoinositide peaks.

1. The chemoattractant gradient required to provoke peak formation decreases with $\kappa_f$, and becomes zero for sufficiently large $\kappa_f$.
2. The width of the peak increases with $\kappa_f$ (Figure 7).

When $\kappa_f > 5$, the peak disappears completely. At such large values of $\kappa_f$, no initial perturbation can provoke the formation of a stable phosphoinositide peak. Similarly, decreasing $\kappa_f$ narrows the peak until at a sufficiently small value ($\kappa_f < 2.2$), peak formation cannot be induced.

These results have the following physical interpretation. The parameter, $\kappa_f$ is the ratio of the characteristic velocities of phosphoinositide synthesis and removal. If $\kappa_f$ is small, phosphoinositide synthesis is rapidly opposed by inhibitory action of inositol phosphates, resulting in high thresholds and narrow peaks. When $\kappa_f < 2.2$, even the maximum possible increment of $\rho$ cannot nullify the threshold at the leading edge, thus making it impossible to provoke a peak. Conversely, if $\kappa_f$ is large, the inositol phosphate pool responds slowly, so that the peak is wider and the threshold lower. At very large values of $\kappa_f$, the threshold is zero and membrane phosphoinositide synthesis is much faster than its removal. Hence, the minutest chemoattractant gradient results in propagation of phosphoinositides throughout the membrane before inositol phosphates can exert the inhibitory effect required for formation of the polarized steady state. Thus, the phosphoinositide distribution ultimately returns to the uniform steady state.

Now, we could just as well assume that the three different distributions of $\kappa_f \rho$ shown in Figure 7 were obtained by keeping the parameter, $\kappa_f$, fixed, and varying the active receptor distribution, $\rho$. In this case, the simulations would imply that the geometry of the phosphoinositide peak varies with the shape of the external chemoattractant.

3 FUTURE WORK

We have shown above that the model captures all the dynamics except adaptation. To be sure, we modeled adaptation in our earlier work by assuming slow desensitization and resensitization of the receptors.
(Narang et al., 2001). In response to uniform increases of the chemoattractant concentration, the model displayed perfect adaptation over several orders of magnitude of chemoattractant concentrations. In response to steady chemoattractant gradients, a phosphoinositide peak developed at steady state, and the shape of the peak was independent of the mean value and slope of the chemoattractant perturbation. This appears to be true in neutrophils, since the same polarization is observed in both uniform environments, when cells polarize spontaneously, and nonuniform environments. However, we have refrained from considering adaptation in order to reconcile the model with two pieces of evidence obtained in Dictyostelium. First, adaptation occurs at some level below the receptors (Kim et al., 1997) and G-proteins (Janetopoulos et al., 2001), which contradicts the mechanism proposed in the model. Second, the shape of the peak does depend on the mean value and the slope of the chemoattractant perturbation, suggesting that in case adaptation is not perfect. The simulations above show that in the absence of adaptation, the geometry of the peak varies with the shape of the external signal. Further attempts to model adaptation must be considered speculative until the mechanism becomes clearer.

Although our local-activator-global-inhibitor model captures the spatiotemporal dynamics observed in experiments, the identity of the local activator and global inhibitor are subjects of considerable debate. Specifically

- Experimental data concerning the polarization of PIP$_2$ is not definitive. It is difficult to discern spatial variations of the PIP$_2$ distribution because its concentration in the plasma is relatively high. It has been argued that the appearance of PIP$_2$ polarization observed in earlier work (Tall et al., 2000) does not reflect a localized increase in the concentration of PIP$_2$—it is a consequence of the high surface area created at the leading edge by the formation of membrane folds (Van Rheenen and Jalink, 2002). On the other hand, it has been shown that PI5K localizes to the ruffles immediately after chemoattractant stimulation (Honda et al., 1999; Doughman et al., 2003).
- The role of PIP$_3$ remains elusive. It has been shown, for instance, that PI3K the enzyme that catalyzes the synthesis of PIP$_3$ is sufficient, but not necessary for polarization. Delivery of exogeneous PIP$_3$ to neutrophils provokes polarity development (Weiner et al., 2002). However, in Dictyostelium, cells lacking both PI3K1 and PI3K2 show only partial defects in chemotaxis (Iijima et al., 2002).
- The influence on polarization of molecules that are downstream of PIP$_2$ and PIP$_3$ is controversial. In Dictyostelium, phosphoinositides are polarized even if actin polymerization is inhibited by latrunculin (Parent et al., 1998). This implies that phosphoinositides are polarized exclusively by reactions upstream of actin polymerization. In neutrophils, on the other hand, the polarization is almost completely abolished when actin polymerization is inhibited by latrunculin or promoted by jasplakinolide (Wang et al., 2002).
- Several potential global inhibitors have been hypothesized, but the evidence supporting their inhibitory role is either lacking or inconclusive. The enzyme, PTEN, which catalyzes the dephosphorylation of PIP$_3$ to PIP$_2$ migrates in a manner that strongly suggests that it may be a global inhibitor. While PIP$_3$ localizes to the “front” of the cell, PTEN translocates to the “back” of the cell (Funamoto et al., 2002; Iijima et al., 2002). However, the mechanism of this translocation is unknown. We have proposed that the cytosolic pool of inositol plays the role global inhibitor (Narang et al., 2001). Others have suggested that cGMP fulfils this role (Rappel et al., 2002). Neither one of these two hypotheses has been subjected to the test of experiment.

The resolution of these outstanding issues will play a crucial role in discriminating between the various models that have been proposed. These issues are likely to be resolved in the near future, spurred in part, at least, by rapid technological advances in imaging (Zhang et al., 2002) and microfluidic technologies (Jeon et al., 2002).

The theory of pattern formation was originally inspired by a desire to explain tissue differentiation (Turing, 1952). The theory showed that in a reaction-diffusion system, chemical inhomogeneities could arise spontaneously, and that these inhomogeneities could confer distinct attributes to different parts of a tissue. Yet, the link between this theory and experiments remains tenuous because of the difficulties encountered in identifying the variables of the theory, namely, the activators and the inhibitors. In recent years, it has become evident that there are chemical inhomogeneities even within a single cell. The chemical and
morphological polarization observed in eucaryotic gradient sensing mechanism is a paradigm of such
subcellular pattern formation. Given our deep understanding of cellular molecular biology, it would not be
surprising if pattern formation theory faces its first rigorous tests of experiment at the level of cells rather
than tissues.

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