Vol. 20, e200032 (2023)
doi: 10.2142/biophysico.bppb-v20.0032

Biophysics and Physicobiology
https://www.jstage.jst.go.jp/browse/biophysico/

Review Article (Invited)

Unveiling the physics underlying symmetry breaking of the actin cytoskeleton: An artificial cell-based approach

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Received June 26, 2023; Accepted August 18, 2023;
Released online in J-STAGE as advance publication August 19, 2023
Edited by Makoto Miyata

Single-cell behaviors cover many biological functions, such as cell division during morphogenesis and tissue metastasis, and cell migration during cancer cell invasion and immune cell responses. Symmetry breaking of the positioning of organelles and the cell shape are often associated with these biological functions. One of the main players in symmetry breaking at the cellular scale is the actin cytoskeleton, comprising actin filaments and myosin motors that generate contractile forces. However, because the self-organization of the actomyosin network is regulated by the biochemical signaling in cells, how the mechanical contraction of the actin cytoskeleton induces diverse self-organized behaviors and drives the cell-scale symmetry breaking remains unclear. In recent times, to understand the physical underpinnings of the symmetry breaking exhibited in the actin cytoskeleton, artificial cell models encapsulating the cytoplasmic actomyosin networks covered with lipid monolayers have been developed. By decoupling the actomyosin mechanics from the complex biochemical signaling within living cells, this system allows one to study the self-organization of actomyosin networks confined in cell-sized spaces. We review the recent developments in the physics of confined actomyosin networks and provide future perspectives on the artificial cell-based approach. This review article is an extended version of the Japanese article, The Physical Principle of Cell Migration Under Confinement: Artificial Cell-based Bottom-up Approach, published in SEIBUTSU BUTSURI Vol. 63, p. 163-164 (2023).

Key words: actomyosin, reconstitution, cytoplasmic extracts, microfluidics, active gels

Significance

Uncovering physical principles underlying diverse biological phenomena is crucial in controlling biological systems. While it has been known that the actin cytoskeleton plays pivotal roles in symmetry breaking of organelle positioning and cell shape, complexities of the living cells often pose challenges in elucidating physical mechanisms that govern the cellular (a)symmetry through cytoskeleton systems. We have constructed a cell model termed “artificial cells” to abstract fundamental symmetry breaking events, including nucleus positioning, shape symmetry breaking, and flow-to-wave state transition of the actin cytoskeleton. The artificial cells developed here can uncover physical principles governing the biological symmetry breaking associated with cell function.

Introduction

Symmetry breaking is a key concept underlying the various biological phenomena from single cell scale to tissue scale.

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Single cells exhibit symmetry breaking of their shape during cell proliferation and developmental processes. For instance, spherical cells undergo either symmetric or asymmetric cell division [1,2] (Fig. 1A). Moreover, symmetry breaking of the direction of the retrograde flow determines the onset of cell migration and chirality of single cells to cell collectives [3-5](Fig. 1B and 1C). In tissue scale, spatial translational symmetry breaking induces various body patterns and head-tail axis [6]. Thus, as the renowned physicist Pierre Curie described, "Asymmetry is what creates phenomena," symmetry breaking serves as a foundation that enables us to understand how biological functions emerge from physical mechanisms. However, the physical mechanism of symmetry breaking is often challenging to elucidate. This is because the rich complexity of the cells represented by the complicated biochemical signaling pathways is entangled with the cellular mechanics, which often obscures the fundamental principles underlying the symmetry breaking-indued cellular functions.

Is there any general physical principle governing the symmetry breaking in cells? To answer this question, reconstituted systems have been developed to decouple the mechanics from biochemical signaling [7-14]. The reconstituted systems mainly comprise the essential components involved in the symmetry breaking of the cells, called actin cytoskeleton, comprising the actin filaments and myosin II motors (Fig. 1D and 1E). Namely, the minimal cell model called 'artificial cells' is the abstraction of cell mechanics and asks if minimal components are sufficient to reproduce the cell-like behaviors and explore the physical principles that govern the cellular symmetry breaking (Fig. 2A). This system allows us to precisely control the size of the compartments, additional protein concentrations, as well as the composition of the lipid membrane, thus suited to study the physical mechanisms behind the cellular behaviors (Fig. 2B). Traditionally, the purified proteins, such as actin filaments and myosin motor proteins, were encapsulated in droplets and giant unilamellar vesicles as simplified cell models to study how the cell-like behaviors can be emerged from the minimal constituents of the actin cytoskeleton (Fig. 2C and 2D) [7-14]. However, because the purified systems usually lack many proteins associated with the actin turnover (i.e., polymerization and depolymerization of filaments), the short duration and low activity of the system limited the biological relevance of the experimental observations.

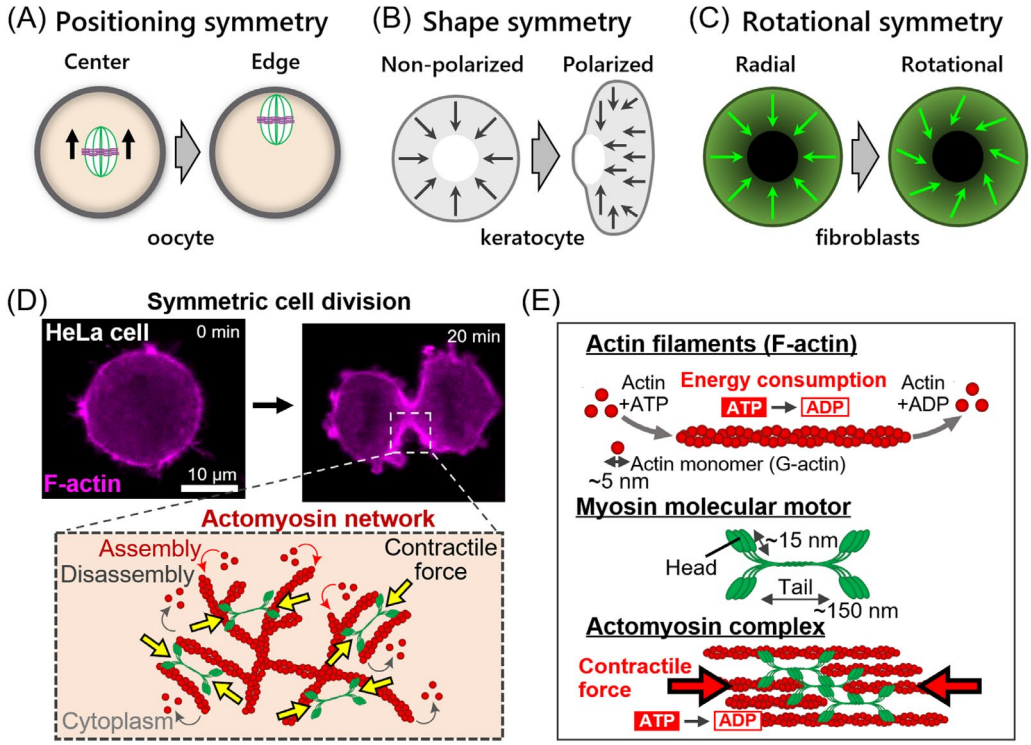

Figure 1 (A) The positioning symmetry breaking of a spindle in mouse oocytes. The spindle migrates from the cell center to beneath the membrane at the onset of the reductional division. (B) The shape symmetry breaking of an adherent keratocyte. The circular cell shape is transformed to a canoe-like shape (i.e., polarized) at the onset of the cell migration. Arrows are the actin flow direction. (C) The rotational symmetry breaking of the radial actin flow in a fibroblast. The radial actin flow undergoes chiral symmetry breaking. (D) Snapshots showing a dividing HeLa cell. The spherical cell undergoes symmetric cell division, which is executed by the contractile force of the actomyosin network. The bottom schematic shows the actomyosin network. (E) Schematic showing the basic properties of the actin filaments and myosin II motors. Actin filaments are polymerized from actin monomers (G-actin) bound to adenosine triphosphate (ATP) while it is depolymerized by ATP hydrolysis. Myosin II motors bound to the actin filaments induce sliding stress on the filaments, resulting in the net contractile force over the connected actin network.

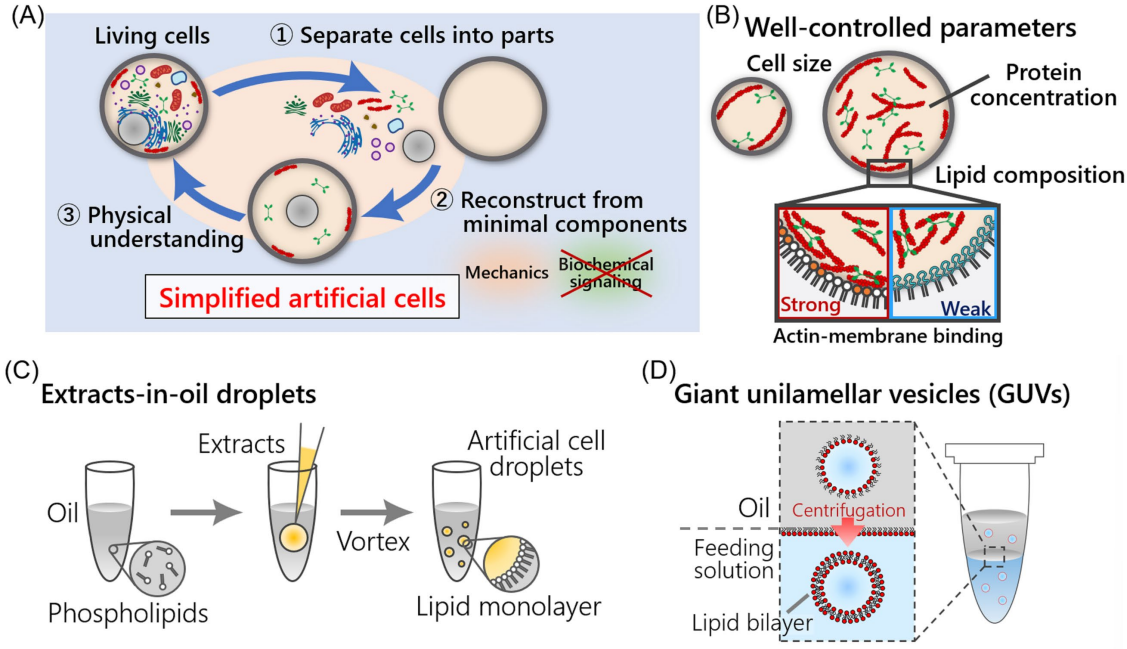

Figure 2 (A) Schematic showing the concept of the artificial cell-based approach that decouples the mechanics from biochemical signaling. (B) Schematic showing the advantages of the artificial cells. The cell size, concentration of proteins, and lipid composition can be precisely controlled. (C) The preparation procedure of extracts-in-oil droplets. Mixing the extracts with the mineral oil containing phospholipids allows one to obtain variously sized droplets covered with a lipid monolayer. (D) The preparation procedure of giant unilamellar vesicles (GUVs) (inverted emulsion method). After preparing water-in-oil droplets, the droplets are transferred to the aqueous phase through a lipid monolayer interface, by which lipid bilayer vesicles (i.e., liposomes) are produced.

To extend artificial cells to more biologically relevant systems, reconstituted systems derived from the cytoplasmic actomyosin networks of the *Xenopus* egg extracts (hereafter called 'extracts') have been developed [15-23]. Because the extracts contain the physiological level of all proteins necessary for highly active polymerization and depolymerization of actin filaments, allowing the realization of physiologically relevant contractile behaviors of the actomyosin networks [24]. Field et al. showed that a droplet of the M-phase cytoplasmic extracts with ~5 mm in diameter exhibits periodic contraction of the actomyosin networks, which they termed the 'gelation-contraction', thought to occur due to the periodic cycle of the following three steps: (i) polymerization of the actin network, (ii) contraction due to the myosin, (iii) and depolymerization of the actin network [15]. The gelation-contraction was observed for more than 6 hours, confirming the effectiveness of the extracts in realizing highly efficient actin turnover. On the other hand, Pinot et al. confined the extracts into cell-sized water-in-oil droplets with ~10 μm in diameter to mimic the spherical boundary like in cells [16]. Notably, confining the extracts within cell-sized droplets showed a continuous flow of the actomyosin network from the periphery of the droplets to the center. They showed that the actin flow did not occur upon either the inhibition of the actin polymerization by cytochalasin D or the inhibition of the actin depolymerization by phalloidin, suggesting that the turnover of actin filaments is necessary to maintain the actin flow. This type of actin flow maintained by actin turnover is observed in migratory cells [3]. Together, these pioneering works have shown the effectiveness of the cytoplasmic extracts in achieving the physiological level of actin turnover and the resulting cell-like actomyosin behaviors.

It should be noted that the extracts contain cellular molecules, including the proteins involved in biochemical signaling. However, we are considering the decoupling of mechanics from biochemical signaling withing the cellular context. For example, in dividing cells, the formation of the contractile ring is controlled through the local activation of RhoA activity via GEF & GAP around the chromosome aligned at the equator of the mitotic spindle [25]. Also, in the positioning of the spindle toward the plasma membrane of the mouse oocytes, the activation of the local F-actin assembly via Arp2/3 is mediated by the Arp2/3 activating chromatin signals at the spindle, which induces the asymmetric actin flow [26]. In these cases, 'biochemical signaling' serves as the driver of symmetry breaking. However, the extracts system is homogenized and uniform. Consequently, F-actin assembly and myosin contractility are expected to be spatially uniform, unlike in cells. In this scenario, actomyosin contractility emerges as the primary driver capable of inducing spatial translational symmetry breaking through mechanical forces. To be specific, we disentangle the contribution of mechanically-driven symmetry breaking from the symmetry breaking induced by biochemical signaling in the extracts-based *in vitro* reconstitution.

It is of note that some types of cells can induce symmetry breaking without requiring myosin contractility. Plant cells

do not have myosin II while they are asymmetric [27]. The cellular slime mold *Dictyostelium discoideum* has myosin II, but a mutant line lacking myosin II can migrate by chemotaxis albeit at a slower speed and divide normally on substrates [28]. However, in those cases, cells often utilize alternative mechanisms to break the cellular symmetry, such as BASL polarity module in stomatal asymmetric division in *Arabidopsis*, reaction-diffusion pattern of PI3K/PTEN in *Dictyostelium* cells, and PAR proteins in *Caenorhabditis elegans* embryo [29,30]. Our objective is not to elucidate a cell-type specific mechanism, but rather to establish a fundamental condition for mechanically inducing actomyosin-driven symmetry breaking without relying on biochemical signaling. Nevertheless, *in vitro* reconstitutions have indicated that actomyosin constitutes a minimal system capable of driving cellular symmetry breaking through mechanical mechanisms.

Earlier works have mainly focused on biophysical aspects of the contraction of the actomyosin network, such as the characterization of the rheological properties of the actomyosin networks in extracts [31,32], detailed quantification of the actin turnover rate upon the addition of different actin polymerization/depolymerization factors [19], connectivity transition-induced change in contractility behaviors upon addition of actin-crosslinking proteins [18]. On the other hand, recent studies focused more on reconstructing cell-like behaviors, including the nucleus positioning and cell migration, trying to elucidate underlying physical principles that govern the symmetry breaking in cells. This review outlines recently developed artificial cells that reconstruct biologically relevant cell-like behaviors accompanied by the dynamics of actomyosin, which uncover the physical principles underlying complex cellular behaviors.

Positioning Symmetry is Determined by the Contractile Behaviors of the Actomyosin Networks

The nucleus positioning plays a pivotal role in the developmental process of oocytes (i.e., premature egg cells before fertilization) [33,34]. Notably, the actomyosin cytoskeleton is involved in the symmetry breaking of the nucleus positioning from the center to the periphery of the cell [35]. If the positioning of the nucleus close to the membrane is failed, the cell dies, or aneuploidy leads to functional disabilities [36-38]. Thus, the actomyosin mechanics and cellular function are tightly coupled in the oocyte. However, the physical mechanism underlying the symmetry breaking of the nucleus positioning has been elusive. Several mechanisms have been proposed in different experimental conditions, such as the actin-comet-like pushing force-driven nucleus motion [39], nucleus positioning by actin flow [40], and actin bridge-driven contractile forces [2]. This is due to the difficulty in obtaining many oocyte samples and the variation of the oocyte quality in different experiments. To overcome these difficulties, Sakamoto et al. have tried to abstract the nucleus positioning behavior using artificial cells [20]. Because the artificial cells are free from the complex signaling pathways, one can study the positioning mechanism that only arises from the actomyosin mechanics. Moreover, the precise control of the cell size and protein concentration in well-defined artificial cells enables a comprehensive characterization of system behavior.

Several minutes after the encapsulation of the actomyosin network within the droplet, the initial contraction of the actomyosin network with the cytoplasmic materials forms a nucleus-like spherical structure at the center of the droplets (hereafter called a ‘cluster’) (Fig. 3A). The cluster takes the center position for the large droplets (Fig. 3A, right, and Fig. 3B). In contrast, it takes the edge position for the smaller droplets (Fig. 3A, left, and Fig. 3B). Because this cluster positioning transition is reminiscent of the nucleus positioning observed in cells, we regarded the cluster as a simplified model of the cell nucleus and studied the mechanism of positioning symmetry breaking.

To study how the actomyosin networks drive the positioning symmetry breaking, the actin filaments were visualized. Notably, we observed the periodic contraction of the ring-like actomyosin networks, which propagates from the periphery of the droplet to the center of the droplet (Fig. 3C). The active gel theory showed that the periodic contraction of the actomyosin network could generate the inward pushing force on the cluster. Therefore, the cluster should be located in the center of the droplets. However, this is true when the droplet size is larger than 100 μm, but when the droplet size is smaller $D_{\mathrm{droplet}} < 100$ μm, most of the cluster is positioned around the periphery of the droplet; in other words, the positioning symmetry is broken for the smaller droplets (Fig. 3B). Even in the presence of the inward contraction of the periodic actin waves, how can the positioning symmetry of the cluster be broken for the smaller droplets? Notably, we observed that the bridge-like structure was connecting the cluster and the periphery of the droplet when the cluster was transported to the boundary of the droplet (Fig. 3C). This result suggested that the bridge-like structure was driving the symmetry breaking of the cluster positioning. Indeed, in molecular perturbation experiments adding the actin crosslinking protein (α-actinin), the probability of the edge-located clusters increased, suggesting that the actin bridge formation is enhanced by increasing the network connectivity. Moreover, elongating the actin filaments using formin (mDia1) increased the probability of the edge-located clusters; in contrast, shortening the actin filaments using actin-severing protein gelsolin resulted in center-located clusters. Together, these results suggested that the actin bridge that connects the

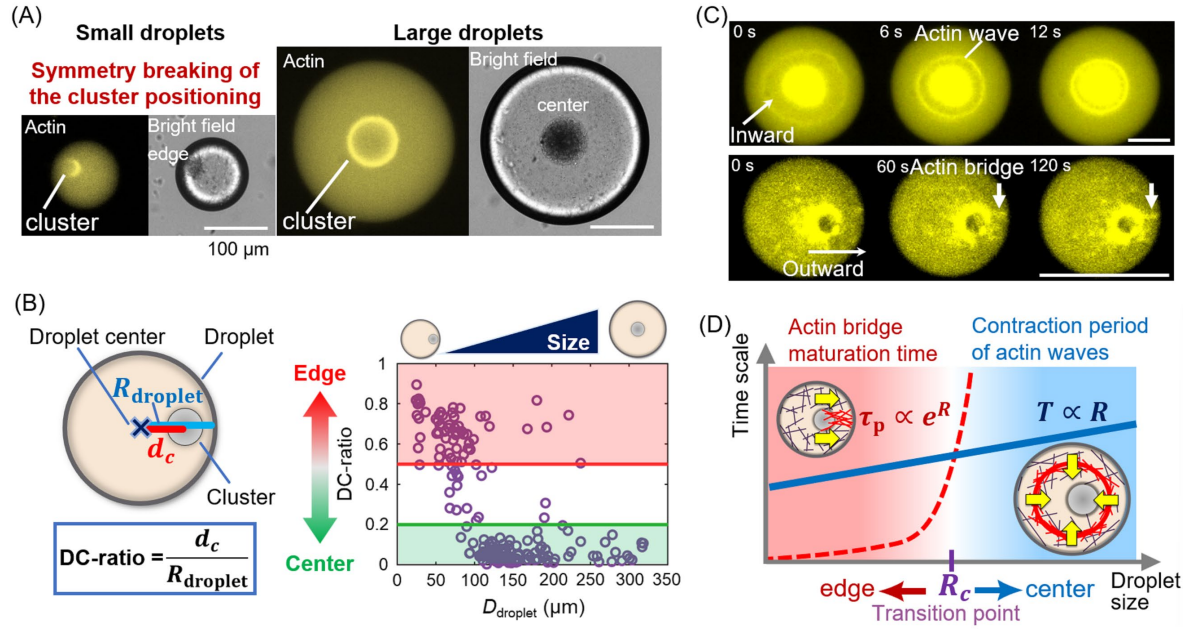

Figure 3 (A) Snapshots showing the droplet size-dependent symmetry breaking of the cluster positioning. The cluster is located at the center of the large droplet, while it is located at the edge of the small droplet. (B) (*Left*) The polarity parameter DC-ratio is defined as the distance from the droplet center to the cluster centroid, d_c, divided by the radius of the droplet, $R_{droplet}$. DC-ratio is classified into two regimes: larger than 0.5 is the 'edge' and smaller than 0.2 is the 'center'. (*Right*) Droplet diameter dependence of the DC-ratio 1 hour after the droplet preparation. (C) Time-lapse images showing the inwardly contracting ring-shaped actin waves and the outwardly contracting actin bridge formation. (D) The mechanism of the droplet size-dependent cluster positioning. The crossover between the two distinct timescales, actin bridge maturation time and the period of actin waves, determines either one of the dominant cluster positioning. This figure is modified from [20].

cluster and the periphery of the droplet drives the symmetry breaking of the cluster positioning in the smaller droplets.

Experimental observations showed that the two antagonistic driving forces control the cluster positioning: (i) inwardly contracting actin waves and (ii) outwardly contracting actin bridge. However, how either of the competing antagonistic forces be dominated, and result in the size-dependent symmetry breaking remained unanswered. To gain insights into the mechanism of the positioning symmetry breaking, we developed a theoretical model called a 'tug-of-war' model based on the following assumptions: (i) Actin waves transport the cluster towards the center with the time period T (wave period); (ii) Once the bridge is formed between the cluster and the droplet periphery with the characteristic bridge maturation time τ_p, the cluster is located at the edge. Thus, this model predicts when $T < \tau_p$, clusters are located at the center (wave-dominated regime); in contrast, for $T > \tau_p$, clusters are located at the edge (bridge-dominated regime).

To estimate the characteristic time of bridge formation τ_p, we adopted a percolation model for actin bridge maturation. In short, we showed that the characteristic bridge maturation time exponentially scales with the size of the droplet, $\tau_p \propto e^R$, because the filaments with length L must connect over the radius R using the total number of filaments $N \sim R/L$. On the other hand, our experiments and theoretical model based on active gel theory showed that the wave period linearly scales with the droplet size $T \propto R$. This was explained by the geometric constraints: because the inward contractility within the actomyosin ring depends on the curvature of the ring, it takes longer time to break the physical link between the growing actomyosin ring and the droplet surface. Taken together, we showed that the crossover of these two distinct time scales, inwardly contracting wave $T \propto R$ and outwardly contracting bridge $\tau_p \propto e^R$, determines the size-dependent positioning symmetry breaking (Fig. 3D). The transition radius R_c was estimated from the equality $T = \tau_p$, leading to the following relation:

$$\frac{R_c}{L} = \log_2\left(\frac{T}{\tau}\right), \tag{1}$$

where τ is the binding/unbinding timescale of the individual crosslinkers. The estimation of the transition point gives $D_c = 2R_c = 73$ μm by using experimentally available parameters $T = 46$ s, $\tau = 0.54$ s, and $L = 5.7$ μm, which is comparable to the experimentally observed transition point $D_c = 85$ μm (Fig. 3B). Thus, the tug-of-war model based on

the competing time scales can explain the size-dependent positioning symmetry breaking. Together, this study uncovers the basic physical mechanism of positioning symmetry breaking solely determined by the mechanics of the self-organized actomyosin structures within the cell-sized confined spaces. The uncovered physical principle of positioning symmetry breaking could be applicable to the living cell's nucleus positioning, which has implications for the physics of the actomyosin network percolation and force generation within the cell-sized confined spaces.

Shape Symmetry Breaking Induces Spontaneous Migration of the Actomyosin Droplets

In positioning symmetry breaking, the internal actomyosin distribution becomes asymmetric while the droplet retains a symmetric circular shape. However, as the Curie principle dictated, breaking the shape symmetry could induce a more complex phenomenon, such as cell division and migration. Notably, the contractility of actomyosin also plays a pivotal role in the shape deformation of cells, in which actin-membrane binding is critical to transmitting the contractility of actomyosin to the cell membrane. To break the symmetry of the circular shape of the actomyosin droplets, Sakamoto et al. employed actin-binding lipids phosphatidylinositol 4,5-bisphosphate (PIP_2), known to localize F-actin beneath the cell membrane via actin-membrane binding [41-43]. Similarly to cells, incorporating PIP_2 in the artificial cells formed a cell-like actin layer beneath the lipid monolayer [22]. Strikingly, the actin-membrane binding led to the local deformation of the droplet interface, inducing the polarized distribution of the actomyosin network within the droplet, triggering the cell-like spontaneous droplet migration (Fig. 4A). Although previous *in vitro* reconstitution works have shown microtubule-kinesin-based motility of droplets [44], or gelation-transition-induced motility of amoeba cell extracts [45], their biological relevance to the migration mechanism of living cells have been limited. In contrast, the present membrane-bound actin network and the polarized (i.e., symmetry broken) actomyosin network is the conserved feature of the migratory cells [46], we regarded this migratory droplet as a simplified model of the cell migration and studied the physical mechanism of the actomyosin droplet migration.

Notably, the actomyosin droplets can only migrate when confined between the two glass substrates, suggesting that the contractile force of the actin flow must be transmitted to the external substrates to propel the droplet forward. To test this hypothesis, we developed force transmission microscopy inspired by the pioneering work of cell migration by Abercrombie [47]. The fluorescent tracer beads were placed at the interface between the droplet and the substrate, and the motion of the beads was observed when actin flow was generated (Fig. 4B). The motion of the beads can be a reporter of the extent to which the contractile forces of the actomyosin network is transmitted to the external environment. Indeed, the beads moved with the actin flow in the presence of actin-membrane binding; in contrast, the beads cannot move without actin-membrane binding. Therefore, this result suggests that the actin flow can generate the friction force at the interface between the droplet and the external substrates in the presence of the actin-membrane binding, termed as the sliding friction force F_{fric}. The sliding friction force propels the droplet forward by balancing with the hydrodynamic resistance from the surrounding fluid environment (Fig. 4C).

To further understand the effect of geometric confinement on droplet migration, we analyzed a theoretical model of droplet migration under confined spaces. In the force-free condition, the force balance between the sliding friction force and the fluid drag determines the migration speed; thus, the migration speed of the droplet V_{drop} can be written as a function of the chamber height h and the droplet size D:

$$V_{\mathrm{drop}} = \frac{\alpha v_{\mathrm{act}}}{2\eta_{\mathrm{oil}}} h \left(1 + \left(\frac{h}{D}\right)^2\right)^{-1} \left(1 - \frac{h}{D} f(\theta)\right)^2, \tag{2}$$

where α is the friction coefficient, v_{act} is the speed of the actin flow, η_{oil} is the viscosity of the surrounding oil, and $f(\theta)$ is the geometric constant determined by the contact angle θ of the droplet. This model predicted that the geometric parameters of the droplet uniquely determine the migration speed of the droplet. We experimentally tested the theoretical model by changing the chamber height h and droplet size D (Fig. 4D). On the one hand, the larger droplet moved faster, suggesting that the larger contact area produces the larger friction force (Fig. 4D, (iii) to (i)). On the other hand, the narrower chamber (smaller h) decreased the droplet speed. This model interprets this behavior that the narrower chamber increases the hydrodynamic resistance (Fig. 4D, (iii) to (ii)). Together, the experimentally observed geometry dependence of the migration speed is consistent with the theoretical model, uncovering the relationship between migration speed and the force transmission constrained by the external environment. Although actomyosin reconstitution has a long history, this study was the first study to realize actomyosin-driven motility in artificial cells. Thus, this versatile platform will help to study how the complex behavior of cell migration can emerge from the simple elements, providing significant understanding from cell biology to active gel physics of the self-organization of the actomyosin systems. An important future direction is to study if this geometry dependence holds for the migration of living cells under confined environments.

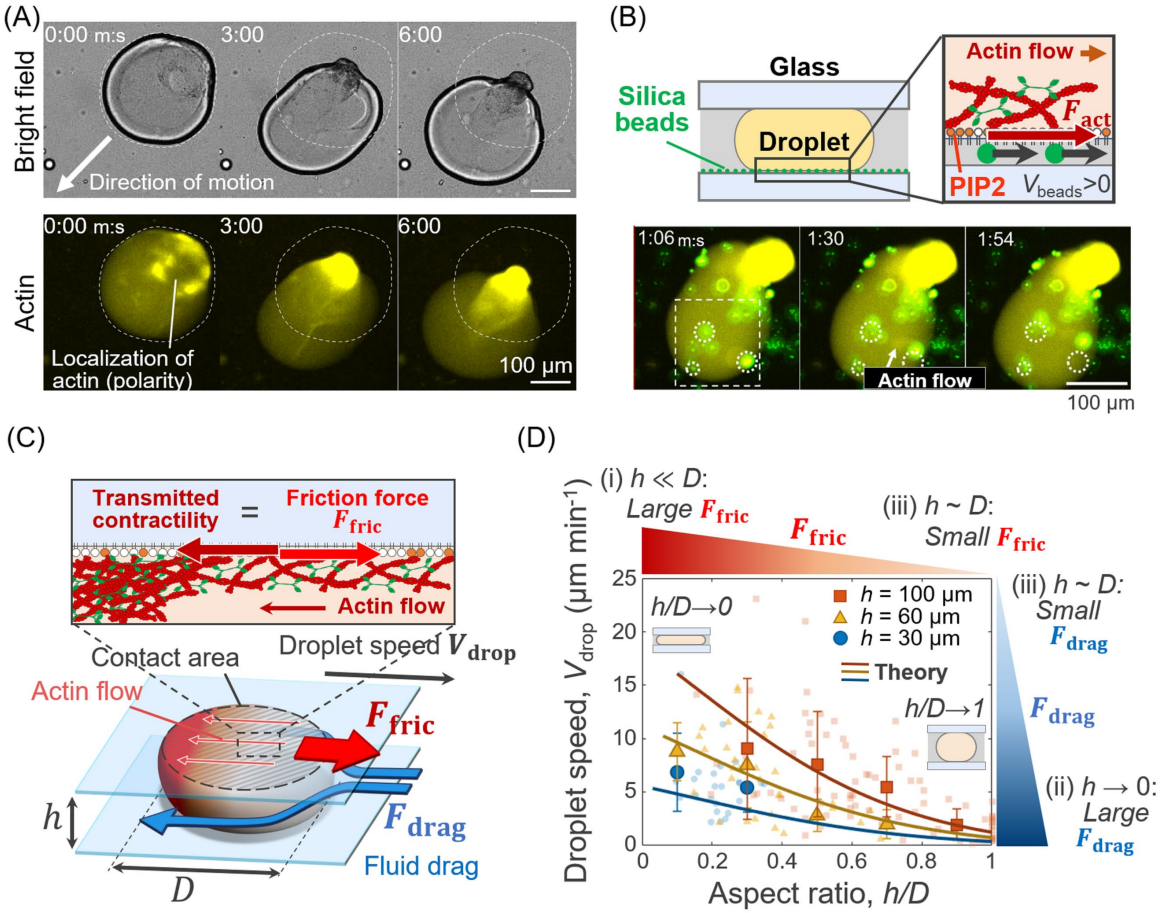

Figure 4 (A) Time-lapse images showing the polarization of the distribution of actin triggered the spontaneous droplet migration under the height of a 60 μm chamber. The white dashed lines represent the initial droplet outlines. (B) Time-lapse images showing the motion of the beads accompanied by the actin flow in the presence of the actin-membrane binding via PIP_2. The white dotted circles represent the initial position of the beads. (C) Schematic showing the droplet migration mechanism. The contractile force of the actin flow is transmitted to the substrate via actin-membrane binding, by which the sliding friction force counteracts the droplet. On the other hand, the migrating droplet experiences fluid drag from the surrounding environment. (D) Aspect ratio h/D dependence of the droplet speed. The scatter plot shows individual data for the different heights, and the larger symbols with error bars are binned-averaged data with mean +/- SD. Solid lines correspond to the theoretical model Eq. (2). This figure is modified from [22].

Symmetry Breaking of the Contractile Patterns of the Confined Actomyosin Networks

Studies in the previous sections mainly focused on the action of actomyosin on cellular objects, such as the nucleus-like cluster inside the droplet and the membrane interface at the droplet boundary. Although the force-generating actomyosin plays pivotal roles in controlling the position of the nucleus and changing the shape as well as driving the motion of the cells, the contractile force of the actomyosin network itself has significant importance in realizing those biological functions. For example, the symmetry breaking of the actin flow within the cell determines the direction of migration [3], and the rotational actin waves stir the mitochondrial network and ensure their equal partition during cell division [48]. Thus, the realization of the cell function crucially relies on the change in the self-organized patterns of the actomyosin networks. However, it still needs to be understood how the actomyosin networks confined within cell-sized spaces change the spatial-temporal behaviors and what are the physical and molecular determinants of the transition between the distinct self-organized patterns.

To understand the physical mechanism of self-organization in the actomyosin networks confined within the cell, we constructed a simple experimental model of the transition among the distinct dynamics of actomyosin contraction, namely the transition between the steady actin flow state and the periodic wave state (Fig. 5A) [21]. We characterized the critical

components determining the transition of the contractile behaviors of the confined actomyosin system. Specifically, molecular perturbation experiments using the actin polymerization inhibitor (Cytochalasin D) revealed that the slower polymerization speed is necessary to transform the steady actin flow to the periodic actin waves, suggesting that a large contrast of the actomyosin density between the bulk region and surface area within the droplet enabling the contractile waves to form. Moreover, the larger contractility induced the transition from the steady actin flow to the periodic actin waves, which is a typical characteristic of the contractile instability of the actomyosin system shown in previous theoretical studies [49-51]. Using these molecular perturbations, we elucidated the physical determinants of the contractile behaviors of the actomyosin system, that is, the (i) contrast between the volume and surface actin polymerization rate, (ii) contractility of myosin (Fig. 5B).

To test if the experimentally found physical determinants are sufficient to induce the transition between the steady actin flow and the periodic actin waves, we developed a simple phase field model of the contractile actomyosin system (active fluid model), in which the actomyosin system confined within the circular boundary is described as a contractile viscoelastic gel. The dynamics of actomyosin contraction in this model is mainly determined by the effective contractility, that is the active Péclet number, Pe (i.e., the ratio of the active transport timescale and passive diffusive relaxation timescale), and the polymerization rate at the surface. The numerical simulation of the active fluid model reproduced the experimentally observed state transition from the steady actin flow to the periodic actin waves with increased contractility and reduced polymerization rate (Fig. 5C). Furthermore, the developed numerical simulation predicted the time delay of the contraction could induce the rotational symmetry breaking of the periodic actin waves, undergoing rotational waves (Fig. 5D). Although some droplets transiently showed rotating spiral wave-like behaviors in experiments, such rotational

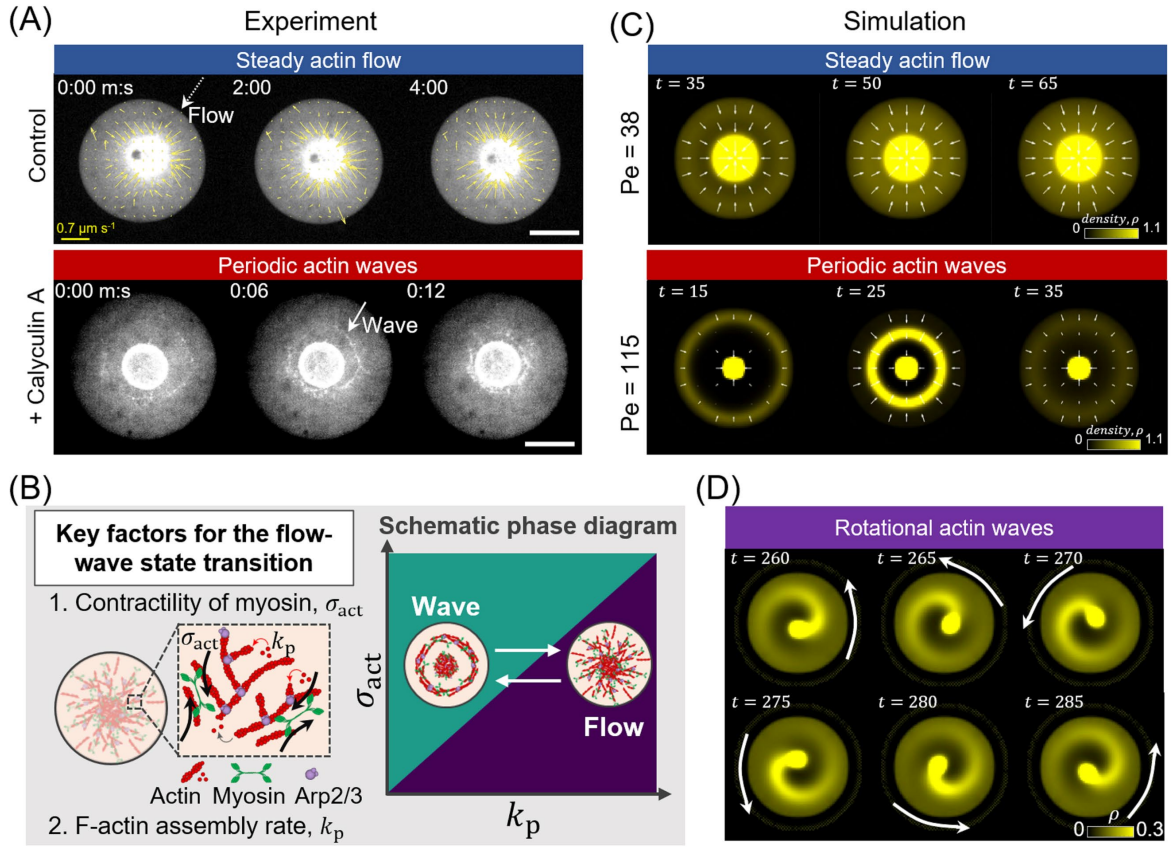

Figure 5 (A) Time-lapse images showing the steady actin flow under a control condition (top), and the periodic actin waves with the addition of Calyculin A enhances myosin motor activity by inhibiting dephosphorylation of myosin regulatory light chain carried out by phosphatases (bottom). Scale bars are 100 μm. (B) Schematic showing the experimentally determined critical factors for the flow-to-wave state transition, myosin contractility σ_{act} and F-actin polymerization rate k_p. The active Péclet number scales with myosin contractility. (C) Numerical simulation of the steady actin flow at the small active Péclet number Pe = 38 (top), and the periodic actin waves at the higher active Péclet number Pe = 115 (bottom). (D) Time-lapse images of the rotational waves in the numerical simulation. This figure is modified from [21].

symmetry breaking has not been experimentally reproduced systematically yet. This could be because the rotational waves are only stable at the phase boundary between the periodic waves and actin flow in numerical simulation. Realizing the rotational waves in the cytoplasmic extracts will be an interesting future challenge.

Conclusion and Perspectives

The artificial cells reproduced various cell-like behaviors exhibiting symmetry breaking. The simplified nature of this experimental system allows one to elucidate the physical principles often hidden by the complexity of living systems. The positioning symmetry breaking is controlled through the two distinct self-organized structures of the actomyosin confined within the cell-sized boundary, in which the different maturation timescales between the actin waves and actin bridge determines the transition point. Furthermore, the autonomous motion of the actomyosin droplet was realized by combining the positioning symmetry breaking with the deformation of the droplet shape. The simplified nature of the migratory actomyosin droplets leads to the relationship connecting the migration speed with the geometric parameters of the confinement, providing a physical principle underlying the actomyosin-driven migration under a confined environment. These symmetry breaking events are ultimately controlled through the self-organization of the actomyosin system confined within the cell boundary. Utilizing simplified artificial systems aids in developing theoretical models that elucidate the emergence of continuous flow, periodic ring-shaped waves, and rotational waves. These physical understanding will help to understand how the symmetry breaking of the actomyosin system can give rise to complex behaviors akin to those observed in living cells, such as nucleus positioning and cell migration. Thus, our findings may pave the way toward an integrative understanding of the rich complexity of living cells based on symmetry and symmetry breaking.

Although the droplet system allows us many sample-size and easy preparations, the droplet surrounded by an oil phase limits the relevance to the situation in living cells, which are covered with a lipid bilayer membrane and surrounded by an aqueous phase. To explore the interplay between the actomyosin dynamics and the deformation of the lipid bilayer membrane, such as cell division and lamellipodia/filopodia protrusions [52], the giant unilamellar vesicles (GUVs) will be a promising future challenge. Using artificial cells will further help us understand universal biological and physical principles underlying complex cell behaviors.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

R.S. and Y.T.M. wrote the manuscript.

Data Availability

Data supporting the findings of this manuscript are available from the corresponding author upon reasonable request.

Acknowledgements

This work was supported by a fellowship from Japan Society for the Promotion of Science (grant no. JP19J20035 to R.S.), Overseas Postdoctoral Fellowships of the Uehara Memorial Foundation (to R.S.), Grant-in-Aid for Scientific Research(B) (Grant No. 20H01872 to Y.T.M.), Grant-in-Aid for Scientific Research on Innovative Areas “Molecular Engines” (Grant No. 18H05427 to Y.T.M., Grant No. 19H05393), Grant-in-Aid for Challenging Research (Exploratory) (Grant No.21K18605 to Y.T.M.) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, Human Frontier Science Program Research Grant (Grant No. RGP0037/2015 to Y.T.M.), Grant for Basic Science Research Project from The Sumitomo Foundation (to Y.T.M.).

References

[1] Sedzinski, J., Biro, M., Oswald, A., Tinevez, J. Y., Salbreux, G., Paluch, E. Polar actomyosin contractility destabilizes the position of the cytokinetic furrow. Nature 476, 462-446 (2011). https://doi.org/10.1038/nature10286

[2] Schuh, M., Ellenberg, J. A new model for asymmetric spindle positioning in mouse oocytes. Curr. Biol. 18, 1986-1992 (2008). https://doi.org/10.1016/j.cub.2008.11.022

[3] Yam, P. T., Wilson, C. A., Ji, L., Hebert, B., Barnhart, E. L., Dye, N. A., et al. Actin-myosin network reorganization breaks symmetry at the cell rear to spontaneously initiate polarized cell motility. J. Cell Biol. 178, 1207-1221 (2007). https://doi.org/10.1083/jcb.200706012
[4] Tee, Y. H., Shemesh, T., Thiagarajan, V., Hariadi, R. F., Anderson, K. L., Page, C., et al. Cellular chirality arising from the self-organization of the actin cytoskeleton. Nat. Cell Biol. 17, 445-457 (2015). https://doi.org/10.1038/ncb3137
[5] Tee, Y. H., Goh, W. J., Yong, X., Ong, H. T., Hu, J., Tay, I. Y. Y., et al. Actin polymerisation and crosslinking drive left-right asymmetry in single cell and cell collectives. Nat. Commun. 14, 776 (2023). https://doi.org/10.1038/s41467-023-35918-1
[6] Kondo, S., Miura, T. Reaction-diffusion model as a framework for understanding biological pattern formation. Science 329, 1616-1620 (2010). https://doi.org/10.1126/science.1179047
[7] Pontani, L. L., van der Gucht, J., Salbreux, G., Heuvingh, J., Joanny, J. F., Sykes, C. Reconstitution of an actin cortex inside a liposome. Biophys. J. 96, 192-198 (2009). https://doi.org/10.1016/j.bpj.2008.09.029
[8] Carvalho, K., Tsai, F. C., Lees, E., Voituriez, R., Koenderink, G. H., Sykes, C. Cell-sized liposomes reveal how actomyosin cortical tension drives shape change. Proc. Natl. Acad. Sci. U.S.A. 110, 16456-16461 (2013). https://doi.org/10.1073/pnas.1221524110
[9] Miyata, H., Hotani, H. Morphological changes in liposomes caused by polymerization of encapsulated actin and spontaneous formation of actin bundles. Proc. Natl. Acad. Sci. U.S.A. 89, 11547-11551 (1992) https://doi.org/10.1073/pnas.89.23.11547
[10] Miyata, H., Kinosita, K., Jr. Transformation of actin-encapsulating liposomes induced by cytochalasin d. Biophys. J. 67, 922-928 (1994). https://doi.org/10.1016/S0006-3495(94)80555-7
[11] Miyata, H., Nishiyama, S., Akashi, K., Kinosita, K., Jr. Protrusive growth from giant liposomes driven by actin polymerization. Proc. Natl. Acad. Sci. U.S.A. 96, 2048-2053 (1999) https://doi.org/10.1073/pnas.96.5.2048
[12] Simon, C., Kusters, R., Caorsi, V., Allard, A., Abou-Ghali, M., Manzi, J., et al. Actin dynamics drive cell-like membrane deformation. Nat. Phys. 15, 602-609 (2019). https://doi.org/10.1038/s41567-019-0464-1
[13] Guevorkian, K., Manzi, J., Pontani, L. L., Brochard-Wyart, F., Sykes, C. Mechanics of biomimetic liposomes encapsulating an actin shell. Biophys. J. 109, 2471-2479 (2015). https://doi.org/10.1016/j.bpj.2015.10.050
[14] Sakamoto, R., Banerjee, D. S., Yadav, V., Chen, S., Gardel, M., Sykes, C., et al. Membrane tension induces f-actin reorganization and flow in a biomimetic model cortex. Commun. Biol. 6, 325 (2023). https://doi.org/10.1038/s42003-023-04684-7
[15] Field, C. M., Wuhr, M., Anderson, G. A., Kueh, H. Y., Strickland, D., Mitchison, T. J. Actin behavior in bulk cytoplasm is cell cycle regulated in early vertebrate embryos. J. Cell Sci. 124, 2086-2095 (2011). https://doi.org/10.1242/jcs.082263
[16] Pinot, M., Steiner, V., Dehapiot, B., Yoo, B. K., Chesnel, F., Blanchoin, L., et al. Confinement induces actin flow in a meiotic cytoplasm. Proc. Natl. Acad. Sci. U.S.A. 109, 11705-11710 (2012). https://doi.org/10.1073/pnas.1121583109
[17] Abu Shah, E., Keren, K. Symmetry breaking in reconstituted actin cortices. eLife 3, e01433 (2014). https://doi.org/10.7554/eLife.01433
[18] Tan, T. H., Malik-Garbi, M., Abu-Shah, E., Li, J. N., Sharma, A., MacKintosh, F. C., et al. Self-organized stress patterns drive state transitions in actin cortices. Sci. Adv. 4, eaar2847 (2018). https://doi.org/10.1126/sciadv.aar2847
[19] Malik-Garbi, M., Ierushalmi, N., Jansen, S., Abu-Shah, E., Goode, B. L., Mogilner, A., et al. Scaling behaviour in steady-state contracting actomyosin networks. Nat. Phys. 15, 509-516 (2019). https://doi.org/10.1038/s41567-018-0413-4
[20] Sakamoto, R., Tanabe, M., Hiraiwa, T., Suzuki, K., Ishiwata, S., Maeda, Y. T., et al. Tug-of-war between actomyosin-driven antagonistic forces determines the positioning symmetry in cell-sized confinement. Nat. Commun. 11, 3063 (2020). https://doi.org/10.1038/s41467-020-16677-9
[21] Sakamoto, R., Miyazaki, M., Maeda, Y. T. State transitions of a confined actomyosin system controlled through contractility and polymerization rate. Phys. Rev. Research 5, 013208 (2023). https://doi.org/10.1103/PhysRevResearch.5.013208
[22] Sakamoto, R., Izri, Z., Shimamoto, Y., Miyazaki, M., Maeda, Y. T. Geometric trade-off between contractile force and viscous drag determines the actomyosin-based motility of a cell-sized droplet. Proc. Natl. Acad. Sci. U.S.A. 119, e2121147119 (2022). https://doi.org/doi:10.1073/pnas.2121147119
[23] Ierushalmi, N., Malik-Garbi, M., Manhart, A., Abu Shah, E., Goode, B. L., Mogilner, A., et al. Centering and symmetry breaking in confined contracting actomyosin networks. eLife 9, 55368 (2020). https://doi.org/10.7554/eLife.55368
[24] Bermudez, J. G., Chen, H., Einstein, L. C., Good, M. C. Probing the biology of cell boundary conditions through

confinement of xenopus cell-free cytoplasmic extracts. Genesis 55, e23013 (2017). https://doi.org/10.1002/dvg.23013
[25] Kelkar, M., Bohec, P., Charras, G. Mechanics of the cellular actin cortex: From signalling to shape change. Curr. Opin. Cell Biol. 66, 69-78 (2020). https://doi.org/10.1016/j.ceb.2020.05.008
[26] Yi, K. X., Rubinstein, B., Unruh, J. R., Guo, F. L., Slaughter, B. D., Li, R. Sequential actin-based pushing forces drive meiosis i chromosome migration and symmetry breaking in oocytes. J. Cell Biol. 200, 567-576 (2013). https://doi.org/10.1083/jcb.201211068
[27] Sparkes, I. Recent advances in understanding plant myosin function: Life in the fast lane. Mol. Plant 4, 805-812 (2011). https://doi.org/10.1093/mp/ssr063
[28] Knecht, D. A., Loomis, W. F. Antisense rna inactivation of myosin heavy-chain gene-expression in dictyostelium-discoideum. Science 236, 1081-1086 (1987). https://doi.org/10.1126/science.3576221
[29] Pillitteri, L. J., Guo, X. Y., Dong, J. Asymmetric cell division in plants: Mechanisms of symmetry breaking and cell fate determination. Cell. Mol. Life Sci. 73, 4213-4229 (2016). https://doi.org/10.1007/s00018-016-2290-2
[30] Arai, Y., Shibata, T., Matsuoka, S., Sato, M. J., Yanagida, T., Ueda, M. Self-organization of the phosphatidylinositol lipids signaling system for random cell migration. Proc. Natl. Acad. Sci. U.S.A. 107, 12399-12404 (2010). https://doi.org/10.1073/pnas.0908278107
[31] Valentine, M. T., Perlman, Z. E., Mitchison, T. J., Weitz, D. A. Mechanical properties of xenopus egg cytoplasmic extracts. Biophys. J. 88, 680-689 (2005). https://doi.org/10.1529/biophysj.104.048025
[32] Bendix, P. M., Koenderink, G. H., Cuvelier, D., Dogic, Z., Koeleman, B. N., Brieher, W. M., et al. A quantitative analysis of contractility in active cytoskeletal protein networks. Biophys. J. 94, 3126-3136 (2008). https://doi.org/10.1529/biophysj.107.117960
[33] Mogessie, B., Scheffler, K., Schuh, M. Assembly and positioning of the oocyte meiotic spindle. Annu. Rev. Cell Dev. Biol. 34, 381-403 (2018). https://doi.org/10.1146/annurev-cellbio-100616-060553
[34] McNally, F. J. Mechanisms of spindle positioning. J. Cell Biol. 200, 131-140 (2013). https://doi.org/10.1083/jcb.201210007
[35] Bezanilla, M., Wadsworth, P. Spindle positioning: Actin mediates pushing and pulling. Curr. Biol. 19, R168-R169 (2009). https://doi.org/10.1016/j.cub.2008.12.026
[36] Brunet, S., Maro, B. Germinal vesicle position and meiotic maturation in mouse oocyte. Reproduction 133, 1069-1072 (2007). https://doi.org/10.1530/rep-07-0036
[37] Levi, M., Ghetler, Y., Shulman, A., Shalgi, R. Morphological and molecular markers are correlated with maturation-competence of human oocytes. Hum. Reprod. 28, 2482-2489 (2013). https://doi.org/10.1093/humrep/det261
[38] Leader, B., Lim, H., Carabatsos, M. J., Harrington, A., Ecsedy, J., Pellman, D., et al. Formin-2, polyploidy, hypofertility and positioning of the meiotic spindle in mouse oocytes. Nat. Cell Biol. 4, 921-928 (2002). https://doi.org/10.1038/ncb880
[39] Li, H. B., Guo, F. L., Rubinstein, B., Li, R. Actin-driven chromosomal motility leads to symmetry breaking in mammalian meiotic oocytes. Nat. Cell Biol. 10, 1301-1308 (2008). https://doi.org/10.1038/ncb1788
[40] Yi, K., Unruh, J. R., Deng, M. Q., Slaughter, B. D., Rubinstein, B., Li, R. Dynamic maintenance of asymmetric meiotic spindle position through arp2/3-complex-driven cytoplasmic streaming in mouse oocytes. Nat. Cell Biol. 13, 1252-1258 (2011). https://doi.org/10.1038/ncb2320
[41] Fukami, K., Endo, T., Imamura, M., Takenawa, T. Alpha-actinin and vinculin are pip(2)-binding proteins involved in signaling by tyrosine kinase. J. Biol. Chem. 269, 1518-1522 (1994). https://doi.org/10.1016/S0021-9258(17)42287-3
[42] Kelley, C. F., Litschel, T., Schumacher, S., Dedden, D., Schwille, P., Mizuno, N. Phosphoinositides regulate force-independent interactions between talin, vinculin, and actin. eLife 9, 56110 (2020). https://doi.org/10.7554/eLife.56110
[43] Saarikangas, J., Zhao, H., Lappalainen, P. Regulation of the actin cytoskeleton-plasma membrane interplay by phosphoinositides. Physiol. Rev. 90, 259-289 (2010). https://doi.org/10.1152/physrev.00036.2009
[44] Sanchez, T., Chen, D. T., DeCamp, S. J., Heymann, M., Dogic, Z. Spontaneous motion in hierarchically assembled active matter. Nature 491, 431-434 (2012). https://doi.org/10.1038/nature11591
[45] Nishigami, Y., Ichikawa, M., Kazama, T., Kobayashi, R., Shimmen, T., Yoshikawa, K., et al. Reconstruction of active regular motion in amoeba extract: Dynamic cooperation between sol and gel states. PLoS One 8, e70317 (2013). https://doi.org/10.1371/journal.pone.0070317
[46] Ruprecht, V., Wieser, S., Callan-Jones, A., Smutny, M., Morita, H., Sako, K., et al. Cortical contractility triggers a stochastic switch to fast amoeboid cell motility. Cell 160, 673-685 (2015). https://doi.org/10.1016/j.cell.2015.01.008
[47] Abercrombie, M., Heaysman, J. E., Pegrum, S. M. The locomotion of fibroblasts in culture. 3. Movements of

particles on the dorsal surface of the leading lamella. Exp. Cell Res. 62, 389-398 (1970). https://doi.org/10.1016/0014-4827(70)90570-7
[48] Moore, A. S., Coscia, S. M., Simpson, C. L., Ortega, F. E., Wait, E. C., Heddleston, J. M., et al. Actin cables and comet tails organize mitochondrial networks in mitosis. Nature 591, 659-664 (2021). https://doi.org/10.1038/s41586-021-03309-5
[49] Bois, J. S., Julicher, F., Grill, S. W. Pattern formation in active fluids. Phys. Rev. Lett. 106, 028103 (2011). https://doi.org/10.1103/PhysRevLett.106.028103
[50] Kumar, K. V., Bois, J. S., Julicher, F., Grill, S. W. Pulsatory patterns in active fluids. Phys. Rev. Lett. 112, 208101 (2014). https://doi.org/10.1103/PhysRevLett.112.208101
[51] Moore, T., Wu, S. K., Michael, M., Yap, A. S., Gomez, G. A., Neufeld, Z. Self-organizing actomyosin patterns on the cell cortex at epithelial cell-cell junctions. Biophys. J. 107, 2652-2661 (2014). https://doi.org/10.1016/j.bpj.2014.10.045
[52] Caswell, P. T., Zech, T. Actin-based cell protrusion in a 3d matrix. Trends Cell Biol. 28, 823-834 (2018). https://doi.org/10.1016/j.tcb.2018.06.003