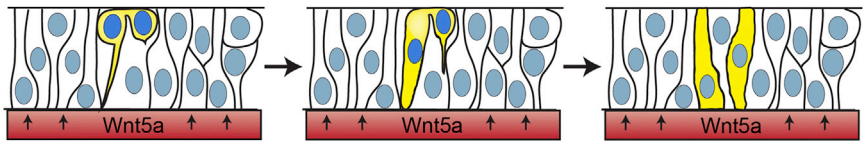

Figure 1. Diagram of Filopodial-Based Pathfinding for Reintegration of Cells in a Pseudostratified Epithelium

The daughter cell that has lost contact with the basement membrane extends a basal process in the direction of a Wnt5a cue and uses this track for subsequent nuclear movement.

basal surface. If the nature of these linkages can be identified, they can then be disrupted to determine whether they are important in epithelial reintegration and/or daughter cell dispersal. Finally, it will be interesting to examine whether the mechanisms identified in this paper are conserved in other proliferative pseudostratified tissues.

REFERENCES

Alves, M.M., Halim, D., Maroofian, R., de Graaf, B.M., Rooman, R., van der Werf, C.S., Van de Vijver, E., Mehrjardi, M.Y., Aflatoonian, M., Chioza, B.A., et al. (2016). Genetic screening of Congenital Short Bowel Syndrome patients confirms CLMP as the major gene involved in the recessive form of this disorder. Eur. J. Hum. Genet. *24*, 1627–1629.

Bergstralh, D.T., Lovegrove, H.E., and St Johnston, D. (2015). Lateral adhesion drives reintegration of misplaced cells into epithelial monolayers. Nat. Cell Biol. *17*, 1497–1503.

Cervantes, S., Yamaguchi, T.P., and Hebrok, M. (2009). Wnt5a is essential for intestinal elongation in mice. Dev. Biol. *326*, 285–294.

Grosse, A.S., Pressprich, M.F., Curley, L.B., Hamilton, K.L., Margolis, B., Hildebrand, J.D., and Gumucio, D.L. (2011). Cell dynamics in fetal intestinal epithelium: implications for intestinal growth and morphogenesis. Development *138*, 4423–4432.

Reed, R.A., Womble, M.A., Dush, M.K., Tull, R.R., Bloom, S.K., Morckel, A.R., Devlin, E.W., and Nascone-Yoder, N.M. (2009). Morphogenesis of the primitive gut tube is generated by Rho/ROCK/myosin II-mediated endoderm rearrangements. Dev. Dyn. *238*, 3111–3125.

van der Werf, C.S., Sribudiani, Y., Verheij, J.B., Carroll, M., O'Loughlin, E., Chen, C.H., Brooks, A.S., Liszewski, M.K., Atkinson, J.P., and Hofstra, R.M. (2013). Congenital short bowel syndrome as the presenting symptom in male patients with FLNA mutations. Genet. Med. *15*, 310–313.

Wang, S., Cebrian, C., Schnell, S., and Gumucio, D.L. (2018). Radial WNT5A-guided post-mitotic filopodial pathfinding is critical for midgut tube elongation. Dev. Cell *46*, this issue, 173–188.

Yamada, M., Udagawa, J., Hashimoto, R., Matsumoto, A., Hatta, T., and Otani, H. (2013). Interkinetic nuclear migration during early development of midgut and ureteric epithelia. Anat. Sci. Int. *88*, 31–37.

The Domino Effect in EGFR-ERK Signaling

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A gradient of EGFR-ERK signaling has been classically implicated in various developmental processes. In this issue of *Developmental Cell*, Ogura et al. (2018) show that in the *Drosophila* tracheal placode, EGFR-ERK signaling propagates via a cell-by-cell relay mechanism rather than a gradient, and this sequential ERK activation controls proper placode invagination.

A classical mechanism in organismal development is the formation of morphogen gradients. Morphogen gradients result from localized secretion and long-range diffusion of signaling molecules, which generate a graded concentration profile along the tissue. Classical works on EGFR-ERK signaling suggested that this signaling pathway exhibits such a morphogen gradient behavior (Gabay et al., 1997; Schweitzer and Shilo, 1997). However, in recent years this picture is challenged by several works showing a wave-like propagation dynamics of ERK activity (Aoki et al., 2017; de la Cova et al., 2017; Lim et al., 2015). In this issue of *Developmental Cell*, Ogura et al. (2018) provide new evidence that EGFR-ERK signaling in the *Drosophila* trachea propagates from cell to cell through a relay mechanism rather than passive diffusion. This relay mechanism is essential for the proper invagination of the tracheal placode.

By using live imaging of an ERK fluorescence resonance energy transfer (FRET) sensor, the authors show that ERK activity propagates in a stepwise manner from the center to the periphery of the tracheal placode. This row-by-row propagation of ERK activity fits a relay model in which each row activates the row next to it. The authors show evidence that the relay works via a feedback mechanism in which ERK activates Rho, which is an endopeptidase that activates the EGF ligand Spitz, which in turn binds and activates EGF receptors (EGFRs) in neighboring cells. Activated EGFR is known to induce ERK activity closing the feedback loop (Figure 1A). It is also shown that mutants that interfere with this feedback (*trh* and *vvl*) lead to a graded rather than stepwise ERK activity.

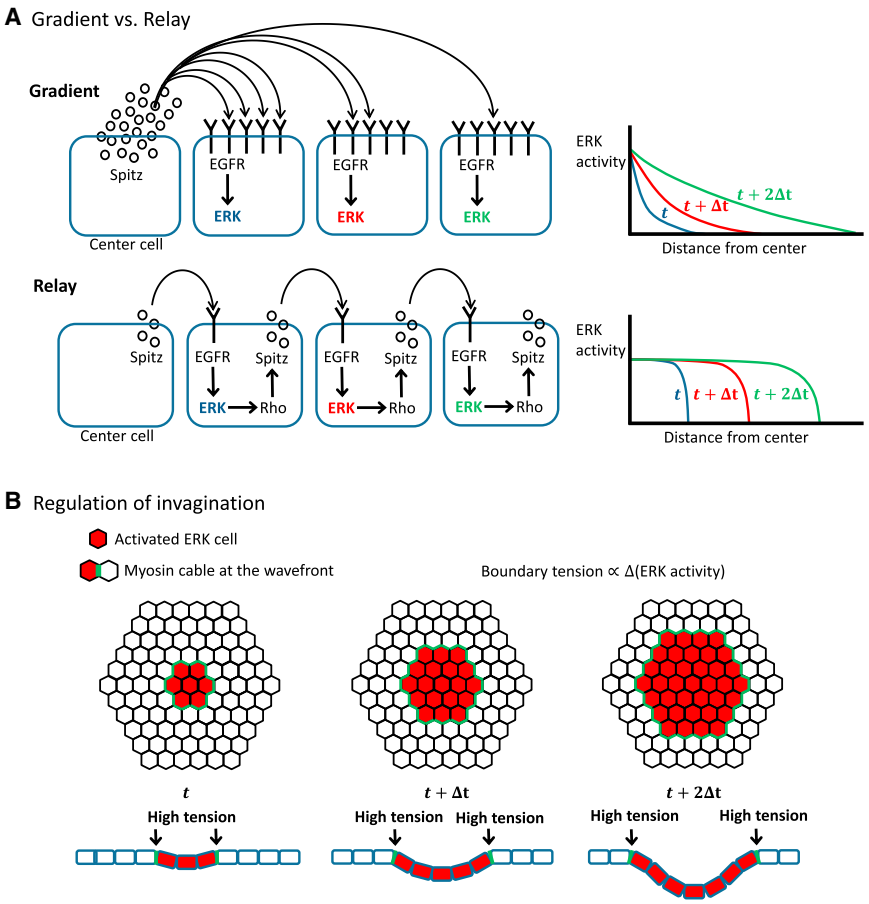

Figure 1. An EGFR-ERK Relay Mechanism Controls Invagination of Tracheal Placode in *Drosophila*

(A) Two alternative models for propagation of EGFR-ERK signaling. Top: a gradient model in which the EGF ligand Spitz (Spi) propagates from the center cell. ERK activity in this model is expected to be graded. Bottom: a relay model in which the EGF ligand activates ERK in a neighboring cell (through EGFR), which in turn activates the release of Spi from that cell (through processing by Rho) that triggers the relay in the next cell over. This model is expected to produce a propagating wavefront.

(B) The relay mechanism controls tracheal placode invagination. Propagating ERK activity (red) triggers the formation of concentric myosin rings (green). Myosin accumulation at the boundary between high and low ERK activity induces high tension, leading to the invagination of the placode.

So what is the role of the relay mechanism in the tracheal placode? It is suggested that it plays an important role in the invagination process of the tracheal placode. It is shown that concentric myosin cables are sequentially formed on the cell boundaries as the wavefront of ERK activity propagates. Moreover, it is shown that myosin accumulation correlates with local differences in ERK activity, namely that myosin accumulates on cell boundaries that separate between cells with high ERK activity and cells with low ERK activity (Figure 1B). The myosin cables that are formed exert tension that drives the invagination process in the placode. The authors further suggest that Sons of Sevenless (Sos), a guanine nucleotide exchange factor (GEF), is the molecular link between EGFR signaling and myosin accumulation. The mechanism that identifies the differences in ERK activity in neighboring cells still remains to be elucidated.

To better understand why a relay mechanism is required for the invagination process, Ogura et al. developed a mathematical model that couples differences in ERK activity to the tension on the boundary between cells (Figure 1B). One part of the model describes the feedback between the EGF ligand and the ERK activity in neighboring cells using coupled dynamic equations. The second part of the model describes the mechanics of the cells in the tracheal placode using a 2D vertex model (Farhadifar et al., 2007) that calculates the morphological configuration with the minimal mechanical energy. Critically, the differences in the levels of ERK activity between adjacent cells control the boundary tensions (by accumulating myosins). Ogura et al. use the mathematical model to compare the relay mechanism, where a feedback between ERK activity and signaling occurs, and a morphogen gradient mechanism, where ERK responds to ligands that are secreted from the central cells but that do not feed back on ligand activity (Figure 1A). It is shown that the sharp front of the ERK activity wave in the relay mechanism is required to explain the sequential formation of the myosin rings and for the proper bending of the placode (Figure 1B). The gradient model, on the other hand, does not capture the observed timing and shape of the invagination.

This type of "domino effect" relay mechanism has been previously observed in other developmental systems and is not restricted to EGFR-ERK signaling. For example, the morphogenetic furrow that sets up the *Drosophila* ommatidia relies on a complex relay mechanism that involves feedback between hedgehog, Dpp, and EGF signaling (Greenwood and Struhl, 1999). Another example is the process of lateral induction that defines the prosensory region in the vertebrate inner ear, in which Notch signaling in each cell promotes the expression of the Notch ligand Jag1, which in turn activates Notch in the next row of cells (Hartman et al., 2010; Petrovic et al., 2014). One thing that is shared between these different processes is the need to generate an organized pattern or a field of cells in a stepwise manner. A relay mechanism has two main features that are important for achieving that: a sharp front and a constant propagation speed. In comparison, an increasing morphogen gradient would not have either of these properties and hence would not work well for defining a stepwise process (Figure 1A). The invagination of the tracheal placode fits well in this picture because it requires the sharp front in ERK activity to generate myosin-mediated tension, as well as the constant propagation speed for the sequential appearance of the concentric myosin cables. It would be interesting to see whether the relay

mechanism identified by this work may also be relevant in other processes controlled by the EGFR-ERK pathway.

REFERENCES

Aoki, K., Kondo, Y., Naoki, H., Hiratsuka, T., Itoh, R.E., and Matsuda, M. (2017). Propagating wave of ERK activation orients collective cell migration. Dev. Cell *43*, 305–317.e5.

de la Cova, C., Townley, R., Regot, S., and Greenwald, I. (2017). A real-time biosensor for ERK activity reveals signaling dynamics during C. elegans cell fate specification. Dev. Cell *42*, 542–553.e4.

Farhadifar, R., Röper, J.C., Aigouy, B., Eaton, S., and Jülicher, F. (2007). The influence of cell mechanics, cell-cell interactions, and proliferation on epithelial packing. Curr. Biol. *17*, 2095–2104.

Gabay, L., Seger, R., and Shilo, B.Z. (1997). In situ activation pattern of Drosophila EGF receptor pathway during development. Science *277*, 1103–1106.

Greenwood, S., and Struhl, G. (1999). Progression of the morphogenetic furrow in the Drosophila eye: the roles of Hedgehog, Decapentaplegic and the Raf pathway. Development *126*, 5795–5808.

Hartman, B.H., Reh, T.A., and Bermingham-McDonogh, O. (2010). Notch signaling specifies prosensory domains via lateral induction in the developing mammalian inner ear. Proc. Natl. Acad. Sci. USA *107*, 15792–15797.

Lim, B., Dsilva, C.J., Levario, T.J., Lu, H., Schüpbach, T., Kevrekidis, I.G., and Shvartsman, S.Y. (2015). Dynamics of inductive ERK signaling in the Drosophila embryo. Curr. Biol. *25*, 1784–1790.

Ogura, Y., Wen, F.-L., Sami, M.M., Shibata, T., and Hayash, S. (2018). A switch-like activation relay of EGFR-ERK signaling regulates a wave of cellular contractility for epithelial invagination. Dev. Cell *46*, this issue, 162–172.

Petrovic, J., Formosa-Jordan, P., Luna-Escalante, J.C., Abelló, G., Ibañes, M., Neves, J., and Giraldez, F. (2014). Ligand-dependent Notch signaling strength orchestrates lateral induction and lateral inhibition in the developing inner ear. Development *141*, 2313–2324.

Schweitzer, R., and Shilo, B.Z. (1997). A thousand and one roles for the Drosophila EGF receptor. Trends Genet. *13*, 191–196.

InterSEPTIN' Kinesins in Dendrites

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Cargo transport to axons and dendrites is essential for maintaining neuronal polarity and function. In this issue of *Developmental Cell*, Karasmanis et al. (2018) identify a septin—SEPT9—in differentially regulating the motility of two kinesin motors, thereby controlling cargo entry into dendrites.

Like workers in the morning commute, every protein in the cell must travel to the location where it exerts its function. This is particularly true in polarized cells, where different membrane domains carry out distinct roles. In neurons, proteins residing specifically in dendrites or axons enable the function of these cellular specializations in receiving and transmitting information, respectively. How polarized protein distribution is controlled is therefore a fundamental question in neuronal cell biology.

Preceding the targeted delivery of cargo to axon or dendrites is the actual establishment of neuronal polarity, which generates distinct cytoskeletal scaffolds in each compartment. These structures will subsequently serve as tracks and regulators for polarized transport. In the axon, microtubules are uniformly oriented with their plus ends pointing away from the soma (plus-end-out). In mammalian dendrites, microtubule polarity is mixed near the soma and becomes plus-end-out distally, whereas in flies and worms the proximal dendrite mostly harbors minus-end-out microtubules (Baas and Lin, 2011). In addition, axons and dendrites acquire distinct sets of microtubule-associated proteins (MAPs) and post-translational modification (PTMs) that further distinguish the tracks on which cargo will be delivered to each compartment. Finally, a unique organization of cytoskeletal proteins in the axon initial segment (AIS) will serve to maintain polarity, at least in part by excluding dendritic cargo from the axon (Nirschl et al., 2017; Sobotzik et al., 2009).

How, then, is this polarized cellular milieu "read" by the cargo transport machinery to deliver transmembrane proteins to their destination? (The transport of cytoplasmic proteins, although as important, remains much less understood). The first step in the process is cargo sorting in the *trans*-Golgi, mediated mostly by recognition of specific protein sequences by adaptor proteins, ensuring that cargo destined for each compartment is packaged into the appropriate vesicle. Microtubule-dependent motors—dynein and kinesins—then transport the vesicles to the axon or dendrites (Bentley and Banker, 2016). Because dynein moves toward the microtubule minus end and kinesins mostly toward the plus end, the uniform plus-end-out orientation of axonal microtubules mandates cargo delivery to this compartment by a kinesin motor. However, the mixed microtubule polarity in the proximal dendrite does not provide a similar clear-cut directional cue. Furthermore, because kinesins can carry both axonal and dendritic cargo, how is dendritic cargo excluded from the axon, and how does axonal cargo reach the correct compartment? These questions have been the focus of intense research, which illuminated the roles of microtubule polarity, MAPs, PTMs, and the AIS in controlling polarized cargo transport.

In this issue of *Developmental Cell*, Karasmanis et al. (2018) uncover a role for a