Universal mechanism of eukaryotic directional sensing

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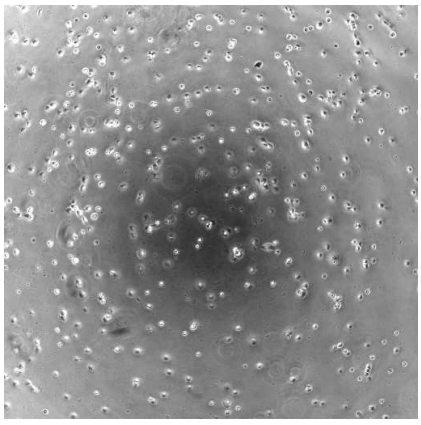
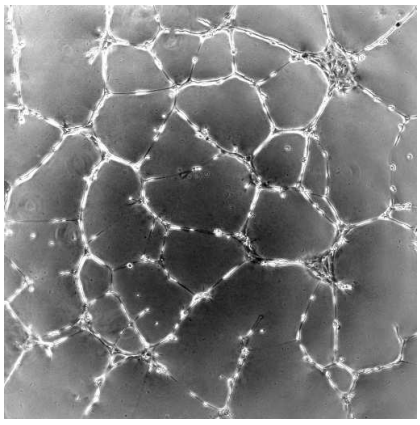
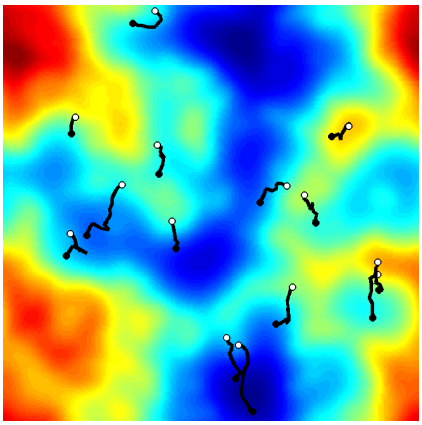
Abstract

Eukaryotic cells possess an extremely sensible chemical compass allowing them to orient toward sources of soluble chemicals. This property is key to the assembly of complex, multicellular organisms. The extracellular chemical signal triggers separation of the cell membrane into two domains populated by different phospholipid molecules and oriented along the signal anisotropy. We propose a universal description of this polarization process, based on the theory of phase ordering in first-order phase transitions. This description implies the existence of two clearly separated polarization regimes depending on the presence or absence of a gradient in the activation pattern produced by the extracellular attractant, and the existence of a sensitivity threshold for the gradient. Simple scaling laws are found: the polarization time t_ϵ depends on the gradient steepness ϵ through the power law $t \propto \epsilon^{-2}$, while the smallest detectable gradient scales as $\epsilon_{\mathrm{th}} \propto R^{-1}$, where R is the size of the cell. Our results are in agreement with existing experimental data.

Motion directed by chemical signals is key to the assembly of complex organisms

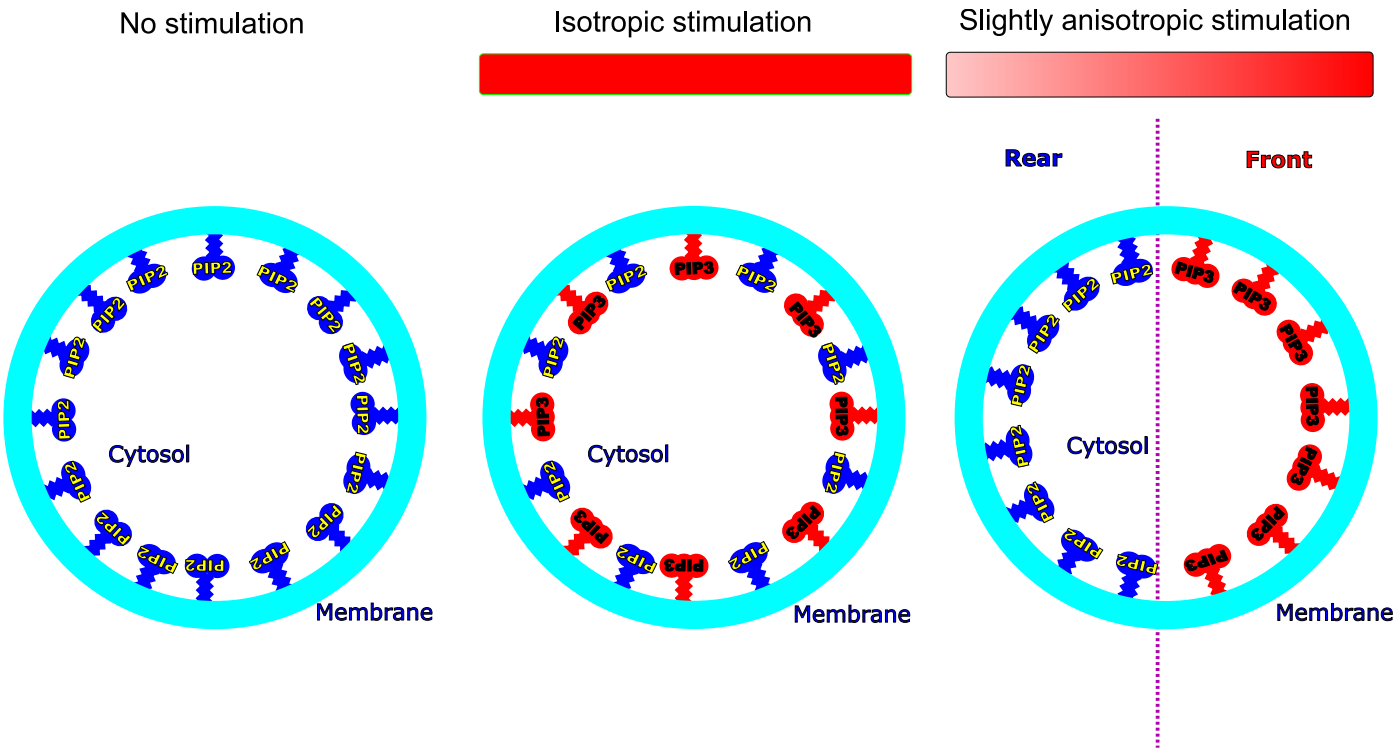
The cells of multicellular organisms are endowed with a chemical compass of amazing sensitivity, which is the result of billions of years of evolution. Concentration differences of the order of a few percent in extracellular soluble attractant chemicals from side to side are sufficient to induce a chemical polarization of the membrane leading to cell migration towards the signal source.
Directional sensing is essential in embryo development, where tissue formation is realized through coordinated migration of specific cells guided by chemical signals, and in the adult organism, where chemical signals guide white blood cells to the sites of inflammation and platelets to sites of wound repair.

a  **b**  **c** 

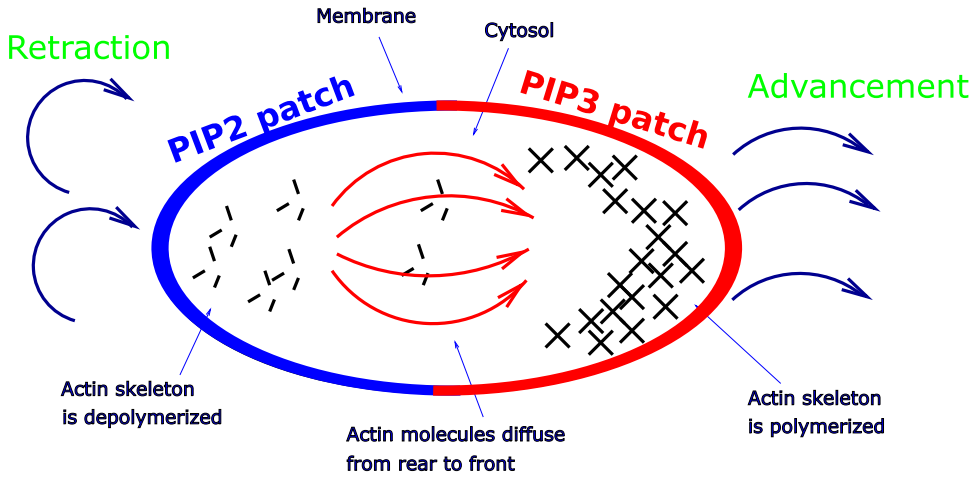
Self aggregation of blood vessels in vitro guided by the exchange of chemical attractants. **a**: cells are randomly seeded on a gel surface; **b**: after 12 hours, cells form a prototype blood vessel network; **c**: experimental cell trajectories move towards attractant sources in a simulated attractant landscape (Gamba et al. *Phys. Rev. Lett.* **90**, 118101, 2003; Serini et al. *EMBO J.* **22**, 1771, 2003)

Directional sensing

Polymerization/depolymerization of the actin cytoskeleton, growth/shrinkage of microtubules, and activation/deactivation of contacts with the extracellular matrix, are coordinated by the directional sensing module located in the cell membrane through a cascade of chemical reactions.
Directional sensing is a phase-ordering process where two phospholipids, PIP_2 and PIP_3, cluster in complementary regions: PIP_3 in the region facing the origin of the stimulation, PIP_2 in the opposing region. Interestingly, phase ordering does not take place under a perfectly isotropic stimulation condition, but only when a slight gradient is present.



This early phase-ordering process then induces cell motion in the direction of the attractant source:



Experimental observation of the patch nucleation process

When a uniform receptor stimulation of a suitable amplitude is switched on, nucleation of phospho-lipid patches is observed on the membrane of live cells.

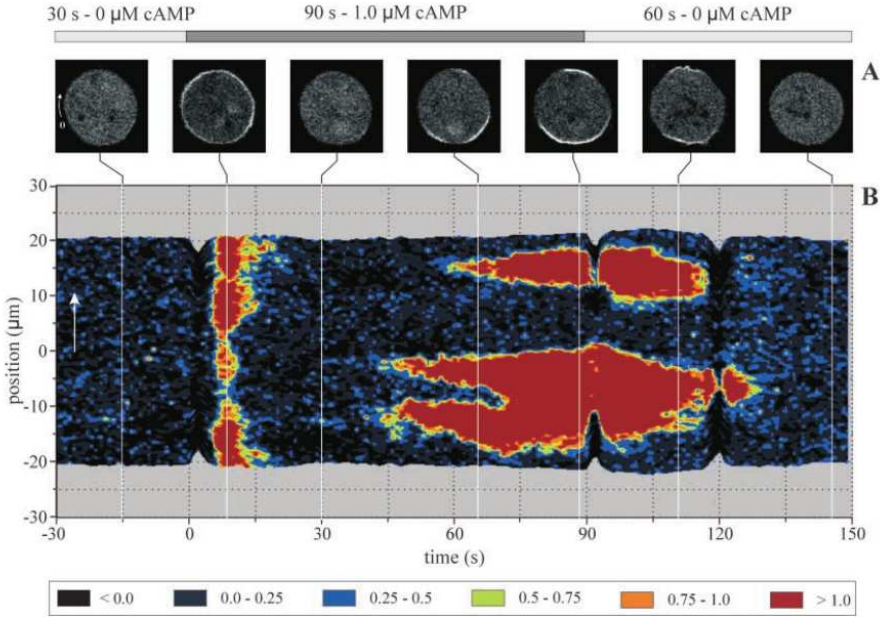
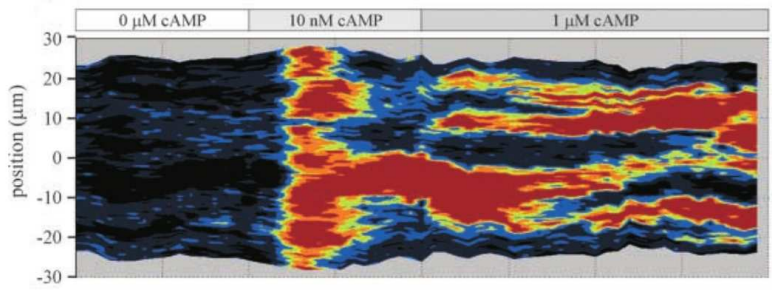


Fig. 5. Patch formation in latrunculin-A-treated cells. Cells in the perfusion chamber were incubated with 1 µM latrunculin A for 15 minutes, subsequently stimulated at t=0 seconds with 1 µM cAMP and washed with buffer at t=90 seconds. (A) The fluorescence intensity and the shape of a representative cell before and after stimulation with cAMP. (B) The fluorescence intensity at the boundary for the same cell. Before mounting the perfusion chamber, cells were overlaid with a thin layer of agarose to prevent elution of these spherical cells. The small indentations at 0 seconds, 90 seconds and 120 seconds are perfusion artefacts; at the onset of perfusion, the cover slip bends for a few seconds and cells move in the plane of focus.



Patch formation in latrunculin-treated Dictyostelium cells (Postma et al., J. Cell Sci. 2004).

Sensitivity Threshold

Two different regimes of membrane polarization may be distinguished. Anisotropy driven polarization induced by the presence of an attractant gradient is realized in a time of the order of a few minutes, and results in the formation of a PIP_3-rich patch on the membrane side closer to the attractant source and of a PIP_2-rich patch in the complementary region. On the other hand, cells exposed to uniform distributions of attractant polarize in random directions, in times of the order of an hour. The existence of two clearly separated polarization regimes is confirmed by the observation of a sensitivity threshold of the order of a few percent difference in the attractant molecule concentration from side to side.

thus replace a dynamically changing concentration distribution with an externally controllable chemical environment.

So far, microfluidic devices have been applied to the migration of neutrophils (Lin et al., 2004), bacteria (Thar and Kuhl, 2003), and cancer cells (Wang et al., 2004). Here, we use microfluidic techniques to study *Dictyostelium* chemotaxis in spatially linear and temporally stable cAMP gradients. In the device, stable gradients can be maintained as long as required. The concentration profile was verified by three-dimensional 2-photon imaging techniques and numerical finite-element simulations.

The experiments showed a threshold for the chemotactic response at a gradient value of $dc/dy \equiv \nabla c \approx 10^{-3}\,nM/\mu m$. Above threshold, the motion was governed by the absolute steepness of the gradient of the chemoattractant, i.e., independent of the local concentration. The chemotactic speed and the motility increased with increasing steepness of the gradient until $\nabla c \approx 10^{-1}\,nM/\mu m$; no further increase of the chemotactic response was observed in steeper gradients up to $\nabla c \approx 1\,nM/\mu m$. In gradients above $\nabla c \approx 10\,nM/\mu m$, the cells lost directionality and the motility returned to the sub-threshold level. An estimate of cAMP receptor occupancy as a function of gradient steepness offers a possible explanation. It shows that in the regime of

(Song et al. *Eur. J. Cell Biol.*, **85**, 981, 2006)

maintain a steady flow through the device. During the recording of experimental data, the flow speed in the main channel was 650 µm/s, which was high enough to ensure a stable concentration gradient over the entire length of the channel but low enough so that the cells in the channel were not washed away. This constant flow

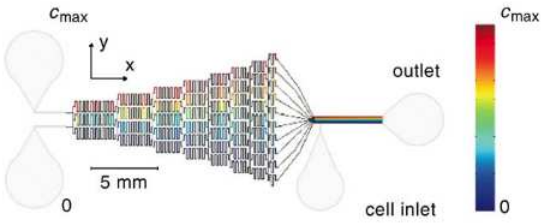
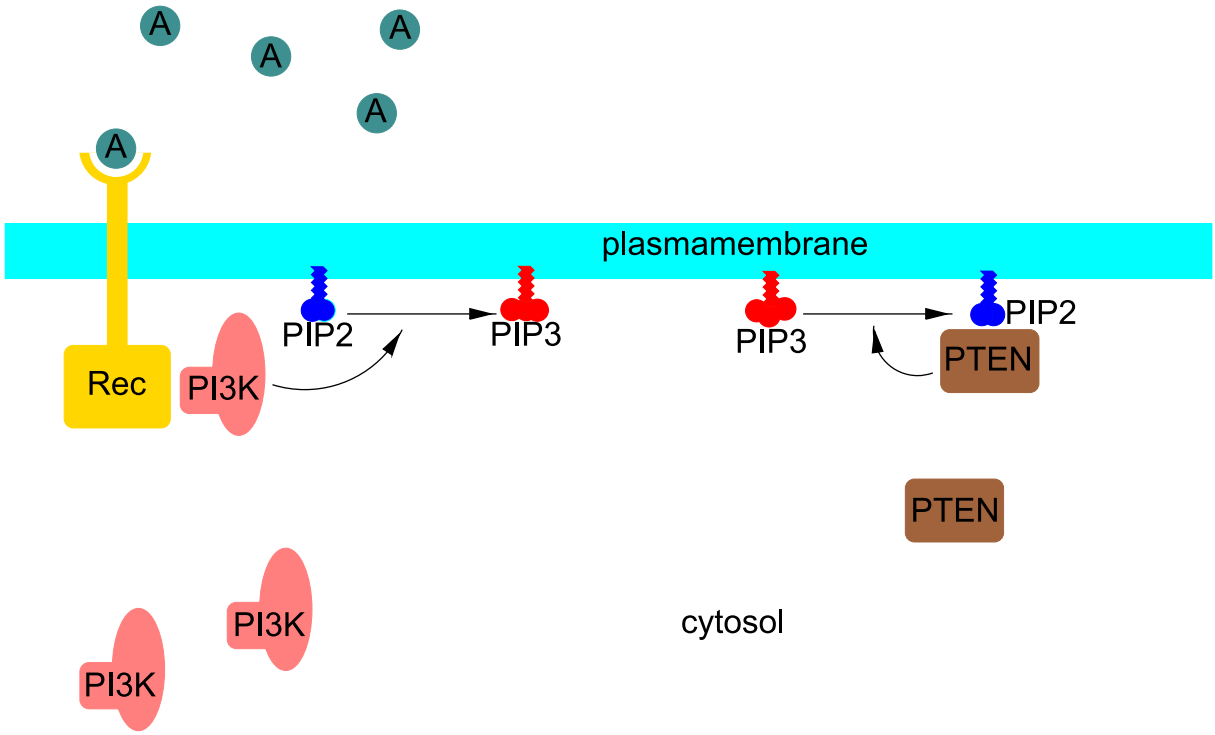


Fig. 1. Layout of the microfluidic channel network used to generate a linear concentration gradient. The color coding displays the concentration from a two-dimensional numerical simulation of the Navier–Stokes and convection-diffusion equation in the shown geometry using FEMlab 3.1. Black lines mark in- and outlets that were not part of the numerical simulation. Parameters: density $\rho = 10^{-12}\,g/\mu m^3$, kinematic viscosity $v = 10^6\,\mu m^2/s$, inflow velocity $v = 3250\,\mu m/s$, inflow concentrations: zero and $c_{max} = 2\,nM$, cAMP diffusivity $D = 400\,\mu m^2/s$ (Bowen and Martin, 1964), no-slip and isolating boundary conditions except for inlet and outlet.

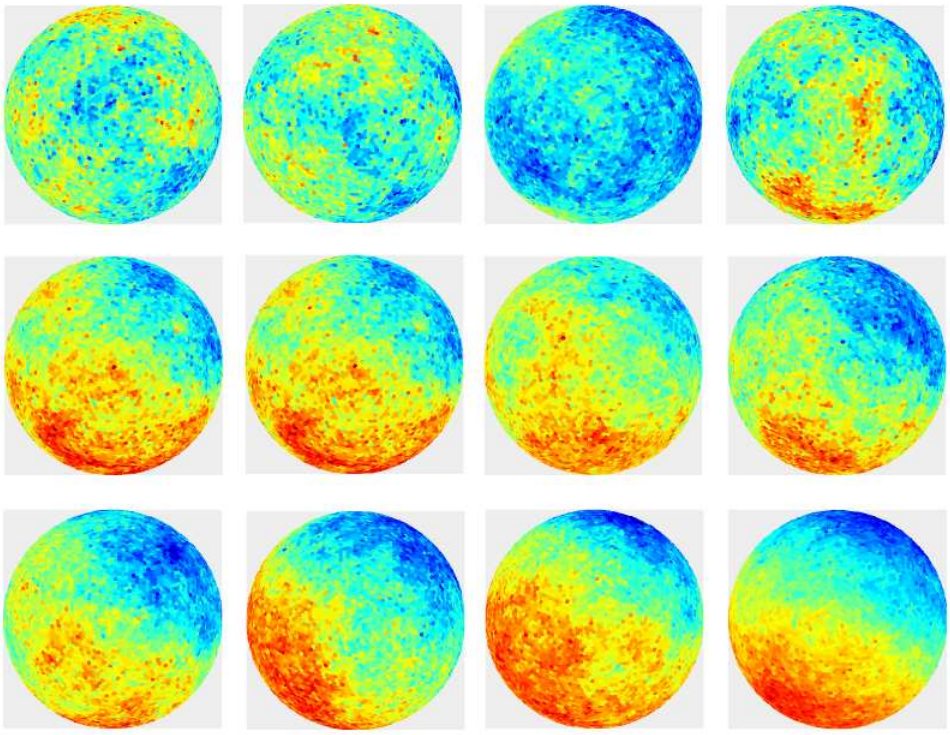
The signaling network

Two enzymes, PI3K and PTEN, respectively transform PIP_2 into PIP_3 and viceversa. The phospholipids are permanently bound to the inner face of the cell membrane, while PI3K and PTEN diffuse in the cell volume and are active only when they are adsorbed by the membrane. PI3K adsorption takes place through binding to receptors activated by the extracellular attractant signal. This way, the external attractant field is coupled to the inner dynamic of the cell. PTEN adsorption takes place through binding to the PTEN product, PIP_2. This process introduces a positive feedback loop in the system dynamics. When the cell is not stimulated by an attractant signal the cell membrane is uniformly populated by PTEN and PIP_2 molecules. When a uniform receptor stimulation of a suitable amplitude is switched on, PI3K molecules bind to the membrane and shift its chemical balance toward a PIP_3-rich phase, while PTEN desorbs. However, the uniform PIP_3-rich distribution is metastable and decays giving rise to a non-uniform state consisting of complementary, PIP_2-rich and PIP_3-rich, clusters.



Monte Carlo simulations

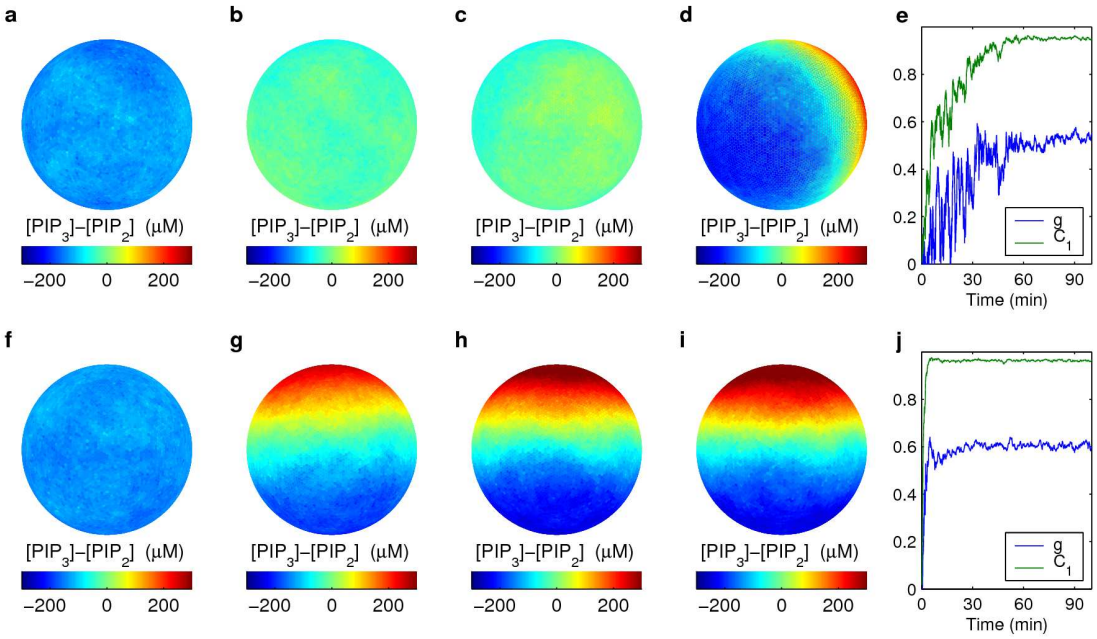
Numerical simulations of the directional sensing network performed with the use of physical and kinetic parameters obtained from the biochemical literature show that under appropriate conditions the biochemical network is indeed bistable, and that it undergoes spontaneous separation in chemically different phases, rich in PIP_2 and PIP_3 , respectively.



Time course of the process of coarsening of PIP_2-rich and PIP_3-ritch patches.
(Gamba et al., *Proc. Nat. Acad. Sci. U.S.A.*, **102**, 16927, 2005; de Candia et al., *Science's STKE*, **378**, pl1, 2007).

Monte Carlo simulations

A 5% anisotropic component in the cell stimulation accelerates cell polarization and correspondingly decreases the characteristic time needed for complete phase separation by more than one order of magnitude: fast, anisotropy driven polarization is realized in times of the order of a minute, while slow, stochastic polarization is realized in times of the order of one hour, in accordance with experimentally observed times.



Time course of cell polarization **a**-**e**: under uniform stimulation, and **f**-**j**: under a 5% stimulation gradient in the vertical direction (Gamba et al., *Proc. Nat. Acad. Sci. U.S.A.*, **102**, 16927, 2005; de Candia et al., *Science's STKE*, **378**, pl1, 2007)

Characteristic time scales

thermal relaxation:	10 μs
catalysis:	1 s
cytosolic diffusion time:	10 s
directed polarization:	100 s
random polarization:	1000 s

Phenomenological description

Taking into account:

- Separation of timescales
- Bistability
- Symmetry breaking is described by $\varphi = [\mathrm{PIP}_3] - [\mathrm{PIP}_2]$
- There is no local conservation law for φ: PIP_2 patches may evaporate freely

we conclude that the correct coarse-grained description of the phase-ordering process is given by a time-dependent Landau-Ginzburg model:

$$\partial_t \varphi(\boldsymbol{r}, t) = -\frac{\delta \mathcal{F}[\varphi]}{\delta \varphi(\boldsymbol{r})} = D\,\nabla^2 \varphi - \frac{\partial V}{\partial \varphi}(\varphi) \tag{1}$$

$$\mathcal{F}[\varphi] = \int_S \left[\frac{D}{2} |\nabla \varphi|^2 + V(\varphi) \right] \mathrm{d}\boldsymbol{r} \tag{2}$$

Model free energy

We can learn something about $V(\varphi)$ from a simple model based on the law of mass action and enzymatic kinetics. We have:

$$\begin{aligned} \varphi &= [\mathrm{PIP_3}] - [\mathrm{PIP_2}] \\ [\mathrm{PIP_2}] + [\mathrm{PIP_3}] &= c = \text{const} \end{aligned}$$

Using local equilibrium:

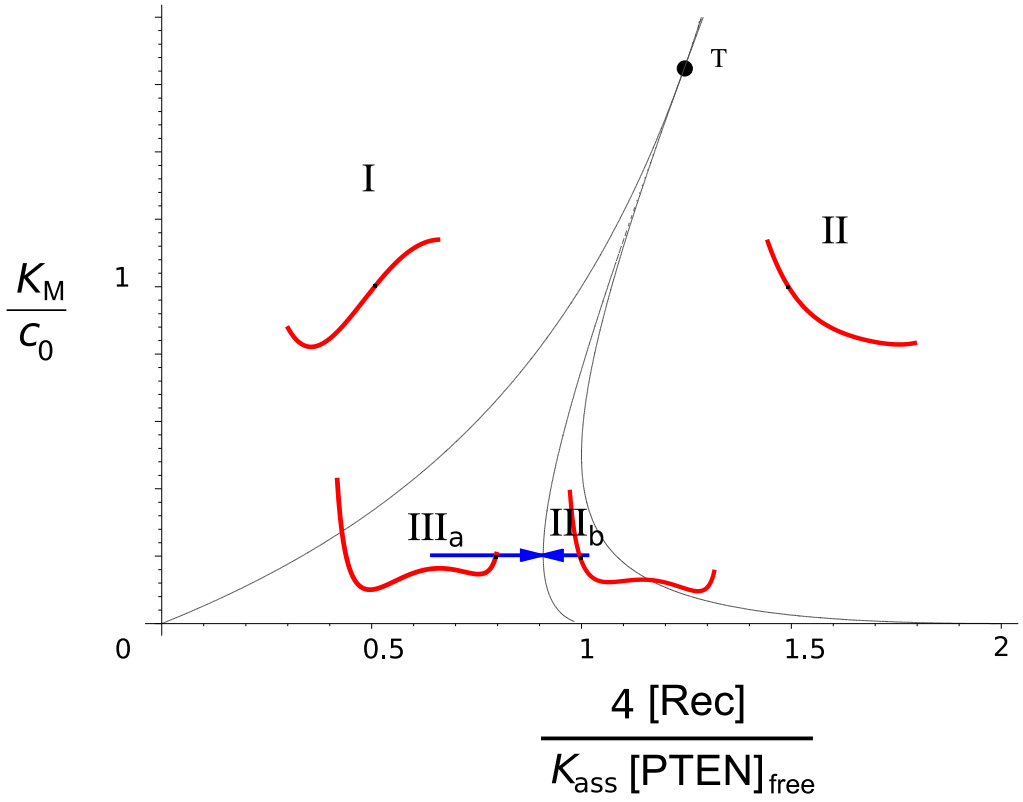
$$[\mathrm{PTEN}]_{\mathrm{bound}} = K_{\mathrm{a}}\,[\mathrm{PTEN}]_{\mathrm{free}}\,[\mathrm{PIP_2}]$$

From the law of mass action and Michaelis-Menten:

$$\partial_t \varphi = D\,\nabla^2\varphi - k_{\mathrm{cat}}\,K_{\mathrm{ass}}[\mathrm{PTEN}]_{\mathrm{free}}\,\frac{c^2-\varphi^2}{2K_{\mathrm{M}}+c+\varphi} + 2\,k_{\mathrm{cat}}\,[\mathrm{Rec}]\,\frac{c-\varphi}{2K_{\mathrm{M}}+c-\varphi}$$

k_{cat} : catalytic rate
K_{ass} : PTEN association constant

$$V(\varphi) = [\mathrm{PTEN}]_{\mathrm{free}}\,V_1(\varphi) + [\mathrm{Rec}]\,V_2(\varphi)$$



Equilibrium phase diagram for the model free energy. In regions $\mathrm{III_a}$ and $\mathrm{III_b}$ the potential is bistable: it has two minima, corresponding to a PIP_2- and a PIP_3-rich phase. Regions $\mathrm{III_a}$ and $\mathrm{III_b}$ are separated by a phase coexistence line, where the two phases have the same energy.

The potential $V(\varphi)$ is a function of receptor activation [Rec] and of the number of free (cytosolic) PTEN's.

If we introduce

$$f \;=\; (\text{free PTENs})$$

we get the following system of equations

$$\partial_t \varphi \;=\; D\,\nabla^2\varphi - \frac{\partial V}{\partial \varphi}(\varphi; f) \tag{3}$$

$$f(t) \;=\; a + b\int_S \varphi(\boldsymbol{x}, t)\,\mathrm{d}^2x \tag{4}$$

since:
$$\begin{aligned}(\text{free PTENs}) + (\text{bound PTENs}) \;&=\; \text{const}\\ (\text{bound PTENs}) \;\propto\; \int_S [\mathrm{PIP_2}]\,\mathrm{d}\sigma \;&\propto\; \int_S (\text{const} - \varphi)\,\mathrm{d}\sigma\end{aligned}$$

The constraint on f drives the system towards phase coexistence. For large times:

$$(\text{free PTEN}) \;\to\; \text{equilibrium value}$$

so we can study (3) in a first approximation as if f was a constant, and in a second approximation take into account the slow variation of f.

We can measure the distance from the line of phase coexistence introducing the
degree of metastability

$$\psi \;\propto\; \Delta V \;=\; \text{potential difference between the two minima}$$

$$\sim\; -\frac{[\mathrm{PTEN}]_{\mathrm{free}} - [\mathrm{PTEN}]^{\mathrm{eq}}_{\mathrm{free}}}{[\mathrm{PTEN}]^{\mathrm{eq}}_{\mathrm{free}}}\, k_{\mathrm{cat}}\,[\mathrm{Rec}]\,c_0$$

The degree of metastability ψ is a function of the excess of free PTEN's with respect to their equilibrium value.

The critical radius

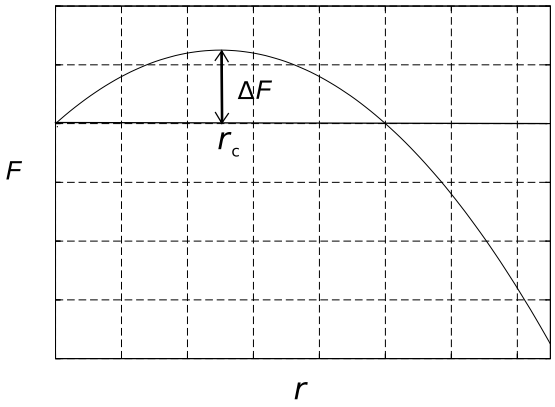
We restrict our considerations to approximately circular patches of the PIP_2-rich phase, which are expected to dominate over different geometries due to the presence of a linear tension between the two phases. The free energy of a PIP_2-rich patch of radius r can be written on phenomenological grounds as

$$\mathcal{F} \;=\; -\psi r^2 + 2\sigma r \tag{5}$$

where σ is the linear tension of the interface with the surrounding PIP_3-rich phase and ψ is the degree of metastability.
According to the kinetic theory of first order phase transitions, the equation of growth of a patch of the new phase is dissipative and can be written as $\Gamma\,\dot{r} = -\,\partial\mathcal{F}/\partial r$, where $\Gamma(r)$ is a damping coefficient. Since energy dissipation occurs mainly along the perimeter of the interface between the two phases, Γ may be written as $2\pi r\gamma$, where γ is a constant, and we get

$$\gamma\dot{r} \;=\; \psi - \frac{\sigma}{r} + \xi \;\equiv\; \sigma\left(\frac{1}{r_c} - \frac{1}{r}\right) + \xi \tag{6}$$

where the noise term ξ represents thermal fluctuations. The fluctuations are responsible for the formation of a population of germs of the new phase with varying radii r.



Graph of the free energy (5).

Domain nucleation

We take into account the effect of thermal agitation by adding a white noise term in the r.h.s. of (1):

$$\begin{aligned} \partial_t\varphi &= -\frac{\delta\mathcal{F}}{\delta\varphi} + \xi \\ \langle\xi(\boldsymbol{x},t)\xi(\boldsymbol{x}',t')\rangle &= 2\Gamma\,\delta^2(\boldsymbol{x}-\boldsymbol{x}')\,\delta(t-t') \end{aligned}$$

The nucleation rate of supercritical germs of the PIP_2-rich phase may be computed as

$$J \;=\; \frac{\Gamma^{1/2}\,\psi^{3/2}}{2\pi\sigma\gamma}\exp\left(-\frac{\pi\sigma^2}{\Gamma\psi}\right)$$

Is nucleation rate the cue to gradient sensing?

The nucleation rate J is highly sensitive to small variations in ψ. In particular, J is highly sensitive to spatial variations in ψ induced by slight activation gradients. This could induce differentiated nucleation rates in different parts of the cell.

However, J is not only sensitive to slight anisotropies, but also to the absolute value of the activation, and to the precise values of all microscopic characteristics, such as kinetic rate constants and diffusivity. If the cell response was controlled by the characteristic nucleation time J^{-1}, a slight variation in any of these characteristics would produce dramatic variations in the response itself.

Since this is not observed, it is reasonable to assume that the cell lives in a region of parameter space where the characteristic nucleation time J^{-1} is much faster than the other relevant timescales, such as the characteristic times for directed and stochastic polarization.

Domain growth

Germs with r smaller than the critical radius

$$r_c \;=\; \frac{\sigma}{\psi}$$

are mainly dissolved by diffusion, while most germs with $r > r_c$ survive and grow because of the gain in free energy. At initial time, r_c is of the order of the thickness δ of the interface between the two phases.

As long as the area occupied by patches of the PIP_2-rich phase phase grows, the degree of metastability ψ decreases, some of the patches that were initially growing become undercritical and shrink, large patches start "feeding"" on smaller ones, and the total number of patches diminishes. In the final stage of this process a single domain of the PIP_2-rich phase coexists with the PIP_3-rich phase. The details of the process leading to this final stage depend on the external conditions, and, particularly, on the degree of anisotropy of the attractant signal.

The population of patches can be described in terms of the size distribution function $n(r,t)$ such that

$$n(r,t)\,\Delta r \;=\; \#\{\text{domains with radius in the interval } (r, r+\Delta r)\}$$

$$\int_0^\infty n(r,t)\,\mathrm{d}r \;=\; N(t) \;=\; \text{total number of domains at time } t$$

An important simplification comes from the fact that for germs with $r > r_c$ the noise term ξ becomes negligible. This means that the stochastic nature of the problem enters mainly in the formation of the initital distribution of germ sizes $n(r)$, while for $r > r_c$ the time evolution of $n(r)$ is dictated by the deterministic part of (6), from which the kinetic equation follows:

$$\gamma\,\frac{\partial n(r,t)}{\partial t} + \frac{\partial}{\partial r}\Big[\Big(\psi(t) - \frac{\sigma}{r}\Big)\,n(r,t)\Big] \;=\; 0 \qquad (7)$$

Scaling solution

To obtain a closed system of equations we need an additional equation for the time evolution of the metastability degree ψ. In the case of isotropic stimulation ψ does not depend on the position on the membrane and is instead only a function of time. Since diffusion of PTEN molecules in the cell volume is faster than phospholipid diffusion on the membrane we can regard the concentration of PTEN molecules in the volume as uniform. Moreover, fast PTEN diffusion also implies that ψ instantaneously adjusts to the changes in the size distribution function. While the total number of patches diminishes as an effect of the coarsening dynamics, the total area occupied by the patches, as well as the total number of PIP_2 molecules found in the patches, monotonically increases towards their respective equilibrium values. The metastability degree ψ is equal to zero in equilibrium, and tends to zero in accordance with

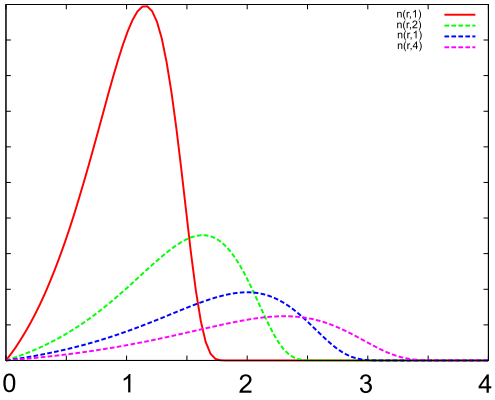
$$\psi \;\propto\; A - \int_0^\infty \pi r^2 n(r,t)\,\mathrm{d}r \tag{8}$$

as the total patch area tends to its limit value A. Eq. (8) reflects the fact that in the asymptotic region ψ is proportional to the excess concentration of PTEN molecules in the volume with respect to the equilibrium value, and therefore to the difference between the area occupied by the PIP_2-rich phase at a given time and at equilibrium.

Asymptotically, (7,8) lead to the self-similar solution

$$\psi(t) \;=\; \frac{\sigma}{r_c(t)}, \qquad r_c(t) \;\sim\; t^{1/2}$$

$$n(r,t) \;=\; r_c^{-3}(t)\, g(r/r_c(t)), \qquad g(\rho) \;=\; \frac{C\rho}{(2-\rho)^4}\exp\!\left(-\frac{4}{2-\rho}\right)$$



Similarly to what happens in Lifshitz-Slyozov theory, the total number of patches decreases in time due to the evaporation of small patches:

$$\int_0^\infty n(r,t)\,\mathrm{d}r \;=\; \int_0^\infty \left[r_c^{-3} g(\rho)\right] r_c\,\mathrm{d}\rho \;\sim\; \frac{1}{r_c^2} \;\sim\; \frac{1}{t}$$

and the average domain size coincides with the critical radius:

$$\langle r\rangle \;=\; \frac{\int_0^\infty r\, n(r,t)\,\mathrm{d}r}{\int_0^\infty n(r,t)\,\mathrm{d}r} \;=\; r_c$$

Isotropic stimulation

The evolution of the size distribution $n(r)$ stops at times of order t_R, where t_R is defined as the instant when the average patch size $\langle r \rangle$ reaches the cell size R. From the scaling law:

$$\langle r \rangle \sim r_0 \, (t/t_0)^{1/2}, \qquad r_0 = \frac{\sigma}{\psi_0}, \qquad t_0 \sim \frac{\gamma r_0^2}{\sigma}$$

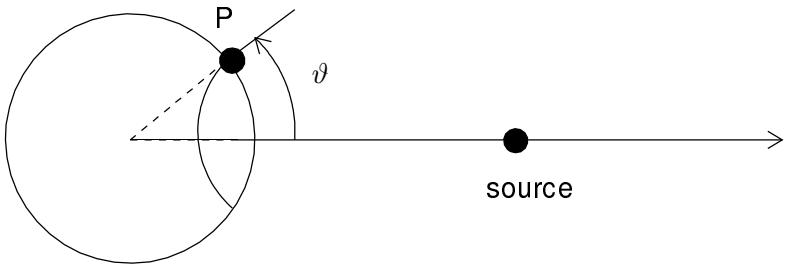
the coarsening process stops when

$$\langle r \rangle \sim R \qquad \Rightarrow \qquad \text{at time} \qquad t_R \sim t_0 \left(\frac{R}{r_0} \right)^2$$

and the cell becomes polarized in a random direction. The direction of polarization is determined by the random unbalance in the initial germ distribution.

Anisotropic stimulation

Let us now consider the case of an inhomogeneous activation pattern. The inhomogeneity of the concentration distribution modifies the degree of metastability, which becomes a function of the position on the membrane surface. Since the distribution of PTEN molecules in the cell volume is homogeneous, it influences only the isotropic part of the metastability degree ψ, which is a function of time, as previously.
In contrast, the anisotropic part of the metastability degree, $\delta\psi$, related to the external attractant inhomogeneity, does not depend on time.

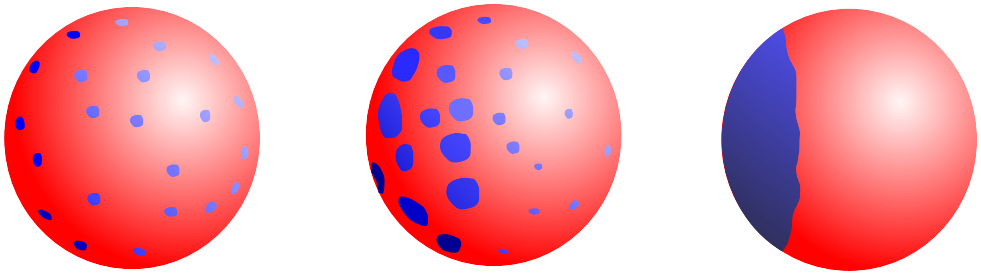


If the cell membrane has a nearly spherical form and a radius R much smaller than the characteristic scale of the extracellular attractant distribution, then

$$\psi \;\rightarrow\; \psi + \delta\psi \qquad \text{with} \qquad \delta\psi \;=\; -\,\epsilon\,\psi_0 \cos\theta$$

$$\text{and we get:} \qquad \gamma\,\dot{r} \;=\; \psi - \frac{\sigma}{r} - \epsilon\,\psi_0 \cos\theta + \xi \tag{9}$$

As long as $\varepsilon\,\psi_0 \ll \psi$, the first stage of patch growth proceeds approximately as in the isotropic case and ψ decreases as $t^{-1/2}$.
However, at a time of order t_ϵ, where t_ϵ is defined by the equation $\psi(t_\epsilon) = \epsilon\,\psi_0$, the perturbation $\epsilon\,\psi_0\,\cos\theta$ becomes comparable to ψ and the process of polarization becomes anisotropic, so that patches in different regions get different average sizes



$t < t_\epsilon$ $\qquad$ $t \sim t_\epsilon$ $\qquad$ $t > t_\epsilon$

Patch growth in the presence of a slight gradient of attractant activation directed from left to right. The PIP_3- and PIP_2-rich phases are respectively red and blue (Gamba, Kolokolov, Lebedev, Ortenzi, *Phys. Rev. Lett.* **99**, 158101, 2007).

Gradient detection

For $t > t_\epsilon$, the leading term in (9) becomes the perturbation $\epsilon\,\psi_0 \cos\theta$, implying that in the region closer to source of the stimulation ($\cos\theta \gtrsim 0$) the PIP_2-rich phase evaporates in a time which is of order t_ϵ, leading to the formation of a single PIP_2-rich patch in the region further from the source of the stimulation ($\cos\theta \lesssim 0$) and realizing complete polarization.

The growth in this second stage is linear and the time needed to reach $r \sim R$ is again of order t_ϵ.

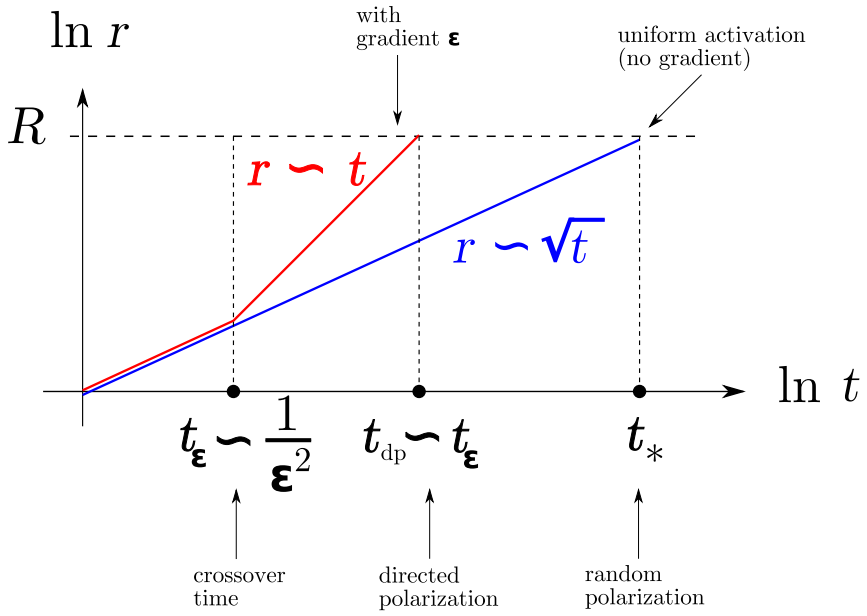
This gives the scaling law

$$t_\epsilon \;\sim\; \frac{t_0}{\epsilon^2}$$

When

$$t_\epsilon \;\sim\; \frac{1}{\epsilon^2} \;\ll\; R^2 \;\sim\; t_* \qquad (\epsilon \gg r_0/R)$$

anisotropy-induced polarization is much faster than random polarization.



Sensitivity threshold

The second stage of patch evolution occurs only if $t_R \gg t_\epsilon$. Otherwise, the presence of a gradient of attractant becomes irrelevant and only the stage of isotropic patch growth actually occurs. This condition implies that a smallest detectable gradient exists, such that directional sensing is impossible below it. The threshold value $\epsilon_{\rm th}$ for ϵ is found by letting $t_\epsilon = t_R$. Since the product $\psi\, r_{\rm c}$ is a time-independent constant, we can simply compare its value at initial and final time when $\epsilon = \epsilon_{\rm th}$, obtaining $\epsilon_{\rm th} = r_0/R$, which gives us the expression for the threshold anisotropy:

$$t_\epsilon \;<\; t_R \;\sim\; t_0 \left(\frac{R}{r_0}\right)^2$$

$$\Rightarrow \qquad \epsilon \;>\; \epsilon_{\rm th} \;\sim\; \frac{r_0}{R}$$

Germ radius

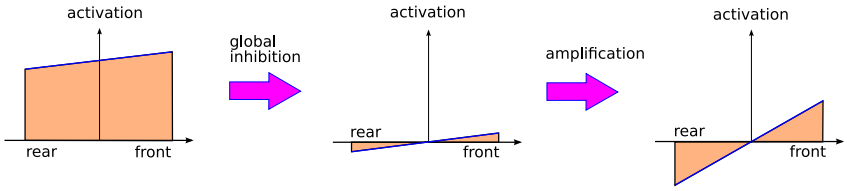
It is interesting to estimate r_0, and, consequently, $\epsilon_{\rm th}$, in terms of observable parameters. Comparing the characteristic patch surface and perimeter energy as a function of the phospholipid diffusion coefficient D, surface phospholipid concentration c, surface concentration of activated receptors h, and the characteristic catalytic time τ, one gets

$$r_0 \;\sim\; \delta \;\sim\; \sqrt{D\tau c/h}$$

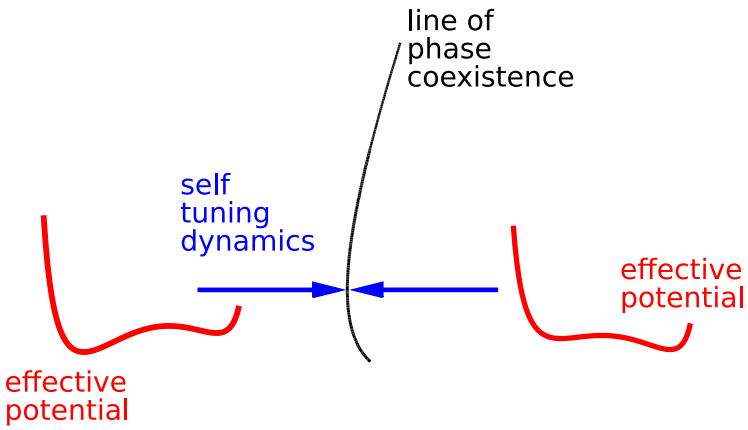
Using realistic parameter values one gets $r_0 \sim 1\,\mu$m and $\epsilon_{\rm th} \sim 10\%$. The value for $\epsilon_{\rm th}$ is compatible with experimental observations.

Self tuning

The constrained phase-ordering dynamics tunes the system towards phase coexistence, similarly to what happens in the case of the precipitation of a supersaturated solution.
This way, the metastability degree at a certain moment in time will become of the order of the free-energy difference induced by the stimulation gradient.
In previous attempts to explain gradient sensing a rapidly diffusing inhibitor was assumed to cancel out the spatial average of the signal, leaving only the anisotropic component:



However, in this scheme one has to fine tune the kinetic rate constants in such a way that the global inhibitor cancels out exactly the average component
Instead, in our scheme the system is seen to self tune dynamically towards the line of phase coexistence in such a way to extract the anisotropic component from the isotropic background.

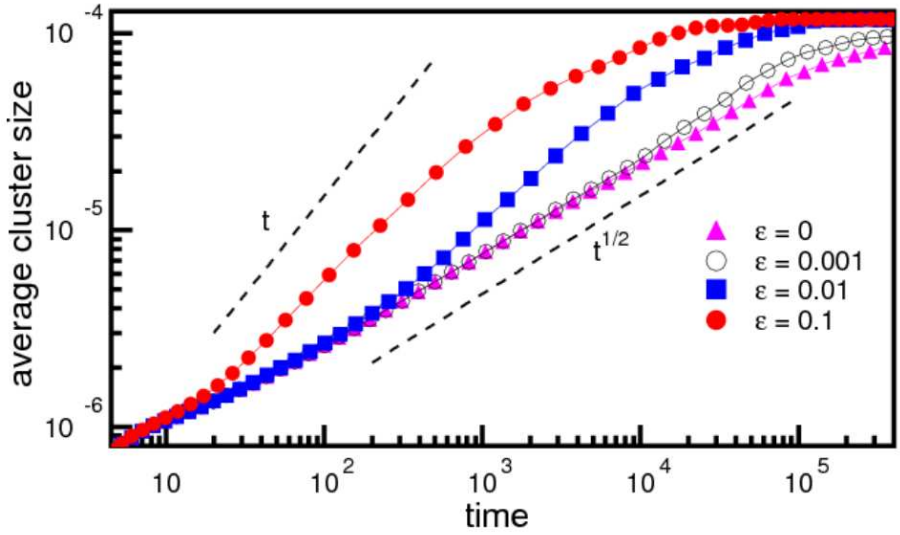


A lattice gas model

Our picture may be concretely realized using a simple lattice-gas model, where the ± 1 value of spins represents predominantly PIP_2 or PIP_3 regions on a 2d lattice, and the external activation field h is coupled to the order parameter to mimick a global feedback mechanism:

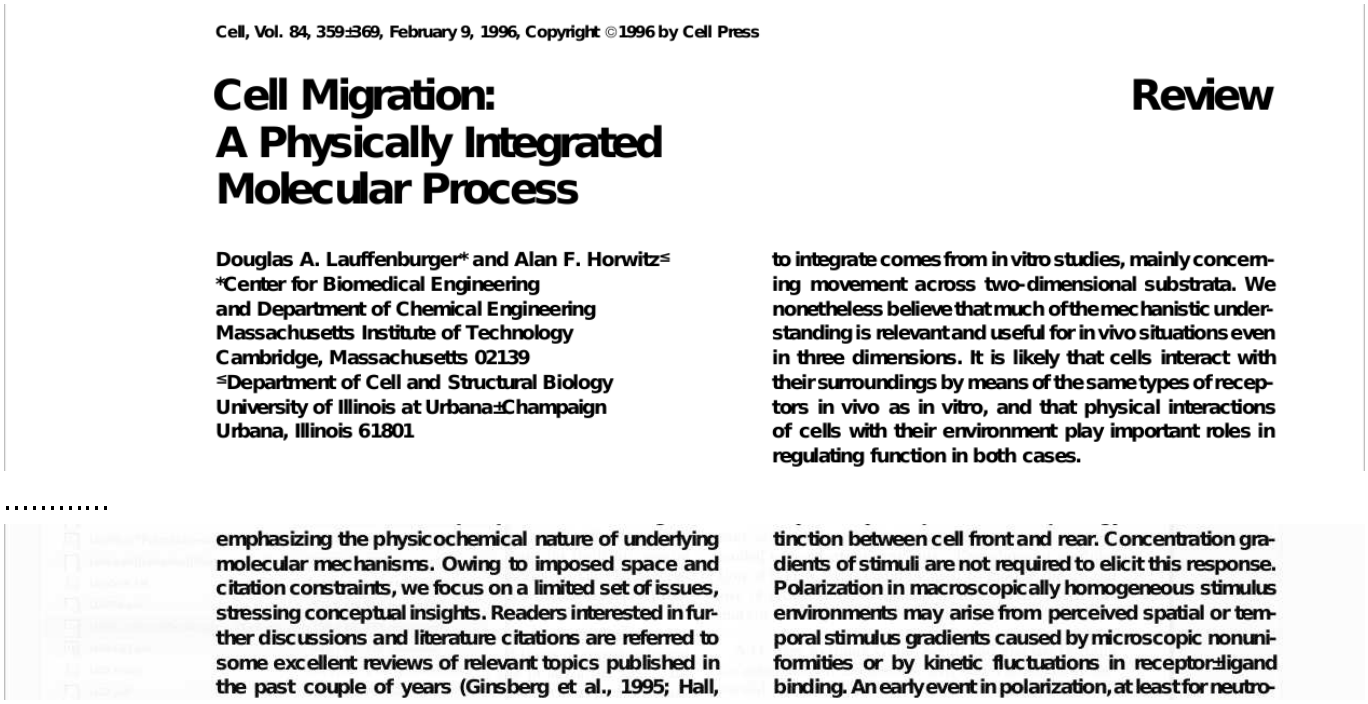
$$H = -J \sum_{\langle i,j \rangle} \sigma_i \sigma_j - \left(h - \frac{1}{N} \sum_j \sigma_j \right)^2$$

The process of phase ordering in the presence of an activation gradient ε shows a double scaling behavior and an ε-dependent crossover time, in agreement with theoretical predictions:



Ferraro, de Candia, Gamba, Coniglio, submitted to PRL

Do fluctuations of extracellular attractant cause random polarization?



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Cell Migration: A Physically Integrated Molecular Process

Review

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to integrate comes from in vitro studies, mainly concerning movement across two-dimensional substrata. We nonetheless believe that much of the mechanistic understanding is relevant and useful for in vivo situations even in three dimensions. It is likely that cells interact with their surroundings by means of the same types of receptors in vivo as in vitro, and that physical interactions of cells with their environment play important roles in regulating function in both cases.

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emphasizing the physicochemical nature of underlying molecular mechanisms. Owing to imposed space and citation constraints, we focus on a limited set of issues, stressing conceptual insights. Readers interested in further discussions and literature citations are referred to some excellent reviews of relevant topics published in the past couple of years (Ginsberg et al., 1995; Hall,

tinction between cell front and rear. Concentration gradients of stimuli are not required to elicit this response. Polarization in macroscopically homogeneous stimulus environments may arise from perceived spatial or temporal stimulus gradients caused by microscopic nonuniformities or by kinetic fluctuations in receptor±ligand binding. An early event in polarization, at least for neutro-

One may wonder whether a cell may become polarized by the anisotropy produced by a spontaneous fluctuation in the extracellular distribution of attractant molecules, or fluctuations in receptor-ligand binding, as has been suggested in the literature. Since eukaryotic cells typically carry 10^4-10^5 receptors for attractant factors, one expects spontaneous fluctuations in the fraction of activated receptors to be of the order of 10^2, a value which is comparable to observed anisotropy thresholds. However, to actually produce directed polarization the fluctuation should sustain itself for several minutes, i.e. for a time comparable to the characteristic polarization time. Such an event has very low probability of being observed since the correlation time of the fluctuations determined by attractant diffusion at the cell scale and the characteristic times of receptor-ligand kinetics are much less than the polarization time. Indeed, the diffusion time is $\sim$1s at the typical cell size 10 μm, and the characteristic times of receptor-ligand kinetics are also $\sim$1s. Therefore, the direction of cell polarization in the case of a homogeneous distribution of attractant can only be determined by the inhomogeneity in the initial distribution of the positions of PIP_2-rich germs produced by thermal fluctuations.

Are bacteria too small for spatial sensing?

PHYSICS OF CHEMORECEPTION

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ABSTRACT Statistical fluctuations limit the precision with which a microorganism can, in a given time T, determine the concentration of a chemoattractant in the surrounding medium. The best a cell can do is to monitor continually the state of occupation of receptors distributed over its surface. For nearly optimum performance only a small fraction of the surface need be specifically adsorbing. The probability that a molecule that has collided with the cell will find a receptor is $Ns/(Ns + \pi a)$, if N receptors, each with a binding site of radius s, are evenly distributed over a cell of radius a. There is ample room for many independent systems of specific receptors. The adsorption rate for molecules of moderate size cannot be significantly enhanced by motion of the cell or by stirring of the medium by the cell. The least fractional error attainable in the determination of a concentration $\bar{c}$ is approximately $(T\bar{c}aD)^{-1/2}$, where D is the diffusion constant of the attractant. The number of specific receptors needed to attain such precision is about a/s. Data on bacteriophage adsorption, bacterial chemotaxis, and chemotaxis in a cellular slime mold are evaluated. The chemotactic sensitivity of *Escherichia coli* approaches that of the cell of optimum design.

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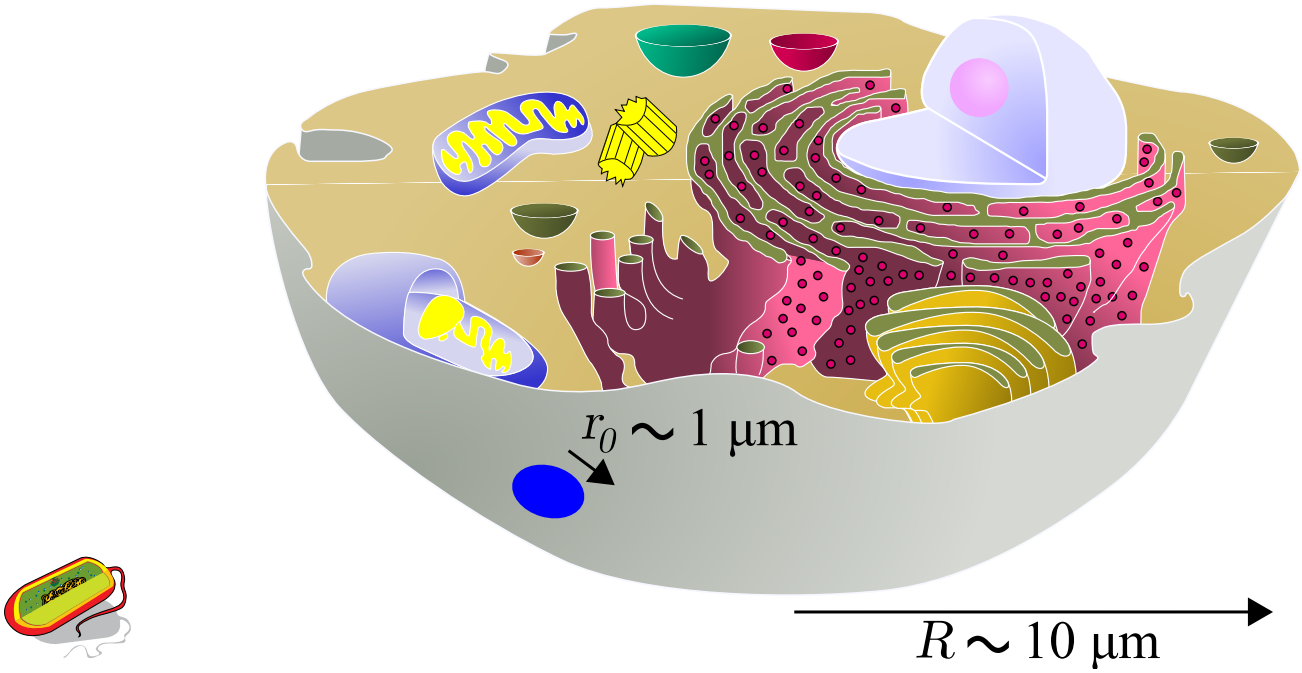
Are bacteria too small for spatial sensing?

An interesting speculation is that the bound $\epsilon_{\rm th} = r_0/R$ may explain why spatial directional sensing was developed only in large eukaryotic cells and not in smaller prokaryotes, whose directional sensing mechanisms rely instead on the measurement of temporal variations in concentration gradients.
Our bound derives from the intrinsic properties of polarization dynamics and is independent of the size criterion formulated in (Berg and Purcell, *Biophys. J.*, **20**, 193, 1977), which is based on estimates of signal-to-noise ratios.

$$R > \frac{r_0}{\epsilon_{\rm th}}$$



Universality

Our picture does not depend on the details of the reactions involved, but only on the general structure of the directional sensing network and on its bistability. This means that the picture is robust not only with respect to variations of the kinetic and physical parameters, but also with respect to the identity of the chemical species involved.

A universal picture of eukaryotic directional sensing

- Our picture derives from universal properties of domain growth
- It depends only on general properties of the biological system: selftuning, bistability, existence of a single-component order parameter, conservation laws
- It is a robust picture: the system self tunes towards the phase-coexistence state
- It explains many observed phenomena, such as:
  - directed and random polarization
  - the sensitivity threshold
  - the role of external fluctuations
  - the role of size in directional sensing