Actomyosin-driven left-right asymmetry: from molecular torques to chiral self organization

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Chirality or mirror asymmetry is a common theme in biology found in organismal body plans, tissue patterns and even in individual cells. In many cases the emergence of chirality is driven by actin cytoskeletal dynamics. Although it is well established that the actin cytoskeleton generates rotational forces at the molecular level, we are only beginning to understand how this can result in chiral behavior of the entire actin network *in vivo*. In this review, we will give an overview of actin driven chiralities across different length scales known until today. Moreover, we evaluate recent quantitative models demonstrating that chiral symmetry breaking of cells can be achieved by properly aligning molecular-scale torque generation processes in the actomyosin cytoskeleton.

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Introduction

An object is said to be chiral, mirror asymmetric or handed, if its mirror image is non-superimposable on itself. Most animal body plans are chiral with left-right (LR) asymmetric internal organ arrangements that are consistent between individuals [1]. Moreover, chirality of biological systems can emerge across multiple length scales. Directional looping of the heart [2] and intestine [3] are common examples of tissue chirality. At the cellular level, LR asymmetric polarization behavior has been observed in various cultured cells [4,5]. Finally, all

biological macromolecules and many interactions between them, for example receptor–ligand binding, display chiral features [6].

Chirality of an organism must derive from the chirality of constituents. Hence Brown and Wolpert hypothesized that embryonic LR symmetry breaking is facilitated by aligning putative chiral molecules (termed F-molecules) with respect to the anteroposterior and dorsoventral axes [7]. In such a way, micro-scale chirality is transformed to chirality at cell-and tissue (macro) scales. For example, during gastrulation in many vertebrate species, a cilia-driven leftward extracellular fluid flow in the ventral node ultimately triggers LR asymmetric gene expression in the entire body [8–10]. In this case the F-molecule takes the form of a cilium, which beats in a chiral fashion [11]. However, for many other instances of LR symmetry breaking it is not as clear how chirality at the molecular scale determines chirality at the cell and tissue scale. Although the generic F-molecule has not been identified yet, common to many chiral symmetry breaking events is a requirement of the cytoskeleton [12]. This raises the question whether cytoskeletal dynamics could fulfill the role of Brown and Wolpert's F-molecule.

In this review, we will focus on chiral morphogenesis of cells, tissues and organisms that are driven by the actomyosin cytoskeleton. At the molecular level, processes in the actin cytoskeleton can generate rotational forces [13–17]. Several recent biophysical studies have linked these molecular torques to chiral organization of the entire actin network [18••,19••]. We now argue that proper alignment of molecular torques generated by the actin cytoskeleton can facilitate chiral symmetry breaking of cells, tissues and organisms.

Actomyosin drives cellular and multicellular chiral processes

Early organismal LR symmetry breaking

In several model organisms, LR symmetries are broken already during early embryogenesis when embryos consist of just a few cells. Here, we discuss examples where the actin cytoskeleton drives these early symmetry breaking events. In the small nematode *Caenorhabditis elegans*, it was recently discovered that the actomyosin cortex plays a crucial role in LR symmetry breaking [19••]. During the 4–6 cell stage transition, two blastomeres, whose spindles are set along the LR axis (see Figure 1a), rotate in a
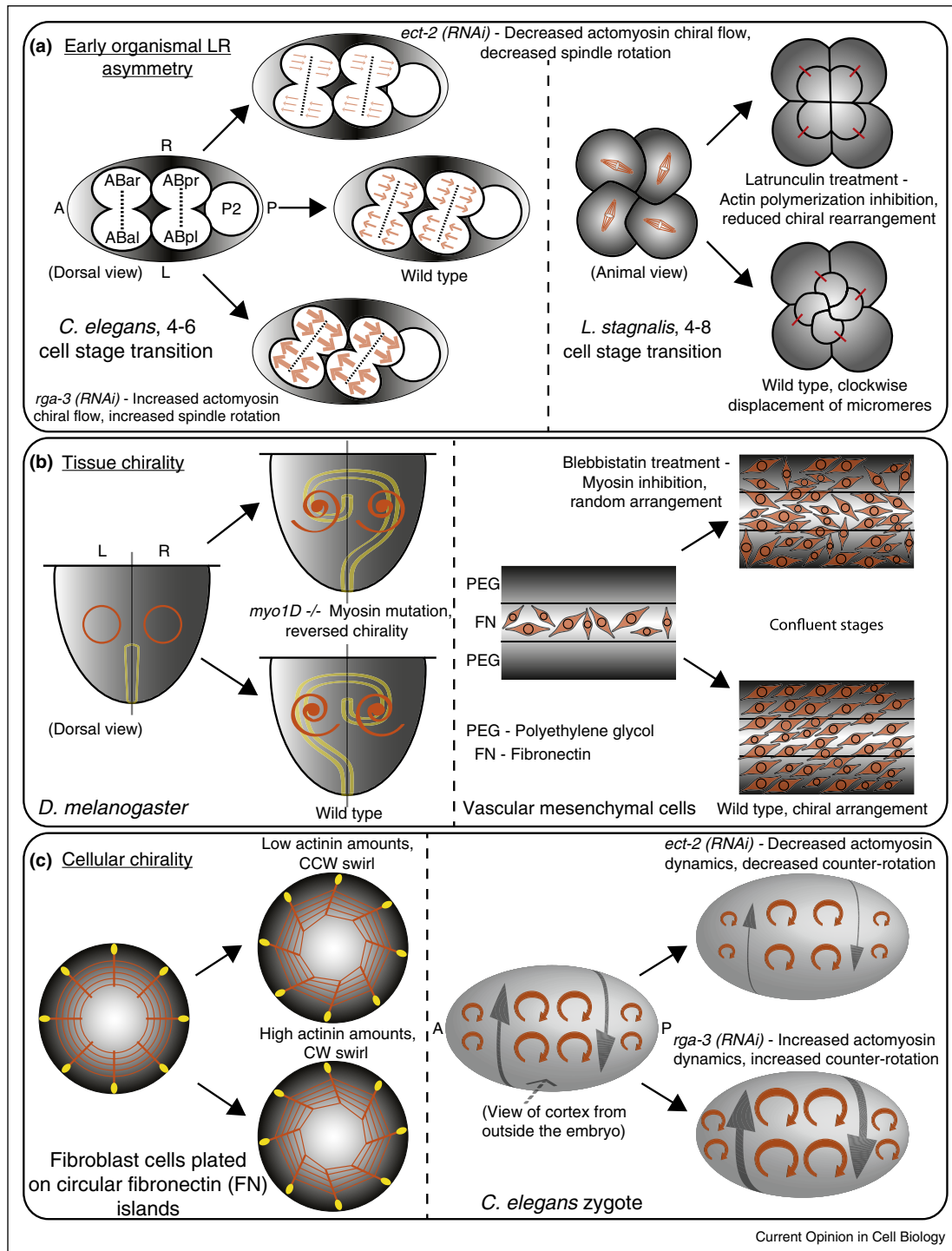
Figure 1

Illustration of left-right (LR) asymmetries across different length scales. **(a)** Early organismal LR asymmetry: *C. elegans* — Two cells at the 4-cell stage, while dividing into ABar-ABal and ABpr-ABpl, exhibit a clockwise spindle rotation (when viewed dorsally). This is driven by counter-rotating cortical flows (beige arrows), the magnitude of which is controlled by the Rho signaling pathway, with *ect-2/rga-3 (RNAi)* leading to Rho inactivation/activation respectively. *Lymnaea stagnalis* — A dextral species exhibits a clockwise displacement of emerging micromeres (red lines indicate corresponding macromeres) at the 4–8 cell stage transition, driven by the actin cytoskeleton. Spindles in beige are arranged in a clockwise fashion. **(b)** Tissue chirality: *Drosophila melanogaster* — Chiral asymmetries of tissues that are under the control of the actomyosin cytoskeleton. Hindgut in yellow, testes in beige. Mutating *myosin 1D* leads to reversed chiralities. Tissue culture — Chiral arrangement of confluent vascular mesenchymal cells plated on polyethylene glycol/fibronectin plates controlled by the actin cytoskeleton. **(c)** Cellular chirality: Cultured fibroblasts — Upon assembly, the actin cytoskeleton displays a chiral swirl

clockwise fashion when viewed dorsally [20,21]. This gives rise to a chiral 6-cell configuration that marks the initiation of LR asymmetry in the embryo. The actomyosin cortex in these rotating blastomeres exhibits chiral counter-rotating cortical flows along the division axes (beige arrows in Figure 1a). Importantly, changing the counter-rotation flow speed changes the degree of blastomere rotation (dashed lines in Figure 1a) [19••], indicating that chiral flows execute LR symmetry breaking in this system.

A particularly interesting chiral pattern of early development is found in spiralians, a clade that includes snails and annelid worms [22,23]. At the 4–8 cell stage transition in snails, all blastomeres rotate their axis of division such that their daughter cells are displaced in either a clockwise (CW) or counter-clockwise (CCW) fashion (Figure 1a). Eventually, this determines handedness of the body plan [24,25]. Mild chemical inhibition of actin assembly abolished these chiral rearrangements, demonstrating that actomyosin plays an important role (Figure 1a) [24]. However, it is unknown whether chiral actomyosin flows, similar to the ones in *C. elegans* embryos, facilitate symmetry breaking in spiralians.

Finally, in the vertebrate *Xenopus laevis*, the actin cytoskeleton is involved in chiral symmetry breaking during the first cell division [26-28]. It was observed that the frog cortex displays a subtle counter-rotating motion that when enhanced by drug treatment, affects LR asymmetry of the adult body plan [29]. Altogether, actin-driven chiral cell movements during early embryogenesis guide organismal LR patterning in various animals.

Tissue chirality

Individual tissues also exhibit chiral asymmetries that are dependent on the actomyosin cytoskeleton. Noël *et al.* revealed that chiral looping of the zebrafish heart was maintained *ex vivo* in cultured heart explants and attenuation of actomyosin activity abolished this chiral behavior [30••]. Moreover, several tissues in *Drosophila* (gut, spermiduct, testes, male terminalia) exhibit LR asymmetries and chiral morphogenesis, which upon mutation of *myosin 1D (myo1D)* exhibit reversed lateralizations [31••,32–35] (Figure 1b). Whether actin or actomyosin dynamics play a direct role in these processes remains to be established. Therefore, the *myo1D* mutant is vital for a further understanding of the mechanisms by which tissue asymmetries emerge. Interestingly, it was suggested that tissue-scale chirality can emerge from chiral shape and chiral polarity of individual cells [36–38,39•].

Chiral behaviors are also observed in tissue culture cells at confluent stages [4,40•,41]. Wan *et al.* showed that numerous cell types, when cultured on circular micropatterns, aligned in either a CW or CCW fashion. Notably, when exposed to actin-interfering drugs (latrunculin A, cytochalsin D, jasplakinolide but not blebbistatin), cell lines that displayed CCW alignment tended to reverse their chirality [42]. Another study [4] showed that vascular smooth muscle cells grown to confluency on micropatterned stripes aligned in a consistent chiral manner that could be abolished by drug treatments targeting non-muscle myosin II or Rho signaling (Figure 1b). Taken together, these findings suggest that tissue-scale chirality likely emerges from actomyosin activity in individual cells interacting with physical boundaries imposed by either the surrounding tissues *in vivo* or the micropatterned surface *in vitro*.

Cell chirality

Individual cells are known to exhibit chiral asymmetries, which also depend on intracellular actomyosin activity [5,18••,43,44]. A recent study spear-headed by the Bershadsky group showed that fibroblasts on micropatterned round surfaces display striking chiral rearrangements [18••]. When the fibroblasts settled on the micropattern, cells displayed a dynamic chiral self-assembly of the actin cytoskeleton with a CCW swirl. The handedness of the pattern is determined by the actin filament crosslinking protein, α-actinin (Figure 1c). The authors suggest that this chiral behavior is driven by rotational movements generated through formin-mediated actin polymerization at peripheral focal adhesions.

Finally, individual cell chirality was also observed in the *C. elegans* zygote [19••,45•]. During anteroposterior polarization, counter-rotating actomyosin flows were observed. Similar to the counter-rotating flows at the 4–6 cell stage, increasing actomyosin activity could enhance chiral flows (grey arrows in Figure 1c). Taken together, these findings clearly suggest that intracellular actomyosin dynamics are driving chiral asymmetries at organismal, tissue and cellular scales.

Chiral self-organization of the actin cytoskeleton

We next discuss the mechanisms by which chirality emerges in the actomyosin cytoskeleton. For this, consider the simplest way of generating a chiral object from an achiral one: apply a torque to twist it. In a developmental context the torque for this must be self-generated. This raises two questions: are there microscopic processes

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(Figure 1 Legend Continued) (clockwise, CW or counter-clockwise, CCW) in fibroblasts plated on circular micropatterns. Actin fibers are shown in orange and focal adhesions in yellow. *C. elegans* zygote — The actomyosin cortex exhibits clockwise rotations (beige arrows). A gradient in myosin activity leads to counter-rotating cortical flows (black arrows) breaking chiral symmetries. Size/width of the arrows represent magnitude of torques/flow velocities.

in the actin cytoskeleton that generate torques? If so, how could these drive cell chirality?

Molecular-scale torque generation in the actin cytoskeleton

Actin filaments are polar right-handed helices with a barbed (+) and a pointed (−) end and a helical pitch of around 72 nm [46]. Their inherent chirality can facilitate the transduction of molecular-scale torque generation during their polymerization, when they interact with motor molecules and while they depolymerize. *In vitro* experiments showed that, when immobilized on a glass surface, formins rotate actin filaments upon polymerization with a full turn per helical pitch [13] (Figure 2a, left). Molecular torques also arise as a result of chiral interactions between myosin motor proteins and actin filaments (Figure 2a, right) [14–17,47–50]. Moreover, myosin generates tension in the network, which can lead to torque generation through non-trivial tension-torque coupling [51,52]. Finally, the actin depolymerizing protein, cofilin, when bound to actin filaments locally modifies the pitch of the actin helix [53,54], possibly generating torques in the process.
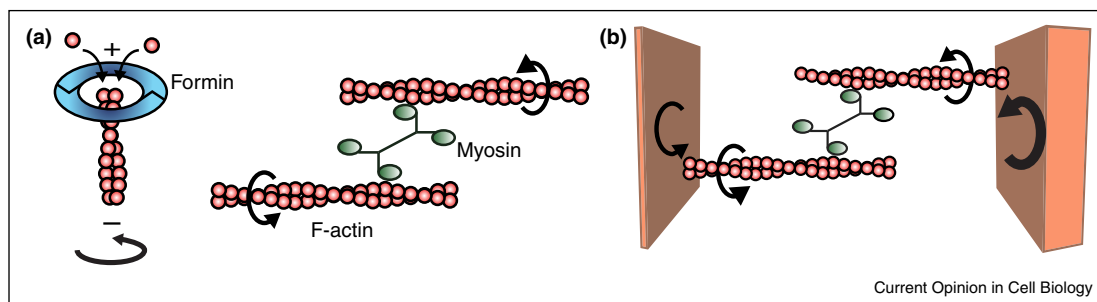
From molecular-scale torque generation to large-scale twist

To unravel how molecular processes can drive chiral self-assembly of the actin cytoskeleton, Tee *et al.* (see section 'Cell chirality' and Figure 1c, left) focused on the molecular scale and characterized both the overall structure and the molecular constituents of the actin cytoskeleton during its assembly [18••,55]. Their analysis showed that contractile forces within transverse fibers together with formin-mediated actin polymerization of radial fibers are key to the dynamic behavior. Since formins generate torques upon actin polymerization [13], they proposed that actin polymerization at the focal adhesions will rotate radial actin fibers. Simulation using a computational model that implemented the actin network topology

together with these forces could faithfully describe the observed chiral self-organization. In this model molecular torques are generated by formins at focal adhesions. Because in these circular fibroblasts focal adhesions are enriched at the cell periphery, molecular torques are thus distributed asymmetrically along the mediolateral axis of the cell (Figure 1c, left). Hence, asymmetric molecular torque generation can result in chiral symmetry breaking.

In many cases where molecular details are not available, a complementary approach is needed to describe large-scale dynamics. Ignoring molecular details, the cortex can be thought of as a thin layer of an active polar gel underneath the cell membrane that has liquid-like properties 56–59. The gel is characterized by a number of large-scale material parameters, which encompass all the details of the underlying molecular processes. Such an approach has been successful for describing large scale rearrangements in the cortical layer [60] or investigating the force balance in the layer [61]. However, in these earlier works there was no chiral component to this thin layer of active liquid material. To investigate how molecular torque generation in this active and liquid-like layer results in chiral flows, Fürthauer *et al.* considered the effects of molecular-scale torque generation in an active gel [62,63•]. Even without detailed knowledge of the microscopic mechanisms, this allows to ask the following question: what are the large-scale effects that emerge from the presence of micro-scale torque generators in the material? It turns out that the constraints imposed by physical conservation laws lead to relatively simple generic equations of motion in which all uncertainties about microscopic interactions are summarized by just a handful of physical parameters that can be measured. For instance, thinking of the *C. elegans* cell cortex as a thin film of an active chiral gel [19••] summarizes the whole complexity of the actin cortex by just its effective viscosity, its tendency to spontaneously contract under the influence of motor proteins, and its chirality, that is its

Figure 2

Torque generation by the actin cytoskeleton. **(a)** Actin polymerization at the barbed end by formin dimers results in rotation of the elongating filament along its axis. While pulling on actin, myosins (green) generate torque dipoles, for example when a myosin filament pulls on two oppositely oriented actin filaments. **(b)** A torque dipole (actin filament) in contact with two unequal surfaces can rotate. In the case of the actomyosin cortex, the two unequal surfaces can be thought of as the cytoplasm and the membrane.

ability to generate torques. Despite its simplicity, this theory quantitatively describes cortical flows using parameters inferred from experiment [19••].

The phenomenological theory [62,63•] allows to identify generic conditions under which microscopic torque generators can produce large-scale twist deformations. The problem boils down to one of symmetry: how to arrange cytoskeletal torque generators such that large-scale chiral flows arise? With regards to the actomyosin cortex, actin filaments are known to be randomly oriented in the cortical layer [64]. However, the inside and outside of the cell are different (cytosol vs. membrane) and the distribution of cortical proteins along the thickness direction is anisotropic. Therefore, the physical boundary conditions between the cortical layer and the inside and the outside of the cell are different. Because of this intrinsic asymmetry along the membrane-cortex-cytosol axis, molecular torque generation can impart angular momentum onto the cortex (Figure 2b) [63•], in a way reminiscent of a skater who pushes against the ice below to make herself spin. This is the first condition for torque generators to cause large scale rotatory motion: a local broken symmetry must exist such that microscopic torque generators can be aligned.

However, if active torques from aligned torque generators are imparted all over the cortical layer homogeneously, no twist deformation could result since all torques cancel globally. This is reminiscent of contractile flow in the cortex, which arises only when some part of the cortex contracts more than the rest, but not when the whole cortex contracts equally [61]. Hence another broken symmetry must exist that specifies the direction around which to twist. In *C. elegans* zygotes, twist deformation is generated by coupling torque generation to the AP axis. Here, a gradient of myosin motor proteins along the AP axis — presumably triggered by the sperm-derived centrosome — leads to a gradient in active torques, which ultimately leads to the observed twist rotation (see Figure 1).

To summarize this section, for molecular-scale torque generators to produce cellular-scale twist deformations two conditions must be met: torque generators must have access to a local asymmetry, for instance the presence of a surface, to allow for local injection of angular momentum into the system, and secondly torque generators must have access to another asymmetry that specifies the global axis of twist. Importantly, this organizing principle needs to hold regardless of the mechanism that generates the torques microscopically. Hence, we expect the same principle to also apply for larger-scale phenomena such as twisting cells organizing into chiral tissues.

Conclusion

When Brown and Wolpert formulated their F-molecule hypothesis they assumed that LR asymmetry emerges due to the alignment of certain chiral molecules along the major body axes [7,11]. We would here like to put forward a slightly modified version and state that an 'F-activity' that is active torques generated by the cytoskeleton, drives those instances of chiral symmetry breaking where actomyosin plays a key role. Given that chiral morphogenesis in many different contexts depends on a functional actin cytoskeleton, the alignment of an F-activity may be a unifying principle guiding diverse LR patterning events. Clearly, the next step is to bridge the gap between molecular torques and large-scale chiral rearrangements, and this requires the development of physical theories that take into account force and torque balances. Finally, the actomyosin cortex is a prominent example of a new class of active chiral materials. Thus, better understanding its physics will be an inspiration not only to biologists but also hopefully to material scientists and engineers.

Intriguingly, even though chirality is an inherent property of cytoskeletal networks, chiral rearrangements appear to mostly occur at specific time points during embryonic development. This means that chiral properties are likely regulated by developmentally significant pathways (such as Wnt signaling as shown in [19••]), which are yet to be characterized with respect to chiral morphogenesis. With respect to chiral deformations in tissues [36–38,39•], an open question is whether the epithelium behaves as a single mechanical entity where active torque generation throughout the actomyosin cortex of the entire epithelium is the cause of all global chiral deformations.

The combination of modern day developmental biology and quantitative physical modeling promises exciting new advancements in our understanding of organismal LR asymmetry and chiral patterning of cells and tissues. Not only will this multidisciplinary approach identify novel components and regulatory principles, it will also yield quantitative mechanistic insights into a phenomenon that has puzzled developmental biologists for decades.

Acknowledgements

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