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An unexpected turn for filopodia

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The origin of chirality in biological systems has long been a major question. Intriguingly, in several cases, the molecular basis of biological chirality depends on cytoskeletal proteins. The handedness of a snail shell, for example, depends on a formin (1), a family of proteins that stimulates polymerization at the barbed end of the actin filament, whereas the left-right positioning of internal organs involves the actin-based motor myosin-Id (Myo1D) (2) and the microtubule-based motor ciliary dynein (3).

In this issue, Li et al. (4) report a fascinating and unexpected form of cellular chirality in the finger-like protrusions known as filopodia. Filopodia are cylindrical extensions of a cell's plasma membrane that typically have a diameter of ∼0.1 μm and can extend 1–10 μm or more (5). A filopodium contains a bundle of actin filaments at its core that endow it with a rod-like shape and structural rigidity. The actin filaments have their barbed ends oriented toward the filopodial tip, and polymerization of actin monomers at the tip creates a pushing force that allows a filopodium to extend. In previous work, Li et al. showed HeLa cells form numerous filopodia when plated on coverslips coated with galectin-8 (6), a secreted lectin that can mediate adhesion by binding to integrins. In control HeLa cells plated on galectin-8, filopodia adhere to the substrate along their length, with over 90% extending straight, and only a few percent turning left (counterclockwise as viewed from above). Li et al. now report the surprising result that when cells are plated onto galectin-8 after being transfected with myosin-X (Myo10), an integrin-binding myosin that induces filopodia and localizes to their tips (7), the fraction of straight filopodia drops to approximately half, with the vast majority of the remaining filopodia turning left.

A striking supplemental video shows that as these filopodia extend, they gradually curve leftward. Interestingly, turning was often preceded by a decrease in the filopodial extension rate, something that could potentially be caused by increased adhesion at the tip. Furthermore, in ∼90% of turns, the spot of fluorescently tagged Myo10 at the filopodial tip split, with one spot continuing to move forward with the other remaining near its original position as it faded away over 10–20 s. To investigate this novel and unexpected form of cellular chirality, Li et al. first show that left-turning filopodia can be induced in other cell types by transfection with myosin-X and plating on galectin-8.

Cryo-EM of the filopodial tips revealed several remarkable results. First, almost half of the bent filopodia had a bulb-like swelling at their tip, a condition present in only ∼5% of straight filopodia. Second, in filopodia that curve left, the filopodial actin bundle was positioned on the right side of the tip swelling. Third, and most remarkably, the filopodial actin bundles in curved filopodia were often bent into an incredibly tight hairpin-like loop within the ∼0.2–0.4 μm diameter of a swollen tip. Pure actin filaments have been reported to break when pulled into a diameter of ∼0.36 μm or less (8), so if the individual actin filaments at the tip are bent this sharply rather than being segmented into shorter pieces, their curvature would be close to or beyond this limit. These cryo-EM results raise a host of fascinating questions, including whether the filaments in the loops have their barbed ends oriented "backward" relative to the other filopodial actin filaments.

To investigate the molecular basis of the filopodial chirality, Li et al. systematically quantified the number, length, and percentage of straight, left-turning, and right-turning filopodia in a variety of conditions. Similar to expressing Myo10, knockdown of endogenous ARP2, an essential component of the complex that nucleates the branched actin arrays in lamellipodia, greatly increased the percentage of filopodia turning left. Much of this increase was abolished when Myo10 was knocked down, indicating that the effects of ARP2 knockdown on filopodial chirality depends in part on endogenous Myo10. Knockdown of fascin, a major actin bundling protein

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in filopodia, also greatly increased the percentage of filopodia that turned left. Interestingly, there was relatively little effect on the percentage of filopodia turning left when either Myo10 or Myo1D, or the filopodial formins Dia1, Dia2, or FMNL2, were knocked down individually.

So what might make filopodia turn left? There is precedent for chirality in filopodia: growth cone filopodia that are not attached to the substrate are reported to rotate or pivot about their base counterclockwise (viewed from outside the cell) at a few degrees per second (9). This rotation was inhibited by overexpressing the head domain of Myo5a, potentially because the myosin head bound to actin filaments and displaced myosins or actin-binding proteins contributing to the rotation. Filopodia and their actin core have also been observed to rotate in nonneuronal cells, with rotation inhibited by knockdown of either Myo5a or Myo10 (10). If Myo10 bound to actin filaments at the tip of a rotating filopodium used its tail to anchor the actin bundle to substrate-attached integrins, twist might build up and displace the actin bundle to one side of the filopodium, eventually leading to a turn. Unfortunately for this model, Li et al. show that cells expressing truncated Myo10 missing most of its tail, including the region that binds to the cytoplasmic domain of integrins, still generate a large fraction of left-turning filopodia. As a possible explanation for this, the authors suggest that if Myo10 were to move up the actin bundle in a left-handed spiral, friction with the cytoplasm or membrane might generate a force to roll the bundle to the right. If adhesion to galectin-8 leaves little space on the bottom of the filopodium between the actin bundle and the plasma membrane, a similar movement of Myo10 could potentially lead it to accumulate on the left and push the bundle right.

Although the exact mechanisms await future work, the author's findings raise a host of fascinating questions. What is special about galectin-8-mediated adhesion, and can filopodia be induced to turn left on other substrates? How do the actin loops form, and are similar structures present physiologically? If the actin filaments at the tips of a turning filopodium are bent and their barbed ends are pointed away from the tip, how do they generate the force to extend the filopodium? Although the system reported by Li et al. is somewhat artificial, it provides a simple and attractive model system to investigate these and many other questions regarding a fascinating and unexpected example of cellular chirality.

DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

1. Abe, M., and R. Kuroda. 2019. The development of CRISPR for a mollusc establishes the formin Lsdia1 as the long-sought gene for snail dextral/sinistral coiling. *Development.* 146, dev175976.

2. Tingler, M., S. Kurz, …, M. Blum. 2018. A conserved role of the unconventional myosin 1d in laterality determination. *Curr. Biol.* 28:810–816.e3.

3. Supp, D. M., D. P. Witte, …, M. Brueckner. 1997. Mutation of an axonemal dynein affects left-right asymmetry in inversus viscerum mice. *Nature.* 389:963–966.

4. Li, W., W. -L. Chung, …, A. D. Bersahsky. 2023. Chiral growth of adherent filopodia. Preprint at Biophys. J. https://doi.org/10.1101/2022.06.09.495577.

5. Mattila, P. K., and P. Lappalainen. 2008. Filopodia: molecular architecture and cellular functions. *Nat. Rev. Mol. Cell Biol.* 9:446–454.

6. Li, W., A. Sancho, …, B. Geiger. 2021. Differential cellular responses to adhesive interactions with galectin-8- and fibronectin-coated substrates. *J. Cell Sci.* 134, jcs252221.

7. Courson, D. S., and R. E. Cheney. 2015. Myosin-X and disease. *Exp. Cell Res.* 334:10–15.

8. Arai, Y., R. Yasuda, …, H. Itoh. 1999. Tying a molecular knot with optical tweezers. *Nature.* 399:446–448.

9. Tamada, A., S. Kawase, …, H. Kamiguchi. 2010. Autonomous right-screw rotation of growth cone filopodia drives neurite turning. *J. Cell Biol.* 188:429–441.

10. Leijnse, N., Y. F. Barooji, …, P. M. Bendix. 2022. Filopodia rotate and coil by actively generating twist in their actin shaft. *Nat. Commun.* 13:1636.