hypomorphic alleles of *Rheb* or *TOR*). Finally, despite the fact that phospho-RpS6 is not detectable in *PTEN* mutant cells at a distance from the morphogenetic furrow, all phenotypic consequences of clones of cells lacking PTEN function in the developing eye require TORC1 activity (Nowak et al., 2013).

A possible explanation comes from another TORC1 activity reporter. Tiebe and colleagues recently described an *unkempt-lacZ* reporter that becomes activated upon TORC1 inhibition (Tiebe et al., 2015). This reporter should produce a negative image of the phospho-RpS6 pattern, but essentially no reporter activity was observed in developing imaginal discs. The reporter gene can, however, be induced by genetically lowering TORC1 activity. These observations suggest a basal TORC1 activation in (growing) cells that is not sufficient to produce detectable levels of RpS6 phosphorylation. It is tempting to speculate that there are different TORC1 activity thresholds: Basal activity—sustained by amino acid signaling and by growth factors such as insulin-like peptides—is probably *permissive* for cellular growth, allowing for cell division in mitotically active cells and cell size increase in post-mitotic cells, respectively. High TORC1 activity—induced by Cyclin D/Cdk4—may facilitate meeting the high metabolic demands caused by nucleotide biosynthesis in S phase.

REFERENCES

Baonza, A., and Freeman, M. (2005). Dev. Cell *8*, 529–539.

Datar, S.A., Jacobs, H.W., de la Cruz, A.F., Lehner, C.F., and Edgar, B.A. (2000). EMBO J. *19*, 4543–4554.

Emmerich, J., Meyer, C.A., de la Cruz, A.F., Edgar, B.A., and Lehner, C.F. (2004). Genetics *168*, 867–875.

Kim, W., Jang, Y.-G., Yang, J., and Chung, J. (2017). Dev. Cell *42*, this issue, 363–375.

Meyer, C.A., Jacobs, H.W., Datar, S.A., Du, W., Edgar, B.A., and Lehner, C.F. (2000). EMBO J. *19*, 4533–4542.

Neufeld, T.P., de la Cruz, A.F., Johnston, L.A., and Edgar, B.A. (1998). Cell *93*, 1183–1193.

Nowak, K., Seisenbacher, G., Hafen, E., and Stocker, H. (2013). eLife *2*, e00380.

Romero-Pozuelo, J.D., Demetriades, C., Schroeder, P., and Teleman, A.A. (2017). Dev. Cell *42*, this issue, 376–387.

Tiebe, M., Lutz, M., De La Garza, A., Buechling, T., Boutros, M., and Teleman, A.A. (2015). Dev. Cell *33*, 272–284.

Wolfson, R.L., and Sabatini, D.M. (2017). Cell Metab. *26*, 301–309.

Optimal Contractile Forces for a Mesenchymal Engine

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The cell and molecular mechanisms that cause a smooth epithelium to become topographically patterned during development are poorly understood. In a recent issue of *Science*, Shyer et al. (2017) show that myosin II contractility drives the smooth dermal mesenchyme into a pattern of surface bumps that triggers epithelial gene expression.

Morphogenetic processes often involve mesenchymal cells in collective motion, sometimes coupled to an overlying epithelium with ridges, bumps, or other topographical features. Fingerprints on our hands, for example, reflect extensions of mesenchyme-rich dermis into ridges in the epidermis. The increased roughness in the contacts between such tissue layers strengthens the bond between the layers, which hints at the physical consequences of patterning, but it is interesting to further consider that biophysical mechanisms could also be key to forming such features. Skin follicles for hair and feathers are dermal extensions that develop from an initially smooth skin in the embryo as a pattern of mesenchymal bumps or condensates below an epithelial sheet and basement membrane. On chick embryos, the bumps are condensed aggregates of cells referred to as "primordia" that arrange in a close-packed array with quasi-6-fold symmetry. The initial formation of this pattern has long been the subject of experimentation and speculation. Patterns of potent diffusible factors such as Bone Morphogenetic Proteins (BMPs) have been hypothesized to be essential, as have patterns of extracellular matrix (ECM) that guide mesenchymal migration. Pioneers in the study of nonmuscle cell contractility also theorized decades ago that the regular patterns of mesenchymal bumps could arise from instabilities intrinsic to the physics of a collection of contractile cells (Oster et al., 1983). Evidence for such a mechanism is now reported in a recent study in *Science* by Shyer et al. (2017), whose experiments show that nuclear translocation of β-catenin in the epithelium and subsequent BMP2 secretion are downstream of a collective, dermal contraction and surface instability driven by an optimal activity of nonmuscle myosin II. The emergence of piston-like bumps on chick embryos suggest a "mesenchymal engine" that is well tuned in its contractile strength and stiffness to drive morphogenesis. The observations also highlight some basic issues

in mechanobiology that no doubt apply to diverse morphogenetic processes.

Shyer et al. (2017) dissected sections of skin from chick embryos at very early stages and first ruled out a role for patterns of BMP2. They stimulated rapid degradation of β-catenin in the smooth section of dissected skin by adding a drug, XAV-939, which inhibits a key regulator of β-catenin's destruction. Transcription mediated by β-catenin was thereby eliminated, which greatly suppressed *bmp2* message in the dermal mesenchyme and the overlying epithelium. Surprisingly, the bumps of nascent follicles formed with seemingly normal kinetics. Image analysis would probably reveal an abnormally broad distribution of bump sizes in a less-regular array; while such defects could reflect the lack of BMP2 signaling, the defects are more likely to reflect physical limits of cell-cell adhesion due to the loss of β-catenin because cadherins bind cytoplasmic β-catenin, which in turn binds to the F-actin binding protein α-catenin, and α-catenin plus high actomyosin tension recruits vinculin and thereby mechanically stabilizes cell aggregates (Ladoux et al., 2015).

The biophysical mechanisms for forming condensed aggregates of cells can in principle be very robust, because they can be seen even in a three-component protein system of purified actin, myosin II, and crosslinker, plus ATP (Schuppler et al., 2016). Although steady-state patterns of aggregating protein nodules lack symmetry, boundary resistance is key to maintaining dispersed nodules. For the same reasons, Shyer et al. (2017) attached their dissected skin sections to underlying gels of controlled stiffness (with fibronectin bound for cell adhesion). Adhesive gels of controlled stiffness are known to control mesenchymal cell spreading and migration (Engler et al., 2006; Raab et al., 2012), and cells tend to migrate from soft gels toward stiff gels—in a process called durotaxis—as cells spread more and polarize more on stiff substrates. When the sections of chick skin are adhering to a very soft gel or else left freely suspended, the cells contract and drive the section of skin into a thick and smooth tissue. In contrast, when adhering to very stiff substrates, dermal cells collectively migrate onto the substrate, which causes the tissue to flatten and once again remain smooth. However, synthetic gels that have a stiffness within the range of various embryonic tissues (~0.1–10 kPa; Majkut et al., 2014) permit arrays of primordial bumps to emerge. Skin stiffness is one crucial factor in the theory that quantitatively predicted the various determinants of bump formation and patterning (Oster et al., 1983). In the absence of a measured value for skin stiffness in chick embryo, and given that skin is rich in collagen (think of leather, which is dried skin), a first estimate is perhaps provided by a typical collagen gel that has a stiffness of ~1 kPa (e.g., Raab et al., 2012). Such a stiffness for the dermis would not only lead to the durotaxis that represses bumps on very stiff gel substrates but would also favor repulsion from very soft gels as observed. It seems surprising nonetheless that the primordial lumps show no sensitivity to underlying substrates that differ in stiffness by 100-fold (~0.1–10 kPa). Other tissues, such as the embryonic chick heart, exhibit a strong maximum in function at the native stiffness (1 kPa), with beating of the heart clearly impeded by softening of the matrix via enzymatic degradation (to 0.1 kPa), as well as by stiffening via crosslinking (to 10 kPa) (Majkut et al., 2014). Shyer et al. (2017) do not perturb the matrix within the dermis, and the effects of the underlying gels that they observe depend entirely on the ability of the bottom-most cells adhering to the gels to strongly influence the overlying mesenchymal cells. However, cell-generated forces decay over microns in distance through homogeneous materials (Engler et al., 2006), and because this length scale is small relative to the thickness of the skin sections (~100 μm), it seems understandable that only with extremes in substrate softness or rigidity can perturbing forces propagate sufficiently into the dermis.

Sensitivity to the softness or the rigidity of an underlying substrate nonetheless indicates a key role for myosin II contractility (e.g., Engler et al., 2006). Like small muscle cells that constantly contract, individual cells have been described in modern theory as a force dipole that interacts with its surrounding matrix as well as other cells (Bischofs et al., 2004). The collection of such interacting force dipoles might thus be referred to as a "mesenchymal engine" that produces net motions in relation to myosin II motor activity. To examine the possible role of myosin-II, Shyer et al. (2017) placed skin sections on a microporous rigid plastic filter that minimizes adhesion and allows drugs to diffuse from below and into the dermal cells. Indeed, inhibition of myosin II ATPase with blebbistatin in this setup caused relaxation of the slab of cells, eliminating both a pre-stress in the slab and the piston-like lumps on top. Diameters of the condensing aggregates of cells increase as height and spacing progressively diminish, analogous to a fluidization or melting process. To increase contractility, Shyer et al. (2017) used an inhibitor of serine/threonine protein phosphatases: Calyculin-A is known to increase phosphorylation of myosin light chain, thereby activating myosin II, and by using this treatment, the authors indeed observed contraction and thickening of skin sections, which once again became smooth. A requirement for a precise level of myosin II activity is consistent with Shyer et al. (2017) finding an optimal stiffness for the underlying adhesive substrate, with high tension generated by myosin II appearing equally potent as low tension in suppressing pattern formation. Although the original theory (Oster et al., 1983) for this mesenchymal engine seems to have predicted that contractile tension monotonically drives pattern formation, the upper limit seems as physically sensible as the lower limit.

Changes in cell shape that result from cell forces are known to trigger translocation of β-catenin into the nucleus (Fernández-Sánchez et al., 2015), which helps explain why Shyer et al. (2017) find that myosin II forces in the dermis are upstream of β-catenin and BMP2. Myosin II is clearly the physical driver, consistent with its known effects in development: knockout of Myh9 indeed produces a flaccid, flattened mass of proliferating mouse embryo cells that exhibit little to no differentiation (Conti et al., 2004). It should be interesting to assess other pathways in the skin sections, and in particular whether and where the Hippo pathway components YAP/TAZ translocate into the nucleus—given the well-established role of this pathway in tissue growth as well as the observations that cell proliferation was not differentially affected by the various myosin-II and substrate perturbations of Shyer et al. (2017). Deeper insight into the inner workings of

the mesenchymal engine, perhaps in terms of active stresses and movements of polar entities (Bischofs et al., 2004), would no doubt deepen our understanding of many other mesenchymal condensation processes that both sculpt and strengthen many organs.

REFERENCES

Bischofs, I.B., Safran, S.A., and Schwarz, U.S. (2004). Phys. Rev. E Stat. Nonlin. Soft Matter Phys. *69*, 021911.

Conti, M.A., Even-Ram, S., Liu, C., Yamada, K.M., and Adelstein, R.S. (2004). J. Biol. Chem. *279*, 41263–41266.

Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. (2006). Cell *126*, 677–689.

Fernández-Sánchez, M.E., Barbier, S., Whitehead, J., Béalle, G., Michel, A., Latorre-Ossa, H., Rey, C., Fouassier, L., Claperon, A., Brullé, L., et al. (2015). Nature *523*, 92–95.

Ladoux, B., Nelson, W.J., Yan, J., and Mège, R.M. (2015). Integr. Biol. *7*, 1109–1119.

Majkut, S., Dingal, P.C., and Discher, D.E. (2014). Curr. Biol. *24*, R495–R501.

Oster, G.F., Murray, J.D., and Harris, A.K. (1983). J. Embryol. Exp. Morphol. *78*, 83–125.

Raab, M., Swift, J., Dingal, P.C., Shah, P., Shin, J.W., and Discher, D.E. (2012). J. Cell Biol. *199*, 669–683.

Schuppler, M., Keber, F.C., Kröger, M., and Bausch, A.R. (2016). Nat. Commun. *7*, 13120.

Shyer, A.E., Rodrigues, A.R., Schroeder, G.G., Kassianidou, E., Kumar, S., and Harland, R.M. (2017). Science. Published online July 13, 2017. http://dx.doi.org/10.1126/science.aai7868.