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Bias in the Gradient Sensing Response of Chemotactic Cells

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Abstract

We apply linear-stability theory and perform perturbation studies to better characterize, and to generate new experimental predictions from, a model of chemotactic gradient sensing in eukaryotic cells. The model uses reaction-diffusion equations to describe 3' phosphoinositide signaling and its regulation at the plasma membrane. It demonstrates a range of possible gradient-sensing mechanisms and captures such characteristic behaviors as strong polarization in response to static gradients, adaptation to differing mean levels of stimulus, and plasticity in response to changing gradients. An analysis of the stability of polarized steady-state solutions indicates that the model is most sensitive to off-axis perturbations. This biased sensitivity is reflected in responses to localized external stimuli as well, and leads to a clear experimental prediction, namely, that a cell which is polarized in a background gradient will be most sensitive to transient point-source stimuli lying within a range of angles that are oblique with respect to the polarization axis. Stimuli at angles below this range will elicit responses whose directions overshoot the stimulus angle, while responses to stimuli applied at larger angles will undershoot the stimulus angle. We argue that such a bias is likely to be a general feature of gradient sensing in highly motile cells, particularly if they are optimized to respond to small gradients. Finally, an angular bias in gradient sensing might lead to preferred turn angles and zigzag movements of cells moving up chemotactic gradients, as has been noted under certain experimental conditions.

Keywords

Gradient-sensing; chemotaxis; phosphoinositides; linear stability theory

1. Introduction

Chemotaxis, the process by which a cell moves in response to a chemical gradient, is essential for many biological functions, including the immune response of neutrophils, growth of blood vessels, embryonic development, and the aggregation of the ameboid cell *Dictyostelium discoideum* (Firtel and Meili, 2000; Isbister et al., 2003; Olson and Ley, 2002; Yang et al., 2002; Zeller et al., 2001). A proper response requires that the spatio-temporal information in a chemotactic stimulus be transduced into a redistribution of the cellular constituents that mediate the mechanical and morphological changes underlying cell movement (Devreotes and

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Janetopoulos, 2003; Parent and Devreotes, 1999). In highly motile cells, such gradient sensing involves adaptation to the spatial average of the experienced stimulus, highly polarized responses to small spatial gradients, and sufficient response plasticity for the cell to follow the direction of a changing gradient (Devreotes and Zigmond, 1988; Fisher et al., 1989; Jin et al., 2000; Moghe and Tranquillo, 1994).

Recent experiments in both Dictyostelium and neutrophils have indicated that spatial distributions of molecules involved in signaling by 3' phosphoinositide (3'PI) lipids at the plasma membrane are correlated with leading edge protrusion and the development of polarity in chemotaxing cells (Iijima et al., 2002; Wang et al., 2002; Weiner, 2002). Similar patterns of molecular localization are seen in chemotactically stimulated cells that are round and immobile due to pharmacological poisoning of the actin cytoskeleton (Janetopoulos et al., 2004; Parent et al., 1998), suggesting that aspects of gradient sensing can be decoupled from motility. These observations have motivated several mathematical models (Gamba et al., 2005; Levchenko and Iglesias, 2002; Levine et al., 2006; Ma et al., 2004; Narang et al., 2001; Postma and Van Haastert, 2001; Rappel et al., 2002; Subramanian and Narang, 2004), including a recent study in which we developed a model of 3'PI-mediated gradient sensing that explicitly includes biochemical mechanisms at an intermediate level of detail (Skupsky, 2005; Skupsky et al., 2005). Our model demonstrates a range of behaviors consistent with observations of characteristic gradient-sensing responses.

The complexity of chemotactic signaling poses many challenges to refined mathematical modeling. Currently, the number of testable model predictions remains small and the available experimental data is insufficient to fully characterize the pathway in any given cell type and distinguish between possible mechanisms. Traditional experiments might measure the steady-state responses to a static gradient of chemoattractant and the response in time to uniform application of the same chemoattractant. Only recently are dynamic and more complex stimuli, such as rotating or alternating gradients, being used to probe gradient-sensing responses and their regulation (Janetopoulos et al., 2004; Jin et al., 2000; Rappel et al., 2002; Subramanian and Narang, 2004; Xu et al., 2005).

Here we demonstrate the use of stability analysis and perturbation studies to identify types of dynamic stimuli to which a cell might be most responsive and which might be best suited to probe cellular behaviors. In particular, our results indicate that a polarized cell should be most sensitive to off-axis perturbations. Such a biased sensitivity might be observed by measuring responses to localized stimuli applied on top of a background gradient, and is potentially a general feature of gradient sensing in highly motile cells.

We begin by introducing our model of eukaryotic gradient sensing and linearizing its equations. An analysis of responses to perturbations in the spatial distributions of *internal* variables about *uniform* steady-state solutions highlights different qualitative behaviors demonstrated by our model in different parameter regimes. Our results suggest that decay rates of normal-mode perturbations about uniform solutions might be measured to characterize cellular gradient-sensing mechanisms, and we comment on the relationship of our results to those found in more general Turing-like systems. An analysis of responses to *internal* perturbations about *polarized* solutions indicates that the least stable modes are localized at an angle with respect to the polarization axis. Motivated by this result, we explore the angular dependence of the responses of the model to localized perturbations in the external stimulus. Our simulations of cellular responses to point source stimuli, applied at different angles with respect to a background linear gradient, demonstrate a maximal response to stimuli applied at an oblique angle with respect to the pre-established cellular polarization axis. We argue that such a biased sensitivity is in fact a general feature of gradient sensing in highly motile cells, and might result naturally if the cells are optimized to detect small chemotactic gradients. Finally, we discuss

how our results might relate to experimentally observed zigzag movements of immune cells moving up a chemotactic gradient (Zigmond, 1974).

2. Mathematical Model

Efficient gradient sensing depends on a complex network of regulatory interactions that couple dynamics on multiple length and time scales. In particular, the amplified response to a gradient involves localization of components to the up-gradient side of the cell, while response adaptation requires components that effectively integrate the external stimulus over the cell surface (Devreotes and Zigmond, 1988; Parent and Devreotes, 1999). In our model, because of differences in characteristic diffusion times, membrane-bound proteins are treated as fixed, and effectively act locally, while cytosolic proteins are treated as uniformly distributed, introducing a global coupling. However, lipid diffusion along the membrane is explicitly calculated. The spatial patterns generated by lipids in our model thus depend on their regulated production and degradation.

The geometry of our model is a 2-D circle (Fig. 1a), which qualitatively captures gradient-sensing responses observed in pharmacologically immobilized *Dictyostelium* cells. Below we briefly describe the particular components of our model and their regulation. Readers interested in a more detailed discussion are referred to (Skupsky, 2005; Skupsky et al., 2005).

2.1 System variables

The scaled variables of our model are indicated in Fig. 1b. They represent molecules that are thought to be important for gradient sensing and that have been visualized in live cells using fluorescent labeling techniques. Other significant regulatory molecules are implicitly included in feedback terms in our equations (see Sec. 2.3 below).

The included lipids are the 3' phosphoinositides, PI(3,4)P₂ and PI(3,4,5)P₃, which are represented by the scaled model variables P_2 and P_3 (scaling factors are given in Appendix A and discussed in (Skupsky et al., 2005)). These 3' phosphoinositides (3'PIs) are thought to act as binding sites for regulators of actin-based structures that generate force at the leading edge of chemotaxing cells (Iijima and Devreotes, 2002; Manahan et al., 2004; Weiner, 2002; Weiner et al., 2002). Their sum, denoted P_n , is a primary output of our model.

Proteins included in our model are 1) the enzyme that generates 3'PIs (a PI 3' kinase, abbreviated as PI3K and denoted by the scaled variable K) and 2) an enzyme that removes them (the PTEN phosphatase, denoted by the scaled variable T). In mammalian cells, phosphorylation is thought to be an important form of regulation for these enzymes (Czapalla et al., 2003; Vazquez et al., 2000). Thus, we will consider three pools of PI3K/PTEN. One pool, which is unphosphorylated and localized on the membrane, acts to generate/remove 3'PIs. A second unphosphorylated pool, which is cytosolic and thus assumed to be uniformly distributed in our model, can bind to the membrane. The third pool is also cytosolic and uniformly distributed, but phosphorylated. Molecules in this pool require dephosphorylation in the cytosol to return to the membrane.

A single scaled variable, R , is used to represent ligand-activated receptors and hetero-trimeric G-proteins (HTGs) at the cell surface, which together act to recruit PI3K from the cytosol to the membrane (Brock et al., 2003; Manahan et al., 2004; Vanhaesebroeck et al., 2001). The 3'PIs, and PI3K and PTEN in different states, are the *internal* variables of our model, for which we will write dynamical equations. The distribution of R along the membrane, on the other hand, is set *externally*; it acts as the input for our simulations and is varied to represent different patterns of chemotactic stimuli.

2.2 Network topology

The topological features of the regulatory network of our model (see Fig. 1b) were inferred primarily from biochemical and microscopy data in *Dictyostelium* and/or neutrophils. Where biochemical details necessary to account for noted observations in gradient-sensing cells are unclear, mechanisms were proposed based on data in related cell types.

Loop I in Fig. 1b represents a positive feedback from 3'PI production to delivery of the substrate upon which PI3K acts to generate more 3'PIs. This feedback is thought to depend on the activities of small G-proteins, whose regulators bind to the membrane in a 3'PI-dependent manner (Jackson et al., 2000; Weiner et al., 2002; Welch et al., 2003). Loop II is a proposed feedback from 3'PIs to localization of both the molecules that generate them and those that degrade them. This feedback accounts for the observation that during chemotactic response in *Dictyostelium*, PI3K translocation to the membrane parallels 3'PI accumulation, while PTEN translocation demonstrates an inverse dynamic (Comer and Parent, 2002; Funamoto et al., 2002; Iijima and Devreotes, 2002). PI3K recruitment to the membrane in response to external stimuli (**R**) drives Loops I and II, which together act to amplify cellular responses in our model.

Response adaptation is necessary for cells to sense chemotactic gradients over many orders of magnitude in the absolute concentration. The mechanisms that account for this adaptation currently are unknown, but are thought to act downstream from receptor and HTG activation (Janetopoulos et al., 2001; Jin et al., 2000; Manahan et al., 2004; Xu et al., 2005). For our model, we have proposed that in response to chemotactic stimuli, PI3K that is recruited from the cytosol to the membrane is subsequently phosphorylated such that it no longer binds to the membrane (negative feedback Loop III in Fig. 1b). The cytosolic pool of unphosphorylated PI3K that can be recruited to the membrane is thus depleted. This adaptation mechanism contains a global element because it involves regulation of a cytosolic variable.

2.3 Model equations and modular interpretation

Our model consists of a set of reaction-diffusion equations describing the dynamics of its scaled model variables. Linear mass-action kinetics have generally been assumed, with more complex terms included to account for feedback regulation by molecules whose dynamics are not well characterized. Integrals in our equations arise because we have treated the cytosol as uniform, exchanging material with the entire membrane simultaneously. The spatial variable, X , is normalized to the range $[0,1]$ and periodic (see Fig. 1a). Here, we briefly discuss the major terms and parameters representing regulatory interaction in our model equations. A more detailed discussion, as well as derivations, may be found in (Skupsky et al., 2005).

In what follows, subscripts m/c denote membrane-bound/cytosolic, and '*' indicates phosphorylated species which are inactive. The parameters χ generally represent scaled forward rate constants, λ represent backwards rate constants, and κ provide saturation concentrations and/or concentrations at which a particular term becomes effective. The parameters ζ designate additional constitutive processes beyond the scope of our model (see, also, Appendix A).

The following equations describe the dynamics of the lipids included in our model:

$$\frac{\partial P_3}{\partial t} = \chi_3 K_m \left(\frac{\Xi}{1 + K_m / \kappa_3} + \zeta_{3/PTP} \right) + \zeta_3 - \lambda_3 P_3 (T_m + \zeta_{3/T}) + D \frac{\partial^2 P_3}{\partial X^2}, \quad (1)$$

where

$$\Xi \equiv \frac{P_n}{\underbrace{P_n/\kappa_m + \int_0^1 P_n dX/\kappa_c + 1}_{\text{Loop I}}} + \zeta_{PTP} \quad (1a)$$

with

$$P_n \equiv P_3 + P_2, \quad (1b)$$

and

$$\frac{\partial P_2}{\partial t} = \zeta_2 + \lambda_3 \zeta_{3/T} P_3 - \lambda_2 P_2 (T_m + \zeta_{2/T}) + D \frac{\partial^2 P_2}{\partial X^2}. \quad (2)$$

Equation 1 states that P_3 is generated by the action of membrane-bound PI3K (K_m) on its substrate. The factor Ξ (defined in Eq. 1a and derived in (Skupsky et al., 2005)) represents 3' PI-mediated enhancement of substrate delivery (Loop I). In the denominators, the term K_m / κ_3 represents a Michaelis-Menten-like saturation of P_3 production at high values of K_m , while the terms $\int_0^1 P_n dX/\kappa_c$ and P_n / κ_m represent saturation at high values of P_n due to depletion of cytosolic and membrane-bound regulatory molecules. Equation 1 also includes degradation of P_3 by PTEN on the membrane (T_m), as well as lateral diffusion of P_3 along the membrane. Equation 2 describes P_2 production from P_3 and its removal by T_m , such that P_2 dynamics generally follow P_3 with a slight lag.

The following equations describe the dynamics of proteins on the membrane:

$$\frac{\partial K_m}{\partial t} = \lambda_K \left(\gamma - \underbrace{\frac{K_m}{1 + P_n/\kappa_K}}_{\text{Loop II}} \right) \quad (3)$$

where

$$\gamma \equiv (\chi_K / \lambda_K)(R+1)K_c; \quad (3a)$$

$$\frac{\partial T_m}{\partial t} = \chi_T T_c - \underbrace{\lambda_T T_m (K_m + \zeta_T)}_{\text{Loop II}}. \quad (4)$$

In Eq. 3, the rate of PI3K recruitment from the cytosol to the membrane (first term) is proportional to the product of cytosolic PI3K (K_c) and the outside stimulus (the factor $(R+1)$ includes both the effect of the outside stimulus and a constitutive activation term). This product, which we label γ (see Eq. 3a), defines a driving parameter for our model (discussed further below), coupling its responses to outside stimuli and driving its positive feedbacks (Loops I

and II, see Fig. 1b). The rate of K_m removal from the membrane by phosphorylation (second term) is decreased with increasing P_n , resulting in a PI3K translocation that parallels 3'PI production (we have postulated a regulatory molecule that binds 3'PIs, as discussed in (Skupsky et al., 2005)). This feedback is represented by Loop II in Fig. 1b, and becomes effective when P_n is of order κ_K . The first term in Eq. 4 describes PTEN translocation from the cytosol to the membrane (i.e., the production of T_m from T_c). PTEN removal from the membrane is enhanced by a postulated K_m -induced phosphorylation (second term), which further enhances 3'PI production and results in a T_m dynamics inverse to that of K_m (also represented by Loop II in Fig. 1b and discussed further in (Skupsky et al., 2005)).

The following equations describe the dynamics of cytosolic proteins in our model:

$$\frac{\partial T_c}{\partial t} = \lambda_{T^*} T_c^* - \chi_T \int_0^1 T_c dX / A_{\text{cell}}, \quad (5)$$

$$\frac{\partial K_c}{\partial t} = \frac{\lambda_{K^*} K_c^*}{\kappa_{K^*} + K_c^*} - \lambda_K \int_0^1 \gamma dX / A_{\text{cell}} \quad (6)$$

$$\sim (\lambda_K / A_{\text{cell}}) \left(\gamma_0 - \int_0^1 \gamma dX \right), \quad (6a)$$

with

$$\gamma_0 \equiv \frac{A_{\text{cell}} \lambda_{K^*}}{\lambda_K}, \quad (6b)$$

where A_{cell} is the area of our two-dimensional cell, equal to $1/4\pi$ in units where the circumference of the cell is 1. Equation 5 describes production of T_c from T_c^* (i.e. dephosphorylation of PTEN in the cytosol, first term) and subsequent recruitment to the membrane (second term). Equation 6 describes production of K_c from K_c^* (PI3K dephosphorylation in the cytosol, first term) and PI3K recruitment to the entire membrane (second term). In what follows, parameters are set such that the reaction that dephosphorylates PI3K in the cytosol is saturated and the approximation in Eq. 6a is valid.

Our system is closed by the following two equations, which fix the total amount of PI3K and PTEN in the cell (scaled to 1).

$$1 = K_c A_{\text{cell}} + K_c^* A_{\text{cell}} + \int_0^1 K_m dX \quad (7)$$

$$1 = T_c A_{\text{cell}} + T_c^* A_{\text{cell}} + \int_0^1 T_m dX \quad (8)$$

Equations 1 – 5 effectively describe an amplification module in our model, coupled to outside stimuli and driven by the product, γ (see Eq. 3a). Equation 6 describes response adaptation and yields an equation for the dynamics of γ at constant $\mathbf{R}$ via multiplication by $\chi_K / \lambda_K (\mathbf{R} + 1)$. The approximation of Eq. 6a thus implies that the average value of the driving parameter over the membrane ($\int_0^1 \gamma dX$) always returns to a baseline value defined by γ_0 (see Eq. 6b).

2.4 Response to uniform stimuli and stationary gradients

Characteristic responses of our model are shown in Fig. 2. A uniform stimulus applied suddenly (Fig. 2a) is represented by a uniform increase in $\mathbf{R}$, and hence an increase in the driving parameter, γ . The components of the amplification module respond approximately in phase: PI3K is recruited to the membrane ($\mathbf{K}_m$), PTEN ($\mathbf{T}_m$) is removed, and 3'PIs ($\mathbf{P}_n$) are generated. Eventually, Loop III-mediated phosphorylation of PI3K (see Fig. 1b and discussion in Sec. 2.2) begins to deplete the cytosolic pool of unphosphorylated PI3K ($\mathbf{K}_c$) that can be recruited to the membrane, such that the driving parameter is returned to its baseline, resting, value and the response subsides (see Eq. 6a). If, however, the stimulus is applied in a gradient (Fig. 2b), the pool of cytosolic PI3K is depleted such that only the *average value* of the driving parameter returns to its resting value. In the front of the cell, however, receptor activity is elevated and the value of γ remains above baseline. The amplification module continues to be driven, generating a localized 3'PI signal. The reverse is true in the back of the cell. These model responses are in qualitative agreement with experimental observations of fluorescently labeled Dictyostelium cells responding to chemotactic stimuli.

2.5 Choice of parameters: Representative Cases

Many details of the mechanisms involved in gradient sensing remain to be elucidated and, even where molecular pathways are known, most of the relevant biochemical parameters have not been directly quantified in cellular contexts. Thus, we have set the parameters of our model empirically to reproduce observations of characteristic gradient-sensing responses such as those that were illustrated in Fig. 2. We study in particular four sets of parameters (given in Appendix A), for which our model demonstrates qualitatively different gradient behaviors (see (Skupsky et al., 2005) for further discussion). The results for these representative parameter sets provide an indication of the generality of our results.

3. Stability Analysis

How does our model respond to perturbations about its steady-state solutions? Will it relax back to the previous state or move to a new one? Will some perturbation shapes dominate responses? To what patterns of stimuli is a gradient-sensing cell likely to be most sensitive? To answer these questions, we now perform a linear-stability and perturbation analysis.

3.1 Linearized equations

We begin by considering *internal* perturbations in our six model variables, whose dynamics are described by Eqs. 1 – 6. For our analysis here, we collect these variables in a vector, $\vec{U}(\mathbf{X}, t)$, which is the sum of a steady-state solution ($\vec{U}_0(\mathbf{X})$) and a perturbation about that solution ($\delta\vec{U}(\mathbf{X}, t)$), i.e.,

$$\vec{U}(\mathbf{X}, t) = \vec{U}_0(\mathbf{X}) + \delta\vec{U}(\mathbf{X}, t). \quad (9)$$

Note that for cytosolic variables, which are assumed to be spatially uniform, the spatial dependence is trivial. In order to collect same-order terms in our perturbations, and in anticipation of a ‘normal-mode’ analysis to follow, we write our perturbations in the form

$$\delta \vec{U}(X, t) = \varepsilon \vec{A}_j(X) \exp(\sigma_j t) \quad (10)$$

where ε is small and the index, j , specifies a normal-mode solution with growth rate, σ_j , and spatially dependent amplitude, $\vec{A}_j(X)$. Solutions of this form are inserted into Eqs. 1 – 6, which are expanded in orders of ε , and only lowest order terms in ε are retained. The resulting system of linearized equations represents an eigenvalue problem, and can be written in the form

$$\sigma_j \vec{A}_j = \vec{\mathfrak{L}} \vec{A}_j. \quad (11)$$

Here, $\vec{\mathfrak{L}}$ is a linear operator, and we must solve for the growth rates, σ_j , and the normal-mode perturbations, $\vec{A}_j(X)$. In terms of our model variables, we find the following system of equations:

$$\begin{aligned} \sigma_j \widehat{P}_{n,j} = & \frac{\chi_3 K_m}{(1 + K_m/\kappa_3)} \widehat{\Xi} + \chi_3 \left(\frac{\Xi}{(1 + K_m/\kappa_3)^2} + \zeta_{3/PTP} \right) \widehat{K}_{m,j} \\ & - (\lambda_3 P_3 + \lambda_2 P_2) \widehat{T}_{m,j} - \lambda_3 T_m \widehat{P}_{n,j} + \left(\lambda_3 T_m - \lambda_2 (T_m + \zeta_{2/T}) \right) \widehat{P}_{2,j} + D \frac{\partial^2 \widehat{P}_{n,j}}{\partial X^2} \end{aligned} \quad (12)$$

where

$$\widehat{\Xi} \equiv \frac{\left(1 + \int_0^1 P_n dX / \kappa_c \right) \widehat{P}_{n,j} - P_n \int_0^1 \widehat{P}_{n,j} dX / \kappa_c}{\left(P_n / \kappa_m + \int_0^1 P_n dX / \kappa_c + 1 \right)^2} \quad (12a)$$

and Ξ was defined in Eq. 1a;

$$\sigma_j \widehat{P}_{2,j} = \lambda_3 \zeta_{3/T} \widehat{P}_{n,j} - \lambda_2 P_2 \widehat{T}_{m,j} - \left(\lambda_3 \zeta_{3/T} + \lambda_2 (T_m + \zeta_{2/T}) \right) \widehat{P}_{2,j} + D \frac{\partial^2 \widehat{P}_{2,j}}{\partial X^2}; \quad (13)$$

$$\sigma_j \widehat{K}_{m,j} = \lambda_K \int_0^1 \widehat{\gamma} dX + \frac{\lambda_K K_m / \kappa_K}{(1 + P_n / \kappa_K)^2} \widehat{P}_{n,j} - \frac{\lambda_K}{1 + P_n / \kappa_K} \widehat{K}_{m,j}; \quad (14)$$

$$\sigma_j \widehat{T}_{m,j} = \chi_T \int_0^1 \widehat{T}_c dX - \lambda_T T_m \widehat{K}_{m,j} - \lambda_T (K_m + \zeta_T) \widehat{T}_{m,j}; \quad (15)$$

$$\sigma_j \widehat{T}_c = -(\lambda_{T^*} - \lambda_T \zeta_T) \int_0^1 \widehat{T}_m dX / A_{\text{cell}} - (\chi_T / A_{\text{cell}} + \lambda_{T^*}) \widehat{T}_c ; \quad (16)$$

$$\sigma_0 \widehat{\gamma} \sim -(\chi_K / A_{\text{cell}})(R+1) \widehat{\gamma}. \quad (17)$$

Equations 12 – 17 are the linearized counterparts of our six model equation (Eqs. 1 – 6). Here, we have eliminated the variable P_3 in favor of P_n via Eq. 1b (see Eq. 12), and we have written K_c in terms of the driving parameter, γ , via Eq. 6a (see Eq. 17). In the above, model variables with a “ $\widehat{}$ ” represent the components of our normal-mode perturbations, while variables without a “ $\widehat{}$ ” represent the steady-state profiles about which we perturb. Both can be spatially dependent; we will find in the next subsection that the system is greatly simplified if we consider perturbations about uniform solutions. It must be emphasized that the stability analyses here are performed with respect to the *internal* distribution of the model variables, and the *external* signal, represented by R , is assumed to be fixed.

3.2 Stability of uniform solutions

Studying the linearized response of our system to *internal* perturbations about uniform steady-state solutions highlights some of qualitative gradient-sensing possibilities demonstrated by our model. At the end of this subsection, we will discuss how the linear stability properties of our equation set might be related to more general models of Turing-type systems, and how they might be studied experimentally.

Consider the case where the unperturbed profile is a uniform steady-state solution, for which a harmonic spatial dependence of the perturbations can be assumed:

$$\vec{A}_j(X) = \vec{a}_j \exp(i2\pi jX). \quad (18)$$

Here, $\vec{a}_j$ is a constant vector of perturbation components, ‘ i ’ is the imaginary unit, and the “wave number” ‘ j ’ must be an integer so that $\vec{A}_j(0) = \vec{A}_j(1)$. Under these conditions, Eqs. 12 – 17 become greatly simplified, such that the linear operator $\mathfrak{L}$ in Eq. 11 can now be written as a 6×6 matrix for each j , and its action on $\vec{A}_j(X)$ in Eq. 11 amounts to matrix multiplication. The matrix, which we now denote as $\mathfrak{L}_j$, is initialized using uniform steady-state solutions to Eqs. 1–6. Normal modes are calculated using SciPy, an open-source scientific toolkit for Python.

For each j , we expect to find 6 eigenvalues and eigenvectors, corresponding to 6 growth rates and normal modes. The mode with the largest growth rate determines the stability of our solutions, and this mode is likely to dominate responses to the most general perturbations. In what follows we only discuss this mode for each j (i.e., we do not include an extra index to enumerate the six solutions at each j), and we will refer to it as the ‘*least stable*’ mode for that j . If the growth rate for this mode is negative, then we can conclude that the system is stable to all perturbations of that wavelength. On the other hand, if the calculated growth rate is found to be positive, then perturbations of that form will grow and the system is unstable to those perturbations.

In Fig. 3 we plot growth rates of the least-stable normal-mode perturbations over a range of j . Results are shown for the four representative sets of model parameters (labeled as Cases 1 – 4) that were discussed in Sec. 2.5 and characterized in previous work (Skupsky et al., 2005).

We note here several general features of our results, as well as several features that highlight the characteristic differences between the analyzed Cases.

Amplification and adaptation become decoupled in our normal-mode analysis (as can be seen from the form of Eq. 17). In particular, the calculated least-stable modes are composed primarily of perturbations in the components of the amplification module, with $\hat{\gamma} \sim 0$ (data not shown). Further, for these least-stable normal modes, the membrane-bound components of the amplification module are always found to be approximately in phase, reinforcing each other as they do in response to external stimuli (see Fig. 2). That is, $\hat{P}_{n,j}$, $\hat{P}_{2,j}$, and $\hat{K}_{m,j}$ all have the same sign, while $\hat{T}_{m,j}$ has the opposite sign. If any of these variables are held fixed, such that the corresponding perturbation component is set to 0, the calculated growth rates are always reduced (not shown).

We see in Fig. 3 that σ_0 is always negative. Thus, the uniform system is always stable to uniform perturbations. More specifically, this is a requirement for the system to demonstrate a transient response followed by adaptation to all uniform stimuli (parameter values were always chosen such that this condition holds). On the other hand, $\sigma_1 > \sigma_0$ is possible because in our linearized equations integrals of perturbation components, which generally account for cytosolic depletion, vanish for $j \neq 0$. Conceptually, cytosolic depletion saturates the activity of positive feedback loops in response to uniform perturbations ($j = 0$), while in response to non-uniform perturbations ($j \neq 0$) this depletion is avoided by a redistribution of molecules on the membrane. Thus, we see in Fig. 3 that $\sigma_1 > \sigma_0$ only for Cases 2 and 4. These Cases were designed to illustrate conditions where depletion of translocating molecules from the cytosol saturates uniform responses, but redistribution of these molecules on the membrane effectively amplifies non-uniform responses. Further, $\sigma_1 > 0$ is possible, as is demonstrated by Case 4 in Fig. 3. This means that the uniform state is unstable to small perturbations that induce polarization (i.e., those with $j = 1$). Case 4 includes both strong, coupled, positive feedbacks and amplification of non-uniform responses by redistribution of translocating molecules; in previous work we had characterized this Case as demonstrating a finite polarization in response to infinitesimal applied gradients in the external stimulus. A model in this regime might relate to the spontaneous polarization in response to uniform stimuli that is demonstrated by some motile cells (this is referred to as chemokinesis, and requires cytoskeletal dynamics for full explanation). Finally, $\sigma_i < \sigma_j$ for $i > j \geq 1$, as is demonstrated by all four Cases in Fig. 3. This means that higher spatial modes, which are subject to greater dissipation from diffusion, decay more quickly. Thus, $\sigma_1 < 0$ means $\sigma_j < 0$ for all j , and we can infer from Fig. 3 that the uniform state is stable to all perturbations for Cases 1 – 3.

We place our analysis in the more general context of pattern forming systems by noting that Case 4 demonstrates a Turing-type instability (Cross and Hohenberg, 1993b; Satnoianu et al, 2000; Turing, 1952). Indeed, gradient sensing responses in our model depend on a competition between activator molecules that drive the response at the leading edge of the cell (i.e. P_3 , P_2 , K_m , and T_m , which are the membrane-bound components of our model), and those whose depletion inhibits that response (i.e. K_c and T_c , as well as other cytosolic components that are implicitly included in feedback terms, see (Skupsky et al., 2005)). Differences between the diffusion coefficients of these components make instability possible. While Cases 1-3 are stable, they are nevertheless defined near bifurcations where instability begins to occur; we generally found gradient sensing to be most efficient in regions of parameter space near such bifurcations (the dependence of model response on variations of key model parameters is analyzed in (Skupsky et al., 2005), though stability is not directly addressed there). For combinations of parameters that do lead to instability in our model, single peaked patterns are always favored (i.e. $j = 1$, see Fig. 3).

If finite diffusion coefficients were to be considered for cytosolic components in our model (recall that we had considered cytosolic diffusion to occur instantaneously), a Turing instability could generate multi-peaked patterns on a length-scale determined by the decay length of the inhibitory effects of the cytosolic depletion near regions of membrane activation. However, robust multi-peaked patterns in our model also require either a decrease in the membrane lipid diffusion coefficient or consideration of a larger cell (see (Levine and Rappel, 2005) for a discussion of Turing patterns in cellular geometries). While simpler Turing-type systems (e.g. those involving only two components) have been well characterized mathematically and find application in diverse areas of study (Cross and Hohenberg, 1993a; Gierer and Meinhardt, 1972; Levine and Rappel, 2005; Meinhardt and Gierer, 2000), our model does not map directly onto these.

In real cells, the values of σ_0 and σ_1 perhaps could be directly measured in response to internal perturbations generated by experimental techniques such as photo-uncaging. Alternately, thermal fluctuations, or fluctuations due to interactions with other cellular components not included in the model, can act as internal perturbations; experimental observations of fluctuations in the concentrations of model components could thus be used to calculate perturbation decay rates. Finally, one might observe cellular responses to small perturbations in the *external* stimulus that act to generate perturbations in the distributions of *internal* cellular components. In what follows, we will continue to see that our model's responses to *internal* and *external* perturbations mirror each other.

3.3 Stability of polarized solutions

How does our model respond to small internal perturbations when the initial profile of signaling molecules is *polarized*, rather than uniform? To investigate this, we initialize our model in its uniform steady state in the absence of stimuli and then externally apply a small, static, spatially linear gradient, whose effect on receptor activity is described by:

$$\{R=0, (t<0); R=S+G(1+S)\cos(2\pi X), (t>0)\} . \quad (19)$$

Here, S measures the average receptor activity that results from the applied stimulus and G measures the relative difference in receptor activity between the front and midline of the cell. In what follows, we consider $G = 0.05$ and $S = 2$, which is sufficient to elicit a highly polarized response in our model (a range of gradients gave the same qualitative results and the steady state is independent of S). The system is evolved to steady-state via Eqs. 1-6 using FiPy, a finite volume partial differential equation solver (developed at the National Institute of Standards and Technology, Gaithersburg MD). The resulting polarized solutions then become the unperturbed profiles in Eqs. 12 – 17, which must now be solved assuming a general spatial dependence for the calculated normal-mode perturbations. The spatial variable, X , is represented by discrete values, a finite difference approximation is used to approximate diffusion terms, and quadrature is used to approximate integrals. In this way, we once again arrive at a matrix representation for our linearized system and solve it by the same methods as in Sec. 3.2. Again, we focus on the features of the least-stable normal-mode perturbations.

Figure 4a shows the P_n distribution for the two least-stable normal-mode perturbations in a representative computation. The calculated growth rates are negative, indicating that the system is stable towards all perturbations about this polarized solution. Further, the second normal-mode perturbation decays significantly faster than the first, indicating that the least-stable mode is likely to dominate responses to general perturbations.

A profile of the model components for the least-stable mode is illustrated in more detail in Fig. 4b. We see here that the system is most sensitive to *internal* perturbations where the model components again reinforce each other in approximately the same relationship that is demonstrated in response to the initially applied *external* gradient (illustrated in Fig. 2b), and as was found in our analysis of internal perturbations about uniform solutions (see discussion in Sec. 3.2). In addition, we note that the peaks in the perturbation profile occur at an oblique angle to the direction of the initial applied gradient (at approximately 60 degrees).

The above-noted features of the response to internal perturbations about polarized solutions were found for all Cases over a range of initial gradients (data not shown), suggesting that they reflect general features of gradient sensing as described by our model. In particular, the off-axis peaks in the spatial profile of the least-stable normal-mode perturbations indicate that the gradient-sensing response of a polarized cell should be most sensitive to perturbations localized at approximately 60 degrees with respect to the polarization axis. Such a biased sensitivity, when coupled to cell motility, might cause a cell to turn at prescribed angles when moving up a shallow gradient, as has been observed for PMN leukocytes undergoing chemotaxis (Nossal and Zigmond, 1976). This will be discussed further in Sec. 4.

3.4 Responses to point source stimuli

How does our analysis of cellular responses to *internal* perturbations about steady-state solutions inform us about responses to *external* stimuli, which might be more easily measured experimentally? Does the enhanced sensitivity to off-axis *internal* perturbations that was demonstrated for the *linearized* behavior of our model in the previous subsection imply an enhanced sensitivity to off-axis *external* stimuli when the full *non-linear* response is considered? We might expect that this is the case because an external stimulus acts to perturb the internal variables for our model, and responses to internal perturbations mirror responses to external stimuli, as was noted in the previous two subsections.

To investigate the responsiveness of polarized cells to localized *external* perturbations, that is, to perturbations in the external stimulus, we simulate responses to a ‘spike’ of chemoattractant, as illustrated in Fig. 5a. A shallow background gradient is applied to initialize the system in a polarized steady-state solution, as in Sec. 3.3. A point-source stimulus, assumed to be a delta function in both space and time, is then released at a point outside of the cell and allowed to diffuse freely. Assuming a linear relationship between chemoattractant concentration at the cell surface and receptor activation in our model, the resulting ‘perturbation’ in R can be written as:

$$\Delta R = (A/t) \exp\left(-\left|\vec{r} - \vec{r}'\right|^2 / 4Dt\right) \quad (20)$$

where $\vec{r}$ specifies a point on the cell surface (in 2 dimensions) and A specifies the ‘strength’ of the point source, released at $t = 0$, at position $\vec{r}'$ (assumed to be in the plane of the cell and measured from its center). The diffusion coefficient, D , was fixed at $300\mu\text{m}^2/\text{sec}$, a value appropriate to a small-molecule chemoattractant such as cAMP (to which *Dictyostelium* responds). A point source stimulus of this type might approximate a bursting vesicle releasing its contents, which has been proposed as a method for cell-cell signaling in *Dictyostelium*.

We considered a range of A , sufficient to cause a peak increase in P_n by 1% to 150% of the background profile maximum. The position of the point source was varied from the cell surface to 15 cell lengths from its center (our model cell can be considered to have a diameter of order $8\mu\text{m}$), and its direction, labeled ϕ (see Fig. 5a), was varied from 0 to 180 degrees with respect

to the direction of the background gradient. For each location, the full response of our model was simulated via Eqs. 1 – 6, again using FiPy, and a time-course was recorded.

In our simulations, the maximum stimulus from the ‘spike’ reaches the cell and is significantly dissipated within a few seconds. The cellular response is generally maximal after approximately 5 – 10 s and decays after approximately 20 -30 s until the initial steady state is reestablished. A sample profile is shown in Fig. 5b for the time point at which the response is maximal. The point source here is described by $|\vec{r}| = 5$ cell lengths, $\varphi = 36$ degrees, and A eliciting approximately a 25% maximal increase in P_n over the background profile. The variable representing the cellular response in the plot, ΔP_n , is the change in P_n from the background profile that is caused by adding the point source over the background gradient. In this instance, the peak in ΔP_n occurs at an angle, $\theta_{\max} \sim 58^\circ$, which is greater than the source angle, φ ; i.e., the cell response overshoots the angle of the external stimulus. The complete time-course of ΔP_n at angle $\theta_{\max}$, at φ , and at two representative off-peak angles, is shown in Fig. 5c for this simulation; the magnitude of the peak response increases with increasing strength of the point source, as shown in the inset. We see that large perturbation responses are possible (a model including cytoskeletal dynamics would be necessary to identify that magnitude of perturbation that would have a significant effect on the path of a moving cell). Other qualitative features of the response, such as its shape and the position of the maximum in ΔP_n , were found to be similar over a range of point-source distances $|r - \varphi|$ and strengths (A) for all four Cases (results not shown).

To investigate any possible angular bias in our model's responses to the above ‘spike’ stimuli, we systematically varied the point-source angle, φ , keeping the point-source distance and strength fixed as in Fig. 5b. Figure 6 records the magnitude and direction of the maximal response from the time-courses for each simulation, as a function of the point-source angle. We see in Fig. 6a that all four Cases respond maximally to stimuli released at angles between approximately 40 and 80 degrees with respect to the background gradient. Figure 6b indicates that when the source angle, φ , is below this range, the maximum response of the cell occurs at an angle, θ , greater than φ , i.e. the response overshoots the direction of the stimulus perturbation. On the other hand, for larger φ , the angle of the maximum response is less than φ , i.e. the response now undershoots the angle of the applied stimulus. Again, the qualitative features of these results were not found to depend on the distance or strength of the point source. As shown in Fig. 7, these features are accentuated when the response is integrated over time; quantifying responses in this way is likely to yield clearer results in experimental investigations of the biased-sensitivity in gradient-sensing response that we are suggesting.

The analysis here indicates that our model is most sensitive to *external* perturbations that are localized at an oblique angle, in the range typically between 40 and 80 degrees, relative to a pre-established polarization axis. The overshooting and undershooting of the response to the stimulus source angle that was observed in our simulations reflects the fact that responses are most easily generated in this angular range even when the stimulus is localized outside of this range. These results are consistent with our finding in the previous subsection that the polarized state of the cell is most sensitive to *internal* perturbations that are peaked at approximately 60 degrees with respect to the polarization axis. Notably, both analyses demonstrate a similar biased sensitivity for *all* four representative Cases, suggesting that this should be a general feature of eukaryotic gradient sensing.

Why should a biased sensitivity be a general feature of gradient sensing in highly motile cells? For the parameters investigated in our model, those parameters that determine the baseline state in the unstimulated cell were always chosen to maximize the polarized response to a small static gradient. Thus, even a very small external gradient applied to a *resting cell* activates the positive feedback loops regulating responses at the front of the cell and suppresses them at the

back. In a *polarized cell*, these feedbacks are *already* activated at the front of the cell and might be functioning near saturation, while at the back of the cell their activity is already suppressed and requires a larger stimulus for reactivation. At the sides of the cell, however, the state of the regulatory loops most resembles that in the resting, unstimulated cell, where responses are easily activated or suppressed in response to even very small perturbations. Thus, a biased sensitivity might be the result of cellular optimization for responses to shallow gradients.

4. Meandering of chemotactic cells

Our analysis addresses only the gradient sensing component of chemotactic response, considering the localization of 3'PIs in response to chemotactic stimuli in a circular geometry. Moreover, it does not include coupling to cellular deformations and/or movements. Nevertheless, if a biased sensitivity is a general feature of gradient sensing in highly motile cells, this bias should affect the paths cells take when they are free to deform and move. Indeed, several key features of our results are consistent with observations of immune cells moving in responses to a chemotactic gradient.

Neutrophils and other amoeboid cells rarely move directly towards a chemotactic source. Rather, their trajectories frequently resemble biased random walks (see, e.g., (Foxman et al., 1997; Korohoda et al., 2002; Tranquillo et al., 1988)), although stochastic elements of their movement may be small compared with intrinsic, internally driven, oscillatory components of their motility (Killich et al., 1993). This was demonstrated quite dramatically, e.g., in a study of PMN leukocytes moving towards a stripe of aggregated protein (nominally γ -globulin) that acted as a source of chemotactic molecules (Zigmond, 1974). In this instance, the trajectories of the cells appeared as essentially straight-line segments, linked by relatively abrupt changes in orientation, as illustrated in Fig. 8a (Nossal and Zigmond, 1976; Zigmond, 1974). The cells seemed to zigzag as they moved up the gradient, having a tendency to overcompensate when they turned, and favoring turn angles of order 40 degrees with respect to the previous morphological polarization axis. In Fig. 8b we show a scatter diagram of the magnitudes of the turn angles demonstrated by the PMN cells as a function of their previous direction of movement with respect to the gradient. We see that cells oriented at angles of less than approximately 40 degrees tend to overshoot the direction of the gradient when they turn, while those oriented at larger angles are likely to undershoot. These data are reminiscent of the angular features of the simulated 3'PI responses to point sources that were analyzed in Fig. 6b.

To match experiment and simulation results one needs to assume that in the experiment the morphological polarization of the cell is aligned with the direction of the internal gradients in our model variables and the external chemical signal acts as a perturbation. These assumptions are not unreasonable: morphological polarization likely feeds back on, and acts to stabilize, the distribution of signaling molecules at the front of a chemotaxing cell, possibly by affecting intracellular trafficking patterns that deliver important regulatory molecules (Eddy et al., 2002; Janmey, 1998; Xu et al., 2003). Thus, if a cell is oriented at an angle with respect to the external gradient, the distribution of signaling molecules will be stabilized on that same axis. The extracellular applied gradient will then generate a perturbation in the profile of signaling molecules, whose shape will reflect the biased sensitivity that we have analyzed in our simulations. To connect bias in the sensitivity to bias in the direction of motion, we need to speculate: for example, if nucleation of new cellular protrusions depends on the change in signaling molecule concentration rather than its absolute value, then a protrusion directed with the same angular bias will be favored. Such a protrusion might then become stabilized via the suggested feedback between signaling molecule localization and cytoskeletal rearrangements. If the old cellular polarization axis eventually becomes destabilized, the new protrusion will define the polarization axis, and its direction will overshoot or undershoot the direction of the gradient in the predicted manner. The cell will then continue to move along this axis until the

external gradient succeeds in nucleating another new extension, whose direction again will reflect a biased sensitivity, overshooting or undershooting appropriately and repeating the process.

We should note that such zigzag motion has only been observed under specific sets of experimental conditions. The tie between our analysis and such movement is quite speculative, and other explanations are possible. For example, the geometry of the dendritic actin cytoskeleton at the leading edge of a cell (Mullins et al., 1998; Svitkina and Borisy, 1999) might lead to preferred angles for the generation of a new lamellapodium. In addition, longer time-scale local inhibition of the cellular signaling apparatus might result in a desensitization of the transduction apparatus at the leading edge of the cell after a characteristic time. Such change in sensitivity may generate a new leading edge in regions adjacent to the old one (Ali et al., 1993; Tranquillo et al., 1988).

5. Summary and Discussion

Eukaryotic gradient sensing is a complex cellular process. Although the general features of the signaling pathways that are involved are known and many of the relevant signaling molecules have been identified, there is still much left for discovery. Thus, a range of qualitative mechanisms that account for characteristic behaviors have been proposed. Novel approaches from multiple disciplines are needed to identify those features of gradient sensing that are general, and to design experiments that might distinguish between possible mechanisms.

Here we have analyzed a model that describes the dynamics of 3' phosphoinositide signaling in eukaryotic gradient sensing. This model is derived from proposed biochemical mechanisms and illustrates a range of possible signal amplification mechanisms. Linear-stability analysis and perturbation studies allow us to characterize responses in a very general way, and to identify patterns of stimulus to which a gradient-sensing cell is likely to be most sensitive. Studying model behaviors for several representative combinations of parameters allows us to start to investigate the generality of our results.

An analysis of the stability of uniform steady-state solutions indicates that the least stable internal perturbations are those where the components linked to signal amplification reinforce each other. We found that we could characterize model responses to such perturbations based on the signs and relative values of rate constants for uniform and first-mode polarizing perturbations (which we labeled as $j = 0$ and $j = 1$, respectively, in Sec. 3.2). We have shown qualitative possibilities that reflect differences in the signal-amplification mechanisms that result from considering the model in different parameter regimes, and we have discussed how the stability properties of our model relate to more general Turing-type systems (see Sec. 3.2). Differences in signal-amplification mechanisms lead to differences in responses to complex stimuli, such as rotating gradients, and may effect patterns of cellular movement in natural settings where stimuli vary in space and time (this is further discussed in our earlier work (Skupsky et al., 2005)). While it may be possible to characterize real cells by directly measuring growth or decay rates of internal perturbations, we have noted that it might be more productive to analyze cellular responses to external stimuli, which are more easily controlled.

Application of our stability analysis to polarized steady-state solutions indicates that these are most sensitive to internal perturbations localized at an oblique angle – of order 60 degrees – with respect to the polarization axis. Here too the relationships among model constituents mirror those found when cells respond to external gradients. These results led us to investigate whether an angular bias exists in cellular responses to external stimuli as well. Indeed, a similar biased sensitivity was found in our simulations of polarized cell responses to ‘spike’ stimuli released at different angles with respect to a steady background gradient.

Our analysis thus leads to the clear experimental prediction that the gradient-sensing response of a polarized cell should be most responsive to point source stimuli released at an oblique angle of order 40 – 80 degrees with respect to a background polarizing gradient. For source angles below this range the direction of the response maximum overshoots the source angle, while for larger source angles it undershoots; in our simulations, we have found this effect to be accentuated if the response on the membrane is integrated over time. Further, we have argued that this biased sensitivity may be a general feature of gradient sensing in highly-motile cells, if developmental conditions exist that optimize their responses to small gradients.

Recently, (Samadani et al., 2006) have published results using an experimental configuration very similar to one that might be used to investigate our model predictions, in which photo-uncaging is used to stimulate rounded *Dictyostelium* cells with localized point-source stimuli. Their results indicate a different kind of biased sensitivity than the one that we have investigated. That is, the data suggests that rounded unstimulated cells retain an intrinsic polarization axis along which their angular response to weak stimuli is biased. Their analysis suggests that this internal polarization can be included in an ‘effective’ stimulus that is a combination of the externally imposed chemotactic gradient and the intrinsic internally generated polarity of the cell. Because our perturbation analysis considers a steady-state polarization, without direct reference to whether that steady state is internally or externally generated, the possible presence of an internal polarization does not necessarily affect our predictions.

The analysis here only considered the gradient sensing component of chemotactic response under isolated conditions – it does not include any coupling to cellular deformation, cytoskeletal dynamics, and cell-cell or cell-self signaling. However, if a biased sensitivity is a general feature of the gradient-sensing apparatus, we expect it to be observable in the full chemotactic response, including motility, under more natural conditions. We have discussed how our results might account for the observed zigzag movements of immune cells moving up a chemotactic gradient, but have also noted several other factors that might account for such movements. These effects should be observable, as well, in other cell types and under other experimental conditions. Further experiments and computational modeling will be necessary to better understand how the various features of gradient sensing mechanisms are expressed in the full chemotactic responses of motile cells.

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Appendix A

Our scaled model parameters were generally set to reproduce characteristic gradient sensing responses. Experimental observations that were considered are discussed in (Skupsky et al., 2005). Table A.1 summarizes the meaning of each model parameter and its value. Those for which a range is indicated differ between the model variants. Table A.2 lists the combinations of parameters that were adjusted to define the model variants. These, together with the conditions $K_c = T_c = 0.5$ in the unstimulated cell, are sufficient to specify the parameters in table A.1 where a range is given. Table A.3 gives the uniform steady state for an unstimulated cell, which is always unique.

Table A.1
Model parameters

Parameter	Equation	Interpretation	Value
χ_3	1	Rate constant for Loop I regulated P_3 production	120.0
κ_m		P_n which begins to saturate Loop I by depleting membrane molecules.	1.18 – 5.0
κ_c		P_n which begins to saturate Loop I by depleting cytosolic molecules.	1.25 – 6.7
ζ_{PITP}		Rate of g-Pindependent P_3 production/rate constant for gPdependent production.	0.025
κ_3		K_m at which PI3K binding to PITP-PI(4,5)P ₂ becomes saturated.	0.05
$\zeta_{3/PITP}$		Rate of PITP independent P_3 production/rate constant for PITP dependent production.	0.025
ζ_3		Rate of unregulated P_3 production.	0.3
λ_3		Rate constant for P_3 removal by T_m .	15.0
$\zeta_{3/T}$		Rate of P_3 conversion to P_2 /rate constant for T_m -mediated removal.	0.13
ζ_2	2	Rate of unregulated production of P_2 .	0.02
λ_2		Rate constant for T_m -mediated removal of P_2 .	8.0
$\zeta_{2/T}$		Rate of unregulated removal of P_2 /rate constant for T_m -mediated removal.	0.05
χ_K	3	Rate constant for unregulated K_c translocation to the membrane.	0.0049 – 0.0076
R		Ligand-induced, receptor-mediated activation, which drives translocation of K_c .	external
λ_K		Rate constant for K_m phosphorylation and removal from the membrane.	0.73 – 4.2
κ_K		P_n at which 3'PI inhibition of K_m return to the cytosol (Loop II) becomes effective.	0.95 – 5.5
χ_T	4	Rate constant for T_c translocation to the membrane.	0.019
λ_T		Rate constant for T_m phosphorylation by K_m .	30.0
ζ_T		Rate of unregulated T_m return to cytosol/rate constant for T_m phosphorylation by K_m .	0.002
λ_T^*	5	Rate constant for T_c^* dephosphorylation in the cytosol.	0.15 – 0.21
λ_K^*	6	Rate constant for K_c^* dephosphorylation in the cytosol.	0.34 – 0.58
κ_K^*		K_c^* for which the reaction that dephosphorylates PI3K begins to saturate.	0.013

Table A.2
Parameters which define the model variants

Parameter	Case 1	Case 2	Case 3	Case 4
κ_K	1.3	5.5	0.95	4.0
κ_C	6.7	1.38	5.0	1.25
γ_0	0.014	0.037	0.011	0.028

Table A.3
Steady-state, uniform profile for each variant at zero stimulus

Variable	Case 1	Case 2	Case 3	Case 4
P_3	0.38	1.16	0.19	0.85
P_2	0.60	2.82	0.25	1.78
$P_n (=P_3+P_2)$	0.98	4.0	0.44	2.63
K_m	0.025	0.064	0.016	0.046
T_m	0.1	0.054	0.15	0.071
$A_{cell}K_c^*$	0.50	0.50	0.50	0.50
$A_{cell}T_c^*$	0.50	0.50	0.50	0.50

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Fig. 1a

Fig. 1b

Figure 1. Idealized model of the cell

a) Geometry and components. The cell is treated as a 2-d circle. The perimeter, marked by the normalized coordinate, X , represents the plasma membrane, along which 3'PI signaling lipids diffuse. Their spatial distribution is a primary output of our model. Regulatory proteins can

exchange between the membrane and the cytosol, where they are considered to be uniformly distributed. A pattern of activated receptor on the cell surface, representing the outside stimulus, is taken as the input for our simulations. b) Variables and network topology. The single variable, R , represents the outside stimulus, which drives PI3K to the membrane. Positive feedback Loops I and II enhance generation of P_n through substrate delivery and enzyme regulation, respectively. Negative Feedback Loop III represents PI3K phosphorylation and removal from the membrane, which lead to adaptation.

Figure 2A

Figure 2B **Figure 2. Characteristic gradient-sensing responses**

a) Transient response to a uniform stimulus. At time $t = 0$, the external variable, R , is uniformly increased, representing a uniform step stimulus. The figure shows the transient, in-phase responses of the model variables, normalized by their values prior to the stimulus. b) Steady-state polarized response to a gradient. The steady-state profile of the model variables (again normalized by their values in the resting cell) arising in response to a small gradient in R that represents a spatially linear gradient in the outside stimulus, where the gradient lies along the $X = 0/X = 1$ direction (the spatial variable, X , is normalized and periodic).

Figure 3. Stability of uniform steady-state solutions

Growth rates, σ_j , are plotted for least-stable perturbations with spatial wave numbers indicated by the index, j , about uniform steady-state solutions. The system is stable towards perturbations with negative growth rate, but unstable when σ_j is positive. The grey scale cartoons indicate the shape of the first few spatial modes around the membrane. The four Cases analyzed represent the qualitatively different response-amplification mechanisms that had been analyzed with the model.

Figure 4A

Figure 4B

Figure 4. Stability of polarized solutions

a) Representative P_n profiles and growth rates (σ) are indicated for the two least stable internal perturbation shapes about the polarized state-state solution that results from application of a small background gradient in receptor activity. The least stable mode (1st mode) is peaked at approximately 60 degrees with respect to the background gradient and the peak in the steady-state profile (identical to that shown in Fig.2b), indicating an enhanced sensitivity to off-axis perturbations. The 2nd mode decays much more quickly than the 1st, indicating that the least stable mode is likely to dominate responses to internal perturbations. Background (steady-state) and perturbation profiles are normalized so that their peak value is 1. b) P_n , for the least stable perturbation (1st mode), shown with the other model variables. Each variable is scaled by its value in the resting cell and then the entire mode profile is scaled so that the peak P_n value is 1. Plotted results pertain to Case 4, but qualitatively similar results were found for all four Cases, over a range of background gradients.

Figure 5A

Figure 5B

Figure 5C

Figure 5C_inset

Figure 5. Point source simulations

a) Simulation geometry. The cell is first allowed to equilibrate to a small external linear gradient, as in Fig. 4. A point source in space and time is then released at an angle, φ , with respect to the background gradient, and the response in time is recorded. θ_{max} measures the direction of the peak response on the membrane. b) Time response. The spatial profile at the time of maximal response to a point source released at $\varphi = 36$ degrees, is plotted. ΔP_n indicates the difference between the instantaneous P_n distribution ('Total P_n ') and the steady-state profile ('Background P_n '). Notice that the peak in ΔP_n , θ_{max} , is at an angle greater than 36 degrees, i.e. the response overshoots. Qualitatively similar results were found for all four Cases, over a wide range of background gradients and point source parameters (results for Case 4 are plotted). c) Time progression of four membrane points for the conditions shown in (b). Here, ΔP_n is normalized by the peak value of the 'Background P_n ' curve in b. Inset: Logarithmic

plot of maximum relative ΔP_n vs point source strength. The response levels off as the source strength increases. The dashed line indicates the strength used in all simulations. Qualitatively similar results were found for all four Cases, over a wide range of background gradients and point source parameters (results for Case 4 are plotted).

Figure 6A

Fig. 6b

Figure 6 C **Figure 6. Angular bias in responses to external stimuli**

Responses of polarized cells to point sources were simulated, as in Fig. 5, and the results plotted as a function of the source angle, φ . a) Peak value of the response (ΔP_n), normalized by the peak value of P_n in the steady-state background profile. The strongest responses occur for point sources at angles of approximately 40 – 80 degrees with respect to the background gradient. b) Direction of the maximal response, θ_{max} (see Fig. 4). The cartoon illustrates the response overshooting that is observed for source angles less than approximately 40 degrees (A), and undershooting that is generally seen for large source angles (C). Similar results were found over a range of point-source parameters.

Figure 7A

Figure 7B

Figure 7C

Figure 7. Angular bias in time-integrated responses to external stimuli

Integrating the response of polarized cells to point sources over time accentuates biased sensitivity in responses. a) The profile of ΔP_n in response to a point-source stimulus released at $\phi = 36^\circ$, integrated over 50 seconds. Now θ_{max} is the direction of maximal time-integrated response. b) Peak value of the time-integrated response for each point source angle, ϕ , analogous to Fig. 6a. Time integration makes the enhanced responses to off-axis stimuli most evident. c) Direction of the maximal response, θ_{max} . Notice that the response overshooting/undershooting sources at small/large ϕ is seen more clearly here than it was in Fig. 6b, where the response was not integrated. Similar results were found over a range of point-source parameters.

Figure 8A

Figure 8B

Figure 8. PMN leukocytes moving in a chemoattractant gradient

a) Idealized representation of cell tracks, showing the angle through which a cell turns after moving on a track directed at an oblique angle with respect the gradient prior to the turn. b) Absolute values of the turn angles, ψ , vs. directions of movement prior to a turn, ω (see (Nossal and Zigmond, 1976)). Solid line indicates the turn angle that would have reoriented the cells exactly towards the source. Note that a curve of the average value of ψ if plotted as a function of ω , would have the qualitative features of the curves shown in Figs. 6b and 7c.