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Patch Coalescence as a Mechanism for Eukaryotic Directional Sensing

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Eukaryotic cells possess a sensible chemical compass allowing them to orient toward sources of soluble chemicals. 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 theory of this polarization process, which is articulated into subsequent stages of germ nucleation, patch coarsening, and merging into a single domain. We find that the polarization time, t_ϵ, depends on the anisotropy degree ϵ through the power law $t_\epsilon \propto \epsilon^{-2}$, and that in a cell of radius R there should exist a threshold value $\epsilon_{\rm th} \propto R^{-1}$ for the smallest detectable anisotropy.

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The cells of multicellular organisms are endowed with a chemical compass of amazing sensitivity, formed as a result of billions of years of evolution. Concentration differences of the order of a few percent in the 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 [1]. This way, a sensible amplifier of slight gradients in the distribution of chemicals in the surrounding environment is realized. Its relevance is easily understood if one recognizes that no multicellular organism could exist without the constituent cells being capable of sensing directional signals. Directional sensing is actually essential both 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. The main steps of the process are as follows (see the reviews [2,3]). As a response to the attractant signal, the cell membrane is polarized, afterwards inducing differentiated polymerization of the cell cytoskeleton in its proximity. The resulting imbalance, triggered by a well characterized cascade of chemical reactions, leads to the formation of a growing head and a retracting tail, in such a way that the cell starts to drift towards the source of the signal. The initial part of this process is constituted by the early chemical polarization of the cell membrane. In this Letter, we propose a simple phenomenological scheme providing a universal description of this fundamental phenomenon.

Membrane polarization can be recognized as a self-organization process governed by a network of diffusion-controlled chemical reactions. It is known that reaction-diffusion networks may become bistable in the presence of chemical feedback loops [4,5]. In spatially extended systems, bistability may lead to the formation of competing phases and to a phenomenology typical of first order phase transitions, such as metastability, nucleation, and coarsening [6,7]. The polarized membrane state observed during directional sensing can therefore be interpreted as the coexistence of domains of two different phases.

Let us briefly describe the chemical reactions which are responsible for directional sensing. The chemical factors clustering in complementary membrane domains are the phospholipids phosphatidylinositol bisphosphate (PIP_2) and phosphatidylinositol trisphosphate (PIP_3). Two enzymes, phosphatidylinositol 3-kinase (PI3K) and phosphatase and tensin homolog (PTEN), respectively, transform PIP_2 into PIP_3 and vice versa. 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 [8,9]. 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. PIP_3-rich germs are then nucleated in the PIP_2-rich sea and PIP_3-rich regions start to coexist with PIP_2-rich ones [10].

Two different regimes of cell 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 domain on the membrane side closer to the attractant source and of a PIP_2-rich domain in the complementary region [8,9]. On the other hand, cells exposed to uniform distributions of an attractant polarize in random directions, in times of the order of an hour (see e.g., [11]). The existence of two clearly separated polarization regimes is confirmed by the recent observation of a sensitivity threshold of the order of a few percent difference in

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the attractant molecule concentration from side to side [1]. Direct observation of the polarization process [[8], Fig. 7(a)] implies the bound <5 s for PTEN diffusion time in the cell volume, which is therefore much less than the polarization time for both regimes. In this process, the amplitude of the cell stimulation is of crucial importance. At very low stimulation levels, PTEN is not desorbed in a significant amount and no directional sensing takes place. At very high stimulation levels, a homogeneous PIP_3-rich phase is realized, and directional sensing again does not take place. There exists therefore an optimal attractant concentration, such that below it, the minority phase is PIP_3-rich and above it is PIP_2-rich.

Numerical simulations of the directional sensing network performed with the use of realistic physical and kinetic parameters have shown 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 [9,12]. 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 1 h, in accordance with experimentally observed times. In the numerical experiments, when PIP_2 is the minority phase, the evolution leading to phase separation consists of an early nucleation regime, resulting in the formation of isolated PIP_2-rich patches and a late coarsening process, where large patches of the PIP_2-rich phase grow at the expense of the evaporation of smaller ones, similarly to what happens in the case of first order phase transitions in a liquid-gas system or in the precipitation of a supersaturated solution [13]. Finally, the patches condense into a single large cluster, leading to a stationary state characterized by the coexistence of a PIP_2- and a PIP_3-rich domain. However, the dynamics of the directional sensing network differs from that of otherwise similar processes, such as the precipitation of a supersaturated solution, under one important respect. When precipitation nuclei in a supersaturated solution dissolve, matter is transferred to larger nuclei through diffusion in the surrounding medium. In contrast, in the directional sensing network, enzyme-substrate patches evaporate through desorption of the PTEN enzyme from the membrane, which is then transferred to other patches through diffusion in the cell volume. Therefore, the transformation of PIP_3 into PIP_2 molecules cannot be described at the membrane level as a local, diffusionlike process as is the case with the adsorption-desorption process from precipitation nuclei; in particular, there is no local conservation of the number of PIP_2 molecules.

The above summarized scenario can be put on a firm analytical ground resorting to the kinetic theory of first order phase transitions [7,13,14]. In this theory, after germ nucleation, larger patches of the stable phase grow at the expense of smaller patches which shrink, leading to scaling laws and universal probability distribution of patch sizes. We shall now show how the ideas of the Lifshitz-Slyozov theory [14] may be adapted to our problem to deduce simple scaling laws for the membrane polarization time and explain most of the observed phenomenology. We discuss here the case when PIP_2 is the minority phase (the other case being symmetric). In this case, PIP_2-rich patches are formed inside the PIP_3-rich sea [see Fig. 1(a)]. We restrict our consideration 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 a can be written on phenomenological grounds as $F = -\pi\psi a^2 + 2\pi\sigma a$, where σ is the linear tension of the interface with the surrounding PIP_3-rich phase and ψ represents the degree of metastability [13], which is a function of the concentration of PTEN molecules in the cell volume and of the concentration of extracellular attractant.

According to the kinetic theory of first order phase transitions, the equation of growth of a patch is dissipative. In the absence of a local conservation law, the equation for a circular patch can be written as $\Gamma\partial_t a = -\partial F/\partial a$, where $\Gamma(a)$ is a damping coefficient [7]. Since energy dissipation occurs mainly along the perimeter of the interface between the two phases, Γ may be written as $2\pi a\gamma$, where γ is a constant, and we get

$$\gamma\partial_t a = \psi - \sigma/a + \xi, \tag{1}$$

where the noise term ξ represents thermal fluctuations. The fluctuations are responsible for the formation of an initial population of patches with varying radii a ([13], §99). Patches with a smaller than the critical radius $a_c = \sigma/\psi$ are mainly dissolved while most patches with $a > a_c$ survive and grow because of the gain in free energy. At initial time, a_c is of the order of the thickness a_0 of the interface between the two phases [7,13]. As long as the area occupied by patches of the PIP_2-rich 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 ([13], §100). In the final stage of this process, a single domain of the PIP_2-rich phase is formed coexisting with the PIP_3-rich

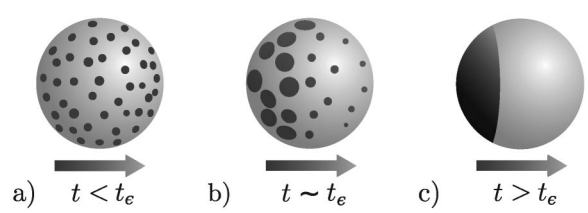

FIG. 1. Patch growth in the presence of a slight gradient of attractant activation directed from left to right. The PIP_3- and PIP_2-rich phases are, respectively, light and dark gray.

phase, see Fig. 1(c). However, 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 $f(a)$ such that $f(a)\Delta a$ gives the number of patches with sizes in the interval $(a, a + \Delta a)$. An important simplification comes from the fact that for patches with $a > a_c$, the noise term ξ in (1) becomes negligible [13]. This means that the stochastic nature of the problem enters mainly in the formation of the initial distribution of patch sizes $f(a)$, while for $a > a_c$, the time evolution of $f(a)$ is dictated by the deterministic part of (1), from which the kinetic equation

$$\gamma \frac{\partial f}{\partial t} + \frac{\partial}{\partial a}\left[\left(\psi - \frac{\sigma}{a}\right) f\right] = 0, \tag{2}$$

follows [7,13]. With the chosen normalization, $\int f(a)da$ represents the total number of patches, a quantity which is monotonically decreasing in time according to the previously described "coarsening" dynamics [14]. Equation (2) is valid as long as $\int f(a)da$ is much larger than 1 [13].

To obtain a closed system of equations, we need an additional equation for the time evolution of the metastability degree ψ [13]. 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 [9]. 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

$$\psi \propto A - \int da \pi a^2 f(t, a), \tag{3}$$

as the total patch area tends to its limit value A. Equation (3) 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 equilibrium and at current time. The law (3) is valid for $t \gg t_0$, where t_0 is the characteristic time needed for the formation of a germ of an alternative phase that can be estimated as $t_0 \sim \gamma a_0^2/\sigma$ [7,13].

Asymptotically, (2) and (3) lead to the self-similar solution

$$\psi(t) = (2\sigma\gamma/t)^{1/2}, \qquad f \propto t^{-3/2} g(\sqrt{\gamma} a/\sqrt{2\sigma t}), \tag{4}$$

where $g(\xi) = \xi(1-\xi)^{-4}\exp[-2(1-\xi)^{-1}]$ if $\xi < 1$ and $g(\xi) = 0$ if $\xi > 1$. Similarly to what happens in Lifshitz-Slyozov theory [14], the total number of patches decreases in time due to the evaporation of small patches: $\int da f(a) \propto t^{-1}$, and from (4) one gets $\langle a \rangle = \sigma/\psi = a_c$. The evolution of the size distribution function f governed by (4) stops at times of order $t_\star$, where $t_\star$ is defined as the instant when the average patch size $\langle a \rangle$ reaches the cell size R. From the scaling law $\langle a \rangle \propto \psi^{-1} \propto \sqrt{t}$, we get $t_\star \sim (R/a_0)^2 t_0$. Eventually, at $t \sim t_\star$, a single PIP_2-rich patch survives. Its orientation is determined by the random unbalance in the initial germ distribution. Notice that in this derivation, following the lines of [7,13,14], isotropy was essential to assume that ψ was uniform along the whole membrane surface.

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 $\delta\psi = -\epsilon\psi_0 \cos\theta$. Here, $\psi_0 = \sigma/a_0$ is the initial metastability degree, ϵ is a dimensionless factor measuring the initial anisotropy degree, and θ is the azimuthal angle on the cell surface. This way we obtain the equation

$$\gamma \partial_t a = \psi - \epsilon\psi_0 \cos\theta - \sigma/a + \xi, \tag{5}$$

generalizing (1). As long as $\epsilon\psi_0 \ll \psi$, 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, see Fig. 1(b). From the scaling law (4) for ψ, one gets $t_\epsilon \sim t_0\epsilon^{-2}$. For $t > t_\epsilon$, the leading term in (5) becomes the perturbation $\epsilon\psi_0 \cos\theta$, implying that in the region closer to the source of the stimulation ($\cos\theta \gtrsim 0$), the PIP_2-rich phase evaporates in a time which is easily estimated as being again 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, as shown in Fig. 1(c).

The above scheme is valid as soon as the initial nucleation time t_0 is significantly smaller than t_ϵ, an assumption which is compatible with the results of numerical experiments [9]. On the other hand, the second stage of patch evolution occurs only if $t_\star \gg t_\epsilon$. 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 $t_\star = t_\epsilon$. Since the product ψa_c is a time-independent constant, we can simply compare its value at initial and final time when $\epsilon = \epsilon_{\rm th}$, obtaining $\epsilon_{\rm th} = a_0/R$, which gives us the expression for the threshold anisotropy.

It is interesting to estimate a_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 $a_0 \sim (D\tau c/h)^{1/2}$. Using parameter values from Ref. [9], one gets $a_0 \sim 1$ μm and $\epsilon_{\rm th} \sim 10\%$. The value for $\epsilon_{\rm th}$ is compatible with the observations (the data from Ref. [1] imply $\epsilon_{\rm th} \simeq 7\%$ for *Dictyostelium*).

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 [3]. 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$1 s at the typical cell size 10 μm, and the characteristic times of receptor-ligand kinetics are also $\sim$1 s (see online supporting information to Ref. [1]). 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.

In conclusion, we have constructed a universal phenomenological description of the mechanism of directional sensing in the eukaryotes based on the process of patch coarsening. This description implies the existence of two clearly separated polarization regimes depending on the presence or absence of an anisotropic component in the activation pattern produced by the extracellular attractant factor, and the existence of a sensitivity threshold for the anisotropic component. Both results are in reasonable agreement with experimental observations. Moreover, we predict that directed polarization time should scale as the inverse square of the relative signal anisotropy, a law that should be verifiable by direct observation. Our picture suggests that directed and stochastic polarization share a common mechanism, and that stochastic polarization should be the result of noise in subcellular and not in extracellular dynamics. Importantly, 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. Indeed, PI3K and PTEN could be substituted by, or synergize with, molecules endowed with similar enzymatic activity. An interesting speculation is that the bound $\epsilon_{\rm th} = a_0/R$ may explain why spatial directional sensing was developed only in the large eukaryotic cells and not in smaller prokaryotes, whose directional sensing mechanisms rely instead on the measurement of temporal variations in concentration gradients [15]. Our bound derives from the intrinsic properties of polarization dynamics and is independent of the size criterion formulated in Ref. [16]. The experimental observation of self-organized phospholipid patches [10] following uniform attractant stimulation provides an initial confirmation of the validity of our scheme. To check the predictions of our theory, similar observations should be performed for the longer times characteristic of random and directed polarization, both under uniform attractant activation and in the presence of accurately controlled concentration gradients. Experimental modulation of PTEN levels could be used to modify the overall size of patches and eventually switch off the patch formation mechanism.

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