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The forces and fates of extruding cells

John Fadul and Jody Rosenblatt

Cell extrusion drives most epithelial cell death while maintaining a functional epithelial barrier. To extrude, a cell produces a lipid signal that triggers the neighboring cells to reorganize actin and myosin basally to squeeze the extruding cell out apically from the barrier. More studies continue to reveal other signals and mechanisms controlling apical extrusion. New developmental studies are uncovering mechanisms controlling basal extrusion, or ingression, which occurs when apical extrusion is defective or during de-differentiation in development. Here, we review recent advances in epithelial extrusion, focusing particularly on forces exerted upon extruding cells and their various later fates ranging from cell death, normal development, and cancer.

Address
Huntsman Cancer Institute, The University of Utah, Salt Lake City, UT, USA

Corresponding author: Rosenblatt, Jody (jody.rosenblatt@hci.utah.edu)

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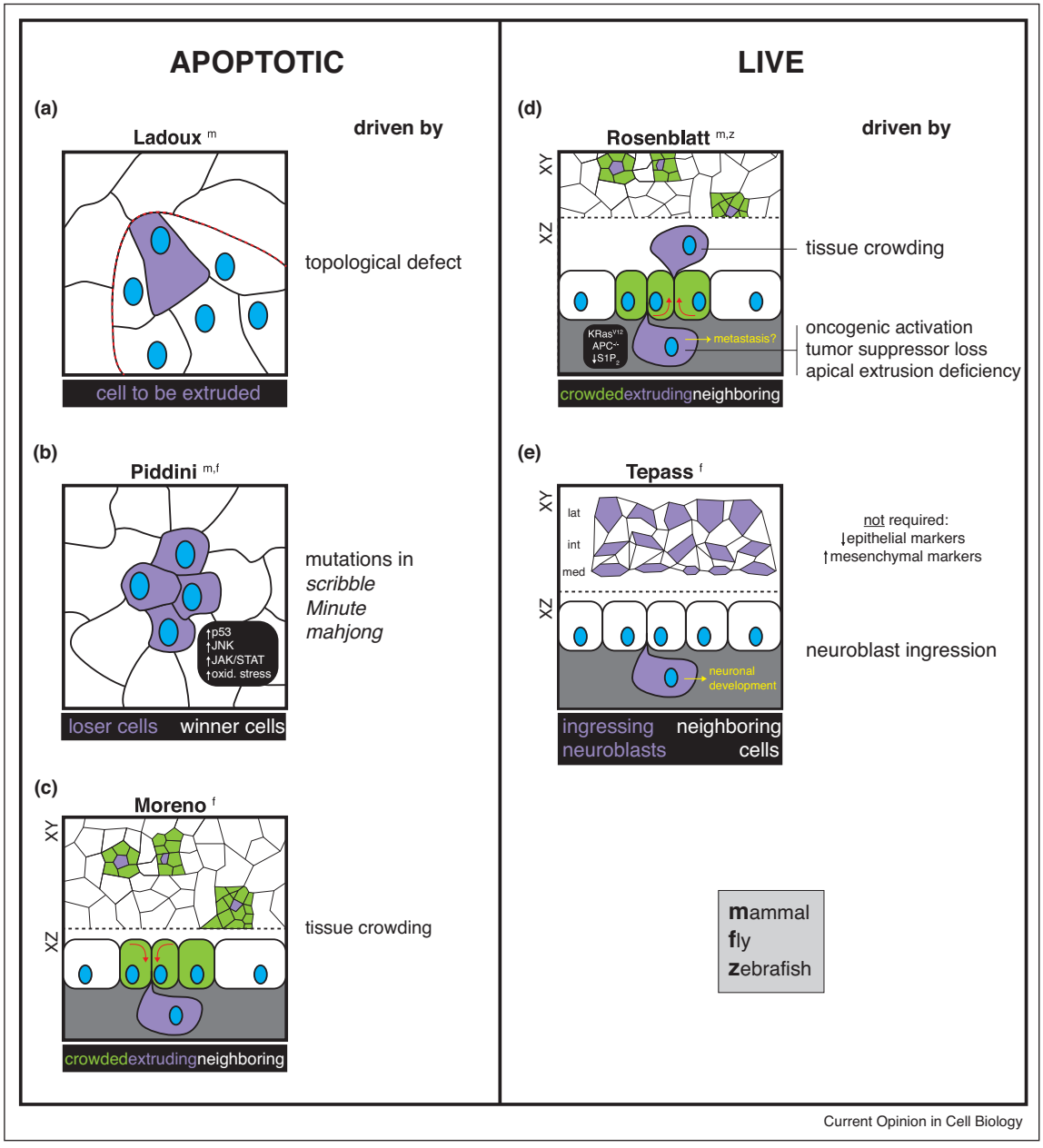
Epithelial cells work together to maintain a tight barrier, yet can turn over rapidly by cell division and death. To help accomplish this feat, epithelia eject cells fated to die by a process called cell extrusion. To extrude, a cell produces and emits the lipid sphingosine-1-phosphate (S1P), which binds to its cognate receptor $S1P_2$ in cells neighboring it to form a intercellular, basolateral, contractile actomyosin ring that squeezes the cell out of the epithelium [1,2]. In this manner, cells triggered to undergo apoptosis [2,3] or, more commonly, supernumerary live cells that later die by anoikis [4], are eliminated without disrupting the barrier [3–7]. Here, we review current cell extrusion literature focusing on its mechanism and signaling, and also highlight emerging new roles for extrusion in driving cell competition and tumor suppression.

One of the most important roles for extrusion is to maintain constant epithelial cell densities by mechanically matching the number of cells that die to those that divide. In vertebrates, mechanical force links cell division with cell death, as crowding triggers apical extrusion of live cells through the stretch-activated channel Piezo1 [4]. Previous work suggested that crowding also drives live cells to basally extrude or delaminate in *Drosophila* notum [8]. However, recent work from Levayer *et al.* shows that crowding causes cells to first undergo apoptosis which, in turn, drives delamination [9••]. Here, inhibition of the apoptotic pathway by *diap1* or p35 overexpression or homozygous loss of *hid*, *grim*, and *reaper* blocks delamination in crowded regions. Additionally, ras^{V12} -overexpressing cells crowd wildtype cells up to three cell diameters away and force their delamination, suggesting that overall tissue crowding causes cell delamination (Figure 1c) [9••]. Differential results in these two systems may be due to differences in the strength of promoters used to overexpress DIAP [8,9••]. In other epithelia at different times during *Drosophila* development, cells activate apoptosis before delaminating, suggesting that flies regulate extrusion of supernumary cells using different signaling pathways to vertebrates [10]. An important reason for this may be due to the fact that extrusion of live cells basally is risky if cells do not later die; for instance, cancer cells or stem cells may use this mechanism to escape their primary epithelial sites and invade or differentiate, respectively. In fact, live neuroblast cells delaminate from the neuroepithelium before becoming neurons [11••]. Thus, activating cell death simultaneously with *basal* extrusion could ensure that supernumerary cells are eliminated.

Although crowding within epithelia drives some cells to extrude, what causes a specific cell within a crowded region to extrude is not well understood. Several recent papers shed light on important signaling and mechanical forces that contribute to one cell extruding. Using Madin-Darby canine kidney (MDCK) epithelial cells grown to confluence on micropatterned, functionalized substrates, Saw *et al.* found that epithelial cells can behave like nematic liquid crystals (liquids comprised of molecules oriented in a crystal-like pattern) that align along their long axes, with cell extrusions occur at sites of patterning defects to relieve cell strain (Figure 1a) [12••]. At these defect sites, extruding cells were not locally crowded but instead the result of perpendicular single-cell collisions. Using elegant experiments where they confined cell growth and movement by plating on matrices of different shapes, they could increase or decrease these defects and collisions, and the extrusion rates, accordingly. Sites of single-cell defects had increased cytoplasmic yes-associated protein (YAP)

Figure 1



The driving forces on and fates of apoptotic and live extruding cells. Depending on species, cell context, and neighboring cell status, cells bound to extrude can have several different fates. **(a,b)** Mammalian cells at the leading edge of a comet-shaped topological defect (a) or harboring 'loser' mutations (b) are destined to die and extrude apically. **(c)** Conversely, cells in the fly notum that are to be eliminated experience crowding forces and delaminate (i.e. extrude basally). In this context, cells necessarily commit to apoptosis, in contrast to a previous report (ref. [4]). **(d)** In mammalian cell cultures, mouse gut epithelia, and the developing zebrafish epidermis, cells that experience crowding forces extrude apically and later die by anoikis. Conversely, cells that harbor oncogenic mutations, lose tumor suppressors, or have deficiencies in apical extrusion machinery extrude basally and may live, revealing a novel path to metastasis. **(e)** Fly neuroblasts ingress (i.e. extrude basally; lateral, intermediate, and medial neuroblasts are shown) in an EMT-independent fashion, and later develop into mature neurons.

and caspase-3 activation [12••], in contrast with live cell extrusions that result from whole epithelial sheet crowding [4,8]. Future work will need to examine if single-cell perpendicular collisions are at the heart of live cell extrusions in crowded regions of tissues, or if this represents another pathway to eliminate cells causing patterning defects in the otherwise regular epithelial fabric that coats organs.

Cell packing density can also result in different modes of extrusion. In a loosely packed environment, dynamic, large-scale cell movements lead to transient increases in local cell density where extrusions occur [13]. At lower densities, some cells can extrude by their neighboring cells' crawling in to replace where a cell exits. Although epithelia *in vivo* exist at high density, in cases where they experience wounds or excessive cell death, this different mechanism would ensure that no gaps form in the epithelial barrier. As cells grow and density increases in mature epithelia, cell movement decreases and the numbers and rates of cell extrusions increase, this time occurring via formation and contraction of the actomyosin cable in surrounding cells, as described previously [2,13]. Here, recent work has found a role for coronin1B in reorganizing actomyosin in adherens junctions at steady-state and during apoptotic cell extrusion. Depletion of E-cadherin or coronin1B compromises the integrity of adherens junctions and consequently hampers cell extrusion [14].

Just as cell crowding due to excess cells can cause cell extrusion and death, cell stretch due to sparse cell populations can drive rapid cell division through a mechanism that also depends on Piezo1 [15••]. Stretch, induced mechanically or by wounding a MDCK monolayer, triggers rapid cell division by Piezo1-dependent activation of a single spike of calcium, which in turn, activates ERK1/2 phosphorylation and cyclin B transcription in cells poised in G2. Loss of Piezo1 in MDCK cells or zebrafish markedly decreased cell divisions. Although Piezo1 activates calcium to control the two opposing processes of cell division and extrusion, the type of force is critical for the outcome: stretch triggers cell division, not extrusion; and crowding triggers extrusion, not division (Figure 1d). Interestingly, the threshold of mechanical strain is ~1.6-fold in both cases [4,15••]. How Piezo1 activates calcium to control two opposing processes is not entirely clear; however, calcium activation alone is sufficient to drive cells poised in G2 to enter mitosis, whereas extrusion requires both calcium and crowding [15••].

Neural development

Although earlier work demonstrated that basal extrusion of cells within the *Drosophila* neuroectoderm gives rise to neuroblasts [16], little was known about the mechanism driving neuroblast delamination (or ingression). Recent work by Simões *et al.* shows that during this ingression, fly neuroblasts lose their apical surfaces anisotropically, with anterior–posterior junctions shrinking before dorsal–ventral junctions, due to polarized myosin distribution, causing a ratchet-like apical constriction of ingressing neuroblasts (Figure 1e) [11••]. Downregulation of epithelial markers such as DEcad and upregulation of mesenchymal markers such as Snail family members are not required for ingression, suggesting that classical mechanisms proposed for epithelial-to-mesenchymal transition (EMT) are dispensable for ingression. Lastly, they show that neuroblasts ingress non-autonomously: experimentally reducing tension in neighboring non-ingressing cells (NICs) by laser ablation of apical junctions hastens ingression, while increasing tension by converting NICs to neuroblasts through *Delta* or *Notch* depletion delays it [11••].

Cell competition

Cell competition is a process whereby fit cells eliminate less fit cells to improve tissue health. Although the genetic drivers of this process have been known for over 40 years, the mechanism for this process has eluded researchers. Now, recent studies suggest that cell extrusion could be a conserved mechanism for removing loser cells during cell competition. Chiba *et al.* showed that constitutively active YAP mutant cells act as losers, which get apically extruded by surrounding wildtype cells [17•]. The TEAD and PDZ domains of YAP, necessary for nuclear localization and gene activation, are required for this phenotype [18–20]. Interestingly, genetic or chemical inhibition of vimentin abrogates the ability of the neighboring cells to extrude YAP-expressing cells, suggesting a role for intermediate filaments in measuring cell fitness. Finally, using co-cultures of different oncogene-expressing or wildtype cells, the authors determined an increasing order of competitiveness, thus: v-Src < $KRas^{V12}$ < YAP (5SA) < wildtype [17•].

Additionally, MDCK cells depleted of the cell polarity gene *scribble* ($scrib^{KD}$) lose to wildtype cells through extrusion in response to mechanical forces, rather than soluble factors. Wagstaff *et al.* showed that $scrib^{KD}$ cells grow to a lower density threshold than wildtype cells: on their own they reach a sparser, less compacted steady state density than wildtype cells; however, when grown mosaically with wildtype cells, they become compacted and extrude (Figure 1b) [21••]. In these cases, wildtype cells corral and then compact $scrib^{KD}$ cells in an E-cadherin-dependent manner. Compaction activates p53 transcription in $scrib^{KD}$ cells, which is necessary and sufficient for their apoptotic extrusion. p53 induction is ROCK-dependent and p38 kinase-dependent, demonstrating a new mechanical mechanism for activating p53 [21••].

Other work from the same group revealed a new role for oxidative stress pathways in loser cells in fly wing imaginal discs. In two different classes of loser cells, either ribosomal mutant alleles of *Minute* or the cell polarity-associated complex component *mahjong*, cells experience oxidative stress and an increase of JNK and JAK/STAT activation (Figure 1b). During competition, the JNK pathway restricts the growth of prospective loser cells whereas JAK/STAT activation drives rapid growth in winner cells. Oxidative stress confers loser status via transcription mediated through Nrf2 [22•]. Interestingly, a fly suppressor screen for *scrib* deficiency-induced loser status identified the Slit-Robo pathway, known for its role

in angiogenesis and axon guidance. Vaughen and Igaki showed that indeed deficiencies in *slit*, *robo* or *ena* rescued elimination of *scrib*$^{-/-}$ eye disc cells, and that the Slit-Robo pathway acts downstream of JNK pathway activation, adding further resolution of the signaling pathway controlling cell loss [23].

Dysregulation of extrusion in disease

Brain defects from excess extrusion

Germline knockout of *Alix* leads to mice with hydrocephaly, smaller cerebrum and hippocampus, and poor epithelial barrier function within the choroid plexus, causing loss of cerebral spinal fluid, compared to control. Further inspection of the choroid plexus epithelia suggests that the poor barrier results from high levels of apical extrusion accompanied by extensive blebbing of the microvilli and abnormal cilia. The extrusion defects and poor barrier of *Alix*$^{-/-}$ mice appear to result from aberrant actomyosin assembly and mislocalization of tight junction proteins at the basolateral membrane, which disrupts proper reformation of cell–cell junctions post-extrusion [24].

Aberrant extrusion and cancer

In cells where certain oncogenes are induced, a program called epithelial defense against cancer (EDAC) causes their expulsion through a process that resembles extrusion but is driven by a separate signaling and mechanism than that occurring in wild-type cells [25–27]. EDAC is an important tumor suppressor mechanism similar to cell competition that can help preserve tissue fitness by eliminating cells with aberrant growth signaling. However, other types of oncogenic mutations can persist in cells and instead drive tumor formation and progression, mainly by switching their direction of extrusion.

As described above, most epithelial cells in vertebrates extrude apically while still alive, whereas in *Drosophila*, cells trigger extrusion basally as they undergo apoptosis, except when delaminating stem cells (Figure 1). Importantly, oncogenic or tumor suppressor mutations can hijack the direction that cells extrude in both vertebrates and in flies. Tamori *et al*. showed that fly wing disc cells without tumor suppressor genes like *lgl* or *scrib* form tumors — but only in 'hotspots,' where JAK/STAT levels are high and structures within the basal membrane and underlying ECM prevent basal extrusion and apoptosis. Instead, cells extrude apically and accumulate into tumor-like masses [28•]. Strikingly, RhoGEF2, the fly ortholog of p115 RhoGEF, necessary for apical extrusion in mammals [31], is critical for basal extrusion in flies: when it and the tumor suppressors are absent, ectopic JAK/STAT activation drives apical extrusion and tumorigenesis [28•].

Similarly, basal extrusion can be exploited by oncogenic mechanisms to promote cancer in vertebrates, enabling transformed cells to invade and metastasize throughout the body [29]. Pancreatic cancers unilaterally downregulate $S1P_2$, the receptor required for apical extrusion (Figure 1d) [30••]. In pancreatic cancer cells, or in cell lines or zebrafish lacking $S1P_2$, cells that cannot extrude apically instead form cell masses that are chemo-resistant, cause poor epithelial barriers, and instead basally extrude. Moreover, labeled pancreatic cancer cells grown orthotopically in nude mice form large metastatic tumors. Rescue of $S1P_2$ in these cells is sufficient to abrogate both tumor growth and metastases [30••].

Two other mutations that drive aggressive tumor types, adenomatous polyposis coli (APC) truncation and $KRas^{V12}$, also hijack apical extrusion (Figure 1d). During apical extrusion, APC and microtubules reorient basally, targeting p115 RhoGEF-mediated Rho activation to contract actomyosin basally and extrude cells apically, disrupts this microtubule targeting, causing actin already at apical junctions to contract and extrude cells basally [31,32]. $KRas^{V12}$, an oncogenic KRas mutation that drives a class of aggressive tumors (pancreatic, lung, and colon cancers [33]) shifts extrusion from apical to basal by degrading the S1P ligand of the $S1P_2$ receptor via increased autophagy. Simply blocking autophagy either genetically or with chloroquine rescues S1P accumulation and apical cell extrusion, suggesting a potential therapeutic approach for shifting cells from a potentially invasive fate to an apoptotic one [34]. Moreover, p120 catenin may also contribute to this aberrant extrusion signaling [35••]. Hendley *et al*. showed that human pancreatic tumors express low levels of p120 catenin, compared to PanIN lesions. Deletion of p120 catenin in a $KRas^{D12}$-pancreatic cancer mouse model reduces S1P signaling via upregulation of the NF-κB pathway, resulting in poorer survival, more basally extruded cells and increased barrier function defects and inflammation [35••]. These studies suggest that invasion of tumor cells may depend on mechanisms independent of classically inferred EMT signaling. Additionally, EMT is also dispensable for lung and pancreatic cancer metastasis in mice [36,37]. Future work will need to determine if basally extruded cells drive the distant metastases linked to the increased mortality (Figure 1d).

In summary, recent studies have highlighted new ways that mechanical forces can drive signaling important for both live and apoptotic cell extrusion. It is unclear why vertebrates tend to extrude cells apically while those in developing *Drosophila* do so basally. However, *Drosophila* genetics could offer further insight to our understanding of the signaling that drives basal extrusion, a point on which little is known. It is particularly noteworthy that shifting the direction that cells normally extrude can impact malignancy in both *Drosophila* and vertebrates. Interestingly, the finding that basal extrusion can cause invasion of both tumor and stem cells in ways that do not implicate normal EMT mechanisms suggest new horizons for our understanding of both metastasis and

development. Further work will need to define if the two processes are mutually exclusive and their exact contributions to cancer progression.

Conflict of interest statement

Nothing declared.

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