Squeezing out in a "tug of war": The role of myosin in neural stem cell delamination

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Neural stem cells or neuroblasts in the *Drosophila melanogaster* embryo delaminate as single cells from the embryonic epidermis to give rise to the nervous system. Using this accessible system to examine the molecular mechanisms of cell ingression at a high temporal and spatial resolution, in this issue, Simões et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201608038) reveal that myosin-driven anisotropic junction loss and apical constriction are the main drivers of this process.

In most animals, the earliest stages of development are characterized by the organization of cells into a polarized epithelial sheet. Several tissues arise from the early embryonic epithelium through a regulated process called the epithelial–mesenchymal transition (EMT), in which single cells or groups of cells leave the epithelial layer in a controlled manner. Regulated loss or extrusion of cells from an epithelial layer also contributes to tissue homeostasis (Fig. 1 A).

EMT can be triggered through numerous signaling pathways and usually involves complex changes to the cellular phenotype through a broad switch at the transcriptional level mediated by transcription factors of the Snail, bHLH (basic helix-loop-helix), and ZEB (zinc-finger E-box binding) families (Lamouille et al., 2014). The changes include a switch from the expression of factors mediating epithelial cell–cell adhesion, such as E-cadherin, to factors mediating cell–matrix interactions and cell migration as well as a change in cell polarity from epithelial apical–basal to a front–rear polarity. Cells during EMT leave the epithelium basally, migrating and contributing to lower tissue layers. The mechanics and dynamics of EMT at an individual cell level are less clear, as they tend to be difficult to image live.

More is known mechanistically about the processes leading to single cell extrusion of either apoptotic or live cells from an epithelial layer. In contrast to EMT, these cells in vertebrates are extruded apically to ensure that unwanted, sick, or dying cells will not receive erroneous survival signals. Both apoptotic and live extruding cells signal to the surrounding tissue through sphingosine-1-phosphate and begin to constrict basally in a cell-autonomous manner. Neighboring cells then assemble an actomyosin ring around the extruding cell, allowing coordination of cell removal and new junction assembly, thereby ensuring that epithelial integrity is maintained (Slattum et al., 2009; Eisenhoffer et al., 2012).

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Homeostatic cell extrusion is also found in *Drosophila melanogaster*, though the direction of extrusion is similar to EMT, i.e., usually toward the basal side of the epithelium. During the development of the adult notum, cell extrusion plays an important role to prevent tissue overcrowding. Here, anisotropic junction losses triggered in a stochastic fashion lead to area reduction, with an accumulation of myosin in the neighboring cells helping the final extrusion. In contrast to EMT, the basally extruded cells quickly undergo anoikis (Marinari et al., 2012).

In this issue, Simões et al. focus on the process by which the first wave of embryonic neuroblasts delaminates from the *Drosophila* embryonic epidermis. The delaminated neuroblasts underneath the epidermis retain aspects of the epithelial polarization and generate all embryonic neurons through a series of asymmetric divisions (Homem and Knoblich, 2012). Although the fate determination of neuroblasts through Achaete–Scute complex transcription factors and Notch–Delta signaling has been extensively studied, much less is known about the actual molecular mechanism of how these cells leave the epithelium. As this process occurs at the surface of the embryo, it is uniquely accessible for quantitative imaging approaches.

Using embryos with fluorescently marked cell outlines, Simões et al. (2017) show that ingressing cells lose over 90% of their apical area within 30 min, whereas neighboring cells form new junctions, filling the space left by the ingressing neuroblast. The apical area loss, when viewed from the apical side of the epithelium, is not isotropic; rather, junctions are lost over time in an anisotropic fashion, with junctions positioned vertically (parallel to the dorsal–ventral axis of the embryo) disappearing first. In contrast to EMT processes of delamination, Snail family transcription factors, although involved in neuroblast specification, are not essential for the neuroblast ingression, and neither is a transcriptional down-regulation of E-cadherin. Simões et al. (2017) also observe that, although junctions of the ingressing cells are shrinking, E-cadherin concentration in those junctions before shrinkage is not reduced, suggesting that processes such as endocytosis drive the reduction rather than a posttranscriptional down-regulation of E-cadherin. In fact, although the classical description of EMT involves transcriptional down-regulation of E-cadherin through Snail family transcription factors to drive junction disassembly, other recent studies suggest that this might not be universal. For instance, mesodermal cells maintain E-cadherin during chick gastrulation, and

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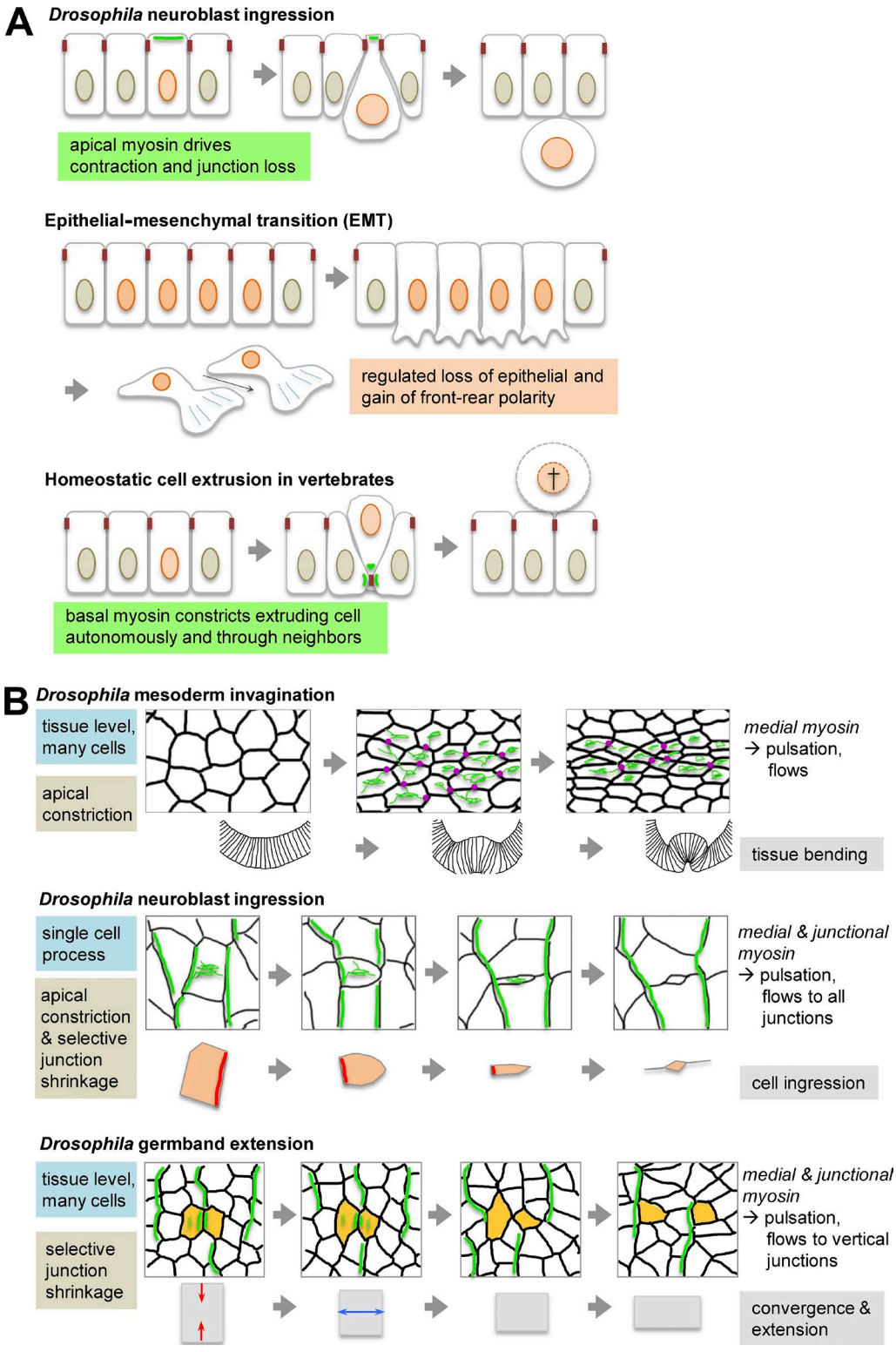

Figure 1. **Mechanisms of epithelial cell delamination and roles of apical myosin in epithelial cells.** (A) Single cell delamination or extrusion from epithelial sheets can occur in different ways: Simões et al. (2017) show that neuroblast ingression depends on myosin-driven apical area and junction loss. EMT involves major transcriptional changes to cell adhesiveness and a shift from apical–basal to front–rear polarity. In both of these processes, cells leave the epithelium basally. Homeostatic cell extrusion leads to apical loss of cells that die and is driven by basal myosin contractions in the extruding as well as the surrounding cells. (B) Junctional and medial myosin morphogenetic modules drive different processes. (Top) During mesoderm invagination in *Drosophila*, medial myosin (green) linked to spot adherens junctions (magenta) in all mesodermal cells drives apical constriction, eventually contributing to tissue bending. (Bottom) During germband extension in the fly, planar polarized myosin enriched at vertical junctions (green; fed by medial myosin flows toward junctions) drives directed junction exchanges (T1 exchange highlighted by yellow cells) to lead to overall convergence and extension of the tissue. (Middle) In ingressing embryonic neuroblasts in the fly, both myosin modules (green) are prominent, with the vertically enriched myosin driving anisotropic junction loss (red junctions in the schematic) and medial myosin driving apical area shrinkage.

Twist-induced migration of mammary cells in organotypic cultures requires retention of E-cadherin in order to occur (Nakaya et al., 2008; Shamir et al., 2014).

Apical area loss in many different morphogenetic contexts is driven by active apical actomyosin networks (Martin and Goldstein, 2014). These can be located either junctionally near apical adherens junctions or in an apical–medial position just underneath the apical cell cortex. Both junctional and apical–medial myosin pools show a pulsatile behavior, undergoing cycles of increase and decrease in intensity. The importance of each pool, junctional versus medial, varies between different processes. Overall, apical–medial actomyosin has been shown to drive apical cell constriction, as seen, for instance, during mesoderm invagination, dorsal closure, and salivary gland tube formation in the fly, where medial myosin is very dominant. Medial myosin is attached to cell–cell junctions in a web-like fashion, thereby pulling the junctions inwards (Martin et al., 2009; Blanchard et al., 2010; Booth et al., 2014). Junctional pools of myosin, however, drive selective apical junction shrinkage, a process essential for neighbor exchanges in *Drosophila* germband elongation, a highly dynamic morphogenetic process of convergence and extension. These neighbor exchanges are polarized because the junctional myosin is preferentially accumulated at the vertical junction, leading to overall tissue convergence along the vertical axis coupled with extension along the orthogonal horizontal (parallel to the anterior–posterior) axis (Rauzi et al., 2010; Simões et al., 2010).

What all of these processes have in common is that they involve large groups of cells, with myosin behavior patterned and to some extent coordinated across a whole tissue, thereby driving large scale events at the tissue level. The behavior of myosin in neuroblast ingression appears to be no different, even though this is a single cell event. Simões et al. (2017) observe and quantify the behavior of both apical junctional and apical–medial myosin in relation to the cycles and duration of apical area contraction and expansion. Periodic pulses of medial and junctional myosin in the neuroblasts drive increasingly stronger cortical contractions that are accompanied by an overall increase in apical myosin levels toward the end of the ingression. Although neuroblast ingression is a single cell event, it occurs in the embryonic epidermis at a time when it is undergoing germband elongation, and therefore when myosin is strongly polarized, with higher levels at vertical junctions compared with horizontal junctions. This planar polarization of myosin appears to be the cause of the preferential shrinkage of vertical junctions at the beginning of ingression.

Despite being surrounded by germband cells undergoing highly dynamic neighbor exchanges, the overall outcome for neuroblasts compared with surrounding germband cells is very different. How is this achieved? There is no complete answer yet, but Simões et al. (2017) show that the transcriptional program downstream of the Achaete–Scute complex primes the whole proneural cluster to be capable of eliciting myosin dynamics driving ingression. Notch–Delta signaling restrains this capacity to a single cell. Simões et al. (2017) show that in the absence of Notch signaling (using RNAi against either the receptor or ligand), the whole cluster of cells ingresses, albeit with changed kinetics. The authors go on to show that, with myosin-driven forces being key for the cell behaviors of both neuroblasts and germband cells, it is the balance of these forces between ingressing neuroblasts and surrounding cells that allows wild-type ingression. A neuroblast freed from connections to surrounding cells (via elegant laser ablation experiments) ingresses faster than a wild-type cell, whereas artificially increased tension in surrounding cells slows down ingression, as is seen when the whole cluster invaginates in the absence of Notch signaling. This "tug of war" also helps to explain the surprisingly small effect that reduction of myosin activity (in hypomorphic mutants or with RNAi) has on the process. Under these conditions, the apical area reduction in the ingressing neuroblast does not have to work against myosin contractility in the surrounding cells, and thus ingression can still progress, albeit slower.

The study by Simões et al. (2017) is a beautiful illustration of how different but concomitant morphogenetic processes are accommodated within a single tissue. It suggests strongly that different morphogenetic modules exist, such as junctional or medial actomyosin dynamics, that can be controlled and deployed in varying combinations to drive very different morphogenetic outcomes. What remains to be discovered is which of the proneural transcription factors responsible for neuroblast specification are upstream of the morphogenetic changes driving neuroblast ingression, and the mechanisms by which their downstream targets modulate the behavior of the junctional and medial myosin modules.

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References

Blanchard, G.B., S. Murugesu, R.J. Adams, A. Martinez-Arias, and N. Gorfinkiel. 2010. Cytoskeletal dynamics and supracellular organisation of cell shape fluctuations during dorsal closure. *Development.* 137:2743–2752. http://dx.doi.org/10.1242/dev.045872

Booth, A.J., G.B. Blanchard, R.J. Adams, and K. Röper. 2014. A dynamic microtubule cytoskeleton directs medial actomyosin function during tube formation. *Dev. Cell.* 29:562–576. http://dx.doi.org/10.1016/j.devcel.2014.03.023

Eisenhoffer, G.T., P.D. Loftus, M. Yoshigi, H. Otsuna, C.B. Chien, P.A. Morcos, and J. Rosenblatt. 2012. Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. *Nature.* 484:546–549. http://dx.doi.org/10.1038/nature10999

Homem, C.C., and J.A. Knoblich. 2012. *Drosophila* neuroblasts: a model for stem cell biology. *Development.* 139:4297–4310. http://dx.doi.org/10.1242/dev.080515

Lamouille, S., J. Xu, and R. Derynck. 2014. Molecular mechanisms of epithelial–mesenchymal transition. *Nat. Rev. Mol. Cell Biol.* 15:178–196. http://dx.doi.org/10.1038/nrm3758

Marinari, E., A. Mehonic, S. Curran, J. Gale, T. Duke, and B. Baum. 2012. Live-cell delamination counterbalances epithelial growth to limit tissue overcrowding. *Nature.* 484:542–545. http://dx.doi.org/10.1038/nature10984

Martin, A.C., and B. Goldstein. 2014. Apical constriction: themes and variations on a cellular mechanism driving morphogenesis. *Development.* 141:1987–1998. http://dx.doi.org/10.1242/dev.102228

Martin, A.C., M. Kaschube, and E.F. Wieschaus. 2009. Pulsed contractions of an actin–myosin network drive apical constriction. *Nature.* 457:495–499. http://dx.doi.org/10.1038/nature07522

Nakaya, Y., E.W. Sukowati, Y. Wu, and G. Sheng. 2008. RhoA and microtubule dynamics control cell–basement membrane interaction in EMT during gastrulation. *Nat. Cell Biol.* 10:765–775. http://dx.doi.org/10.1038/ncb1739

Rauzi, M., P.F. Lenne, and T. Lecuit. 2010. Planar polarized actomyosin contractile flows control epithelial junction remodelling. *Nature.* 468:1110–1114. http://dx.doi.org/10.1038/nature09566

Shamir, E.R., E. Pappalardo, D.M. Jorgens, K. Coutinho, W.T. Tsai, K. Aziz, M. Auer, P.T. Tran, J.S. Bader, and A.J. Ewald. 2014. Twist1-induced dissemination preserves epithelial identity and requires E-cadherin. *J. Cell Biol.* 204:839–856. http://dx.doi.org/10.1083/jcb.201306088

Simões, S.M., J.T. Blankenship, O. Weitz, D.L. Farrell, M. Tamada, R. Fernandez-Gonzalez, and J.A. Zallen. 2010. Rho-kinase directs Bazooka/Par-3 planar polarity during *Drosophila* axis elongation. *Dev. Cell.* 19:377–388. http://dx.doi.org/10.1016/j.devcel.2010.08.011

Simões, S., Y. Oh, M.F.Z. Wang, R. Fernandez-Gonzalez, and U. Tepass. 2017. Myosin II promotes the anisotropic loss of the apical domain during *Drosophila* neuroblast ingression. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201608038

Slattum, G., K.M. McGee, and J. Rosenblatt. 2009. P115 RhoGEF and microtubules decide the direction apoptotic cells extrude from an epithelium. *J. Cell Biol.* 186:693–702. http://dx.doi.org/10.1083/jcb.200903079