

# Cell polarity: mechanochemical patterning

Nathan W. Goehring<sup>1,2,3</sup> and Stephan W. Grill<sup>1,2</sup>

<sup>1</sup> Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Pfotenauerstraße 108, 01307 Dresden, Germany

<sup>2</sup> Max Planck Institute for the Physics of Complex Systems (MPI-PKS), Nöthnitzer Straße 38, 01187 Dresden, Germany

<sup>3</sup> Cancer Research UK London Research Institute, Lincoln's Inn Fields Laboratories, 44 Lincoln's Inn Fields, London WC2A 3LY, UK

**Nearly every cell type exhibits some form of polarity, yet the molecular mechanisms vary widely. Here we examine what we term ‘chemical systems’ where cell polarization arises through biochemical interactions in signaling pathways, ‘mechanical systems’ where cells polarize due to forces, stresses and transport, and ‘mechanochemical systems’ where polarization results from interplay between mechanics and chemical signaling. To reveal potentially unifying principles, we discuss mathematical conceptualizations of several prototypical examples. We suggest that the concept of local activation and global inhibition – originally developed to explain spatial patterning in reaction–diffusion systems – provides a framework for understanding many cases of cell polarity. Importantly, we find that the core ingredients in this framework – symmetry breaking, self-amplifying feedback, and long-range inhibition – involve processes that can be chemical, mechanical, or even mechanochemical in nature.**

## Ubiquity and universality during cell polarization

Polarity, the ability of a cell to define a geometric axis, is critical to a broad variety of cell functions, including cell migration, directional cell growth, and asymmetric cell division. It is likely to be an ancient property of cells, perhaps having initially evolved to ensure differential inheritance at cell divisions, a role maintained to this day [1]. Polarity is also critical for cells in multicellular environments. In epithelial tissue, for example, apical–basal polarity drives the opposing surfaces of the cell to acquire distinct functions and molecular components [2], whereas planar cell polarity (PCP) aligns cells and cellular structures such as hairs and bristles within the epithelial plane [3]. Consistent with the fundamental importance of polarity in the organization of cells, the ability to polarize is ubiquitous in prokaryotes, plants, fungi, protozoa, and animals [4] and there are even reports of asymmetric cell divisions in *Archaea* [5].

The end point of cell polarity networks is to differentiate molecularly one side of the cell from the other. This molecular differentiation both defines a polarity axis and allows cellular process to be regulated differentially along this axis. In this way, cells can target specific proteins to a growing tip (e.g., yeast) or to leading edge (e.g., crawling cells). They

can set up intracellular signaling gradients or selectively segregate molecules, such as fate determinants, during cell division (e.g., stem cells).

Despite its ubiquity and potentially ancient appearance, the ability to polarize appears to have evolved repeatedly, yielding a diversity of seemingly unrelated molecular mechanisms. Some polarity pathways rely on the ability of interactions between diffusible species to drive pattern formation. We will refer to these as chemical systems because they can be understood primarily within the language of reaction rates and molecular diffusion [6]. We distinguish these from mechanical systems, in which polarization requires consideration of force, stress, and elastic and viscous properties of biological materials such as cytoskeletal elements and membranes. In many cases, polarity may be considered mechanochemical, requiring complex interplay between both chemical and mechanical networks [7,8].

How can we reconcile the universality of cell polarity as a concept in biology with such complexity and diversity of molecular mechanisms? Here we examine a broad range of cell polarity systems to highlight one basic paradigm for cell polarity based on coupling symmetry breaking to local signal amplification and long-range inhibition. This paradigm is a robust, general mechanism for inducing and maintaining cell polarity and can convert potentially weak, transient, or even stochastically arising signals into persistent cellular asymmetry. A central point of this review is to demonstrate that this conceptual framework applies equally well to mechanical, chemical, and even coupled mechanochemical polarity networks, thereby providing a shared framework for understanding a diverse range of polarization processes despite wide variation in their molecular mechanisms. We argue that in the current drive towards a systems level understanding of development, uncovering such key organizing principles of morphogenetic processes will increasingly require consideration of both chemical and mechanical processes.

## Symmetry breaking and pattern formation in chemical systems

The paradigm of pattern formation through local symmetry breaking, signal amplification, and long-range inhibition traces its roots to seminal work by Alan Turing [6]. It was he who first described how chemical patterns can emerge from a uniform starting condition through an instability that arises from stochastic fluctuations combined with

Corresponding authors: Goehring, N.W. (nate.goehring@cancer.org.uk); Grill, S.W. (grill@mpi-cbg.de).

Keywords: cell polarity; mechanics; reaction–diffusion; pattern formation.

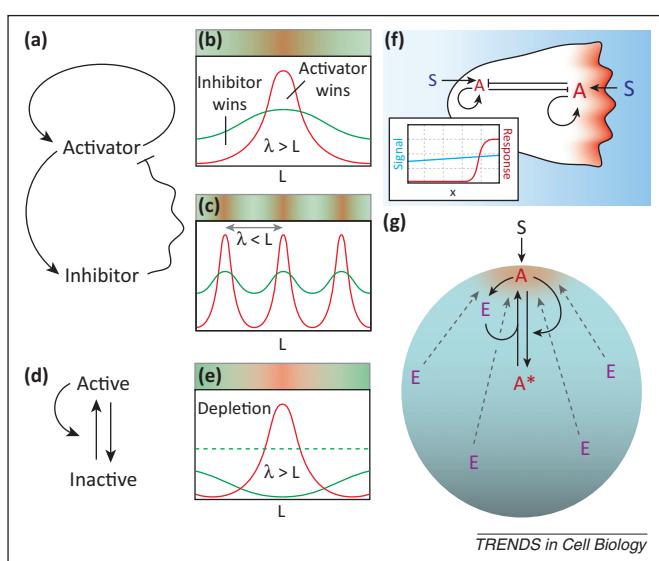
## Review

interactions between chemical species (morphogens) that diffuse at different rates; hence, pattern formation by reaction–diffusion. Subsequent work by Gierer and Meinhardt codified the idea of competing short- and long-range feedback in the stabilization of patterns, including polarization [9]. Local activation typical takes the form of self-enhancing feedback, which amplifies local, potentially random increases in signaling activity (Figure 1a). On its own, such self-amplifying feedback will tend to convert more and more of a system to an ‘on’ state. This activation must therefore be coupled to an inhibitory process that is long range. Long-range inhibition is typically provided by a rapidly diffusing inhibitor produced by the activator (Figure 1a). The inhibitor spreads faster than the activator, allowing it to suppress the activator outside the immediate region of activation (Figure 1b). In classic Turing-like systems, the resulting spatial pattern is specified by differences in the diffusivities of the slower activating molecule and the faster inhibitor, leading to polarized (Figure 1b) or repeating patterns (Figure 1c). In fact, a diffusible inhibitor is not strictly required, because its role can be served by a freely diffusing component that is depleted as part of the signal amplification process (Figure 1d,e) [9,10]. The diffusivity of this

component effectively specifies the extent of the region over which the component is depleted and thus signal amplification inhibited. As long as the region of inhibition extends beyond that of activation, the region of activation will be constrained.

Although initially stimulated by a need to understand spontaneous pattern formation, the basic principle of local activation and global inhibition underlies a broad range of models to describe chemically polarizing systems (by which we mean polarization in biochemical signaling pathways, in contrast to mechanical; see below) [10]. Indeed, a system that can amplify infinitesimally small fluctuations should also be able to amplify signals emerging from dedicated spatial cues. At the same time, by suppressing amplification everywhere except at the initial location of the cue, the combination of local amplification and global inhibition can transform a weak or noisy chemical asymmetry into a steep signaling gradient (Figure 1f). Numerous variations on this theme exist and include mechanisms that are highly reversible, persisting only as long as the signal persists, as well as those that are highly irreversible, locking in a stable response to a transient cue.

Polarization during budding by the yeast *Saccharomyces cerevisiae* is a particularly illustrative case of the principles of local activation and global inhibition in chemical pattern formation (Figure 1g). In *S. cerevisiae*, reproduction involves formation of a bud on the surface of the mother cell that grows to become the new daughter cell. Bud site selection is under control of the small Rho-like GTPase Cdc42, which localizes in highly polarized fashion to a cap near the bud site and directs recruitment of several downstream pathways involved in bud growth [11]. Symmetry breaking can occur either in response to a localized cue or spontaneously if this cue is absent [12]. In most cases, the GTPase Rsr1 acts as a localized cue [13], specifically recruiting Cdc42-GTP near the remnant of the previous budding event, the so-called ‘bud scar’. In cells lacking Rsr1, Cdc42 undergoes spontaneous polarization, forming a cap at a random position through a classic Turing-like mechanism [14]. In both cases, the formation of a stable, highly enriched patch of Cdc42-GTP requires that the initial symmetry-breaking event be reinforced through a Cdc42-dependent positive feedback circuit. Positive feedback requires a complex containing the GTP exchange factor (GEF) for Cdc42, Cdc24, the scaffold protein Bem1, and the Cdc42 effector PAK [15]. By binding active Cdc42, PAK recruits this complex to the existing patch, where Cdc24 can catalyze the local activation of additional Cdc42 [16]. Spreading of the Cdc42 cap and the formation of additional caps are inhibited by depletion of free cytoplasmic Bem1 as it is incorporated into the Cdc42 cap [14,17,18]. Because free Bem1 diffuses rapidly, local membrane recruitment of Bem1 rapidly depletes Bem1 from the rest of the cell. In other words, rapid Bem1 diffusion ensures that the effect of local membrane accumulation is global suppression of further membrane recruitment. Thus, the basic paradigm of local amplification and global inhibition within a chemical network serves to reinforce and sustain an initial asymmetry, whether spontaneous or induced, to yield a robustly polarized system.



**Figure 1.** Pattern formation in chemical systems. (a) In an activator-inhibitor system, a slow-diffusing activator stimulates production of both itself via autocatalysis and a fast-diffusing inhibitor. (b) Because the inhibitor spreads faster than the activator, it allows for activation in the central zone, but suppresses activation beyond. The diffusivities of the two molecules together with the reaction kinetics define the scale of periodicity of the pattern. Typically, this length scale is related to the distance over which the inhibitor can effectively exert its activity. If this length scale is larger than the system ( $\lambda > L$ ), inhibition will spread throughout the system, leading to a single activation peak. (c) Conversely, if  $\lambda < L$ , a new region of activation can emerge at a distance  $\lambda$  from the original zone of activation, resulting in a periodic pattern. (d) Long-range inhibition can also occur through depletion of a diffusible molecule; for example, if a molecule that stimulates its own conversion from an inactive, rapidly diffusing state to an active, slowly diffusing state. Rapid diffusion ensures that local conversion events result in system-wide reduction in the concentration of inactive molecules, again restricting activation to a single zone (e). (f) Activation-inhibition can amplify weak signals. The signal, S, induces autocatalytic accumulation of activator, A. Competition between zones of activation result from A-dependent long-range inhibition. Weak signal asymmetries ensure that the zone of activation at the front wins, resulting in a sharp front-to-back response. (g) An activator-depletion mechanism underlies actin-independent polarity in *Saccharomyces cerevisiae*. The bud scar signal, Rsr1 (S), stimulates local recruitment of active membrane-associated Cdc42-GTP(A). A autocatalytically stimulates activation of Cdc42-GDP(A\*) via the GTP exchange factor (GEF) complex (E). Long-range inhibition is provided by depletion of E from the cytoplasm.

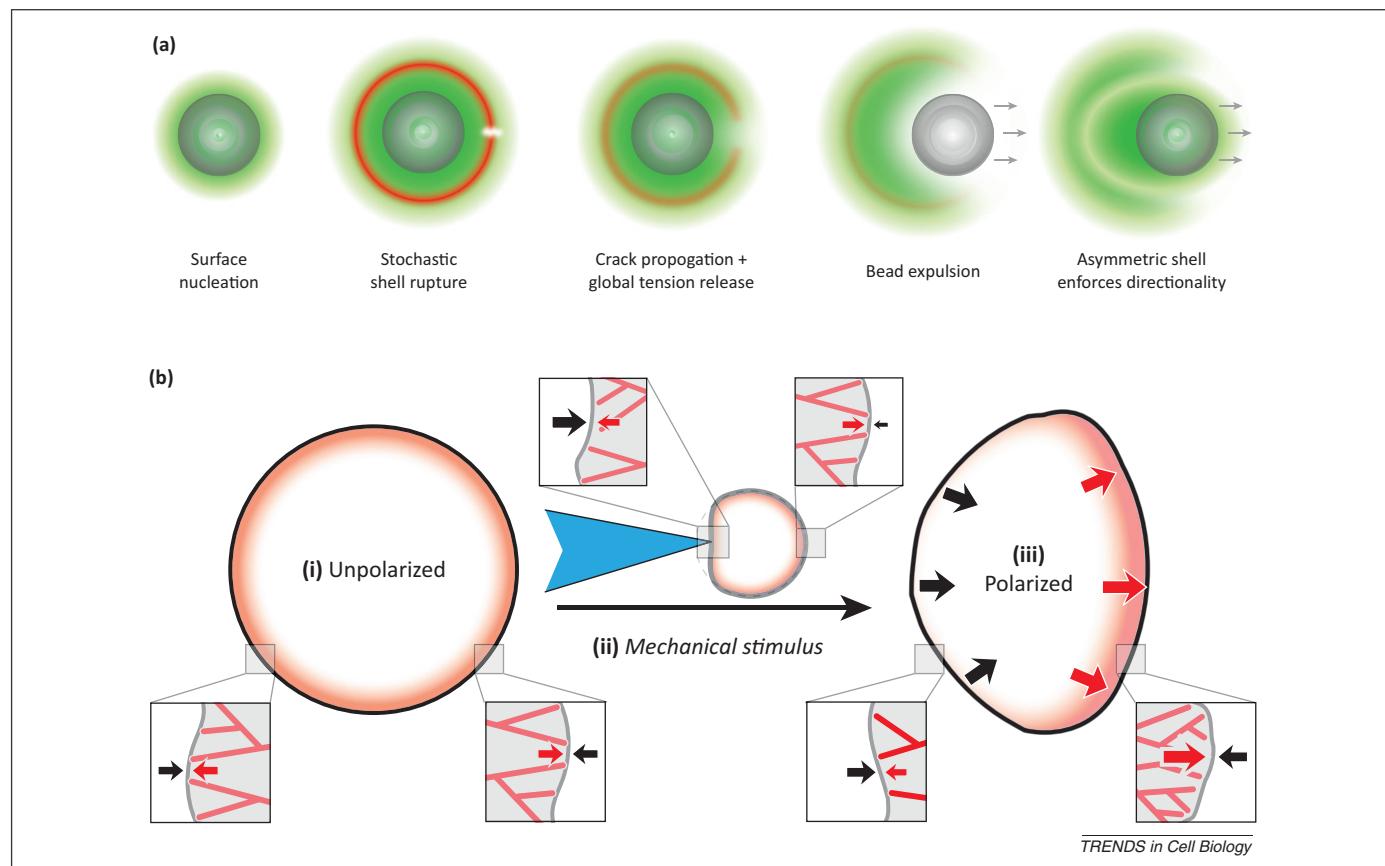
Self-organizing chemical systems can be both much simpler and much more complex. For example, theory suggests that one can achieve polarity with a single molecular species if there is a positive feedback loop in which an active membrane-associated molecule recruits additional copies of itself from a cytoplasmic pool, provided the system is operating within a stochastic regime and molecule number is limited [19]. Positive feedback effectively stabilizes local fluctuations, allowing them to persist for long periods of time. Limiting molecule numbers are required to ensure that multiple fluctuations compete with one another for unbound molecules, thus yielding a single dominant front. At the other end of the spectrum is *Dictyostelium*, where the core of the polarity pathway is a complicated set of positive and negative feedback loops that involve multiple phosphatidylinositol lipid signaling molecules and a set of phosphatidylinositol lipid-modifying enzymes that interconvert these two species [20,21]. Moreover, because cells can still polarize when this core system is compromised, polarity in *Dictyostelium* is likely to reflect interaction among multiple, partially redundant pathways, each potentially tuned to particular cellular or environmental conditions [22,23].

### Mechanically polarizing systems

For reasons of simplicity, Turing omitted mechanics in his formulation of pattern formation by reaction–diffusion. However, as he noted at the time, force, stress, active movement, and material properties such as viscosity and

elasticity cannot be ignored when seeking to understand morphogenetic processes [8]. Indeed, examples of purely mechanical polarizing systems exist. Here we describe two classic cases involving polarized actin networks: the formation of actin comet tails by intracellular pathogens (Figure 2a) and the directional motility of keratocytes (Figure 2b) [24]. In both cases, the mechanical properties of an actin cytoskeletal network appear sufficient for polarization, which can be triggered by stochastic or induced asymmetries in the mechanical network. What are the organizing principles of these systems and how do they relate to the principles espoused by Turing?

Polarized actin tails are used by intracellular pathogens such as *Listeria*, *Rickettsia*, *Shigella*, and vaccinia virus to promote their intracellular transport and spread between cells [25]. In each case, formation of comet tails relies on a pathogen-encoded protein that nucleates branched actin networks on the pathogen's surface. Reconstitution of the process *in vitro* by coating beads with actin nucleators proved that uniform surface nucleation is sufficient to promote motility and provided insight into the mechanisms of symmetry breaking and actin tail formation, summarized in Figure 2a [26,27]. Actin nucleation on the bead surface forms a shell. As additional actin is nucleated at the bead's surface, the outer layers expand isotropically, inducing stress in the actin network. Stochastic variation in the shell results in spontaneous local breakage of actin filaments, the so-called symmetry-breaking event. This initial site of



**Figure 2.** Polarization in mechanical systems. (a) Actin tail-mediated propulsion. Stochastic instability in the shell of actin nucleated from the surface of nucleator-coated bead results in shell rupture. Shell remnants asymmetrically stabilize the newly synthesized shell at the rear, ensuring that subsequent rupture always occurs at the front. (b) Polarization of keratocytes. Protrusive force generated by actin polymerization at the membrane (red arrows) is opposed by membrane tension (black arrows). Stochastic or induced spatial asymmetry in polymerization is reinforced and propagated by the self-amplifying nature of actin polymerization (local positive feedback) and membrane tension, which introduces competition between polymerization and protrusion at different sites in the cell (long-range inhibition).

## Review

weakening is then amplified: As filaments begin to break, the local actin network weakens, leaving it less able to bear stress and favoring further expansion of the existing rupture. This expansion of the rupture is coupled to a relaxation of tension elsewhere in the actin shell. Importantly, because mechanical stress in a material propagates at roughly the speed of sound, tension relaxation will occur nearly simultaneously with shell rupture, making ruptures of the shell at secondary sites immediately less likely. Thus tension relaxation acts as a long-range inhibitor to confine shell rupture to a single site.

Fish epithelial keratocytes are highly motile cells characterized by a well developed lamellipodium and rapid, persistent, directional migration along surfaces. Like actin tails, the actin network in keratocytes is mechanically self-organizing (Figure 2b). Theoretical models suggest that two components are critical for polarization. Arp2/3-mediated branched actin assembly at the cell membrane is thought to be autocatalytic and thus locally self-amplifying, leading to a protrusive force on the membrane due to polymerization [24,28]. This protrusive force is resisted by plasma membrane tension. Membrane tension is critical for two things. First, it is required to use some of the protrusive force generated by actin polymerization to move the cell body. By resisting protrusive force at the leading-edge membrane, tension causes the actin network to be displaced rearward as it polymerizes, a phenomenon known as retrograde flow. Physical linkage between the flowing actin network and sites of adhesion to the substrate allow retrograde flow to exert traction forces on the substrate to push the cell body forward [29]. Second, membrane tension is essential for cell polarity itself due to its role as a long-range inhibitor of protrusion [30]. As discussed above, mechanical stress propagates extremely rapidly. Therefore, a local increase in tension due to membrane protrusion at the leading edge will induce a near-simultaneous increase in membrane tension

throughout the rest of the cell. This increased tension makes it increasingly difficult for actin polymerization to induce protrusion of the membrane. Thus, membrane tension serves as a long-range mechanical inhibitory signal to limit protrusion to a single leading edge. Ultimately, additional factors are at play beyond these minimal ingredients, including myosin-based contraction at the cell rear, which may play a critical role in initial symmetry breaking [24,31,32]. Notably, though, mechanical stimuli alone are sufficient to trigger polarization [33].

In conclusion, what appear to be largely mechanical polarity systems share core organizing principles with the chemical reaction–diffusion systems described above, including symmetry breaking, local self-amplification, and long-range inhibition, despite not relying on chemical diffusion. Perhaps one should not be surprised that these basic principles apply to mechanical systems, because examples of pattern formation based on these principles exist throughout the physical world (Box 1) [34].

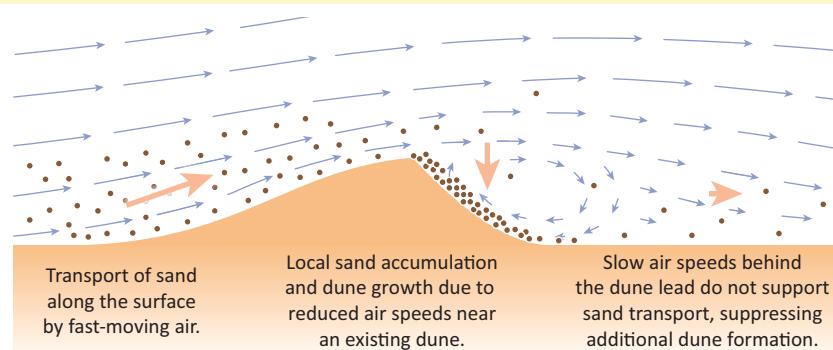
### Coupled mechanochemical systems

Although cell polarity can emerge from systems that are largely either chemical or mechanical, in many, if not most, cases, cell polarization depends critically on the interplay between the two. Numerous mechanisms exist for coupling mechanical and chemical processes (examples of which are shown in Boxes 2 and 3). In the simplest cases, asymmetry in one network acts to induce or reinforce asymmetry in the other. In other cases, asymmetry in the two networks is interrelated, with each contributing links in the core feedback circuits that drive polarization. In the following section we consider several examples of coupled mechanochemical systems that highlight the complexity of this interplay during polarization. Only by considering both can the core mechanisms that induce and reinforce asymmetry be understood.

#### Box 1. Sand dunes form via local activation and long-range inhibition

Because sand transport is highly sensitive to wind speed, local changes in wind velocity will alter the pattern of sand deposition (Figure 1). Local accumulation may be triggered by small stochastic variations, a larger object, or even local surface variation, which affect air speed. Once an accumulation forms, it creates a wind shadow – a region of suppressed wind velocity. As sand particles transported by the wind encounter this region, their velocity slows and they settle

onto the surface, thereby ‘amplifying’ the dune. Dune growth in turn increases the wind-shelter effect and enhances sand deposition even further. Long-range inhibition arises because slowed air currents downstream of the dune cannot drive significant sand transport. Thus, the presence of a dune suppresses formation of additional dunes in its immediate vicinity, contributing to their characteristic spacing.



**Figure 1.** Sand dunes form via local activation and long-range inhibition.

## Review

**Box 2. Chemical cues shape cellular mechanics**

Both the structure of a cell's mechanical elements and their activity can be shaped by local biochemical signals (Figure I). Signaling molecules can polarize the mechanical elements of a cell through local control of the assembly of cytoskeletal polymers. In polarized cells (a), the activity of membrane-associated Rho-GTPases such as Cdc42 and Rac (red) simultaneously stimulate actin nucleators such as formin (green) and Arp2/3 (purple) and suppress destabilizing factors (blue) [50,64]. Similarly, microtubules are nucleated from microtubule organizing centers (MTOCs) such as the centrosome, resulting in polarity of the outgrowing network [65], and in migrating cells leading edge signals stimulate the capture and stabilization of microtubule plus ends by microtubule-associated proteins (MAPs) such as EB1 and APC (purple) [66]. These signals may also inhibit molecules that promote microtubule destabilization (orange), further focusing microtubule networks toward the leading edge. Asymmetries in mechanical networks can also arise through spatial regulation of cytoskeletal motor activity. For example, in the *C. elegans* one-cell embryo (b), membrane-anchored dynein pulling motors (blue motors) are regulated by the PAR polarity proteins (red/cyan), which are distributed asymmetrically along the anterior-posterior axis. Consequently, the number of active force generators at the posterior exceeds that at the anterior, resulting in an asymmetry of force applied to the spindle [67–69].

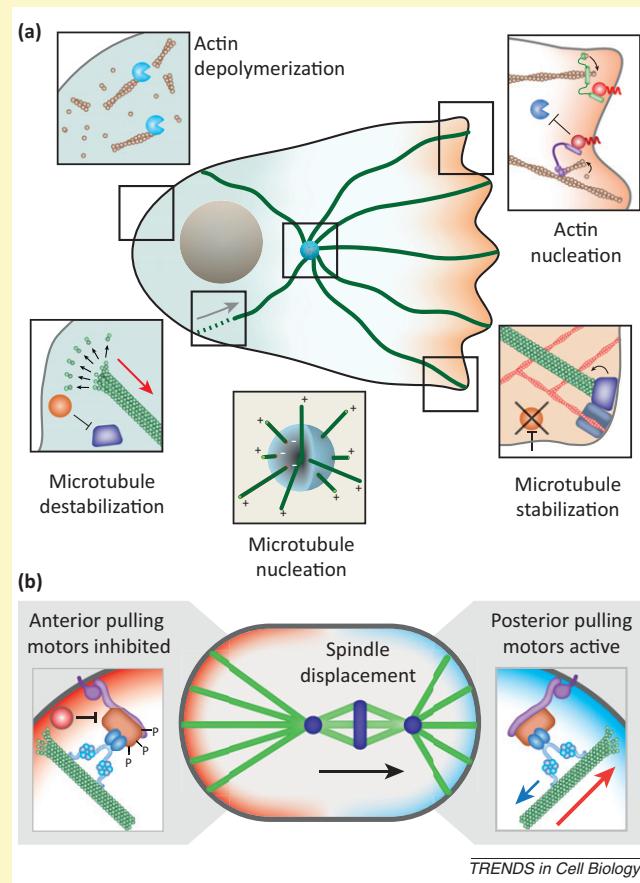


Figure I. Chemical cues shape cellular mechanics.

**Mechanical symmetry breaking**

One well-studied example of mechanochemical polarization is the establishment of the anterior-posterior (AP) axis in one-celled *Caenorhabditis elegans* embryos, which depends on both mechanical (actin/myosin) and biochemical (partitioning-defective [PAR] protein) networks.

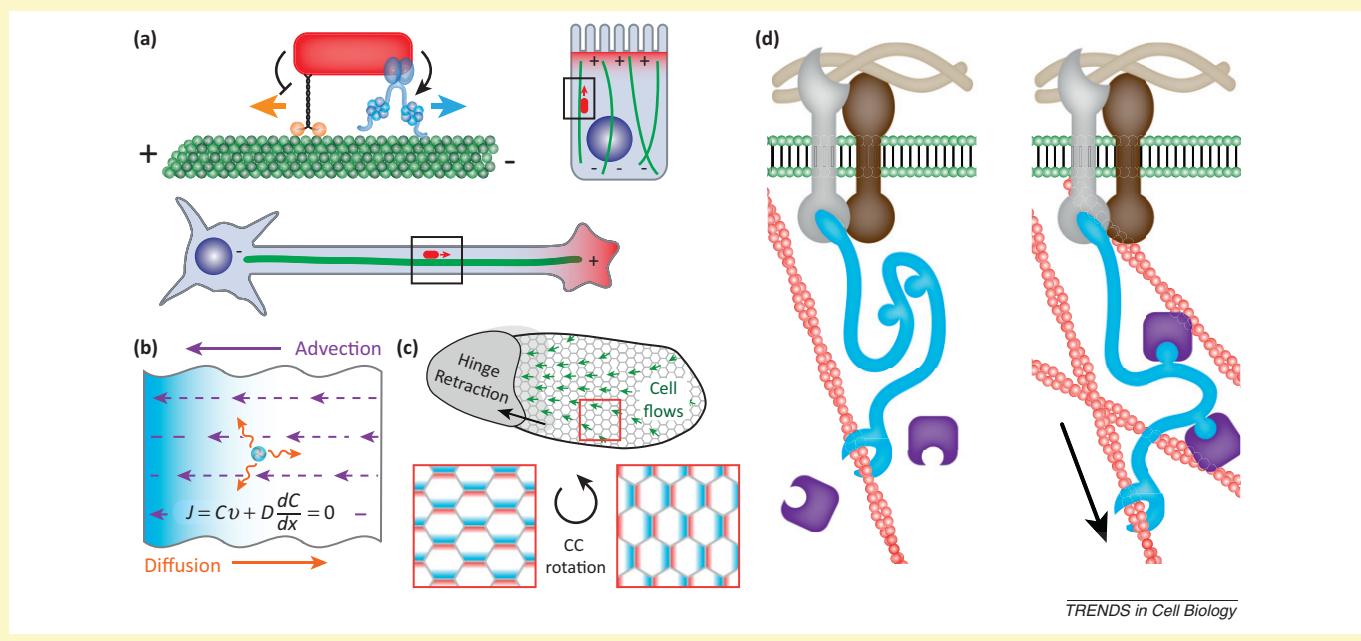
At the core of the polarization machinery are the so-called PAR proteins [35]. In a given cell, there are typically two groups of antagonistic PAR proteins that are segregated to opposite halves of the cell. In *C. elegans*, these include PAR-3, PAR-6, and an atypical protein kinase C (aPKC), which localize to the anterior membrane (anterior PARs), and PAR-1, PAR-2, and lethal giant larva (LGL), which localize to the posterior (posterior PARs) [36]. Recent experimental and theoretical analysis suggests that two ingredients are critical for the ability of PAR proteins to form patterns in the cell: the ability of each group of PAR proteins to displace the other from the membrane, so-called mutual antagonism, and the presence of limiting amounts of PAR protein in the cell (Figure 3a) [37–39]. Mutually antagonistic negative feedback between the anterior and posterior PARs yields a locally self-amplifying feedback loop, because a molecule's inhibition of its own inhibitor effectively constitutes an auto-activation pathway. This self-amplifying feedback ensures that the membrane will tend to be dominated locally by one or the other PAR complex, but cannot on its own specify whether domains will exist or where the boundaries between domains will occur. Such spatial organization requires long-range inhibition, which in this case is provided by the limiting pools of PAR proteins [38]. As PAR proteins are recruited into a membrane domain, the pool of free cytoplasmic PAR proteins is decreased, thereby reducing the tendency of the domain to grow further. A stably polarized state results when the sizes of the two domains are such that their tendency to grow is balanced.

But how is symmetry broken? In other words, why does the system proceed to a polarized state rather than remaining in its initial unpolarized state in which anterior PARs are enriched throughout the cell membrane and posterior PARs are confined to the cytoplasm, a situation equally supported by the antagonistic process described above. Symmetry breaking in the PAR system relies on the mechanical activity of a thin, contractile cytoskeletal layer under the cell membrane known as the actomyosin cortex (Figure 3b). Recent work points to a contractile asymmetry in this network along the AP axis [40] that is induced by the centrosome, most likely via local modulation of Rho activity [41–44]. This contractile asymmetry results in a long-range flow of cortex from posterior to anterior [40,42], which in turn entrains the motion of the cell cytoplasm, creating a fluid flow towards the anterior along the inner surface of the membrane [45]. As it turns out, the diffusive properties of anterior PAR proteins at the membrane are such that these flows can induce a significant redistribution of PAR proteins within the cell through a process known as advection (Box 3) [38]. Consequently, anterior PARs, which are initially enriched at the membrane, will be preferentially transported towards the anterior, thus depleting anterior PARs from the posterior membrane (Figure 3b). Freely diffusing posterior PARs in the cytoplasm can then take advantage of this local depletion to associate with the posterior membrane. Once asymmetry is established, biochemical reaction-diffusion processes can take over to drive the system to the stably polarized state. Thus, PAR polarity in *C. elegans* can be understood as a largely chemical pattern-forming system based on

**Box 3. Cell mechanics shape spatial signaling patterns**

The activity of molecular motors and the assembly dynamics of the cytoskeleton itself can create patterns of inhomogeneous stresses and asymmetrically distributed molecular forces that can shape the local distribution or activity of signaling molecules [70] (Figure I). Motors are typically guided by intrinsically polarized cytoskeletal filaments [24,71] (a). In the presence of even subtle biases in the polarity of the cytoskeleton, motor-driven transport can yield robust asymmetries [48,72]. An alternative to direct, motor-driven transport is advection, the nonspecific transport of material due to bulk flow, similar to the transport of objects in a flowing river (b). Within cells, such bulk-material transport can be driven by flows of cytoplasm [45,73–75]. Bulk flow also occurs at the tissue scale, resulting from changes in cell position, orientation, and shape that are induced by applied stress (c). Such cell flows can have significant effects on the local orientation of polarized cells relative to their environments

[59,76]. Bulk flows can have very molecule-specific effects, because the efficiency of advection depends on the relative effect of material flow versus the diffusivity and turnover of the molecules of interest. Direct coupling between applied mechanical stress and local biochemical activity can also be achieved through stress-sensitive molecules that undergo tension-induced conformational change [77]. For example, at focal adhesions, talin molecules crosslink extracellular matrix (ECM)-associated integrins and actin (d). Force applied to talin-bound actin filaments induce a conformational change in talin, exposing previously cryptic vinculin-binding sites. Newly bound vinculin can then recruit additional actin filaments, strengthening the mechanical linkage between the ECM and the cell cytoskeleton. Such ‘stress sensing’ is not limited by individual stress-sensitive molecules, but may also arise from more complex mechanisms of feedback within the cell cytoskeleton [78,79].



**Figure I.** Cell mechanics shape spatial signaling patterns.

local self-amplification and long-range inhibition processes that are intrinsic to the PAR network, combined with a mechanical symmetry-breaking event.

#### Mechanochemical self-amplifying feedback

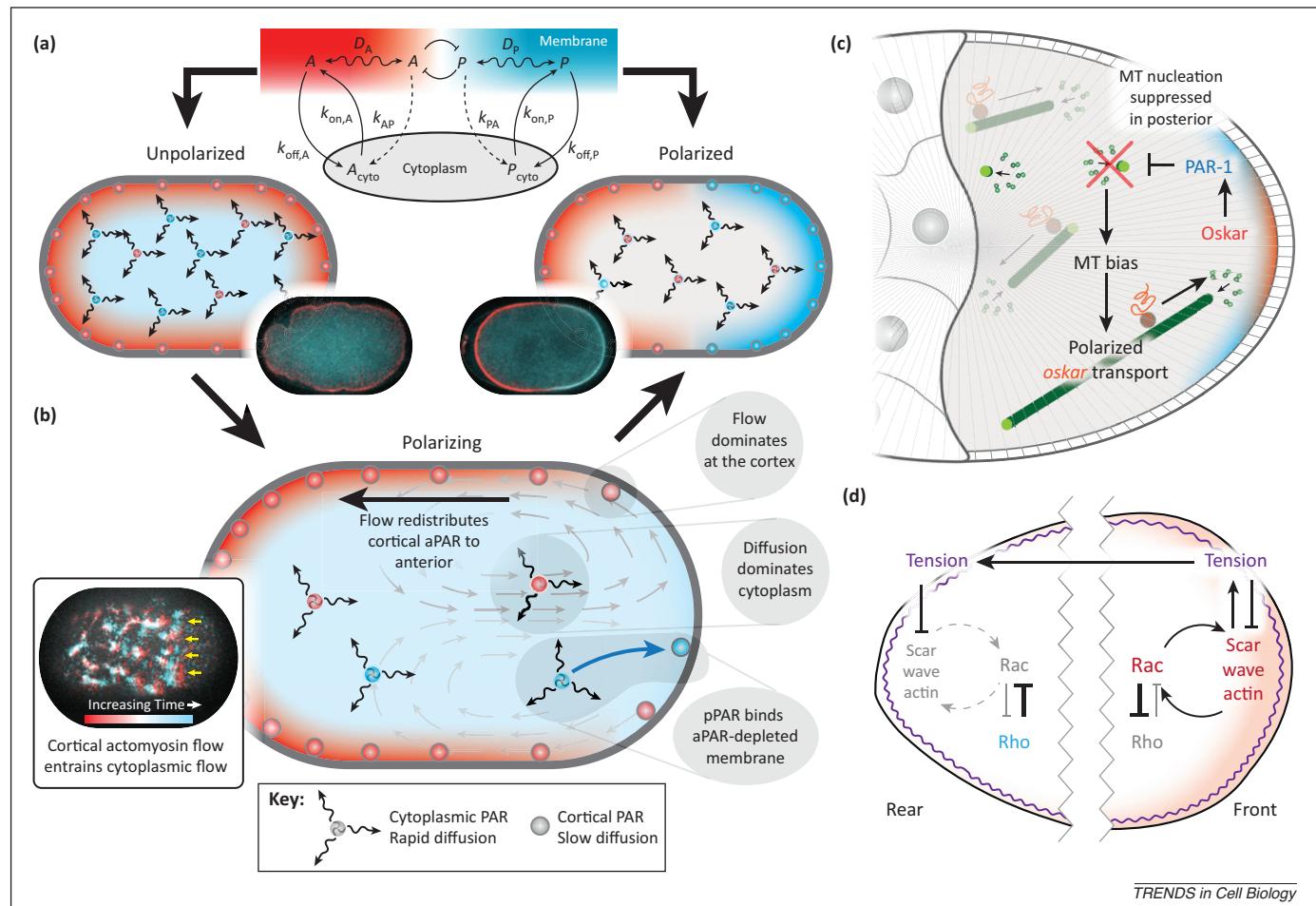
A key step in the establishment of AP polarity during mid-oogenesis in *Drosophila* is the targeted accumulation of oskar mRNA to the posterior pole, which relies on a self-enhancing feedback loop involving polarized microtubule (MT) arrays, the polarity protein PAR-1, and both mRNA and protein derived from the *oskar* gene [46] (Figure 3c). PAR-1 promotes polarization of the MT cytoskeleton by locally suppressing MT nucleation [47]. This underlying MT polarity combined with kinesin-mediated transport of *oskar* mRNA towards MT plus ends drives accumulation of *oskar* mRNA at the posterior [48]. Local translation of these mRNAs yields Oskar protein, which promotes further recruitment of PAR-1, thereby completing the self-amplifying positive feedback loop [49]. Thus, in *Drosophila*, the central self-amplifying feedback loop involved in AP polarity comprises both mechanical (MT transport) and chemical elements (PAR-1, Oskar activity). In contrast to *C. elegans*, symmetry breaking is not mechanical but

chemical: A small group of posterior follicle cells, specified earlier in development, signal into the oocyte to promote the initial recruitment of a small pool of PAR-1 to the posterior pole, thereby initiating the feedback loop [46]. However, similar to *C. elegans*, in the *Drosophila* oocyte depletion of a core component, in this case *oskar* mRNA, is likely to provide for long-range inhibition, because overexpression of *oskar* results in ectopic localization [49].

#### Long range mechanical inhibition of signaling networks

In neutrophils, asymmetry in a biochemical signaling network of Rho-family GTPases, specifically asymmetric Rac activity, is critical for locally promoting the formation of branched actin networks and stimulating leading edge protrusion [50]. This asymmetry is thought to arise through coupled positive and negative feedback between Rho-family GTPases as well as phosphoinositide-based pathways. These chemical feedback circuits amplify asymmetries that arise from extracellular chemical gradients and/or stochastic fluctuations to yield a robustly polarized cell with a clear front and back. For example, Rac is thought to suppress Rho activity at the front, whereas Rho suppresses Rac activity at the rear. Similar to what

## Review



**Figure 3.** Coupling of mechanical and chemical elements in cell polarization. **(a)** Polarization of the *Caenorhabditis elegans* embryo. A reaction-diffusion network comprising two antagonistic groups of partitioning-defective (PAR) polarity proteins results in a self-organizing system capable of sustaining either an unpolarized or a polarized state. The polarized pattern relies on local reciprocal negative feedback between the PAR species (local self-enhancing feedback) and depletion of the pool of rapidly diffusing inactive PAR proteins in the cytoplasm as they are converted into their active, membrane-associated forms (long-range inhibition). PAR scheme modified with permission from [38]. **(b)** Pattern formation in the embryo is induced by advection of membrane-associated anterior PAR proteins by actomyosin-dependent cytoplasmic flow. This allows membrane binding of posterior PARs, resulting in an asymmetry that can be amplified by the self-organizing characteristics of the PAR reaction-diffusion system described in (a). **(c)** Polarization of the *Drosophila* oocyte. Posterior localized PAR-1 locally inhibits microtubule (MT) nucleation, leading to an enrichment of MT plus ends at the posterior. Due to this bias, polarized transport of *oskar* mRNA along MTs results in accumulation of *oskar* mRNA and, consequently, Oskar protein at the posterior pole. Oskar, in turn, stimulates additional recruitment of PAR-1, completing formation of a locally self-amplifying mechanochemical feedback circuit. **(d)** Long-range inhibition through tension. Rac activity and leading edge actin polymerization comprise a self-amplifying positive feedback loop resulting in membrane protrusion. Protrusion increases membrane tension (purple), which propagates through the cell to suppress Rac activity. Presumably, the inhibitory effect of tension is global, but is not strong enough to counter the self-amplifying feedback loop that already exists at the leading edge.

we have seen for Cdc42 polarity in yeast and for PAR proteins in *C. elegans*, long-range inhibition has been proposed to be due to chemical, diffusion-mediated processes such as a fast-diffusing ‘global’ inhibitor or depletion of a critical effector such as the pool of inactive GTPase [51,52]. Indeed, such schemes are sufficient to yield polarization in theoretical models [10]. However, in highly elongated but still polarized cells, such diffusive processes are too slow to serve as viable long-range cues [53]. Rather, in these cases long-range inhibition may be transmitted via changes in membrane tension: Asymmetric actin-driven membrane protrusion at the cell front leads to a global increase in membrane tension, which suppresses Rac activity [53]. Consistent with this hypothesis, disruption of tension resulted in cells with multiple protrusions. Thus, similar to keratocytes, neutrophils rely on the propagation of membrane tension from the leading edge to the rest of the cell to mediate long-range inhibition. However, in this case, tension appears to exert its effects not solely through

physical inhibition of actin polymerization, but via suppression of Rac signaling, implying that a mechanosensitive pathway must exist to transduce the long-range mechanical cue into a local chemical signal. Interestingly, Rac activity and SCAR/WAVE-mediated actin polymerization appear to be coupled, possibly comprising their own positive feedback loop. By physically resisting SCAR/WAVE-dependent actin protrusion, tension could prevent activation of this Rac activation feedback loop. The fact that Rac remains active at the leading edge under conditions of high tension reinforces how important balance between feedback circuits is for polarization: tension must be able to suppress activation of the Rac activation feedback loop at the cell rear, yet not overcome the already activated feedback loop at the front.

### Concluding remarks

By extracting core features of prototypical examples of polarizing systems, we have shown here how the general

## Review

framework of symmetry breaking, self-amplifying feedback, and long-range inhibition, originally postulated by Turing to account for pattern formation in chemical systems, can be used to understand many examples of cell polarity, including those that rely in part or entirely on mechanical processes in the cell.

Examples certainly do exist that fall outside the paradigm that we have focused on in this review. For example, diffusion barriers can prevent the spread of molecules produced within or delivered to a particular region of the cell [54]. However, on close examination, other seemingly unrelated phenomena can be found to share core features. For example, one could imagine a physical phase-transition model for polarity in which membrane constituents segregate into distinct phases on opposite sides of the cell, much like oil and water. Mixtures of saturated and non-saturated lipids in membrane bilayers tend to segregate into liquid-ordered (saturated) and liquid-disordered (non-saturated) phases under appropriate conditions, segregating the membrane into two or more domains [55]. Segregation depends on molecular attraction and repulsion. Similar lipids group together driven by their packing order, simultaneously excluding dissimilar lipids; hence, phase segregation is effectively locally self-reinforcing. Long-range inhibition comes into play here as well, in the form of depletion: in a closed system, the fraction of the membrane occupied by a given lipid species will ultimately be limited by the relative amount of that lipid in the system.

We have also neglected some level of complexity in certain cases to highlight core features. For example, in *C. elegans*, there is a secondary chemical cue that operates in parallel to the mechanical flow-dependent cue that relies on local modulation of the antagonism between the two groups of PAR proteins to drive symmetry breaking [56]. In *Dictyostelium*, although actin is dispensable for certain aspects of cell polarity and direction sensing, actin-dependent processes are required to shape the polarity response and drive directed cell motility and thus must be integrated into any comprehensive model of cell polarity [21].

Given the diversity of polarizing systems driven by an equally diverse and complex set of often molecularly unrelated mechanisms, a comprehensive survey here would be impossible. Rather, we hope that we have highlighted how combining a consideration of both cell signaling and cell mechanics together with analysis of mathematical conceptualizations of core features has helped to identify some broadly applicable design principles for polarizing systems.

We should note that, in morphogenesis, many of the concepts discussed here repeat at scales much larger than the cellular scales discussed so far. Chemical signaling networks pattern embryos [57]. Molecules are secreted by cells and can diffuse or be actively transported through tissues [58]. Cells also exert forces on their neighbors and the environment, giving rise to patterns of stresses and forces that can be used to restructure tissue and drive cell flows [59]. Importantly, both signals and forces operate at a range of length and time scales, which is crucial for defining morphogenic processes.

Some of these tissue-scale phenomena fall into the paradigm of symmetry breaking reinforced by the self-amplification and long-range inhibition discussed above. In fact,

Alan Turing introduced the basic concepts of chemical pattern formation to explain precisely such types of large-scale developmental patterns and recent work identified at least several cases of such systems where a Turing-like framework appears to apply [60–62]. Given that morphogenesis is an inherently mechanical process, it is also not surprising that mechanics can provide both local and long-range signals during developmental pattern formation [63].

The complexity and variety of developmental forms ensures that the underlying principles that govern morphogenesis will be significantly more diverse than those governing polarity. Yet, just as combined analysis of chemical reaction–diffusion systems and mesoscale mechanics at the intracellular level has provided a richer understanding of cell polarization, the identification of both the biophysical laws by which tissues deform, reshape, and flow, as well as the overlaying chemical processes that govern and are in turn governed by such mechanical processes, will undoubtedly be critical to uncovering general principles that define the mechanochemical basis of morphogenesis.

### Acknowledgments

We thank J. Bois and several anonymous referees for their critical comments on the manuscript. Financial support was provided by the Alexander von Humboldt Foundation (N.W.G.), a Marie Curie Grant (219286) from the European Commission (N.W.G.), the Max Planck Society (N.W.G. and S.W.G.), the ARCHES Minerva Foundation (S.W.G.), the European Molecular Biology Organization Young Investigator Programme (S.W.G.), and the European Research Council (S.W.G.).

### References

- 1 Macara, I.G. and Mili, S. (2008) Polarity and differential inheritance—universal attributes of life? *Cell* 135, 801–812
- 2 St Johnston, D. and Ahringer, J. (2010) Cell polarity in eggs and epithelia: parallels and diversity. *Cell* 141, 757–774
- 3 McNeill, H. (2010) Planar cell polarity: keeping hairs straight is not so simple. *Cold Spring Harb. Perspect. Biol.* 2, a003376
- 4 Li, R. and Bowerman, B. (2010) Symmetry breaking in biology. *Cold Spring Harb. Perspect. Biol.* 2, a003475
- 5 Hamamoto, T. *et al.* (1988) Asymmetric cell division of a triangular halophilic archaebacterium. *FEMS Microbiol. Lett.* 56, 221–224
- 6 Turing, A.M. (1952) The chemical basis of morphogenesis. *Philos. Trans. R. Soc. Lond. B: Biol. Sci.* 237, 37–72
- 7 Bois, J.S. *et al.* (2011) Pattern formation in active fluids. *Phys. Rev. Lett.* 106, 028103
- 8 Howard, J. *et al.* (2011) Turing's next steps: the mechanochemical basis of morphogenesis. *Nat. Rev. Mol. Cell Biol.* 12, 392–398
- 9 Gierer, A. and Meinhardt, H. (1972) A theory of biological pattern formation. *Kybernetik* 12, 30–39
- 10 Jilkine, A. and Edelstein-Keshet, L. (2011) A comparison of mathematical models for polarization of single eukaryotic cells in response to guided cues. *PLoS Comput. Biol.* 7, e1001121
- 11 Johnson, J.M. *et al.* (2011) Symmetry breaking and the establishment of cell polarity in budding yeast. *Curr. Opin. Genet. Dev.* 21, 740–746
- 12 Wedlich-Soldner, R. *et al.* (2003) Spontaneous cell polarization through actomyosin-based delivery of the Cdc42 GTPase. *Science* 299, 1231–1235
- 13 Chant, J. and Herskowitz, I. (1991) Genetic control of bud site selection in yeast by a set of gene products that constitute a morphogenetic pathway. *Cell* 65, 1203–1212
- 14 Goryachev, A.B. and Pokhilko, A.V. (2008) Dynamics of Cdc42 network embodies a Turing-type mechanism of yeast cell polarity. *FEBS Lett.* 582, 1437–1443
- 15 Irazoqui, J.E. *et al.* (2003) Scaffold-mediated symmetry breaking by Cdc42p. *Nat. Cell Biol.* 5, 1062–1070
- 16 Kozubowski, L. *et al.* (2008) Symmetry-breaking polarization driven by a Cdc42p GEF-PAK complex. *Curr. Biol.* 18, 1719–1726
- 17 Howell, A.S. *et al.* (2009) Singularity in polarization: rewiring yeast cells to make two buds. *Cell* 139, 731–743

## Review

Trends in Cell Biology xxx xxxx, Vol. xxx, No. x

- 18 Howell, A.S. *et al.* (2012) Negative feedback enhances robustness in the yeast polarity establishment circuit. *Cell* 149, 322–333
- 19 Altschuler, S.J. *et al.* (2008) On the spontaneous emergence of cell polarity. *Nature* 454, 886–889
- 20 Arai, Y. *et al.* (2010) Self-organization of the phosphatidylinositol lipids signaling system for random cell migration. *Proc. Natl. Acad. Sci. U.S.A.* 107, 12399–12404
- 21 Iglesias, P.A. and Devreotes, P.N. (2012) Biased excitable networks: how cells direct motion in response to gradients. *Curr. Opin. Cell Biol.* 24, 245–253
- 22 King, J.S. and Insall, R.H. (2009) Chemotaxis: finding the way forward with *Dictyostelium*. *Trends Cell Biol.* 19, 523–530
- 23 Afonso, P.V. and Parent, C.A. (2011) PI3K and chemotaxis: a priming issue? *Sci. Signal.* 4, pe22
- 24 Mullins, R.D. (2010) Cytoskeletal mechanisms for breaking cellular symmetry. *Cold Spring Harb. Perspect. Biol.* 2, a003392
- 25 Haglund, C.M. and Welch, M.D. (2011) Pathogens and polymers: microbe-host interactions illuminate the cytoskeleton. *J. Cell Biol.* 195, 7–17
- 26 Loisel, T.P. *et al.* (1999) Reconstitution of actin-based motility of *Listeria* and *Shigella* using pure proteins. *Nature* 401, 613–616
- 27 Dayel, M.J. *et al.* (2009) In silico reconstitution of actin-based symmetry breaking and motility. *PLoS Biol.* 7, e1000201
- 28 Carlsson, A.E. (2010) Dendritic actin filament nucleation causes traveling waves and patches. *Phys. Rev. Lett.* 104, 228102
- 29 Gardel, M.L. *et al.* (2010) Mechanical integration of actin and adhesion dynamics in cell migration. *Annu. Rev. Cell Dev. Biol.* 26, 315–333
- 30 Bershadsky, A.D. and Kozlov, M.M. (2011) Crawling cell locomotion revisited. *Proc. Natl. Acad. Sci. U.S.A.* 108, 20275–20276
- 31 Mogilner, A. and Keren, K. (2009) The shape of motile cells. *Curr. Biol.* 19, R762–R771
- 32 Yam, P.T. *et al.* (2007) Actin-myosin network reorganization breaks symmetry at the cell rear to spontaneously initiate polarized cell motility. *J. Cell Biol.* 178, 1207–1221
- 33 Verkhovsky, A.B. *et al.* (1999) Self-polarization and directional motility of cytoplasm. *Mol. Cell* 9, 11–20
- 34 Meinhardt, H. (1982) *Models of Biological Pattern Formation*, Academic Press
- 35 Goldstein, B. and Macara, I.G. (2007) The PAR proteins: fundamental players in animal cell polarization. *Dev. Cell* 13, 609–622
- 36 Nance, J. and Zallen, J.A. (2011) Elaborating polarity: PAR proteins and the cytoskeleton. *Development* 138, 799–809
- 37 Goehring, N.W. *et al.* (2011) PAR proteins diffuse freely across the anterior-posterior boundary in polarized *C. elegans* embryos. *J. Cell Biol.* 193, 583–594
- 38 Goehring, N.W. *et al.* (2011) Polarization of PAR proteins by advective triggering of a pattern-forming system. *Science* 334, 1137–1141
- 39 Dawes, A.T. and Munro, E.M. (2011) PAR-3 oligomerization may provide an actin-independent mechanism to maintain distinct par protein domains in the early *Caenorhabditis elegans* embryo. *Biophys. J.* 101, 1412–1422
- 40 Mayer, M. *et al.* (2010) Anisotropies in cortical tension reveal the physical basis of polarizing cortical flows. *Nature* 467, 617–621
- 41 Jenkins, N. *et al.* (2006) CYK-4/GAP provides a localized cue to initiate anteroposterior polarity upon fertilization. *Science* 313, 1298–1301
- 42 Munro, E. and Bowerman, B. (2009) Cellular symmetry breaking during *Caenorhabditis elegans* development. *Cold Spring Harb. Perspect. Biol.* 1, a003400
- 43 Motegi, F. and Sugimoto, A. (2006) Sequential functioning of the ECT-2 RhoGEF, RHO-1 and CDC-42 establishes cell polarity in *Caenorhabditis elegans* embryos. *Nat. Cell Biol.* 8, 978–985
- 44 Schonegg, S. and Hyman, A.A. (2006) CDC-42 and RHO-1 coordinate acto-myosin contractility and PAR protein localization during polarity establishment in *C. elegans* embryos. *Development* 133, 3507–3516
- 45 Hird, S.N. and White, J.G. (1993) Cortical and cytoplasmic flow polarity in early embryonic cells of *Caenorhabditis elegans*. *J. Cell Biol.* 121, 1343–1355
- 46 Roth, S. and Lynch, J.A. (2009) Symmetry breaking during *Drosophila* oogenesis. *Cold Spring Harb. Perspect. Biol.* 1, a001891
- 47 Parton, R.M. *et al.* (2011) A PAR-1-dependent orientation gradient of dynamic microtubules directs posterior cargo transport in the *Drosophila* oocyte. *J. Cell Biol.* 194, 121–135
- 48 Zimyanin, V.L. *et al.* (2008) In vivo imaging of oskar mRNA transport reveals the mechanism of posterior localization. *Cell* 134, 843–853
- 49 Zimyanin, V. *et al.* (2007) An oskar-dependent positive feedback loop maintains the polarity of the *Drosophila* oocyte. *Curr. Biol.* 17, 353–359
- 50 Ridley, A.J. (2011) Life at the leading edge. *Cell* 145, 1012–1022
- 51 Parent, C.A. and Devreotes, P.N. (1999) A cell's sense of direction. *Science* 284, 765–770
- 52 Meinhardt, H. (1999) Orientation of chemotactic cells and growth cones: models and mechanisms. *J. Cell Sci.* 112, 2867–2874
- 53 Houk, A.R. *et al.* (2012) Membrane tension maintains cell polarity by confining signals to the leading edge during neutrophil migration. *Cell* 148, 175–188
- 54 Caudron, F. and Barral, Y. (2009) Septins and the lateral compartmentalization of eukaryotic membranes. *Dev. Cell* 16, 493–506
- 55 Lingwood, D. and Simons, K. (2010) Lipid rafts as a membrane-organizing principle. *Science* 327, 46–50
- 56 Motegi, F. *et al.* (2011) Microtubules induce self-organization of polarized PAR domains in *Caenorhabditis elegans* zygotes. *Nat. Cell Biol.* 13, 1361–1367
- 57 Rogers, K.W. and Schier, A.F. (2011) Morphogen gradients: from generation to interpretation. *Annu. Rev. Cell Dev. Biol.* 27, 377–407
- 58 Yu, S.R. *et al.* (2009) Fgf8 morphogen gradient forms by a source-sink mechanism with freely diffusing molecules. *Nature* 461, 533–536
- 59 Eaton, S. and Jülicher, F. (2011) Cell flow and tissue polarity patterns. *Curr. Opin. Genet. Dev.* 21, 747–752
- 60 Kondo, S. and Miura, T. (2010) Reaction-diffusion model as a framework for understanding biological pattern formation. *Science* 329, 1616–1620
- 61 Nakamura, T. *et al.* (2006) Generation of robust left-right asymmetry in the mouse embryo requires a self-enhancement and lateral-inhibition system. *Dev. Cell* 11, 495–504
- 62 Müller, P. *et al.* (2012) Differential diffusivity of Nodal and Lefty underlies a reaction-diffusion patterning system. *Science* 336, 721–724
- 63 Mammoto, T. and Ingber, D.E. (2010) Mechanical control of tissue and organ development. *Development* 137, 1407–1420
- 64 Li, R. and Gundersen, G.G. (2008) Beyond polymer polarity: how the cytoskeleton builds a polarized cell. *Nat. Rev. Mol. Cell Biol.* 9, 860–873
- 65 Sugioka, K. and Sawa, H. (2012) Formation and functions of asymmetric microtubule organization in polarized cells. *Curr. Opin. Cell Biol.* 24, 517–525
- 66 Jiang, K. and Akhmanova, A. (2011) Microtubule tip-interacting proteins: a view from both ends. *Curr. Opin. Cell Biol.* 23, 94–101
- 67 Grill, S.W. *et al.* (2001) Polarity controls forces governing asymmetric spindle positioning in the *Caenorhabditis elegans* embryo. *Nature* 409, 630–633
- 68 Colombo, K. *et al.* (2003) Translation of polarity cues into asymmetric spindle positioning in *Caenorhabditis elegans* embryos. *Science* 300, 1957–1961
- 69 Galli, M. *et al.* (2011) aPKC phosphorylates NuMA-related LIN-5 to position the mitotic spindle during asymmetric division. *Nat. Cell Biol.* 13, 1132–1138
- 70 Howard, J. (2001) *Mechanics of Motor Proteins and the Cytoskeleton*, Sinauer Associates
- 71 Mallik, R. and Gross, S.P. (2004) Molecular motors: strategies to get along. *Curr. Biol.* 14, R971–R982
- 72 Amrute-Nayak, M. and Bullock, S.L. (2012) Single-molecule assays reveal that RNA localization signals regulate dynein-dynactin copy number on individual transcript cargoes. *Nat. Cell Biol.* 14, 416–423
- 73 Gutzeit, H. and Koppa, R. (1982) Time-lapse film analysis of cytoplasmic streaming during late oogenesis of *Drosophila*. *J. Embryol. Exp. Morphol.* 67, 101–111
- 74 Wolke, U. *et al.* (2007) Actin-dependent cytoplasmic streaming in *C. elegans* oogenesis. *Development* 134, 2227–2236
- 75 Verchot-Lubicz, J. and Goldstein, R.E. (2010) Cytoplasmic streaming enables the distribution of molecules and vesicles in large plant cells. *Protoplasma* 240, 99–107
- 76 Aigouy, B. *et al.* (2010) Cell flow reorients the axis of planar polarity in the wing epithelium of *Drosophila*. *Cell* 142, 773–786
- 77 Hoffman, B.D. *et al.* (2011) Dynamic molecular processes mediate cellular mechanotransduction. *Nature* 475, 316–323
- 78 Kee, Y-S. *et al.* (2012) A mechanosensory system governs myosin II accumulation in dividing cells. *Mol. Biol. Cell* 23, 1510–1523
- 79 Trichet, L. *et al.* (2012) Evidence of a large-scale mechanosensing mechanism for cellular adaptation to substrate stiffness. *Proc. Natl. Acad. Sci. U.S.A.* 109, 6933–6938