Soft Matter

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Dynamic membrane patterning, signal localization and polarity in living cells

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November 18, 2014

Abstract

We review molecular and physical aspects of the dynamic localization of signaling molecules on the plasma membrane of living cells. At the nanoscale, clusters of receptors and signaling proteins play an essential role in the processing of extracellular signals. At the microscale, "soft" and highly dynamic signaling domains control the interaction of individual cells with their environment. At the multicellular scale, individual polarity patterns control the forces that shape multicellular aggregates and tissues.

Introduction

Living cells process information and respond to environmental changes by exploiting a complex circuitry of interacting signaling molecules. Many of these molecules localize either at the interface between the cell and its environment (the plasma membrane) or on the surface of inner compartments delimited by lipid membranes (endosomes). Improvements in molecular imaging and gene manipulation techniques provide increasing evidence that such membrane-bound molecules self-organize dynamically in a multitude of nano- and micro-domains. However, the function and mechanisms of formation of these signaling domains are still poorly understood.

The cell transfers information across widely separated space and time scales, and ultimately converts molecular signals that pertain to the nanoscale into mesoscopic effects, such as the morphological changes that take place during the processes of migration and cell division.[1,2] At the nanoscale, lipid-protein clusters process extracellular signals that are afterwards transmitted downstream.[3] At the microscale, signaling domains that control the interaction of cells with their environment self-organize on the plasma membrane through a process of symmetry breaking that can be either spontaneous or induced by signal anisotropies.[4] At the multicellular scale, chemical polarity patterns on the surface of individual cells transduce positional information and guide the distribution of forces that shape multicellular aggregates and tissues.[5,6]

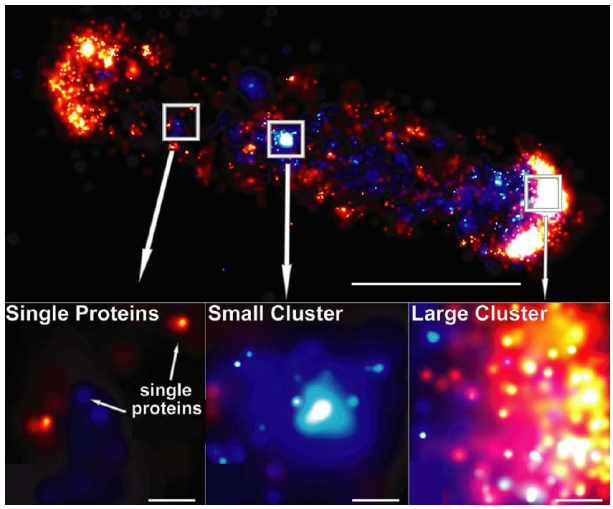

Figure 1 Chemoreceptor clusters on the surface of *E. coli* cells imaged with ultrahigh-resolution light microscopy (photoactivated localization microscopy). Scale bars: 1 μm and 50 nm. From Ref. 7.

Signaling domains contribute to cell order by providing distinct lipid membrane regions with unique functional identities. Being enriched in specific molecules characterized by precise (e.g. active vs. inactive) signaling states, they locally promote alternative transformations, such as growth vs. retraction, or adhesion vs. detachment.[1,2,5,6]

Mechanisms that lead to the formation of signaling domains on the cell lipid membranes can be divided in two main classes: those driven by contact interactions, and those driven by a combination of diffusive and autocatalytic processes, that give rise to effective (non-contact) interactions. Contact interactions generate "hard" signaling domains: oligomers, clusters, and other supramolecular aggregates.[8] Effective interactions sustained by positive and negative chemical feedback loops generate "soft" signaling domains: membrane regions that are significantly enriched in specific, but highly diluted, molecular factors.[9] In both cases, reaction-diffusion processes on the surface of lipid membranes are accompanied by shuttling processes involving the exchange of signaling molecules with the cytosol. Such shuttling processes in their turn can have a purely diffusive character,[10] or be driven by directed vesicle traffic.[11]

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Diffusion plays a crucial role in domain formation as it makes possible the encounter of different molecular factors. At the same time, it opposes self-organization by smoothing down spatiotemporal structures. Self-reinforcing feedback loops contrast the homogenizing effect of diffusion by consuming energy, mainly provided by ATP hydrolysis.[12] Therefore, domain formation should be regarded as an intrinsically irreversible out-of-equilibrium process.

Here we review some of the present knowledge about the molecular and physical mechanisms that lead to the formation of spatiotemporally localized signaling domains on the lipid membranes of living cells. In a first section we summarise some important biological examples, and in a second section we discuss some of the mathematical and physical models that were recently introduced to provide a mechanistic understanding of the observed properties.

Signal localization on lipid membranes

Receptor clustering in bacterial chemotaxis

Cells use chemotactic receptors to detect sources of chemoattractant factors or nutrients. The chemotactic signal is elaborated by the cell circuitry and transduced into a migratory movement. This process has been thoroughly studied in bacteria. Possibly due to their tiny size,[13] bacteria evolved a chemotactic strategy that relies on the temporal (rather than spatial) comparison of chemoattractant concentrations.[13] Most studies of bacterial chemotaxis have been performed on the model organism *E. coli*. When *E. coli* moves towards higher nutrient concentrations, it upregulates the duration of its forward swim and downregulates the frequency of random reorientations, thus performing a biased random walk towards nutrient sources.[13–15]

The chemoreceptors of *E. coli* self-organize in clusters that have been observed at the cell poles and along the cell body by a variety of techniques: immuno-electron microscopy, immuno-fluorescence microscopy,[16] cryo-electron microscopy,[17] electron cryo-tomography,[18,19] and super resolution light microscopy (Fig. 1).[7] These macromolecular clusters can contain thousand of receptors, arranged in roughly hexagonal arrays. Molecules that establish contacts between the cytoplasmic tail of receptors contribute to the formation of an ordered lattice.[19–21]

How does receptor clustering influence the efficiency of chemotactic sensing? It has been proposed that clustering provides increased sensitivity by exploiting some cooperative mechanism.[22] Activation of an individual receptor would favor activation of neighboring receptors, possibly by sharing transducing enzymes.[23,24] Experiments appear to confirm that clusters of bacterial chemoreceptors work in a highly cooperative manner.[25] The role of cooperativity has been investigated theoretically by mapping the activities of receptors on a set of Ising spins, the spread of activity onto a local exchange interaction, and ligand binding onto a random magnetic field.[26–28] In this analogy, the response of the receptor system corresponds to magnetization. In the strong coupling limit, this receptor model reduces to a cooperative model where all receptors in a cluster are either active or inactive together.[24,25,29,30] Computations show that receptor clustering provides enhanced sensitivity to signal variations.[27] However, cooperativity also amplifies both extra- and intracellular noise, so that the best signal-to-noise ratio is achieved by a system of uncoupled receptors.[31–33] Cooperativity therefore appears as a convenient strategy for systems that require high amplification of weak signals, even at the expense of fidelity in signal transduction.

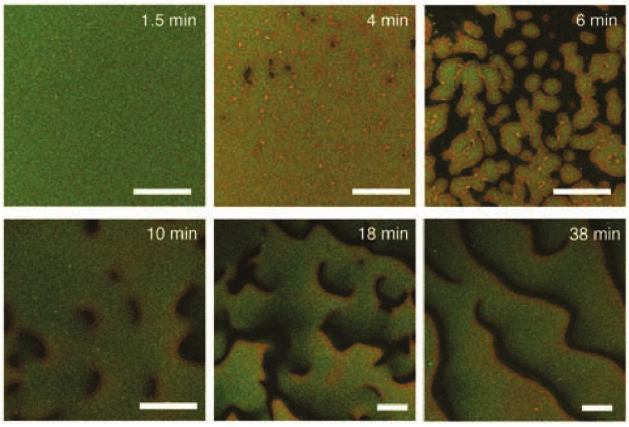

Figure 2 Protein surface waves *in vitro*. Starting from a homogeneous distribution of MinD, addition of MinE leads to a dynamic instability: First, MinD detaches from the membrane; after reattachment, protein-free ripples in the protein layer synchronize to a regular pattern of parallel surface waves. Green: MinD (1 μM), Red: MinE (1.5 μM). Scale bar: 50 μm. From Ref. 35.

Signaling domains in bacterial cell division

The division of *E. coli* cells starts with the formation at the midpoint of the cell body of a ring ("Z ring") enriched in specific proteins.[34] The Z ring forms at a node of a standing wave of enrichment in the proteins MinC, MinD and MinE, that periodically shuttle between the cytosol and the inner bacterial membrane.[34] MinD binds to the membrane originating a self-reinforcing, autocatalytic process that induces the formation of a MinD-enriched signaling domain. MinE binds to MinD and triggers MinD release from the cell membrane. Released MinD binds again to the membrane where MinE concentration is lower, thus forming a new MinD-enriched domain. This chase-and-run behavior leads to persistent oscillations. MinC, an inhibitor of Z ring formation, is recruited by MinD and follows the periodical oscillations of MinD and MinE. Z ring formation takes place at the central node of the oscillating system, where Z ring inhibition is lower.[34,36–40] The dynamics of Min proteins has been reproduced *in vitro*, where the spontaneous emergence of spatiotemporally organized protein patterns has been observed (Fig. 2).[35]

Protein nanoclusters in eukaryotic signaling

A central role in the signal processing circuitry of eukaryotic cells is played by *small GTPases*, proteins that can exist in two complementary states (active/inactive), whose switch is

regulated by specific enzymes (Fig. 3a). Prominent members of the small GTPase family are the Ras, Rho, and Rab proteins. Studies based on immuno-electron microscopy have shown that Ras proteins reside in distinct non-overlapping nanoclusters, according to their isoform and active/inactive state.[3,41] On average, a nanocluster comprises ∼10 Ras proteins, has a radius of about ∼10 nm and a lifetime of ∼1 sec. Clustering is apparently required for correct signal processing,[3,42] but a complete mechanistic understanding of its role is still lacking.

It has been suggested that Ras nanoclusters digitize the signals coming from the environment.[3] In this scenario, each single nanocluster operates in a digital manner in response to stimulation from an extracellular signal, providing an all-or-none response, whereas the number of activated Ras nanoclusters is directly proportional to the stimulus.[3] This mechanism could generate linear signal amplification over a wide range of signal strengths.[3]

Another evidence of the role of signaling nanoclusters in eukaryotic signal processing comes from the study of the immune system. T lymphocytes are white blood cells that coordinate the immune response. They express antigen receptors that bind to antigens on the surface of antigen-presenting cells. In the synaptic contact between the two cells, antigens induce the formation of receptor nanoclusters containing ∼10 receptors each.[43] Proteins in the synapse self-organize also on a larger spatial scale: integrins, the proteins that maintain the mechanical contact between the two cells, concentrate in an outer ring, whereas receptor clusters concentrate in the central synaptic zone.[44,45]

Proposed mechanisms leading to the self-assembly of protein nanoclusters include protein-protein, protein-lipid, and lipid-lipid contact interactions. The reciprocal affinities between different lipid constituents of the cell membrane can lead to phase separation, i.e. to the dynamic formation of homogeneous nanodomains, or *lipid rafts*, enriched in specific lipids proteins.[46] Recent technical advances in imaging and spectroscopic methods allow the experimental observation of such nanoscale assemblies, having 10–50 nm sizes and sub-second half lives.[47,48] Increasing evidence points at their crucial role in molecule sorting, vesicle trafficking, cell migration, cell polarity, and signal processing.[42,48]

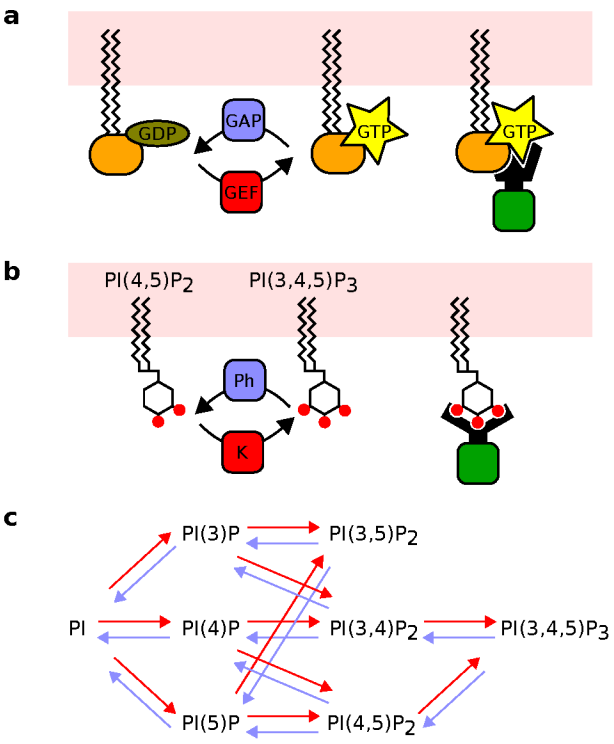

Figure 3 Multistate signaling molecules. **a:** Small GTPases (orange) can exist in either an inactive (GDP-bound) or an active (GTP-bound) state. Specific enzymes (GEFs and GAPs) catalyze the switch between the two states. A lipid tail anchors the small GTPase to the inner leaflet of the plasma membrane (pink). Cytosolic proteins (green) endowed with specific binding domains recognize and bind to small GTPases depending on their activation state. **b:** Phosphoinositides can host a variable number of phosphate groups (red circles) on the corners of an inositol ring (corners are numbered starting from the upper vertex of the ring). Kinases (red) and phosphatases (blue) catalyze the switch between different phosphorylation states. Cytosolic proteins (green) endowed with specific binding domains recognize and bind to phosphoinositides depending on their phosphorylation state. **c:** The addition of phosphate groups on different corners of the inositol ring is exploited combinatorially to generate multiple states of the phosphoinositide molecule. The switch between different states is controlled by specific kinases and phosphatases (red and blue arrows).

Signaling domains in eukaryotic cell division

The process of cell division has been mainly studied on model unicellular organisms such as *S. cerevisiae* (budding yeast). In *S. cerevisiae* the site of budding of a daughter cell is initially identified by the formation on the cell membrane of a circular domain enriched in the active form of Cdc42, a member of the Rho family of small GTPases. In normal yeast the location of this domain is correlated with the position of scars produced by previous divisions. However, genetic modifications allow to observe the spontaneous breaking of the cell symmetry, with the formation of the Cdc42-enriched domain in a random position.[11] This symmetry breaking event relies on self-reinforcing feedback loops that convert an initial random distribution of active Cdc42 into a single localized membrane domain. Two parallel shuttling mechanisms cooperate to this purpose. The former involves the active transport of vesicles enriched in Cdc42 to regions that are already enriched in active Cdc42.[11,49] The latter involves reinforced recruitment of diffusing Cdc42 from the cytosol to these same membrane regions.[10,49] The interplay and relative role of the two mechanisms are still matter of investigation.[50,51]

In multicellular organisms, the maintenance of living tissues requires continuous self-renewal by the generation of new tissue-specific, differentiated cells from the undifferentiated stem cell pool. This regeneration takes place by asymmetric cell division, whereby the mother cell retains stem cell potential, while the daughter cell differentiates into the cell type that is characteristic of the given tissue.[52,53] This markedly asymmetric cell division takes place by a spontaneous or induced symmetry breaking leading to the formation of opposite, polar signaling domains on the plasma mem-

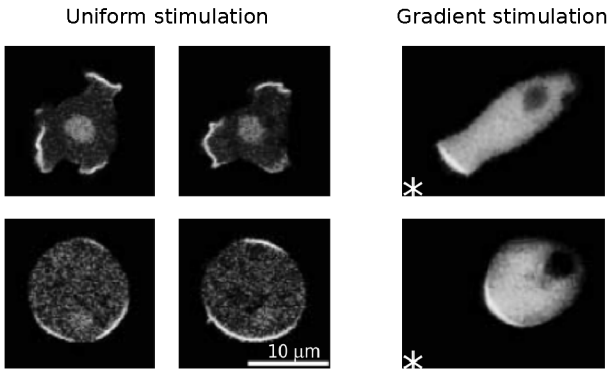

Figure 4 Spontaneous and directed formation of $PI(3,4,5)P_3$ domains. The panels show transverse sections of cells imaged through a confocal microscope by the use of a fluorescent reporter for $PI(3,4,5)P_3$. Left panels: cells of *D. discoideum* form $PI(3,4,5)P_3$-enriched domains in response to uniform stimulation with a chemoattractant factor. Rightmost panels: cells form a $PI(3,4,5)P_3$-enriched domain at the leading edge in response to the chemotactic signal released by a micropipette (the position of the micropipette is indicated by an asterisk). Bottom panels: Cells treated with inhibitors of the actin cytoskeleton maintain a spherical shape, but still form $PI(3,4,5)P_3$-enriched domains in response to the chemotactic stimulation. From Ref. 54.

brane of the mother cell.[52,53] Afterwards, distinct molecular "fate determinants" segregate on the two polar signaling domains. Lastly, the mitotic spindle aligns with the polar signaling domains so that the mother and daughter cell become enriched in different fate determinants.[52,53]

Signaling domains in eukaryotic chemotaxis

In eukaryotic cells, chemoattractant gradients induce a symmetry breaking in the molecular content of the plasma membrane, with the formation of signaling domains that identify the front and back of the cell.[1] This front-back polarity axis promotes directed cell migration by determining the molecular identity and activity of both the protruding leading edge and retracting trailing edge. A central role in the formation of the front-back polarity axis is played by *phosphoinositides*, a class of membrane phospholipids equipped with an inositol ring that can host several phosphate groups (Fig. 3b).[55] Specific enzymes (kinases and phosphatases) catalyze the addition or removal of a phosphate group (Fig. 3b,c). Different proteins specifically recognize and bind to phosphoinositides carrying different numbers of phosphate groups (Fig. 3b). The resulting network of interactions includes self-reinforcing feedback loops that ultimately lead to the selective enrichment of the phosphoinositide species $PI(3,4,5)P_3$ at the leading edge (Fig. 4, top right). In its turn, $PI(3,4,5)P_3$ guides the recruitment of the molecular machinery required for the polymerization of the actin cytoskeleton.[56–58] The formation of a $PI(3,4,5)P_3$-enriched signaling domain at the migrating front is part of a "molecular compass" that allows the cell to move directionally towards chemoattractant sources.[1] The complex dynamics of $PI(3,4,5)P_3$ signaling domains includes spontaneous symmetry breaking induced by uniform chemoattractant stimulation (Fig. 4, left), the formation of both intermittent and stable patches, and the generation of propagating waves.[58,59]

Signaling domains and tissue architecture

In higher organisms, cellular polarity plays a crucial role in the development of tissues by allowing individual cells to find and maintain their place in the framework of a well ordered multicellular architecture. A paradigm for this kind of multicellular structure are *epithelia*, the ordered layers of cells that line the inner organs and the outer surface of animal bodies. Signaling domains enriched in specific molecules assign to epithelial cells the correct placement and orientation in the epithelial layer. For instance, the interface of epithelial cells with the outer world (apical membrane) is identified by an enrichment in the phosphoinositide $PI(4,5)P_2$, whereas enrichment in $PI(3,4,5)P_3$ characterizes the interface with the surrounding parts of the organism (basolateral membrane).[6] The interconversion between the two phosphoinositide species is regulated by specific enzymes. For instance, exclusion of $PI(3,4,5)P_3$ from the apical surface is induced by membrane recruitment of the lipid phosphatase PTEN, that converts $PI(3,4,5)P_3$ into $PI(4,5)P_2$. This kind of well-regulated polarity cue controls the distribution of mechanical forces in the tissue and the orientation of cell division axes, so that a well-formed tissue architecture can be maintained.[6,60–63] Genetic modifications (such as PTEN mutations) that subvert the localization of $PI(4,5)P_2$ and $PI(3,4,5)P_3$ disrupt the epithelial architecture and may lead to severe pathologies (Fig. 5).[62,64,65]

Cooperative and self-organized effects in the activation of polarity cues are believed to play a relevant role during embryonic development, as suggested by the study of early symmetry-breaking events in simple multicellular organisms.[66,67]

A dynamic membrane code

While the experimental study of signaling domains has been initially concerned with a small number of specific cellular functions, such as migration and proliferation, a large

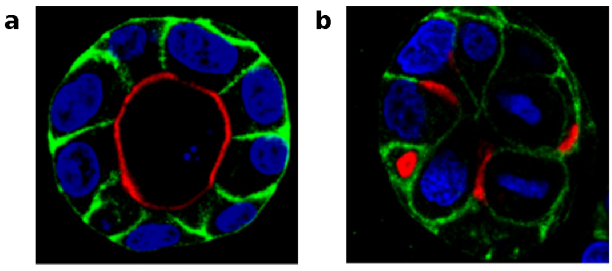

Figure 5 Silencing of PTEN disrupts the epithelial architecture. **a:** Epithelial cells self-organize *in vitro* in a spherical cell monolayer with a central cavity (a section obtained by confocal microscopy is shown here). Red, green, blue: molecular markers for the apical membrane, basolateral membrane, and nuclei. **b:** Silencing of PTEN leads to disruption of the well-ordered epithelial architecture observed in control cells. From Ref. 68.

scale analysis of the organization of plasma membrane has recently shown that the compartmentalization of signaling molecules in spatially localized regions is a quite universal feature.[9] Visualizing a set of fluorescent markers in real time by total internal reflection microscopy it was shown that ~ 50 different plasma membrane proteins, with such diverse functions as transport, signal processing, metabolism and sensing, localize in highly dynamic signaling domains with morphologies ranging from isolated patches to percolating networks (Fig. 6).[9]

Localized enrichments in specific molecular factors are not limited to the cell plasma membrane, but regard also the membranes of internal organelles (endosomes), as the two compartments constantly exchange lipid-membrane patches: parts of the plasma membrane are internalized in the form of vesicles and transported to endosomes (endocytosis), while vesicles traveling in the opposite way fuse with the plasma membrane (exocytosis), thus allowing a constant recycling of molecular cargos.[69,70] This way, for instance, many types of receptors are internalized, pass through "early" endosomes, then are either sent to recycling endosomes to be freed of their ligands and later re-exposed, or sent to "late" and degradative endosomes to be destroyed.[71,72] Vesicles are targeted to specific endosomal or plasma membrane regions that are recognized by the presence on their surface of signaling domains enriched in specific molecular factors. A central role is played here by phosphoinositides and small GTPases of the Rab family.[73–75] Specific enzymes (GEFs and GAPs) convert the inactive form of Rab proteins into the active form, and vice versa (Fig. 3a). For instance, the transformation from "early" to "late" endosomes is accompanied by a switch of the endosome membrane from a state enriched in the protein Rab5 to a state enriched in the protein Rab7.[76–78] The conversion from the Rab5 to the Rab7 stage can be seen as the transition between the two stable states of a chemical network involving the two Rab proteins, their activators and their inhibitors.[78]

Extended and highly dynamic, "soft" lipid-protein signaling domains found either on the plasma membrane or on the membranes of endosomes consist of local enrichments in highly diluted molecules that rarely participate in contact interactions. For instance, phosphoinositides constitute only $\sim$10% of the total phospholipids, and the typical concentration of particular phosphoinositide species, such as $PI(3,4,5)P_3$, can be 2 to 3 orders of magnitude lower.[79] Signaling domains constituted by such diluted and evanescent information carriers are maintained into existence by a sequence of binding and enzyme-driven transitions in an incessant stochastic ballet.[80] Endless recycling keeps signaling structures in approximately stationary out-of-equilibrium states and allows the cell to plastically adapt to changing environmental conditions.

Theory and models of signal localization

Basic circuitry of signal localization

"Soft" signaling domains originate from the nonlinear effective interactions of their components in the context of a diffusive environment. Some of the biochemical mechanisms that generate the nonlinearities are:

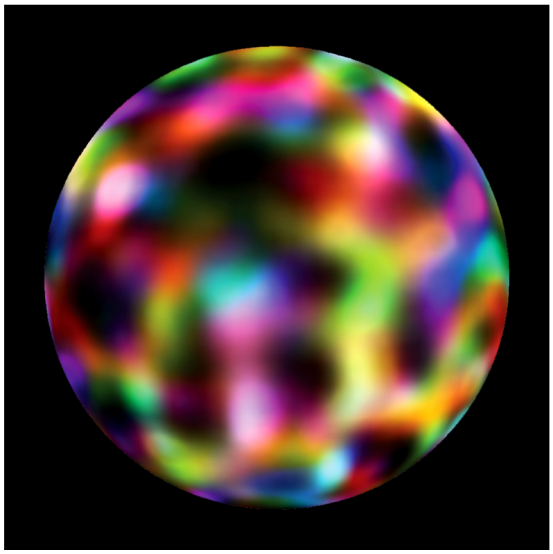

Figure 6 Dynamic signaling domains on the plasma membrane of a eukaryotic cell. The image is a projection on a spherical surface of six independently acquired snapshots of plasma-membrane domains enriched in different proteins. Courtesy of R. Wedlich-Söldner.[9]

Saturation. Most biochemical reactions require catalysts (enzymes) to advance with proper rates. Enzymes catalyze the transformation of a substrate into a product via the formation of an intermediate complex: $E^\dagger + X \rightleftharpoons E^\dagger X \to E^\dagger + X^\dagger$ (Fig. 7a). If the process is quasi-stationary, the rate of production of $X^\dagger$ is given by Michaelis-Menten's law[81–89] $\partial_t X^\dagger \propto E^\dagger X/(K+X)$, that describes an approximately first-order kinetics when X is below the threshold concentration K, and a saturation (zero-order) kinetics above it (Fig. 7a). This and similar saturation effects are a primary source of nonlinearity in signaling networks.

Ultrasensitivity. Cycles of reversible chemical modifications driven by counteracting enzymes can provide step-like (sigmoidal) responses to stimuli.[90,91] In such "futile" cycles the switch between the inactive form X and the active form $X^\dagger$ of some molecule is catalyzed by the counteracting action of two enzymes E and $E^\dagger$ (Fig. 7b). Here $X^\dagger$ and X could be for example the active and inactive forms of a small GTPase, and $E^\dagger$ and E the corresponding GEF and GAP (Fig. 3a). The enzyme-driven switch of a phosphoinositide molecule betwen different phosphorylation states could be a second example (Fig. 3b). If the process is quasi stationary, the rate of production of $X^\dagger$ is given by $\partial_t X^\dagger \propto E^\dagger X/(K+X) - \lambda E X^\dagger/(K^\dagger + X^\dagger)$. If the activity of E is proportional to a stimulus S, the steady-state response $X^\dagger$ is a sigmoidal function of S when the system works close to saturation, i.e. when $X + X^\dagger \gg K + K^\dagger$ (Fig. 7b). This behavior has been described as zero-order ultrasensitivity.[92] Cascades of covalent cycles may result in even stronger ultrasensitivity[92–94] and corresponding attenuation of the noisy components of the signal S.[95]

Adaptation. A commonly observed signaling pattern involves the synchronized activation of the two counteracting enzymes that control a futile cycle (Fig. 7c). If E and $E^\dagger$ are both proportional to the stimulus S, the steady-state response $X^\dagger$ is S-independent. It is at first sight surprising that many vital stimuli are actually processed by such "incoherent feedforward" modules[96,97] that are so perfectly forgetful of stimulation levels. However, if the characteristic time of E activation is slower than the time for the activation of $E^\dagger$, the circuit responds to step-like changes in the stimulus S by a fast activation peak, followed by a slower return to the stimulus-independent steady-state (perfect adaptation, Fig. 7c).[98] Such biochemical circuits are therefore suitable to extract temporal fold changes from a temporally varying stimulation pattern.[99,100]

Bistability. Chemical circuits characterized by multiple steady states can be obtained by combining ultrasensitive switches and amplifying feedback loops (Fig. 7d).[101–108] Such multistable circuits have the potential to store information. In a cell controlled by a bistable signaling pathway the transition between different states may be driven by an external stimulation that exceeds a threshold level. After the transition, the cell may persist in the new stable state even after withdrawal of the stimulation. The discontinuous transition between the two states resembles a first-order phase transition, with chemical noise instead of thermal noise as the driving force.[109] Bistability may arise even in the absence of amplifying feedback loops.[110] On the other hand, noise may induce bistability in systems that would appear monostable in the mean-field approximation,[111,112] while on the contrary, spatiotemporal correlations may lead to the loss of bistability.[113] Bistable circuits have been identified in living cells[109] and have also been created synthetically by ad hoc genetic modifications.[114] A bistable circuit has been proposed for instance to control the maturation of *Xenopus* egg cells (oocytes), where a continuously variable stimulus (the concentration of the maturation-inducing hormone progesterone) is converted into an all-or-none biological response (oocyte maturation).[115]

Linear instabilities

Autocatalytic reactions coupled to diffusion can induce the formation of spatiotemporal patterns. This was earlier shown by Turing in an abstract system constituted by two diffusive chemical factors, an activator and an inhibitor.[116] In that system, arbitrarily small perturbations of the homogeneous state are amplified by a linear instability and may lead to various types of dynamically developing patterns. Gierer and Meinhardt later observed that such patterns can be stabilized if a "global" inhibiting factor diffuses and equilibrates much faster than a "local" activator. The slow activator promotes its own synthesis and simultaneously induces the synthesis of an inhibitor, that in its turn, by rapidly diffusing, suppresses the formation of new activator-rich regions in the proximity of existing ones.[117,118] The stabilizing effect of the global inhibitor could be played also by the depletion of some chemical factor present in only a finite amount.[118]

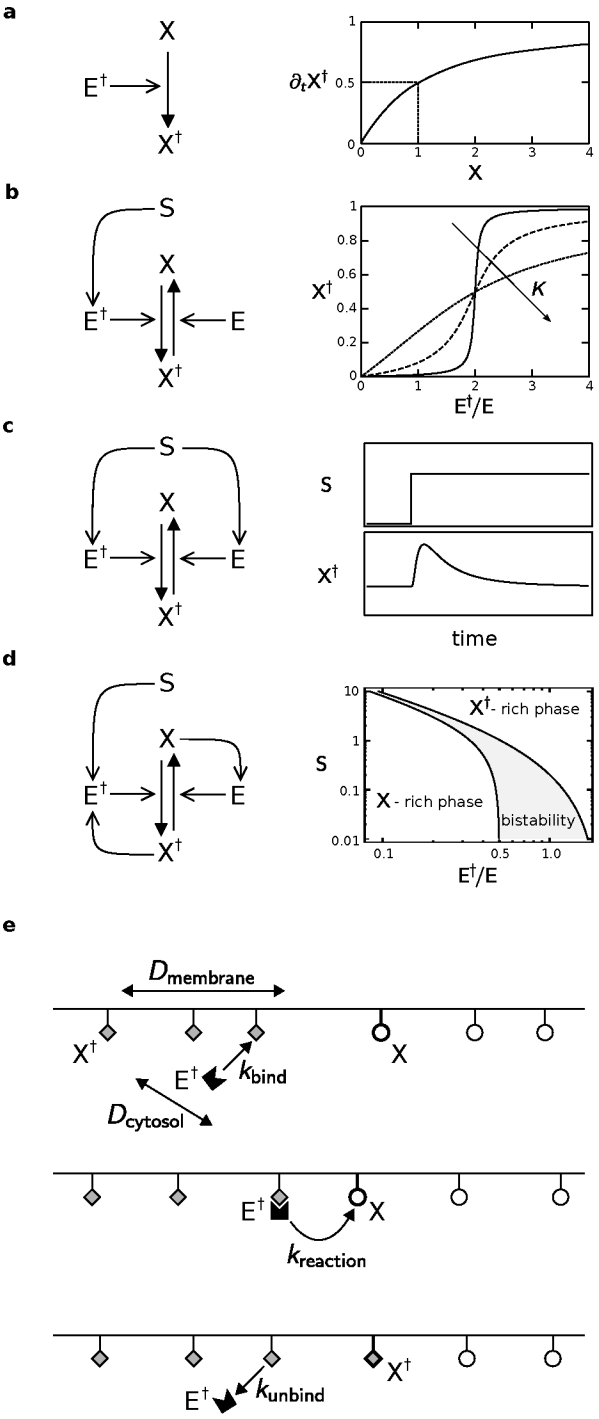

Figure 7 Polarity circuitry. **a:** Enzyme-catalyzed activation of protein X and Michaelis-Menten response ($K = 1$). **b:** Futile cycle and sigmoidal response ($X + X^\dagger = 1$, $\lambda = 2$, $K = K^\dagger = 0.01,\ 0.1,\ 1$). **c:** Incoherent feedforward and adaptation. **d:** Futile cycle with reinforcing feedback loops and phase diagram showing the bistability region.[105–108] Normalized units. **e:** Abstract example of spatially distributed reinforcing feedback loop. A lipid membrane is populated by diffusing signaling molecules that can switch between two states, X and $X^\dagger$. In the $X^\dagger$ state they function as anchors for the cytosolic enzyme $E^\dagger$. When anchored, the enzyme drives the switch $X \to X^\dagger$ and creates new anchors for other $E^\dagger$ molecules. This way, a localized region enriched in $X^\dagger$ can propagate into the X-enriched "sea". Propagation stops when the extension of the $X^\dagger$-enriched region has caused a sufficient depletion in the cytosolic content of $E^\dagger$.[105–107]

Such principles have been invoked to explain the formation of extended signaling domains in eukaryotic chemotaxis.[119–126] In particular, it has been suggested that a rapidly diffusing inhibitor could cancel the uniform signal component and enhance the detection of the gradient component in gradient-driven chemotaxis.[127] As the corresponding globally diffusing molecular counterpart has not been clearly identified, it is likely that pattern stabilization is due to the depletion of signaling molecules that shuttle between the cytosol and the plasma membrane.[105] Experiments also suggest that a relevant role is played here by *local* inhibitory mechanisms.[128]

Turing-type schemes have also been proposed to explain the formation of signaling domains in budding yeast[129] and in neurological synapses.[130]

Stochastic effects

Deterministic Turing systems do not account satisfactorily for some aspects of the formation of signaling domains on the cell plasma membrane. Indeed, if the formation of these domains could be triggered by arbitrarily small perturbations of a uniform plasma membrane state the cell would be permanently polarized and patterns would live indefinitely. On the contrary, the formation of chemotactic signaling domains is a rare event at low levels of stimulation[131] and spontaneously formed signaling domains have a stochastic character, consisting in transient appearances with finite lifetime. Stochasticity originates from thermal fluctuations and its role is enhanced by the small copy number of signaling molecules.

In order to recover these aspects of domain formation, non-linear reaction-diffusion systems with noise have been considered.[105–108,132–135] Positive feedbacks in combination with stochastic effects have been identified as a possible mechanism for the spontaneous establishment of localized sites of polarity. Different paradigms of domain formation as a stimulus-activated process have been proposed, prompted by the intuition that the above described features of domain formation may be the signature of an excitable or bistable behavior, where some barrier has to be overcome to make the nucleation of a new signaling domain possible.[105–108,132–135]

Excitability and bistability

Nonlinearities in signaling pathways, coupled to diffusion on the inner leaflet of cellular membranes, diffusion in the cytosolic volume, and shuttling between the two compartments, generate an excitable physico-chemical system that can support both spontaneous and induced pattern formation. In zero-dimensional excitable systems, an over-threshold perturbation can induce a wide excursion away from a stable fixed point.[136] When local excitability is coupled with diffusion in a spatially distributed system, intermittent, spatially localized excitations are possible.[136] Distributed excitability has been proposed as a mechanism for the spontaneous polarization of eukaryotic cells.[137–139]

Local bistability in signaling circuits naturally follows from the ubiquitous presence of two-state molecules, such as phosphoinositides or small GTPases (Fig. 3) involved in futile cycles of covalent modifications (Fig. 7). Such cycles can generate bistable systems when "closed" with reinforcing feedback loops that sustain a local increase in the concentration of one of the two forms of the molecule (Fig. 7d). Spatially distributed bistability follows from the coupling of locally bistable signaling circuits with membrane and cytosolic diffusivity (Fig. 7e).[105–107,140–146] This coupling may induce spontaneous symmetry breaking, i.e. the separation of the cell membrane into signaling domains populated by chemically distinct signaling phases.[4,105–107,145–153]

Bistability has been shown to satisfactorily account for the membrane polarization of fertilized *Xenopus* oocytes.[151] In that case, polarization is coupled with a global process of membrane advection.[151] Bistability has been proposed to explain the formation of the immunological synapse[154,155] and of Ras microclusters.[149] Distributed bistability appears also to satisfactorily describe the formation of signaling domains in budding yeast[156]. Models combining excitable and bistable components have been proposed for the polarization of *D. discoideum* cells.[138,157,158]

Phase separation

Under simplifying assumptions, the bistable behavior of cell polarization can be described by a Landau-Ginzburg system for an appropriate order parameter $\varphi(\mathbf{x},t)$ describing a concentration of signaling molecules:

$$\partial_t \varphi = D\Delta\varphi + V'(\varphi) + \xi(\mathbf{x},t) \qquad (1)$$

with D the diffusivity of signaling molecules on the cell membrane, $V(\varphi)$ an effective potential, and ξ a white noise term.[106–108] If $V(\varphi)$ is a double-well potential, the microscopic counterpart of (1) is the Ising model with Glauber dynamics.[159] In this kind of system two chemical phases are separated by an effective energy barrier that has to be overcome either by a spontaneous large fluctuation, or by a sizable external perturbation. This situation is quite different from the Turing scheme of pattern formation, where the uniform state is unstable with respect to arbitrarily small perturbations.

The shuttling of signaling molecules between the cytosol and the cell membrane $\mathscr{M}$ can be described by the *global* particle conservation law:

$$\int_{\mathscr{M}} \varphi \, \mathrm{d}\sigma = \text{const} \qquad (2)$$

taking into account that, since diffusion in the cytosol is often much faster than diffusion on membranes, signaling molecules can unbind from the cell membrane, fast diffuse in the cytosol, and bind again at a different place.[105–108] The constraint (2) introduces a long range competition for a finite amount of signaling molecules. The finiteness in the number of molecules that can be exchanged between the two phases enforces polarization, i.e., the coexistence of two signaling phases on complimentary domains.[105,106,108,151,159,160]

If $V(\varphi)$ is a suitable single-well potential, Eqs. (1,2) can describe a distributed excitable system, where spatially localized, intermittent excitations are generated by the white-noise term.

Universality

The phenomenological approach based on Eqs. (1,2) suggests that simple principles, such as local bistability and particle conservation, may be sufficient to provide a universal description of cell polarization.[106] The property of universality, i.e. independence on microscopic details, is here of the utmost importance, as the reaction rates and molecular concentrations involved in cell signaling are poorly known and variable, while polarization processes are a robust feature of living cells. Eqs. (1,2) are a non-trivial variation of the well-known model A from the Hohenberg-Halperin classification of dynamical field theories.[106,107,161] A putative universality class for the polarization of cell membranes is therefore model A with global particle conservation[106,107,162,163] (and not the locally conserved model B, although the two are easily confused[164]).

A few universal predictions stem from model (1,2): the existence of a line tension that is minimized during domain formation, leading to the predominance of circular domains; competitive domain growth (coarsening, Fig. 8a); and scaling laws for the time evolution of domain sizes.[106] The predominance of circular domains has been observed in several experiments on cell polarization.[165,166] The competitive growth of signaling domains has also been observed in recent years (Fig. 8b).[166] On the other hand, the observation of dynamic scaling laws is likely to be complicated by the difficulty of detecting sub-micrometric domains with the adequate space and time resolution.

Winner-take-all mechanism

Several cellular functions, such as that of developing a migratory front, or determining the site of budding of a daughter cell, require that the corresponding signaling domain be unique.[50,166,167] This means that, in addition to mechanisms that start the formation of signaling domains, there must be some mechanism that stops their uncontrolled spread. Several global mechanisms have been proposed to this purpose: fast diffusing inhibitors, sequestering of essential polarity components, and membrane tension.[168] Depletion of molecules from a finite cytosolic pool is probably the simplest mechanism that can stop the spread of an energetically favored phase, leading to competitive growth, where larger domains grow at the expense of the smaller ones.[106,107,160] The recent observation of domain coarsening in budding yeast[166] supports the following scenario: random fluctuations generate a multiplicity of polarity germs; as a finite amount of molecules has to be shared between different domains, and molecules have a higher probability to stick to larger domains, a unique domain wins in the long run.[106,166] Diffusive and active mechanisms are likely to cooperate in the process of global molecule redistribution, but their reciprocal roles need to be more thoroughly investigated.

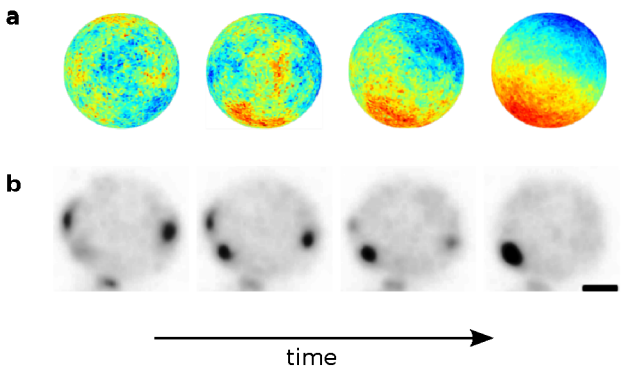

Figure 8 Domain coarsening. **a:** Numerical simulations of cell polarization. Larger domains feed on top of the smaller ones, leading to the survival of a unique domain that minimizes the line tension.[105–108] **b:** Experimental observation of domain coarsening in budding yeast (scale bar: 2μm; time interval: 135 s); from Ref. 166.

Conclusion

A hectic activity of dynamic clustering and patterning is continuously taking place on the membranes of living cells, mostly below the limits of optical resolution and with functions yet to be understood. Patterning cascades span over a wide range of scales, with molecular clusters at the smallest scales providing the material for larger signaling domains. The peculiar molecular composition of such domains suggests the existence of a still largely unknown code that endows localized membrane regions with distinct functional identities. Clustering and partitioning are an important part of the process of molecule sorting, that incessantly counteracts the homogenizing effect of diffusion. Signaling domains provide cells with polarity patterns that coordinate their movement, localization and mechanical interactions with the complex architectural patterns of living multicellular organisms. The destruction of polarity leads to for severe pathologies.

Self-reinforcing feedback loops drive the growth of localized signaling domains whose size and uniqueness is controlled by the depletion of molecular factors that shuttle between the cytosolic reservoir and lipid membranes. The corresponding dynamics can be described at the microscopic level by lattice-gas cellular automata and at the mean-field level by reaction-diffusion equations, where excitable, bistable or multistable systems are coupled by diffusion. Within this setting, a universal description of domain formation in living cells may emerge, analogous to the classical theory of first-order phase separation. For instance, bistability and global constraints that take into account the shuttling of a finite number of molecules between the cytosol and the cell membrane lead to a globally conserved version of Hohenberg and Halperin's model A, independently of molecular details. Such a framework could also include active transport processes.

Several questions however ask for further investigation.

Targeted experiments are required to understand whether domain formation in each given biological system is better described by Turing instabilities, excitability, or phase-separation, i.e. whether domain formation is driven by a linear instability, or some sort of activation barrier has to be overcome for cell polarity to start; and whether the process is transient and intermittent, or leads to the formation of unique domains by a coarsening process.

The interplay between patterning mechanisms based on diffusion and on active vesicular traffic is still unclear, although it is likely that the different mechanisms are designed to operate on different time and length scales, and to provide varying degrees of stability of polarity patterns.

Lastly, it would be highly desirable to attain a universal theory of domain formation on cell membranes, where the main properties are independent of molecular details, and cellular polarization phenomena are classified in universality classes based on the number of components of suitable order parameters and local or global conservation laws.

Acknowledgements. AG thanks Antonio Celani, Günther Gerisch, Igor Kolokolov, Vladimir Lebedev, Hans Meinhardt, Keith Mostov, Guido Serini, and Roland Wedlich-Söldner for fruitful discussions and useful comments.

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