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Cytoskeletal Chirality: Swirling Cells Tell Left from Right

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A new study reports that dynamic actin fibers in cells on circular islands self-organize into a swirling counter-clockwise pattern and describes a basic cytoskeletal mechanism for the establishment of left–right asymmetry that is based on myosin contraction and twisting of the formin–actin filament.

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]. A growing number of observations have uncovered fascinating cytoskeletal chiral processes, such as: torque generation in the *C. elegans* cortex [10]; right-handed rotation of the *Listeria* pathogen [11]; steering of nerve growth cones by right-screw filopodia rotation [12] (Figure 1C); spiral actin network organization and movement in platelet and melanophore cells [13,14]; and chiral collective migration of cells on adhesive rings [15].

What has been missing from these studies of chirality is a mechanistic understanding of the link between chiral molecular events and handedness on the cellular scale. The recent work from Tee *et al.* [5] gives us an appealing model of such a link. The authors investigated cells confined to circular adhesive islands, which eliminated the complex asymmetries of cell shape and movement and allowed them to focus on the actin network's remarkable ability to

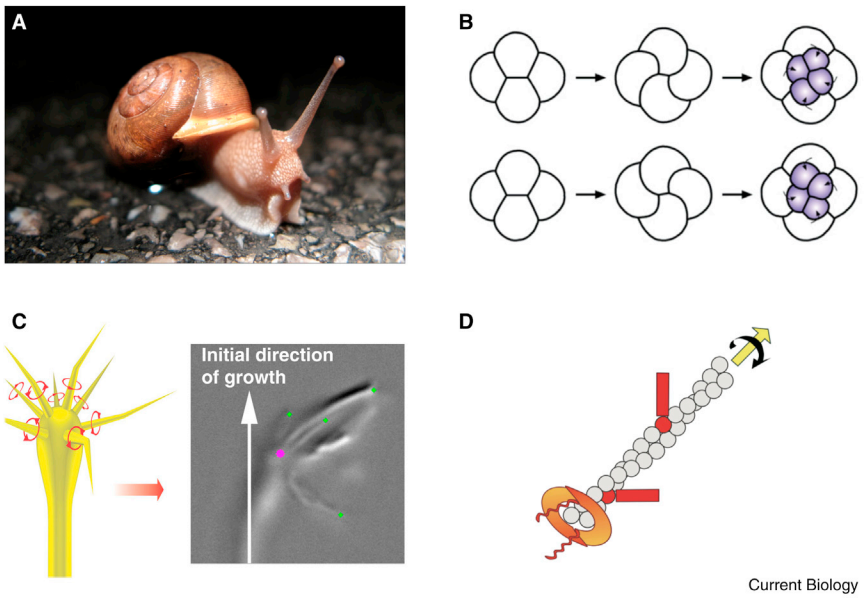

Figure 1. Chirality at different scales.
(A) Chiral structures are apparent in whole organisms, such as snails (photo: David Huth). (B) In many embryos, the earliest chiral event occurs at the third cell division, when daughter cells self-organize into a clockwise (top row) or anticlockwise (bottom row) 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).

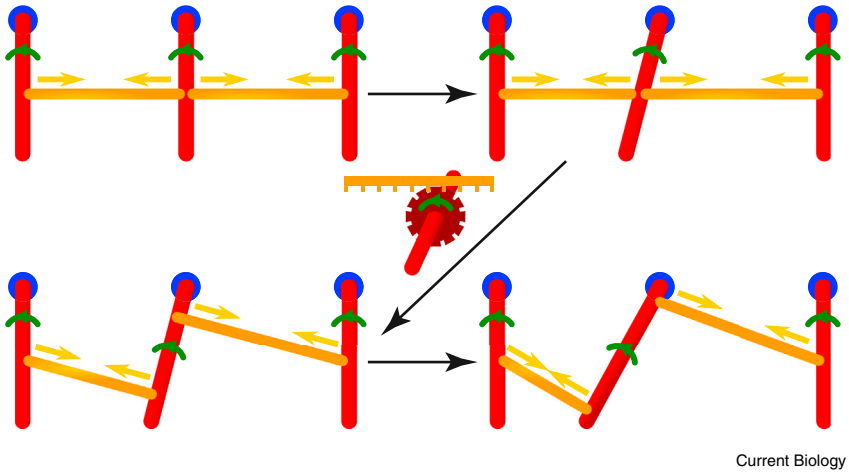

Figure 2. Mechanical chiral instability.
Anticlockwise rotation of radial actin filaments (green arrow) develops via a 'rack and pinion' mechanism (center inset) from an initially radial structure (top left), when formin-nucleated actin filaments (red) are rotated by formins immobilized at focal adhesions (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).

self-organize into a beautiful array of patterns [16].

Tee *et al.* [5] observed a familiar cobweb-like system of radial and transverse fibers (RFs and TFs, respectively) [17]. The RFs grew centripetally from the cell periphery, like spokes on a wheel. These fibers — which resemble the 'dorsal' stress fibers of migrating cells — extended inward from focal adhesions, were rich in the crosslinking protein α-actinin-1 and did not contain myosin II. TFs — which resemble previously described 'transverse arcs' — ran parallel to the cell edge, linking the RFs together. An elegant experiment showed that nanoparticles conjugated with myosin V molecules travelled bidirectionally along TFs, indicating that there was no left–right asymmetry in these fibers. The nanoparticles did not bind to the RFs, so the organization of actin in RFs remains unclear. Electron microscopy showed that the two systems of fibers passed through each other, hinting at a likely physical interaction. Photobleaching and pharmacological perturbations demonstrated that RFs grew inward, likely from formins at the focal adhesions, and that the formin-independent TFs moved inward more quickly than the RFs, meaning that TFs were sliding relative to the RFs. Inhibition of myosin revealed that this TF sliding was generated by myosin-powered contraction.

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 α-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 α-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 their barbed ends outward? Is the TF sliding fast enough to explain the tilting instability? 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

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

1. Coutelis, J.-B., González-Morales, N., Géminard, C., and Noselli, S. (2014). Diversity and convergence in the mechanisms establishing L/R asymmetry in metazoa. EMBO Rep. *15*, 926–937.
2. Grande, C., and Patel, N.H. (2008). Nodal signalling is involved in left-right asymmetry in snails. Nature *457*, 1007–1011.
3. Nonaka, S., Tanaka, Y., Okada, Y., Takeda, S., Harada, A., Kanai, Y., Kido, M., and Hirokawa, N. (1998). Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. Cell *95*, 829–837.
4. Rose, L., and Gönczy, P. (2014). Polarity establishment, asymmetric division and segregation of fate determinants in early C. elegans embryos. WormBook, 1–43.
5. Tee, Y.H., Shemesh, T., Thiagarajan, V., Hariadi, R.F., Anderson, K.L., Page, C., Volkmann, N., Hanein, D., Sivaramakrishnan, S., Kozlov, M.M., *et al*. (2015). Cellular chirality arising from the self-organization of the actin cytoskeleton. Nat. Cell Biol. *17*, 445–457.
6. Pyrpassopoulos, S., Feeser, E.A., Mazerik, J.N., Tyska, M.J., and Ostap, E.M. (2012). Membrane-bound myo1c powers asymmetric motility of actin filaments. Curr. Biol. *22*, 1688–1692.
7. Shemesh, T., Otomo, T., Rosen, M.K., Bershadsky, A.D., and Kozlov, M.M. (2005). A novel mechanism of actin filament processive capping by formin: solution of the rotation paradox. J. Cell Biol. *170*, 889–893.
8. Mizuno, H., Higashida, C., Yuan, Y., Ishizaki, T., Narumiya, S., and Watanabe, N. (2011). Rotational movement of the formin mDia1 along the double helical strand of an actin filament. Science *331*, 80–83.
9. Fürthauer, S., Strempel, M., Grill, S.W., and Jülicher, F. (2013). Active chiral processes in thin films. Phys. Rev. Lett. *110*, 048103.
10. Naganathan, S.R., Fürthauer, S., Nishikawa, M., Jülicher, F., and Grill, S.W. (2014). Active torque generation by the actomyosin cell cortex drives left-right symmetry breaking. eLife *3*, e04165.
11. Robbins, J.R., and Theriot, J.A. (2003). Listeria monocytogenes rotates around its long axis during actin-based motility. Curr. Biol. *13*, R754–R756.
12. Tamada, A., Kawase, S., Murakami, F., and Kamiguchi, H. (2010). Autonomous right-screw rotation of growth cone filopodia drives neurite turning. J. Cell Biol. *188*, 429–441.
13. Hagmann, J. (1993). Pattern formation and handedness in the cytoskeleton of human platelets. Proc. Natl. Acad. Sci. USA *90*, 3280–3283.
14. Yamanaka, H., and Kondo, S. (2015). Rotating pigment cells exhibit an intrinsic chirality. Genes Cells *20*, 29–35.
15. Wan, L.Q., Ronaldson, K., Park, M., Taylor, G., Zhang, Y., Gimble, J.M., and Vunjak-Novakovic, G. (2011). Micropatterned mammalian cells exhibit phenotype-specific left-right asymmetry. Proc. Natl. Acad. Sci. USA *108*, 12295–12300.
16. Vignaud, T., Blanchoin, L., and Théry, M. (2012). Directed cytoskeleton self-organization. Trends Cell Biol. *22*, 671–682.
17. Burnette, D.T., Shao, L., Ott, C., Pasapera, A.M., Fischer, R.S., Baird, M.A., Der Loughian, C., Delanoe-Ayari, H., Paszek, M.J., Davidson, M.W., *et al*. (2014). A contractile and counterbalancing adhesion system controls the 3D shape of crawling cells. J. Cell Biol. *205*, 83–96.
18. Lacayo, C.I., Soneral, P.A.G., Zhu, J., Tsuchida, M.A., Footer, M.J., Soo, F.S., Lu, Y., Xia, Y., Mogilner, A., and Theriot, J.A. (2012). Choosing orientation: influence of cargo geometry and ActA polarization on actin comet tails. Mol. Biol. Cell *23*, 614–629.
19. Prager-Khoutorsky, M., Lichtenstein, A., Krishnan, R., Rajendran, K., Mayo, A., Kam, Z., Geiger, B., and Bershadsky, A.D. (2011). Fibroblast polarization is a matrix-rigidity-dependent process controlled by focal adhesion mechanosensing. Nat. Cell Biol. *13*, 1457–1465.
20. Xu, J., Van Keymeulen, A., Wakida, N.M., Carlton, P., Berns, M.W., and Bourne, H.R. (2007). Polarity reveals intrinsic cell chirality. Proc. Natl. Acad. Sci. USA *104*, 9296–9300.

Palaeontology: In a Flap About Flaps

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An anomalocaridid from the Ordovician exposes a second set of body flaps and reopens the question of how the two branches of arthropod legs evolved.

Sorting out the evolutionary transformations of the legs of arthropods is a vexing problem. 1.2 million known living species and a vast diversity of fossils that span 520 million years demonstrate that legs on different segments have been modified for walking, swimming, feeding, breeding and breathing in ways that make