Three-ring circus without a ringmaster: Self-organization of supracellular actin ring patterns during epithelial morphogenesis

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Formation of patterns during development has been a long-standing puzzle. Alan Turing proposed chemical gradients as a solution to the problem (1), and many chemical signals that pattern cells have since been found. However, only recently have roles for mechanical forces in patterning become apparent (2–6). In PNAS, Hannezo et al. (7) present and test a biophysical model involving three key elements—actin, myosin II, and anisotropic "effective friction" arising from interactions with the extracellular matrix (ECM)—that recapitulates the formation of the periodic subcellular actin bundles that coherently span several cells to form rings in the developing *Drosophila* tracheal (airway) tubes (Fig. 1*A*). Strikingly, although the mechanism of ring formation was previously unknown, Hannezo et al.'s model predicts that formation of the bundles, as well as their periodicity and orientation, will arise within each cell through self-organization. Experimental tests of predictions of the model show that it correctly describes multiple unexpected behaviors of the system in vivo, including imperfections in the actin rings (Fig. 1 *B* and *C*) and the formation of only a single unanchored actin ring per cell when the ECM is eliminated. Hannezo et al.'s work provides a mechanistic basis for understanding formation of patterned actin ring structures in *Drosophila* and other species, and highlights the potential of the ECM to influence actin organization through mechanical rather than biochemical signaling interactions.

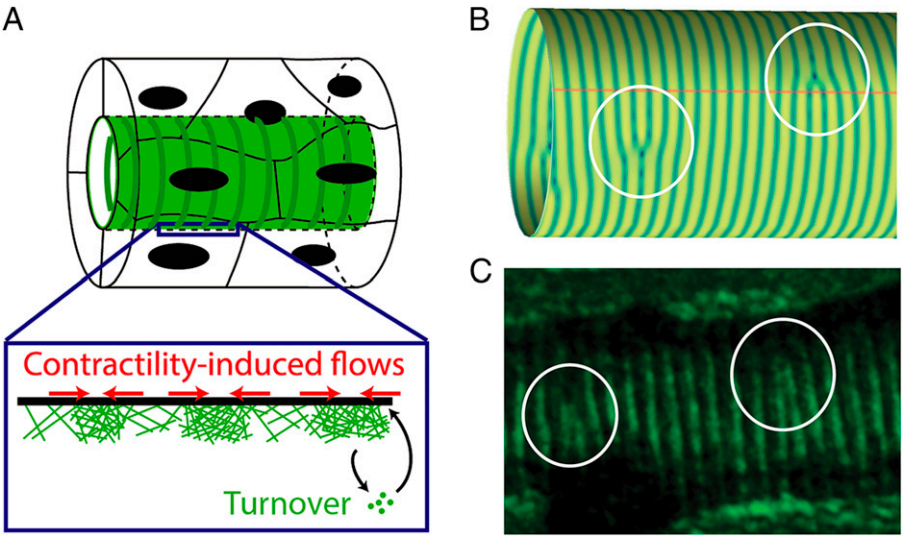

Fig. 1. A biophysical model of actin, myosin and ECM interactions recapitulates the formation of supracellular actin rings in the *Drosophila* tracheal system (7). (*A*, *Upper*) The epithelial cells of the *Drosophila* tracheal system form a tube approximately four cells in diameter. During embryogenesis, the tracheal lumen is filled with a chitin-based ECM (lighter green). Cortical actin bundles at the cells' apical surfaces (darker green) form supracellular rings that continuously encircle the lumen. (*Lower*, *Inset*) The model predicts that actin rings self-assemble within each cell as a consequence of actin polymerization, myosin II contractility, and anisotropic "effective friction" from interactions with the ECM. Notably, the influence of the ECM is proposed to be strictly mechanical and does not involve biochemical signaling. Intercellular coupling that aligns actin bundles across cell boundaries requires only small coupling constants that could be achieved by mechanically induced junctional maturation. (*B*) An example of actin ring patterns predicted by the biophysical model. Importantly, the model predicts defects in the patterns (white circles) that are remarkably similar to those observed in vivo (*C*). (*C*) Confocal image of actin staining in the tracheal system. Note that actin bundles maintain near perfect registry across cell–cell boundaries (not shown). White circles highlight defects the ring pattern.

To a large extent, the actin cytoskeleton determines a cell's mechanical properties and greatly influences its shape, mobility, and intracellular signaling (8). The actin network at the cell's plasma membrane (referred to as cortical actin) is especially dynamic, because actin nucleators that promote actin polymerization are often recruited to and activated at the plasma membrane. Consequently, cortical actin polymerization drives formation of many subcellular structures, the most visible of which are cellular protrusions. Simultaneously, myosin II molecular motors attach to actin filaments and induce contractile forces and motions that play crucial roles in cellular polarization, morphogenesis, and cell division. The model reported in Hannezo et al. (7) uses the same building blocks of actin polymerization and myosin II contractility, but also includes interactions with an external substrate, which in this case is a chitin-based ECM that transiently fills the lumen of the trachea during development (9; reviewed in ref. 10). Remarkably, a strictly mechanical interaction between the ECM and cortical actin flow, represented as friction in the model, can control the periodicity, orientation, and dynamics of the actin patterns, without invoking the biochemical signaling typically induced by cell–matrix interactions.

Although the ability of Hannezo et al.'s (7) model to reproduce central aspects of the actin bundles in individual tracheal cells is impressive, an even bigger accomplishment

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of the model is its ability to account for the supracellular nature of the actin structures. As seen in Fig. 1 *B* and *C*, actin bundles appear to seamlessly cross cell–cell junctions, keeping perfect registration despite intervening pairs of cell membranes and adherens junctions. Indeed, in the absence of junctional markers, the location of cell–cell boundaries cannot be discerned (Fig. 1*C*). Although one might expect such coordination to require molecular feedback loops or specialized junctional complexes, to date no mutations have been described that specifically disrupt continuity of the supracellular tracheal rings. Consistent with the lack of a specific "ringmaster," Hannezo et al. find that even very small coupling interactions, such as those that would be produced by known ability of some cell–cell junctions to mature in response to stresses (11), are capable of producing correlated patterns. Thus, the model predicts not only that individual cells can produce periodic linear actin structures using "off the shelf" components found in most epithelial cells, but that groups of cells can spontaneously coordinate their actin patterns using standard junctional components. However, further work is required to test models of coordination, as it remains conceivable that elastic interactions across the ECM core of the tube can give rise to long-range registry (12), as well as interactions involving the curvature of the rings (13).

What is the function of actin rings in tracheal tubes and how do tracheal actin rings correspond to rings observed in other species? During tracheal development, the actin rings template formation of cuticular ECM ridges that, like the ridges of a vacuum cleaner hose, are thought to serve as circumferential buttresses to prevent tube collapse. In other organisms and tissues, actin rings serve a wide variety of purposes. In *Caenorhabditis elegans*, actin rings form at the epidermal surface just before molting, presumably to resist the outward turgor pressure of the worm body that would otherwise be unopposed upon cuticle shedding (14, 15). The mechanism by which these actin rings arise has not been determined, but the parallels with the *Drosophila* trachea strongly suggest a common underlying mechanism. Perhaps the best-known example of actin ring formation is that of the single actin ring that forms at the cleavage furrow of all dividing eukaryotic cells. Intriguingly, during division of some cell types, including the *C. elegans* germ line and preimplantation mouse embryos, successful cell division requires the ECM protein hemicentin (16). The precise role of hemicentins in furrow maturation is unclear, but the results of Hannezo et al. (7) suggest that mechanical as well as biochemical signaling from hemicentins to the actomyosin contractile ring could be important. Notably, not all actin rings may use the actin-mysoin-EMC assembly model. For example, in the case of the actin rings that were recently identified within mammalian axons, ring formation and periodicity may be controlled by intervening complexes containing the cytoskeletal protein spectrin (17). Regardless of whether actin rings form by one or more mechanisms, the work by Hannezo et al. (7) makes a critical contribution by proposing the first mechanism by which periodic, supracellular actin rings form and by creating a detailed biophysical framework for understanding the process.

The work of Hannezo et al. illuminates a new role of mechanical interactions in forming cytoskeletal patterns that span entire tissues.

With the model in hand, what remains to be done in understanding the biology of the actin rings? Further validation of the model in *Drosophila* and other species will be critical. A deeper probing into underlying patterning issues is also needed. In particular, Hannezo et al. (7) show that anisotropy in the ECM can orient the actin rings, but how is the anisotropy of the chitin-based matrix created and oriented? And what mechanisms allow chitin fibrils to span many cells and form a supracellular structure oriented orthogonally to the actin rings? Another frontier will be to understand how the actin rings pattern the ECM. The tracheal actin rings have been predicted to control secretion of the cuticle, and although there is some evidence for actin rings controlling secretion in *C. elegans* (18), the nature of these mechanisms in trachea remains to be determined. Considerable research will be needed to answer these and other questions, but the work of Hannezo et al. (7) illuminates a new role of mechanical interactions in forming cytoskeletal patterns that span entire tissues.

1 Turing AM (1952) The chemical basis of morphogenesis. *Philos Trans R Soc Lond B Biol Sci* 237(641):37–72.
2 Brouzés E, Farge E (2004) Interplay of mechanical deformation and patterned gene expression in developing embryos. *Curr Opin Genet Dev* 14(4):367–374.
3 Ingber DE (2006) Mechanical control of tissue morphogenesis during embryological development. *Int J Dev Biol* 50(2-3):255–266.
4 Driquez B, Bouclet A, Farge E (2011) Mechanotransduction in mechanically coupled pulsating cells: Transition to collective constriction and mesoderm invagination simulation. *Phys Biol* 8(6): 066007.
5 Guillot C, Lecuit T (2013) Mechanics of epithelial tissue homeostasis and morphogenesis. *Science* 340(6137):1185–1189.
6 Heisenberg CP, Bellaïche Y (2013) Forces in tissue morphogenesis and patterning. *Cell* 153(5):948–962.
7 Hannezo E, Dong B, Recho P, Joanny J-F, Hayashi S (2015) Cortical instability drives periodic supracellular actin pattern formation in epithelial tubes. *Proc Natl Acad Sci USA* 112:8620–8625.
8 Salbreux G, Charras G, Paluch E (2012) Actin cortex mechanics and cellular morphogenesis. *Trends Cell Biol* 22(10):536–545.
9 Dong B, Hayashi S (2015) Shaping of biological tubes by mechanical interaction of cell and extracellular matrix. *Curr Opin Genet Dev* 32:129–134.
10 Swanson LE, Beitel GJ (2006) Tubulogenesis: An inside job. *Curr Biol* 16(2):R51–R53.
11 Yonemura S, Wada Y, Watanabe T, Nagafuchi A, Shibata M (2010) Alpha-catenin as a tension transducer that induces adherens junction development. *Nat Cell Biol* 12(6):533–542.
12 Friedrich BM, Buxboim A, Discher DE, Safran SA (2011) Striated acto-myosin fibers can reorganize and register in response to elastic interactions with the matrix. *Biophys J* 100(11):2706–2715.
13 Shlomovitz R, Gov NS (2009) Membrane-mediated interactions drive the condensation and coalescence of FtsZ rings. *Phys Biol* 6(4): 046017.
14 Priess JR, Jr, Hirsh DI (1986) *Caenorhabditis elegans* morphogenesis: The role of the cytoskeleton in elongation of the embryo. *Dev Biol* 117(1):156–173.
15 Costa M, Draper BW, Priess JR (1997) The role of actin filaments in patterning the *Caenorhabditis elegans* cuticle. *Dev Biol* 184(2): 373–384.
16 Xu X, Vogel BE (2011) A secreted protein promotes cleavage furrow maturation during cytokinesis. *Curr Biol* 21(2): 114–119.
17 Xu K, Zhong G, Zhuang X (2013) Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons. *Science* 339(6118):452–456.
18 Liégeois S, Benedetto A, Michaux G, Belliard G, Labouesse M (2007) Genes required for osmoregulation and apical secretion in *Caenorhabditis elegans*. *Genetics* 175(2):709–724.