Cytoskeletal Chirality: Swirling Cells Tell Left from Right

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How developing embryos acquire left–right asymmetry is a tantalizing question of great physiological importance [1,2]. Asymmetric organ morphogenesis follows asymmetric signaling cascades, which in turn follow asymmetric events on the cellular scale — for example, rotating cilia that generate a right-to-left fluid flow in mouse embryos [3]. Underlying such cell-scale asymmetries must be some molecular event that generates chirality — the asymmetric property of a structure whereby it is distinguishable from its mirror image. A prime candidate for the molecular system governing chirality is the cytoskeleton, which contains innately handed molecules, such as the right-handed actin helix. Indeed, cytoskeleton-dependent chiralities have been identified in many organisms, from Drosophila to Caenorhabditis elegans to snails [4] (Figure 1A,B). Unclear, however, is how those individual handed molecules lead to large-scale chirality. A new study by Tee et al. [5] now proposes a concrete mechanical model linking individual cytoskeletal components to cellular left–right asymmetries.

Chirality can emerge at the molecular level, for instance, from anchored myosin motors walking along the helical actin pitch, thereby rotating actin filaments and resulting in the filaments turning in leftward circles [6]. Similarly, the actin nucleator formin rotates actin filaments as it extrudes them, much like the swirl of a soft-serve ice-cream dispenser [7,8] (Figure 1D). The theory of active polar gels predicts that these types of microscopic asymmetries can drive chirality on the cellular and multicellular scales [9].

Figure 1. Chirality at different scales.
(A) Chiral structures are apparent in whole organisms, such as snails (photo: David Huth). (B) In many embryos, the earliest chiral event occurs at the third cell division, when daughter cells self-organize into a clockwise (top row) or anticlockwise (bottom row) spiral structure preceded by asymmetric positioning of the mitotic spindles and division plane (reprinted by permission from [2] Macmillan Publishers Ltd, Nature © 2008). (C) At the leading edge of nerve growth cones, filopodia rotate against the substrate (as shown on the left) and bias the cone’s growth direction to the right, as shown by the positions of the growth cone center (magenta dot) and filopodia tips (green dots), which were tracked relative to the cone’s original direction of growth (image on right reproduced with permission from [12] © 2010). (D) Immobilized formin (orange disc) rotates the helical actin filament in the clockwise direction relative to formin. Red marks indicate the right-handed axis of the actin filament. (From [8], reprinted with permission from AAAS. Adapted from Sase, I., Miyata, H., Ishiwata, S., Kinosita, K. (1997) Axial rotation of sliding actin filaments revealed by single-fluorophore imaging. Proc. Natl. Acad. Sci. USA 94, 5646–5650 © 1997 National Academy of Sciences, USA).
myosin-powered contraction.

Figure 2. Mechanical chiral instability. Anticlockwise rotation of radial actin filaments (green arrow) develops via a ‘rack and pinion’ mechanism (center inset) from an initially radial structure (top left), when formin-nucleated actin filaments (red) are rotated by formins immobilized at focal adhesions (blue circles). A small leftwards tilt (top right) of radial actin filaments, pivoting at the focal adhesions, causes contractile transverse fibers (yellow) on either side of the tilting radial fiber to slide relative to one another (bottom left; yellow arrows illustrate contractile force). This sliding generates a torque imbalance which amplifies the tilt (bottom right).

Unexpectedly, a couple of hours after the cobweb radial pattern emerged, the RFs broke radial symmetry and started to swirl. This swirling lasted for a few hours before giving way to a linear pattern of fibers spanning the whole cell with both ends associated with focal adhesions at the cell edge. Swirling almost always happened in an anticlockwise direction. Serendipitously, Tee et al. [5] noticed that when they overexpressed the crosslinker -actinin-1, swirling was less frequent but could also go in the opposite, i.e. clockwise, direction. These results raise two burning questions: why do the RFs tilt, and why do they overwhelmingly tilt in the anticlockwise direction? Since these questions could not be answered by cell biological or biophysical methods, the authors turned to a simple computational model of TFs as contractile springs that can slide along RFs. The simulations predicted an instability: when RFs undergo a slight tilt relative to the radius of the cell, the tilt tends to increase (Figure 2). Understanding this instability is highly nontrivial, which is why modelling was so crucial. If the TFs were not sliding relative to RFs, then the non-chiral symmetric position of the RFs would have been stable. However, if TF sliding is fast and a RF pivots to the left with respect to the focal adhesion (as shown in Figure 2), then the TFs pulling it to the left slide down to the turning tip of the RF, while the TFs to the right slide closer up towards the focal adhesion. This sliding changes the lever arms for the left- and right-pulling TFs, generating a net torque that tilts the RF further from the radial direction.

This model explains the emergence of swirling, but not its predominantly anticlockwise direction. The authors hypothesized that, as RF filaments grow inwards from formins embedded in focal adhesions, the filaments would rotate in a clockwise direction (if one looks at the filament from its barbed end at the focal adhesion; Figures 1D and 2). If these rotating radial filaments act like gears driving the TFs (Figure 2), then the RFs will propel the TFs in an anticlockwise direction in the cell. Note that in order for this ‘rack and pinion’ explanation to work, TFs have to be located higher than the RFs relative to the substrate, which is not explicitly stated in the new study [5]. What about the clockwise swirling when the -actinin crosslinkers are overexpressed? With high levels of crosslinking, formin-generated twist is stored elastically in the filament tips rather than rotating filaments like a gear, and from time to time this twist is relieved abruptly by quick anticlockwise rotations of the filament, pushing the RFs in the clockwise direction in the cell. Note that the formin-generated twist does not have to be significant — even a slight bias can tweak the tilting instability into a preferred direction. One of the lessons from the model is that torque, a mechanical factor that remains underappreciated but is gathering attention [8,9,18], plays an important role in cells.

Many open questions remain. Are the TFs really located higher than the RFs relative to the substrate? Electron microscopy images are inconclusive. Are actin filaments in RFs really oriented with the substrate? From time to time this twist is relieved abruptly by quick anticlockwise rotations of the filament, pushing the RFs in the clockwise direction in the cell. These gaps in our understanding will no doubt be filled via follow-up studies. There are larger questions: when the swirling actin pattern evolves into the linear pattern, does some part of the chirality survive? What happens to chirality in cells that are geometrically unconstrained? Does this self-organization work in a more physiologically relevant setting, for cell adhesion; Figures 1 D and 2). If these rotating radial filaments act like gears driving the TFs (Figure 2), then the RFs will propel the TFs in an anticlockwise direction in the cell. Note that in order for this ‘rack and pinion’ explanation to work, TFs have to be located higher than the RFs relative to the substrate, which is not explicitly stated in the new study [5]. What about the clockwise swirling when the -actinin crosslinkers are overexpressed? With high levels of crosslinking, formin-generated twist is stored elastically in the filament tips rather than rotating filaments like a gear, and from time to time this twist is relieved abruptly by quick anticlockwise rotations of the filament, pushing the RFs in the clockwise direction in the cell. Note that the formin-generated twist does not have to be significant — even a slight bias can tweak the tilting instability into a preferred direction. One of the lessons from the model is that torque, a mechanical factor that remains underappreciated but is gathering attention [8,9,18], plays an important role in cells.

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example, with cells of highly irregular shape embedded into a 3D extracellular matrix?

Most importantly, is this self-organization an epiphenomenon or does it have a function? Alignment of linear actin fibers, similar to that observed when the swirling ends, is an important stage of spontaneous polarization in some cell types [19]. A few years ago it was reported that neutrophils polarize to the left of an arrow drawn from the center of the nucleus of an unpolarized cell to its centrosome [20]. Thus, although the mechanism reported in the new study [5] depends on actin dynamics and is independent of microtubules, it could be that the transient handedness of the actin network is only able to sense left and right in a polarized cell, which could be useful for motile cells navigating complex chemical and physical gradients. It is also possible that the tilting and swirling enable the cell or its organelles to rotate [12–14], or mechanically ease the transition from the ‘spokes-on-a-wheel’ pattern into the linear fiber arrangement, or create skewed transportation tracks or stresses in the cell.

Even with all these questions unanswered, for the first time we have a concrete physical understanding of how actin self-organization could use chiral molecular motors to give the cell a sense of right and left.

REFERENCES