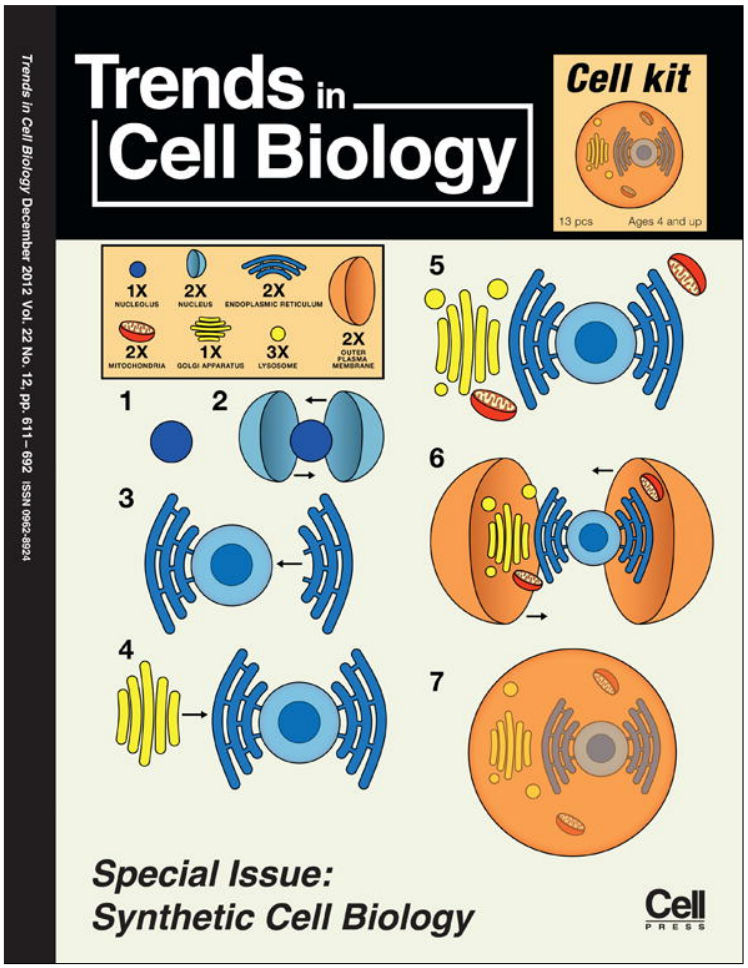

Review

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Directed cytoskeleton self-organization

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The cytoskeleton architecture supports many cellular functions. Cytoskeleton networks form complex intracellular structures that vary during the cell cycle and between different cell types according to their physiological role. These structures do not emerge spontaneously. They result from the interplay between intrinsic self-organization properties and the conditions imposed by spatial boundaries. Along these boundaries, cytoskeleton filaments are anchored, repulsed, aligned, or reoriented. Such local effects can propagate alterations throughout the network and guide cytoskeleton assembly over relatively large distances. The experimental manipulation of spatial boundaries using microfabrication methods has revealed the underlying physical processes directing cytoskeleton self-organization. Here we review, step-by-step, from molecules to tissues, how the rules that govern assembly have been identified. We describe how complementary approaches, all based on controlling geometric conditions, from *in vitro* reconstruction to *in vivo* observation, shed new light on these fundamental organizing principles.

Setting boundaries

The reproducible shape and spatial organization of organs imply the existence of deterministic rules directing the assembly of complex biological structures. Organ shape depends on cell architecture, which is supported by cytoskeleton networks. The formation of defined and geometrically controlled intracellular structures relies on the self-organization properties of the cytoskeleton. The contribution of self-organization in cell biology is vast and now well documented [1]. Cytoskeleton self-organization is a process in which the consumption (physicists would say dissipation) of energy brings the cytoskeleton away from its thermodynamic equilibrium (i.e., a disordered mixture of poorly dynamic filaments) toward defined and reproducible steady states. This differs from the process of self-assembly, in which components assemble spontaneously – without an external energy source – to form a structure corresponding to the thermodynamic equilibrium. Depending on the rules regulating the interaction of cytoskeleton components, complex structures may self-organize in a robust manner. The purpose of much of the research described in this review has been to identify and formulate these rules to understand how physical principles direct biological morphogenesis.

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Cytoskeleton self-organization is partially regulated by the action of proteins modulating the biochemical rules of filament growth and interactions. The combination of simple biochemical rules can lead to the formation of complex structures [2]. Robust patterns can emerge from oriented displacements of cytoskeleton filaments by molecular motors in the absence of any external guidance [3–5]. However, these autonomous self-organization processes are extremely sensitive to the presence of spatial boundary conditions (SBCs). An SBC is an external geometrical cue, within or at the periphery of the network, that can locally affect the self-organization of the network. For tissues, an SBC can be a frontier with an external fluid or a contact with bone, muscle, or other organ. For a cell, an SBC can be a neighboring cell or extracellular matrix (ECM). For intracellular cytoskeleton networks, an SBC can be a cell adhesion for the actin network, a centrosome for the microtubule (MT) network, or a frontier such as the plasma membrane or an intracellular organelle.

How an SBC can direct an autonomous self-organization process is the subject of this review. We describe recent advances in the understanding of the role of SBCs in the self-organization of actin networks and MT arrays, how these processes are integrated in the internal organization of a cell, and how this in turn affects tissue architecture. In the formation of cytoskeleton networks, an SBC can bias monomer diffusion and thereby the assembly process ([6] and references therein). Here, we focus on the role of geometrical constraints on the growth, orientation, anchorage, and production of mechanical forces during cytoskeleton assembly.

Actin network self-organization

Actin is an asymmetric protein that can self-assemble to form polarized actin filaments [7]. This spontaneous process can be accelerated and temporally regulated by the energy liberated from the release of a phosphate group from the nucleotide triphosphate bound to actin [8]. Actin filaments can interact to form actin networks. Actin networks can self-organize into several types of structures in cells: bundles comprising aligned long filaments and meshworks comprising branched and intermingled short filaments. Bundles and meshworks form such complex intricate networks in cells [9] that it is difficult to identify the principles of their self-organization.

Biochemists have developed alternative methods to analyze self-organization in controlled conditions *in vitro* by mixing, in defined proportions, the individual components (either purified from tissues or from recombinant

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bacteria or yeasts). The kinetic parameters of actin polymerization measured *in vitro* and how these parameters vary in response to the presence of actin-associated proteins has provided key information about the regulation of actin assembly dynamics [10]. However, the rules guiding the spatial organization of the network can be identified only by using controlled geometric boundary conditions.

Symmetry break

Mechanical constraints in an actin network can induce a symmetry break (i.e., the sudden occurrence of a singular axis in isotropic conditions in which all directions were previously equivalent). This propensity for symmetry breaking in actin networks was elegantly revealed using a spherical glass bead coated with actin nucleation factors as a simple SBC [11,12]. Actin nucleation is induced from the bead, and the presence of capping proteins, which block filament elongation from their fast growing end, ensures that the actin filaments are short and form a dense branched meshwork. As the actin filaments grow at the bead surface, material accumulates and the stress increases in the network up to a critical value inducing its rupture [13]. The rupture creates an asymmetry in the pressure applied on the bead such that the bead is displaced (Figure 1a). Repetition of this sequence of events induces saltatory propulsion of the bead [14,15].

In this experimental system, the SBC can easily be manipulated by changing its dimensional parameters. For example, the larger the bead, the shallower the curvature of the bead surface, leading to an increase in the critical value of network thickness before rupture [16,17] and the periodicity of the saltatory propulsion (Figure 1b). An asymmetric SBC can be created using ellipsoidal beads. The difference in surface curvature of the bead biases the location of network rupture, which occurs preferentially in line with or orthogonal to the long axis of the bead [18] (Figure 1c). Higher aspect ratios, obtained by actin nucleation on small glass rods, further increase the spatial bias and branched network growth is restricted to being orthogonal to the long axis of the rod [15]. As the rod length increases, several independent networks can form, revealing the existence of a critical length for subnetwork interconnections (Figure 1d). Interestingly, symmetry break and asymmetric force production are not restricted to branched meshworks of actin, but can also be induced by the bundling and alignment of individual filaments polymerizing against the bead surface [19].

Filament alignment

Several self-organization processes can induce actin filament alignment in response to an SBC. Filaments can become aligned by steric interactions. When two long filaments come close to each other, they prevent the insertion of a short filament between them. Long filaments will be further forced to align by the steric interactions of short filaments around them. Steric interactions between long filament bundles will then promote their orientation in line with the long axis of the volume in which they are confined [20] (Figure 1e). Steric interaction of filaments freely moving on a layer of molecular motors can also result in their alignment along each other [4] and along the SBC [21]. Filaments can become aligned by membrane tension. Two filaments pushing orthogonally to a deformable membrane will coalesce and align to reduce the elastic energy of the membrane [22]. Preassembled filaments can become aligned by defining the anchorage positions with regular arrays of beads or micropillars and adding filamin to crosslink filaments [23,24].

Filaments can become aligned in parallel or antiparallel configurations by controlling the orientation of their growth. Surface micropatterning can be used to manipulate precisely the geometrical boundary conditions of filament growth and orientation [25]. Selective adsorption of actin nucleation-promoting factors on micropatterned regions induces localized formation of a branched meshwork. Only non-branched filaments grow out of the micropattern, with their barbed ends reproducibly oriented outward. Steric interactions force growing filaments to align parallel to each other, orthogonal to the nucleation region (Figure 1g). Distant from the nucleation region, two filaments growing toward each other in nearly opposite directions tend to form antiparallel bundles; whereas two filaments growing toward each other but at an oblique angle tend to form parallel bundles (Figure 1g). However, these tendencies can be biased because adjacent filaments sterically affect each other. The reorientation of filaments during bundle formation guides adjacent filaments also to align with the bundle (Figure 1g). Bundle formation is thus a combination of local probabilistic events, governed by filament flexibility, and the propagation of the alignment configuration to adjacent filaments by steric interactions [25].

In egg extracts, biochemical conditions are less well defined but closer to intracellular conditions. Encapsulation of egg extracts in membrane vesicles revealed that filaments nucleated at the periphery move inward and align to form a central ring. Interestingly, the ring can form only when nucleation is restricted to the vesicle periphery and not distributed evenly throughout the entire volume. A scaling law appears to regulate the ring size in proportion to the vesicle diameter [26] (Figure 1f).

Network contraction

Myosins are oriented motors moving toward a defined extremity of actin filaments. Thus, they have specific actions depending on actin network architecture. They walk along parallel filaments, whereas they slide along antiparallel filaments in opposite directions relative to each other and thus contract the network [27] (Figure 1i). Myosins can also induce the contraction of branched meshworks, because these networks also contain antiparallel filaments. However, the rate of contraction is reduced due to the resistance associated with branches and network anchoring to nucleation regions [27]. It has been shown, based on the use of actomyosin bundles connecting beads, that the contraction rate is proportional to bundle length [28]. In more complex structures comprising various types of network, the contraction rate is determined by the local proportion of parallel and antiparallel bundles and branched meshwork [27]. Variations of these proportions in a given architecture will induce anisotropic contraction, although myosins are present throughout the network (Figure 1i). Therefore, an SBC

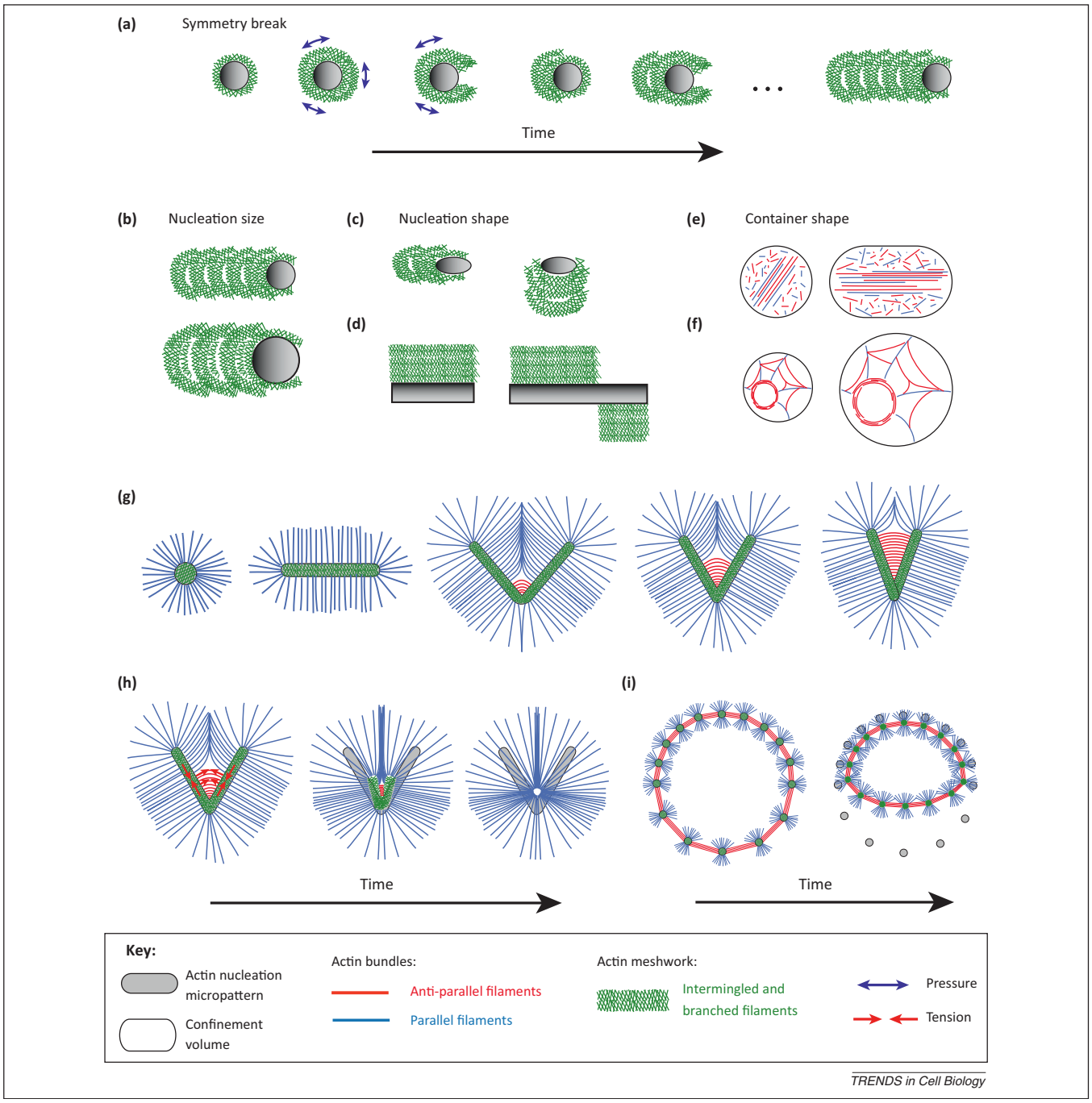

Figure 1. Actin network self-organization. **(a)** Actin meshwork polymerization around beads leads to symmetry breaking, meshwork rupture, and bead propulsion. **(b)** Bead size regulates the period and size of meshwork rupture. **(c)** Bead asymmetry orients meshwork growth. **(d)** Bar length affects network coherence. **(e)** Long filaments self-align to form bundles, which become oriented along the long axis of the container. **(f)** Inward flow of filaments nucleated at the vesicle periphery leads to the formation of a ring, the size of which is in proportion with the vesicle diameter. **(g)** Filament nucleation and growth of micropatterned branched meshworks. The filament interaction angle modulates the probability of association in either parallel (blue filaments) or antiparallel (red filaments) configurations. **(h)** Myosins induce the specific contraction and disassembly of antiparallel bundles and branched meshworks while leaving parallel bundles unaffected. **(i)** Asymmetric distribution of the ratio between branched and antiparallel networks leads to asymmetric contraction.

can define the type of network architecture, which in turn can define its pattern of contraction.

MT network self-organization

Similar to the formation of actin filaments from the self-assembly of actin monomers, tubulin forms asymmetric dimers that can self-assemble into MTs. However, the release of tubulin-bound nucleotide triphosphate is required to accelerate the process [29]. Compared with actin filaments, MTs are much more rigid and almost straight in the dimensions of a single cell. MTs can sustain higher compression forces than actin filaments. They can form bundles, but they cannot form branched networks. MTs are not as numerous as actin filaments in the cell cytoskeleton. MT growth is characterized by long growth phases alternated with short periods of rapid shortening.

The 'plus-end' of the MT is much more dynamic than the 'minus-end', which can be attached to a MT-organizing center (MTOC). In most animal cells, the MT network forms as an aster in which MTs radiate from the MTOC. As cells divide, the MTOC is duplicated and the network forms a bipolar spindle.

Centering

The most straightforward way to investigate MT aster positioning in response to an SBC has been to purify MTOCs from cells and place them in microfabricated chambers of defined dimensions [30]. Hence, the boundaries of the chamber can serve as an external SBC. As MT plus-ends grow and push against the edges of a square chamber, MTs are subjected to compression forces that push the aster toward the geometrical center of the chamber [30] (Figure 2a). When fluctuations cause the MTOC to become off-center in a given direction, MT curvature and pressure increases in that direction and pushes the MTOC back toward the center. Thus, an isotropic array of MTs pushing on peripheral barriers is sufficient to maintain the aster at the center of the volume in which it is confined. However, MTs sliding along the periphery could affect the stability of this centering mechanism by reorienting MTs. In such conditions, both pushing and pulling forces, by minus-end-directed motors attached to the periphery, are necessary to ensure efficient stabilization of the MT aster at the geometrical center of the SBC [31] (Figure 2a).

By contrast, asters with opposite polarities (i.e., with MT plus-ends at the center of the aster) cannot adopt the same steady state. As long as MTs contacting the periphery are short enough to release their elastic energy by straightening, they gently push the aster toward the center. As they get longer, the compression forces in bent MTs increase. The clustering of dynamic plus-ends by kinesins at the aster center is not strong enough to resist these forces and so the aster fragments. The MT network then switches to highly robust vortex-like structures [32] (Figure 2b).

Symmetry break

When an aster is trapped in a water droplet encapsulated in oil, MTs cannot attach to the periphery. The spherical water–oil interface has minimal tangential resistance and is an effective SBC along which MT can slide easily. In these conditions, symmetry breaks in the aster configuration can occur [33] (Figure 2c). In a relatively large spherical volume, few MTs reach the boundaries and the aster is stabilized close to the geometrical center. As the size of the spherical volume is reduced, MTs tend to be longer than the container radius. To minimize their curvature and relax their elastic energy, MTs slip along the edges and align with the SBC [33,34]. This produces an asymmetric redistribution of MTs that pushes the MTOC to the periphery of the droplet [33] (Figure 2c). Interestingly, when the rigidity of the SBC is reduced, clustered MTs push and deform it to the extent that a tubular protrusion can be formed [33,35,36].

Alignment and spindle formation

The formation of bipolar mitotic spindles also depends on geometrical boundary conditions defined by DNA and cell shape. Two mechanisms contribute to mitotic spindle assembly around DNA: the focusing of the minus-ends of MTs that are associated with large DNA clusters to form spindle poles, and the antiparallel alignment of the plus-ends of MTs that are anchored at the two MTOCs such that the aster MTs overlap [37].

Multimeric minus-end-directed motors, such as dyneins, induce the formation of spindle poles. DNA provides guidance cues for initial MT alignment and thus biases bipolar spindle formation [38,39]. MTs tend to align parallel to the surface of a DNA-coated bead. The intrinsic molecular machinery supporting spindle pole focusing and mitotic spindle spatial organization is robust and initially appeared insensitive to configuration of the DNA complex [40]. However, extensive manipulations of the amount of DNA and its spatial distribution using microcontact printing revealed the DNA directing role in spindle assembly [41]. The increase in size of DNA aggregates induces spindle lengthening (Figure 2d). Above a critical size, large DNA aggregates can induce the formation of multiple poles [41] (Figure 2d). Moderately asymmetric distribution of DNA is sufficient to orient spindle formation [40,41] (Figure 2e). Long bars coated with DNA result in the formation of multiple repeats of spindles along the length of the bar and thus revealed the existence of an intrinsic spindle width (Figure 2f). This intrinsic spindle width seems to be defined by the balance between motors forcing the focusing MT ends and the elastic reaction force due to MT bending. In a certain range of parameters defined by the ratio between the DNA aggregate width and MT length, the symmetry is broken and all MTs collapse on one side of the DNA, resulting in an asymmetric configuration of spindles with respect to the long axis of the bar [41] (Figure 2f). Below this critical range, antiparallel MTs from opposite poles (with the bar in between) interact to stabilize the formation of symmetric bipolar spindles; above this range, the two spindle configurations on opposing sides of the DNA bar are independent and both form independent monopolar spindles.

Cellular self-organization

In cells, the organizing principles described above appear applicable but more difficult to reveal and investigate. Both actin and MT networks are regulated by hundreds of different types of binding protein. In addition, the assembly of actin filaments and MTs are affected by each other through physical and biochemical interactions. Cytoskeleton network assembly is regulated at the scale of a cell and is no longer solely dependent on local biochemical and geometrical conditions. The implication of biochemical signals forces the system to break its symmetry and define an axis of polarity. Although actin or MT network assembly is more complex in the cellular context than *in vitro*, some self-organizing principles have been identified.

In simple conditions as near to cellular conditions as can be achieved in experiments *in vitro*, similar self-organized structures can be observed. Cytoskeleton networks in cells from lymphatic lines or in other cells or cell fragments on non-adhesive substrates are subjected to no other geometrical constraints than the flexible plasma membrane. In the absence of MT networks, the actin network contracts and breaks symmetry after a local rupture occurs in the

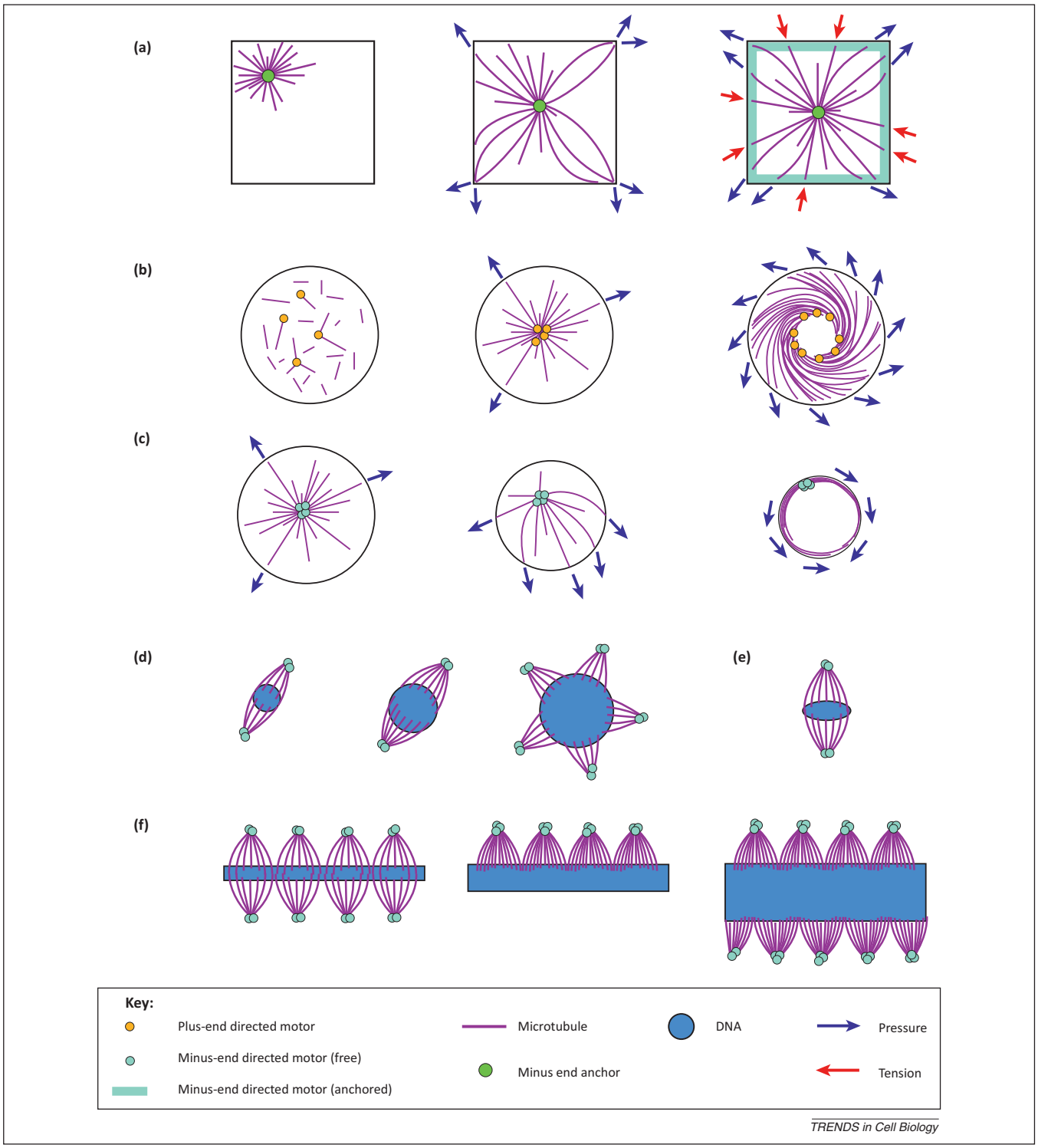

Figure 2. Microtubule (MT) network self-organization. **(a)** Aster off-centering with short MTs in a large container (left). Aster centering by MT sliding and pushing on the container corners (middle). Highly efficient aster centering by pushing and pulling forces (right). **(b)** MT length-dependent aster formation and centering. Short MT 'plus-end' coalescence by motors (left). Aster centering by a few MT 'minus-ends' reaching and pushing on container edges (middle). Aster fragmentation and vortex formation by pushing forces exerted by long MTs on container edges (right). **(c)** MT length-dependent aster off-centering. Aster centering by few MT plus-ends reaching and pushing on container edges (left). Symmetry break and aster off-centering by a few, sliding MTs pushing on container edges (middle). Cortical alignment of MTs and peripheral localization of MT-organizing center (MTOC) due to numerous MTs sliding and pushing on container edges (right). **(d)** DNA cluster size regulates spindle size and pole formation. **(e)** DNA cluster asymmetry regulates spindle orientation. **(f)** DNA cluster width regulates spindle symmetry.

network. With the symmetry breaking, an over-contracted region propagates in the network [42–44]. The process of rupture is similar to what happens in branched meshworks around beads [12], except the occurrence of the rupture results from the contractile force generated by myosins rather than by the pushing force associated with actin polymerization. In the absence of actin networks, MTs pushing on a deformable membrane coalesce, align, and

break symmetry by forming a long tubular protrusion [42,43], reminiscent of their behavior in vesicles [33,35,36]. However, in cellular conditions, actin and MT networks interact and the SBCs are more complex that a freely fluctuating plasma membrane. The ECM and cell neighbors can represent adhesive SBCs. Hence, the precise control and manipulation of cell adhesions, which are cellular structures that interact with the cell's structural microenvironment, reveal how these SBCs could direct intrinsic cytoskeleton self-organizing properties.

Directed shape

Cells spreading on a defined regular array of adhesion spots revealed that the size and spacing between spots was a critical regulator of cell shape. Cells need a minimum spot size to assemble focal adhesions and cannot extend over a maximal distance between these spots [45–48]. Cell shape appears to result from the competition between the force from adhesion-induced spreading and a reaction force from the cell's elasticity and other internal contraction forces [49]. However, some cells, such as fibroblasts, have an intrinsic mechanism to regulate the length of their long axis regardless of their width, which seems to implicate tight crosstalk between actin and the MT network [50,51].

Although cell shape elongation, cytoskeletal alignment, and internal cell polarity orientation are usually correlated, cell shape does not determine actin and MT organization. Modifying the actin network by fluid flow while maintaining constant shape reorients the MT network [52]. Similarly, modifying the MT network independently of cell shape reorients the actin network [53]. Rather, there is an intricate coupling between actin and MT networks that affects their respective spatial organizations and the axis of cell polarity.

Directed actin network architecture

The cellular actin network is organized by a balance between the assembly of a contractile network of aligned filaments and the polymerization of a non-contractile branched meshwork. This balance appears to be finely regulated by the degree of cell adhesion [54].

The branched meshwork assembles at the cell periphery. It is preferentially developed along convex rather than concave cell edges [55]; thus, it promotes the formation of larger membrane deformations at a cell apex [56] (Figure 3a), the size of which increases as the angle of the apex is reduced [57].

Contractile bundles of antiparallel filaments are present throughout the cytoplasm. Peripheral bundles and more interior bundles have distinct dynamics and contraction properties. Components of peripheral bundles move toward the bundle center, whereas components of interior bundles remain static with respect to the bundle organization [58] (Figure 3b). This probably reveals key differences in the polarity of filaments and thus specific contraction properties of these two types of bundle. As cell spreading or the cell aspect ratio increases, cell contraction increases [59–61]. The cell aspect ratio increase induces the alignment of contractile bundles, which form structures such as stress fibers or myofibrils (Figure 3c). Aligned stress fibers and the associated anisotropic contraction along the cell's basal surface are coupled to the assembly of similar structures and force distribution along the cell's apical surface [62,63]. Aligned myofibrils tend to organize their banding patterns in register [64].

Asymmetric SBCs, defined in cell culture by micropatterned adhesion sites, can lead to the development of asymmetric actin networks. Bundles accumulate preferentially along concave rather than convex cell edges [65]. As the cell spreads over an adhesive region, conspicuous contractile bundles are formed that connect this region to other adhesive regions separated by non-adhesive regions [47,66,61], revealing the development of larger traction forces [67] (Figure 3d). A relatively larger distance between adhesion sites leads to a reduced edge curvature and thicker bundles and so probably reflects a larger force between these sites [66,68] (Figure 3e).

Directed MT network

The MT network adapts its dynamics to the various configurations of the actin network. MTs bend and grow along actin contractile bundles, but stop growing when they reach a branched actin meshwork [69]. Interestingly, although an asymmetric actin network will lead to asymmetric organization of MTs, the MTOC remains at its central location (Figure 3f). Centrosome positioning appears to depend on generation of forces by dyneins on MTs [70,71], but also on the forces generated by the less characterized connections with the actomyosin network [71,72]. Centrosome central positioning is even more remarkable given that a large part of the cytoplasm is occupied by the nucleus, on which MTs can also push and pull. The robust mechanism by which the centrosome becomes positioned at the geometrical center of the contour that describes the cell shape, where the actin network is asymmetric and the nucleus occupies a large part of the cytoplasmic volume, remains to be elucidated.

The positioning of the nucleus in a cell in culture is off-center and distal from the cell's adhesion to the ECM and the actin branched meshwork, but is proximal to the contractile bundles [69]. Therefore, the internal polarity, as revealed by the nucleus–centrosome vector, is oriented with respect to ECM and actin network asymmetries [55,69]. Biochemical disruption of the actomyosin network, the MT network or the nucleus–cytoskeleton connections can perturb polarity orientation with respect to cell–ECM SBCs [71,73,75].

The centrosome and nucleus are often described as being in the cell spreading plane in culture, but they can be positioned on an axis orthogonal to this. Moreover, their relative positions can be switched on this axis, depending on the degree of confinement imposed by the available spreading area. Indeed, the centrosome is positioned toward the apical curved surface, above the nucleus, in confined cells and below the nucleus in cells that have spread extensively [76] (Figure 3g).

Directed migration

The asymmetric cytoskeleton organization in response to an asymmetric SBC can affect the direction of a motile cell. The relationship between external asymmetry and oriented motility is not straightforward, because it seems to

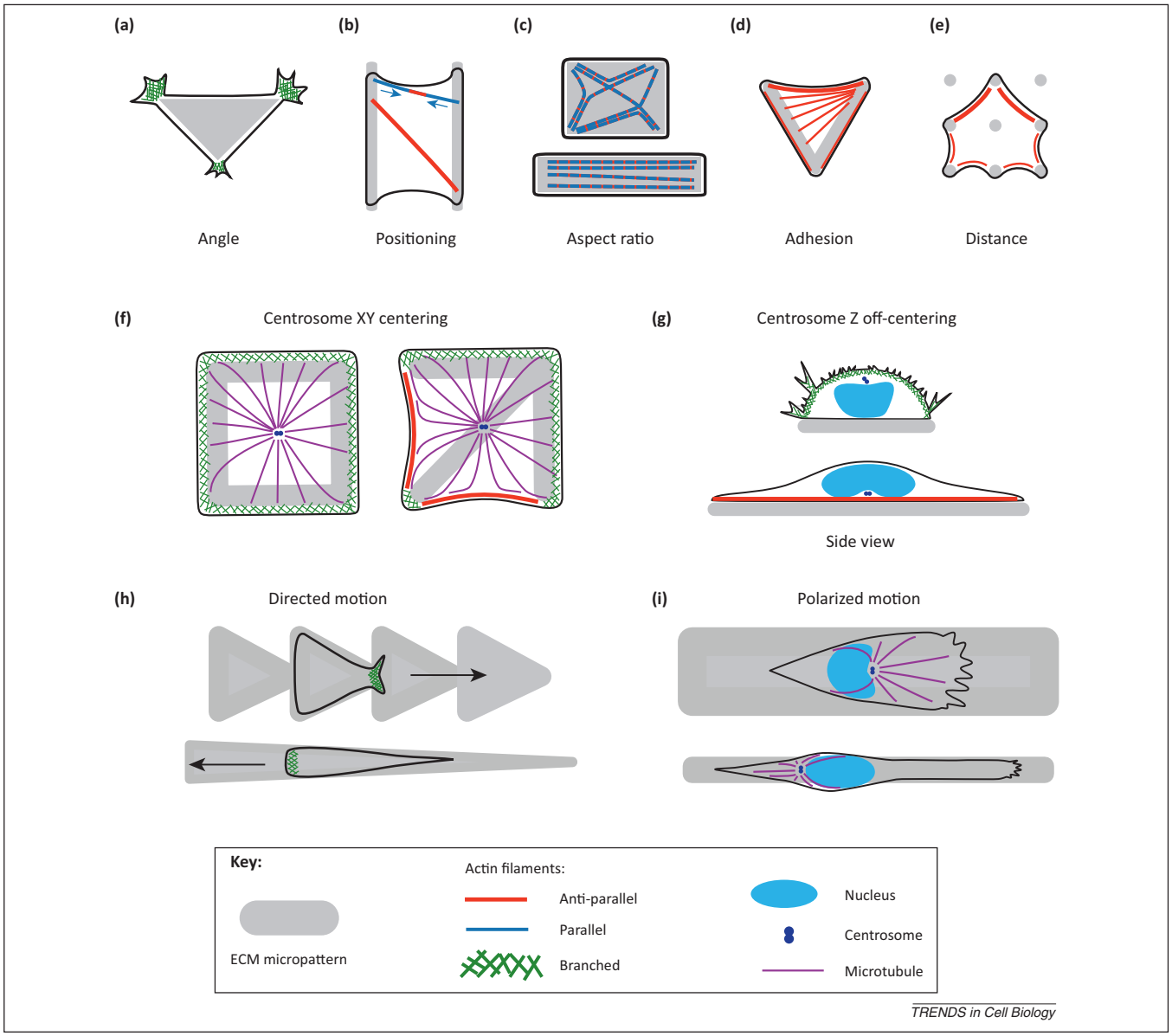

Figure 3. Cellular self-organization. **(a)** Branched meshwork polymerization in acute-angled regions of the cell periphery. **(b)** Inward treadmilling (arrows) in peripheral actin bundles and absence of treadmilling of internal bundles may reveal differences in filament polarities. **(c)** Alignment of myofibrils in response to cell shape elongation. **(d)** Formation of conspicuous actin bundles along non-adhesive edges and thin actin bundles along adhesive edges. **(e)** Longer peripheral bundles are also thicker. **(f)** Microtubules (MTs) adapt their growth to local actin structures. The centrosome maintains its central position in symmetric (left) and asymmetric environments (right). **(g)** Centrosome positioned above the nucleus, close to branched actin meshwork, in spatially confined cells (top). Centrosome positioned below the nucleus, close to actin bundles, in spread cells (bottom). **(h)** Cells move toward confined spaces above a certain threshold (top) and toward open spaces below that threshold (bottom). **(i)** Spread cells move with the centrosome toward the front (top), whereas confined cells move with the centrosome toward the back (bottom).

depend on cell type and the degree of asymmetry. A linear track of repeated micropatterns in the shape of isosceles triangles can lead cells to move toward the acute angle apices of these triangles [77,78]. By contrast, an elongated isosceles triangle with a very acute angle (several degrees only) can lead cells away from the acute angle apex [79] (Figure 3h).

Without an external bias such as those created by asymmetric SBC, the direction of motility is defined by intrinsic cell polarization mechanisms and can be observed in motile cells on adhesive micropatterns in the shape of bars. However, bar width affects actin network organization. Variations in actin network assembly in response to bar width are cell type-specific because keratocytes need large transversal spreading to move relatively fast [80], whereas fibroblast speed is greater when the bar width is narrower [81]. Interestingly, the orientation of internal cell polarity, revealed by the position of the centrosome with respect to the nucleus, also depends on the width of the adhesive micropattern. A cell migrates on a wide bar with the centrosome nearer the leading edge, whereas on a narrow bar, the centrosome is nearer the trailing edge [82] (Figure 3i). How this centrosome positioning is related to the different types of actin organization remains to be investigated.

The speed of migrating cells and their persistence in moving in a given direction are both affected in cells whose internal polarity orientation process is defective [75]. The systematic connection between the actin network machinery powering cell migration and the degree of stability of

spatial organization of the internal cell polarity was further supported by the observation in around a hundred different cell types of a correlation between cell speed and the persistence of the cell in maintaining their direction of migration [83].

Directed cell division

The adaptation of MT network architecture in relation to the actin network architecture and to cell shape is manifest during cell division. The tensions in astral MTs, radiating from the spindle pole toward the cell cortex, exert a torque on the spindle and direct its orientation. The tension in these astral MTs is regulated by the presence of cortical cues associated with the actin network, which orient the cell division axis with respect to cell adhesion cues and the architecture of the actin network [84–87]. Tension can also be exerted throughout the cytoplasm and therefore be proportional to astral MT length; differences in astral MT length can differ with respect to cell shape elongation and these variations can direct the orientation of the division axis accordingly [88,89].

Tissue self-organization

At the level of tissue organization, the complexity of the system increases with greater numbers of components. Nevertheless, precise manipulation of the geometries of SBCs has proven useful in identifying consistent self-organization rules.

Directed cell positioning

Self-organization of cells in a given space depends on the balance of mechanical forces between the cells and the surrounding matrix. Two cells in contact constitute a minimal multicellular structure where cells can form cell–matrix adhesions (CMAs) and cell–cell adhesions (CCAs). When confined on a homogeneous micropattern (i.e., when the cell basal surface is in contact with a continuous layer of ECM), endothelial cells forming CCAs move regularly around each other in the plane of the culture dish, whereas fibroblasts, which cannot form CCAs, do not [90]. Therefore, the formation of CCAs appears to modulate the capacity of the two cells to reach a mechanical balance. The two adhesive systems – CCA and CMA – within a cell can mutually affect their respective localizations [91]. Two cells of a given epithelial cell type confined on micropatterned ECM within a defined area can move or adopt a stationary position in response to subtle changes in ECM geometry [92] (Figure 4a). Indeed, the production of tensional forces on the CCA depends on the spatial organization of the ECM. Intercellular force is higher when the CCA is close to the ECM. This directs the CCA away from the ECM and stabilizes the cell position in this configuration, which corresponds to global minimization of the overall contractile energy [92] (Figure 4a). Conversely, the formation of a CCA prevents the formation of proximal CMAs [74,93]. The mutual exclusions of the two adhesion systems lead to their spatial segregation [91] and directs cell positioning.

Directed collective motion

The collective motion of a large multicellular group depends on the production of intercellular forces, the spatial distribution of which directs the migration of cells with respect to their neighbors. The sudden removal of SBCs, allowing a previously confined group to migrate, revealed that intercellular forces propagate from the migrating front to the group's rear [94] (Figure 4b). Intercellular forces did not appear to pull cells forward but rather to orient the traction force field they develop on the ECM to migrate.

An intriguing recent work revealed that global coherence can emerge in the spatial organization and collective motion of large cell groups [95]. Cells plated as multicellular groups on micropatterned discs do not display any coherent global motion nor specific cell orientation. However, on a torus-shaped micropattern, there is a clear asymmetry in cell orientations such that the long cell axes tilt at similar angles with respect to the torus center (Figure 4c). This appears to be reflected in the direction cells adopt when motile on the torus. The angular direction of cell motility at the peripheral edge of the torus (with positive curvature) tends to be opposite to that at the interior edge of the torus (with negative curvature) (Figure 4c). Therefore, it appears that the symmetry break imposed by the torus arises from this directional property of cell motility at the edges of the torus that is propagated throughout the entire group of cells. Surprisingly, the angular bias of endothelial cell orientation is clockwise, whereas with myoblasts it is counterclockwise. Thus, variations in intracellular parameters presumably can be manifested as specific asymmetries for different cell types. However, no explanation has yet been proposed for this geometrically simple organization resulting from a probably quite complex mechanism. One area where a mechanism may be identified is in the regulation of cell polarity and its relationship to oriented cell motility.

Directed cell polarity

The relationship between the locations of CCAs and CMAs affects nucleus–centrosome axis orientation. The centrosome, with respect to the nucleus, tends to adopt a more distal position from CCAs and a more proximal position to CMAs [73,74,96] (Figure 4d). Thus, the asymmetric locations of both CCAs and CMAs are sufficient to bias the nucleus–centrosome axis [73,74]. CCAs seem to regulate centrosome positioning [73,96], whereas CMAs seem to regulate nucleus off-centering [69,73]. Both actin filaments [96] and MTs [73] have been shown to be involved in the regulation of centrosome positioning away from CCAs. Therefore, the mechanisms by which the cytoskeleton affects centrosome and nucleus positioning remain unclear. In addition, the orientation of cell polarity not only depends on the position of CCAs, but also on the orientation of intercellular force fields [97].

Given that the self-organization of actin filament and MT networks is highly sensitive to SBCs and to the distribution of mechanical constraints, and that both types of network have intrinsic capacities to break symmetry, perhaps biased collective directional motility [95] results from symmetry break in the intracellular actin networks and the consequent asymmetric orientation of internal cell organization [98] (Figure 4e). How these polarized signals propagate to adjacent cells and result in collective oriented motility remains to be elucidated. Particularly, the role of

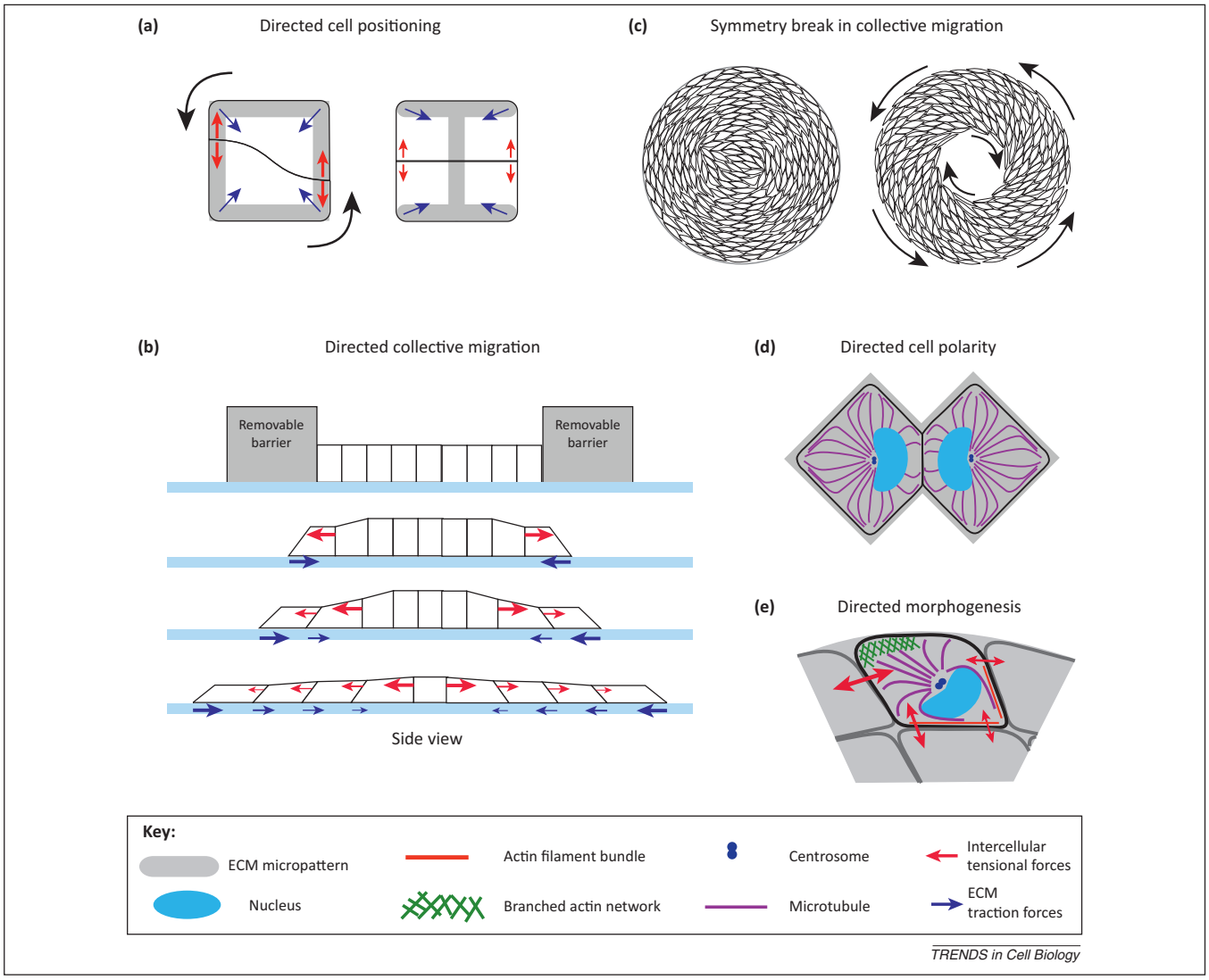

Figure 4. Tissue self-organization. **(a)** Two cells move regularly around each other (black arrows) when extracellular matrix (ECM) is present all along the periphery (left), whereas they stop moving when the extremities of their ECM contact plane reach a region without ECM (right). The presence and absence of ECM regulate intra- and intercellular forces in opposite ways. **(b)** Intercellular forces propagate from the front to the center of a migrating cell group. **(c)** A large multicellular group on a disk of ECM displays no geometrical bias (left), whereas on a torus, symmetry is broken and cells bias their orientation and move (black arrows) in a coherent fashion (right). **(d)** Two adherent cells orient their internal polarity away from their contact plane. **(e)** Speculation on coherent tissue polarity establishment. Symmetry break first occurs in the actin network, followed by microtubule (MT) rerouting and internal polarity reorientation. The asymmetric distribution of internal forces associated with these changes is counterbalanced by asymmetric intercellular forces, which further affect polarity in adjacent cells and propagate asymmetric orientation cues.

internal mechanics and intercellular force transmission could be the key elements supporting intracellular integration of spatial signals and the establishment of coherent cell polarities in dynamic multicellular structures.

Concluding remarks

SBCs play a major role in directing intrinsic cytoskeleton self-organization properties, from the architecture of macromolecular structures to the distribution of cells in tissues. Investigations at each scale – on isolated cytoskeleton components, more complex cell extracts, or entire cells – provide complementary information. All contribute to the establishment of a working framework, which should ultimately allow us to formulate the exact rules of cytoskeleton self-organization during morphogenesis. However, our understanding of the self-organization of minimal molecular systems *in vitro* is not sufficient to account for genuine cellular architectures and dynamics. Additional efforts need to be initiated to connect *in vitro* and *in vivo* self-organized cytoskeleton networks and fully to benefit from the former in understanding the latter.

There is currently a gap between the few self-organized structures that have been characterized *in vitro* and the myriad different structures observed in cells. Efforts should be made to reconstitute all of these structures *in vitro*. This will become possible by: (i) using more complex protein mixtures *in vitro* to recapitulate their effects on cytoskeleton networks observed in cells; (ii) identifying ways to engineer controlled SBCs mimicking actual biological membrane; and (iii) modulating biochemical signaling.

The regulation of network disassembly is as important as the regulation of assembly in network dynamics. There is a critical need to further understand how this network

disassembly is modulated by SBCs. Progress in this direction should allow the reconstitution of dynamic steady states in which manipulation of SBCs and network assembly–disassembly could lead to conditions in which the network persistently self-renews, with its overall structure remaining unaffected. Technological developments are also required to modulate SBCs in real time [47], especially for analyzing dynamic systems and cytoskeleton adaptation to external changes.

However, the considerable efforts made to understand the regulation of the self-organization properties of actin filament or MT networks will not be sufficient to understand their self-organization in a cellular context, because the two networks are not independent of each other. Instead, the two networks are physically and biochemically coupled. It is necessary to design new, controlled *in vitro* biochemical assays in which the two networks can interact and regulate each other. Such assays should offer the possibility to manipulate the geometry of network interactions as well as the spatial distribution of crosslinking proteins and regulating enzymes such as Rho-GTPases. Physical SBCs need to be completed by biochemical SBCs comprising surface-grafted, but also soluble and diffusible, cues.

Notably, understanding of the basic laws governing cytoskeleton assembly can not only provide insights into cell and tissue morphogenesis, but may also have technological applications in the development of microdevices requiring complex and dynamic architectures. A structure whose precise architecture is regulated by deterministic assembly rules, that can grow and self-repair because it self-renews, has advantages over a fixed structure that would have to be repaired or replaced by a prefabricated static component. This new sort of manufacturing would be a useful way to prepare novel biomaterials and should find promising applications in microelectronics and robotics.

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References

1 Karsenti, E. (2008) Self-organization in cell biology: a brief history. *Nat. Rev. Mol. Cell Biol.* 9, 255–262
2 Huber, F. and Käs, J. (2011) Self-regulative organization of the cytoskeleton. *Cytoskeleton (Hoboken)* 68, 259–265
3 Surrey, T. *et al.* (2001) Physical properties determining self-organization of motors and microtubules. *Science* 292, 1167–1171
4 Schaller, V. *et al.* (2010) Polar patterns of driven filaments. *Nature* 467, 73–77
5 Sumino, Y. *et al.* (2012) Large-scale vortex lattice emerging from collectively moving microtubules. *Nature* 483, 448–452
6 Cortès, S. *et al.* (2006) Microtubule self-organisation by reaction-diffusion processes in miniature cell-sized containers and phospholipid vesicles. *Biophys. Chem.* 120, 168–177
7 Pollard, T.D. and Cooper, J.A. (1986) Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. *Annu. Rev. Biochem.* 55, 987–1035
8 De La Cruz, E.M. *et al.* (2000) Polymerization and structure of nucleotide-free actin filaments. *J. Mol. Biol.* 295, 517–526
9 Xu, K. *et al.* (2012) Dual-objective STORM reveals three-dimensional filament organization in the actin cytoskeleton. *Nat. Methods* 9, 185–188
10 Pollard, T.D. (2007) Regulation of actin filament assembly by Arp2/3 complex and formins. *Annu. Rev. Biophys. Biomol. Struct.* 36, 451–477
11 Yarar, D. *et al.* (1999) The Wiskott-Aldrich syndrome protein directs actin-based motility by stimulating actin nucleation with the Arp2/3 complex. *Curr. Biol.* 9, 555–558
12 Oudenaarden, A.V. and Theriot, J.A. (1999) Cooperative symmetry-breaking by actin polymerization in a model for cell motility. *Nat. Cell Biol.* 1, 493–499
13 van der Gucht, J. *et al.* (2005) Stress release drives symmetry breaking for actin-based movement. *Proc. Natl. Acad. Sci. U.S.A.* 102, 7847–7852
14 Bernheim-Groswasser, A. *et al.* (2005) Mechanism of actin-based motility: a dynamic state diagram. *Biophys. J.* 89, 1411–1419
15 Achard, V. *et al.* (2010) A "primer"-based mechanism underlies branched actin filament network formation and motility. *Curr. Biol.* 20, 423–428
16 Noireaux, V. *et al.* (2000) Growing an actin gel on spherical surfaces. *Biophys. J.* 78, 1643–1654
17 Bernheim-Groswasser, A. *et al.* (2002) The dynamics of actin-based motility depend on surface parameters. *Nature* 417, 308–311
18 Lacayo, C.I. *et al.* (2012) Choosing orientation: influence of cargo geometry and ActA polarization on actin comet tails. *Mol. Biol. Cell* 23, 614–629
19 Michelot, A. *et al.* (2007) Actin-filament stochastic dynamics mediated by ADF/cofilin. *Curr. Biol.* 17, 825–833
20 Soares e Silva, M. *et al.* (2011) Self-organized patterns of actin filaments in cell-sized confinement. *Soft Matter* 7, 10631
21 Månsson, A. *et al.* (2012) Self-organization of motor-propelled cytoskeletal filaments at topographically defined borders. *J. Biomed. Biotechnol.* 2012, 647265
22 Liu, A.P. *et al.* (2008) Membrane-induced bundling of actin filaments. *Nat. Phys.* 4, 789–793
23 Roos, W.H. *et al.* (2003) Freely suspended actin cortex models on arrays of microfabricated pillars. *Chemphyschem* 4, 872–877
24 Uhrig, K. *et al.* (2009) Optical force sensor array in a microfluidic device based on holographic optical tweezers. *Lab Chip* 9, 661–668
25 Reymann, A-C. *et al.* (2010) Nucleation geometry governs ordered actin networks structures. *Nat. Mater.* 9, 827–832
26 Pinot, M. *et al.* (2012) Confinement induces actin flow in a meiotic cytoplasm. *Proc. Natl. Acad. Sci. U.S.A.* 109, 11705–11710
27 Reymann, A-C. *et al.* (2012) Actin network architecture can determine myosin motor activity. *Science* 336, 1310–1314
28 Thoresen, T. *et al.* (2011) Reconstitution of contractile actomyosin bundles. *Biophys. J.* 100, 2698–2705
29 Shelanski, M.L. (1973) Chemistry of the filaments and tubules of brain. *J. Histochem. Cytochem.* 21, 529–539
30 Holy, T.E. *et al.* (1997) Assembly and positioning of microtubule asters in microfabricated chambers. *Proc. Natl. Acad. Sci. U.S.A.* 94, 6228–6231
31 Laan, L. *et al.* (2012) Cortical dynein controls microtubule dynamics to generate pulling forces that position microtubule asters. *Cell* 148, 502–514
32 Nedelec, F. *et al.* (1997) Self-organization of microtubules and motors. *Nature* 389, 305–308
33 Pinot, M. *et al.* (2009) Effects of confinement on the self-organization of microtubules and motors. *Curr. Biol.* 19, 954–960
34 Cosentino Lagomarsino, M. *et al.* (2007) Microtubule organization in three-dimensional confined geometries: evaluating the role of elasticity through a combined in vitro and modeling approach. *Biophys. J.* 92, 1046–1057
35 Emsellem, V. *et al.* (1998) Vesicle deformation by microtubules: a phase diagram. *Phys. Rev. E* 58, 4807–4810
36 Fygenson, D. *et al.* (1997) Mechanics of microtubule-based membrane extension. *Phys. Rev. Lett.* 79, 4497–4500
37 Karsenti, E. and Vernos, I. (2001) The mitotic spindle: a self-made machine. *Science* 294, 543–547

38 Heald, R. *et al.* (1996) Self-organization of microtubules into bipolar spindles around artificial chromosomes in *Xenopus* egg extracts. *Nature* 382, 420–425
39 Halpin, D. *et al.* (2011) Mitotic spindle assembly around RCC1-coated beads in *Xenopus* egg extracts. *PLoS Biol.* 9, e1001225
40 Gaetz, J. *et al.* (2006) Examining how the spatial organization of chromatin signals influences metaphase spindle assembly. *Nat. Cell Biol.* 8, 924–932
41 Dinarina, A. *et al.* (2009) Chromatin shapes the mitotic spindle. *Cell* 138, 502–513
42 Bornens, M. *et al.* (1989) The cortical microfilament system of lymphoblasts displays a periodic oscillatory activity in the absence of microtubules: implications for cell polarity. *J. Cell Biol.* 109, 1071–1083
43 Bailly, E. *et al.* (1991) The cortical actomyosin system of cytochalasin D-treated lymphoblasts. *Exp. Cell Res.* 196, 287–293
44 Paluch, E. *et al.* (2005) Cortical actomyosin breakage triggers shape oscillations in cells and cell fragments. *Biophys. J.* 89, 724–733
45 Cavalcanti-Adam, E.A. *et al.* (2007) Cell spreading and focal adhesion dynamics are regulated by spacing of integrin ligands. *Biophys. J.* 92, 2964–2974
46 Schvartzman, M. *et al.* (2011) Nanolithographic control of the spatial organization of cellular adhesion receptors at the single-molecule level. *Nano Lett.* 11, 1306–1312
47 Vignaud, T. *et al.* (2012) Reprogramming cell shape with laser nano-patterning. *J. Cell Sci.* 125, 2134–2140
48 Lehnert, D. *et al.* (2004) Cell behaviour on micropatterned substrata: limits of extracellular matrix geometry for spreading and adhesion. *J. Cell Sci.* 117, 41–52
49 Vianay, B. *et al.* (2010) Single cells spreading on a protein lattice adopt an energy minimizing shape. *Phys. Rev. Lett.* 105, 3–6
50 Levina, E.M. *et al.* (2001) Cytoskeletal control of fibroblast length: experiments with linear strips of substrate. *J. Cell Sci.* 114, 4335–4341
51 Picone, R. *et al.* (2010) A polarised population of dynamic microtubules mediates homeostatic length control in animal cells. *PLoS Biol.* 8, e1000542
52 Vartanian, K.B. *et al.* (2008) Endothelial cell cytoskeletal alignment independent of fluid shear stress on micropatterned surfaces. *Biochem. Biophys. Res. Commun.* 371, 787–792
53 Terenna, C.R. *et al.* (2008) Physical mechanisms redirecting cell polarity and cell shape in fission yeast. *Curr. Biol.* 18, 1748–1753
54 Bergert, M. *et al.* (2012) Cell mechanics control rapid transitions between blebs and lamellipodia during migration. *Proc. Natl. Acad. Sci. U.S.A.* 666, 1–7
55 James, J. *et al.* (2008) Subcellular curvature at the perimeter of micropatterned cells influences lamellipodial distribution and cell polarity. *Cell Motil. Cytoskeleton* 65, 841–852
56 Parker, K.K. *et al.* (2002) Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces. *FASEB J.* 16, 1195–1204
57 Brock, A. *et al.* (2003) Geometric determinants of directional cell motility revealed using microcontact printing. *Langmuir* 19, 1611–1617
58 Rossier, O.M. *et al.* (2010) Force generated by actomyosin contraction builds bridges between adhesive contacts. *EMBO J.* 29, 1055–1068
59 Tan, J.L. *et al.* (2003) Cells lying on a bed of microneedles: an approach to isolate mechanical force. *Proc. Natl. Acad. Sci. U.S.A.* 100, 1484–1489
60 Rape, A.D. *et al.* (2011) The regulation of traction force in relation to cell shape and focal adhesions. *Biomaterials* 32, 2043–2051
61 Kilian, K. *et al.* (2010) Geometric cues for directing the differentiation of mesenchymal stem cells. *Proc. Natl. Acad. Sci. U.S.A.* 107, 4872–4877
62 Hu, S. *et al.* (2004) Mechanical anisotropy of adherent cells probed by a 3D magnetic twisting device. *Am. J. Physiol. Cell Physiol.* 287, C1884–C1191
63 Khatau, S.B. *et al.* (2009) A perinuclear actin cap regulates nuclear shape. *Proc. Natl. Acad. Sci. U.S.A.* 106, 19017–19022
64 Bray, M-A. *et al.* (2008) Sarcomere alignment is regulated by myocyte shape. *Cell Motil. Cytoskeleton* 651, 641–651
65 Xu, J. *et al.* (2011) Effects of micropatterned curvature on the motility and mechanical properties of airway smooth muscle cells. *Biochem. Biophys. Res. Commun.* 415, 591–596
66 Théry, M. *et al.* (2006) Cell distribution of stress fibres in response to the geometry of the adhesive environment. *Cell Motil. Cytoskeleton* 63, 341–355
67 Tseng, Q. *et al.* (2011) A new micropatterning method of soft substrates reveals that different tumorigenic signals can promote or reduce cell contraction levels. *Lab Chip* 11, 2231–2240
68 Bischofs, I.B. *et al.* (2008) Filamentous network mechanics and active contractility determine cell and tissue shape. *Biophys. J.* 95, 3488–3496
69 Théry, M. *et al.* (2006) Anisotropy of cell adhesive microenvironment governs cell internal organization and orientation of polarity. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19771–19776
70 Wu, J. *et al.* (2011) Effects of dynein on microtubule mechanics and centrosome positioning. *Mol. Biol. Cell* 22, 4834–4841
71 Hale, C.M. *et al.* (2011) SMRT analysis of MTOC and nuclear positioning reveals the role of EB1 and LIC1 in single-cell polarization. *J. Cell Sci.* 124, 4267–4285
72 Zhu, J. *et al.* (2010) finding the cell center by a balance of dynein and myosin pulling and microtubule pushing: a computational study. *Mol. Biol. Cell* 21, 4418–4427
73 Dupin, I. *et al.* (2009) Classical cadherins control nucleus and centrosome position and cell polarity. *J. Cell Biol.* 185, 779–786
74 Camand, E. *et al.* (2012) N-cadherin expression level modulates integrin-mediated polarity and strongly impacts on the speed and directionality of glial cell migration. *J. Cell Sci.* 125, 844–857
75 Lombardi, M.L. *et al.* (2011) The interaction between nesprins and sun proteins at the nuclear envelope is critical for force transmission between the nucleus and cytoskeleton. *J. Biol. Chem.* 286, 26743–26753
76 Pitaval, A. *et al.* (2010) Cell shape and contractility regulate ciliogenesis in cell cycle-arrested cells. *J. Cell Biol.* 191, 303–312
77 Mahmud, G. *et al.* (2009) Directing cell motions on micropatterned ratchets. *Nat. Phys.* 5, 606–612
78 Kushiro, K. *et al.* (2012) Modular design of micropattern geometry achieves combinatorial enhancements in cell motility. *Langmuir* 28, 4357–4362
79 Yoon, S-H. *et al.* (2011) A biological breadboard platform for cell adhesion and detachment studies. *Lab Chip* 11, 3555–3562
80 Csucs, G. *et al.* (2007) Locomotion of fish epidermal keratocytes on spatially selective adhesion patterns. *Cell Motil. Cytoskeleton* 64, 856–867
81 Doyle, A.D. *et al.* (2009) One-dimensional topography underlies three-dimensional fibrillar cell migration. *J. Cell Biol.* 184, 481–490
82 Pouthas, F. *et al.* (2008) In migrating cells, the Golgi complex and the position of the centrosome depend on geometrical constraints of the substratum. *J. Cell Sci.* 121, 2406–2414
83 Maiuri, P. *et al.* (2012) The world first cell race. *Curr. Biol.* 22, R673–R675
84 Théry, M. *et al.* (2007) Experimental and theoretical study of mitotic spindle orientation. *Nature* 447, 493–496
85 Fink, J. *et al.* (2011) External forces control mitotic spindle positioning. *Nat. Cell Biol.* 13, 771–778
86 Samora, C.P. *et al.* (2011) MAP4 and CLASP1 operate as a safety mechanism to maintain a stable spindle position in mitosis. *Nat. Cell Biol.* 13, 1040–1050
87 Kiyomitsu, T. and Cheeseman, I.M. (2012) Chromosome- and spindle-pole-derived signals generate an intrinsic code for spindle position and orientation. *Nat. Cell Biol.* 14, 311–317
88 Minc, N. *et al.* (2011) Influence of cell geometry on division-plane positioning. *Cell* 144, 414–426
89 Minc, N. and Piel, M. (2012) Predicting division plane position and orientation. *Trends Cell Biol.* 22, 193–200
90 Huang, S. *et al.* (2005) Symmetry-breaking in mammalian cell cohort migration during tissue pattern formation: role of random-walk persistence. *Cell Motil. Cytoskeleton* 61, 201–213
91 Burute, M. and Thery, M. (2012) Spatial segregation of cell–cell and cell–matrix adhesions. *Curr. Opin. Cell Biol.* 24, 628–636
92 Tseng, Q. *et al.* (2012) Spatial organization of the extracellular matrix regulates cell-cell junction positioning. *Proc. Natl. Acad. Sci. U.S.A.* 109, 1506–1511

93 McCain, M.L. *et al.* (2012) Cooperative coupling of cell-matrix and cell-cell adhesions in cardiac muscle. *Proc. Natl. Acad. Sci. U.S.A.* 109, 9881–9886
94 Serra-Picamal, X. *et al.* (2012) Mechanical waves during tissue expansion. *Nat. Phys.* 8, 628–634
95 Wan, L.Q. *et al.* (2011) Micropatterned mammalian cells exhibit phenotype-specific left-right asymmetry. *Proc. Natl. Acad. Sci. U.S.A.* 108, 12295–12300
96 Desai, R.A. *et al.* (2009) Cell polarity triggered by cell-cell adhesion via E-cadherin. *J. Cell Sci.* 122, 905–911
97 Reffay, M. *et al.* (2011) Orientation and polarity in collectively migrating cell structures: statics and dynamics. *Biophys. J.* 100, 2566–2575
98 Mullins, R.D. (2010) Cytoskeletal mechanisms for breaking cellular symmetry. *Cold Spring Harb. Perspect. Biol.* 2, a003392