Cell polarity and extrusion: how to polarize extrusion and extrude misspolarized cells?

Ralitza Staneva, Romain Levayer

Cell polarity and extrusion: how to polarize extrusion and extrude misspolarized cells?

Ralitza Staneva[1] and Romain Levayer[1*]

1. Department of Developmental and Stem Cell Biology, Institut Pasteur, Université de Paris Cité, CNRS UMR 3738, 25 rue du Dr. Roux, 75015 Paris, France

* Correspondance to: romain.levayer@pasteur.fr

Contents

Abstract

The barrier function of epithelia is one of the cornerstones of the body plan organisation of metazoans. It relies on the polarity of epithelial cells which organises along the apico-basal axis the mechanical properties, signalling as well as transport. This barrier function is however constantly challenged by the fast turnover of epithelia occurring during morphogenesis or adult tissue homeostasis. Yet, the sealing property of the

tissue can be maintained thanks to cell extrusion: a series of remodelling steps involving the dying cell and its neighbours leading to seamless cell expulsion. Alternatively, the tissue architecture can also be challenged by local damages or the emergence of mutant cells that may alter its organisation. This includes mutants of the polarity complexes which can generate neoplastic overgrowths or be eliminated by cell competition when surrounded by wild type cells. In this review, we will provide an overview of the regulation of cell extrusion in various tissues focusing on the relationship between cell polarity, cell organisation and the direction of cell expulsion. We will then describe how local perturbations of polarity can also trigger cell elimination either by apoptosis or by cell exclusion, focusing specifically on how polarity defects can be directly causal to cell elimination. Overall, we propose a general framework connecting the influence of polarity on cell extrusion and its contribution to aberrant cell elimination.

Keywords: cell extrusion, apico-basal polarity, cell competition, epithelium, cell death, planar cell polarity

Approximate words number: 10,000

1. Introduction

The barrier function of epithelia is intimately related to the diversification of body plans in metazoans. By mechanically and chemically separating compartments, they provided the unique possibility to diversify and specialised the functions of organs. This barrier function is based on the tight coupling between epithelial cells, which through adherens junctions and tight junctions maintain the cohesion and sealing properties of the tissue. This organisation relies on stereotypical distribution of various proteins and lipids along the apico-basal axis of the cell. This polarity has been studied extensively in cell culture or model organisms such as *Drosophila* and is based on mutual exclusive relationship between apical complexes (Crumbs, Stardust, aPKC, Par6, PatJ), apical junctional complexes (Par3) and lateral complex composed of Scribbled (Scrib), Disc-large (Dlg) and Lethal giant larvae (Lgl) (Flores-Benitez and Knust, 2016) as well as Par1, Yurt and septate junction components (Buckley and St Johnston, 2022). This relatively static view of epithelia is challenged by the very plastic behaviours observed during development and adult homeostasis. Epithelial organisation can be dramatically remodelled during morphogenesis, involving modifications of cell shape and cell-cell junctions (Guirao and Bellaiche, 2017), while some epithelia are constantly renewed

through cell division and cell death during adult organ homeostasis (Patterson and Watson, 2017). As such, the mechanisms maintaining apico-basal polarity and sealing properties during cell division has become an intensive field of study (Osswald and Morais-de-Sa, 2019). Similarly, the mechanisms maintaining the sealing property during cell death has become another central question of epithelial biology. Cell extrusion is defined by the series of remodelling steps leading to epithelial cell exclusion from the tissue layer without impairing the sealing properties of the tissue (Rosenblatt et al., 2001). While it has been historically associated with cell death, it is nowadays associated with a wide variety of cellular events that either exclude aberrant cells (Kajita and Fujita, 2015), promote cell differentiation (An et al., 2017; Miroshnikova et al., 2018; Simoes et al., 2017) or promote tumoral cell invasion (Gu et al., 2015). Cell extrusion is by essence a polarised process which relies on the tight coordination between cell constriction, junction disassembly and *de novo* junction formation between the neighbouring cells. How these different remodelling steps are coordinated remains an essential and unresolved question. Similarly, the direction of cell expulsion (apically or basally) can have drastic consequences on the fate of the extruding cell which will either be expelled from the body into the lumen or remain on the basal side to die, invade or undergo differentiation. What is then driving the direction of cell expulsion and which conditions can perturb this directionality? How can core components of the apico-basal polarity complexes influence cell extrusion?

While these questions are related to the orchestration of cell extrusion by polarity cues, the perturbations of polarity can also be a driving force for extrusion and death induction. Polarity mutants such as *scrib*, *dlg* or *lgl* have been initially associated with neoplastic growth in *Drosophila* larvae where the period of imaginal tissues growth is overextended and associated with a global disorganisation of epithelia (Bilder, 2004; Bilder et al., 2000b). However, mosaic induction of some polarity mutants is associated with cell death and clone elimination through cell competition (Brumby and Richardson, 2003; Igaki et al., 2006; Igaki et al., 2009; Norman et al., 2012), a context-dependent cell elimination process which can remove aberrant, misspecified or unfit cells (Baker, 2020; Ohsawa et al., 2018). Works over the last decades have outlined the large range of genetic contexts and tissues in which cell competition can be observed. The mechanism of cell competition however remains largely not understood and seems to rely on a variety of recognition and elimination processes that can coexist in the same

tissue. This includes the elimination of mis-polarised cells, which can either be driven by extracellular diffusive factors (de Vreede et al., 2022; Katsukawa et al., 2018), direct contact-dependent communication (Yamamoto et al., 2017), or differential sensitivity to mechanical stress (Bove et al., 2017; Gradeci et al., 2021; Wagstaff et al., 2016a). Is there then any intrinsic relationship between the local perturbation of apico-basal polarity of cells and competition-driven elimination? More generally, can spatial heterogeneity of cell polarity cues across an epithelium influence cell survival and the stability of epithelial cells?

In this review, we will provide an overview of how polarity may influence cell extrusion and how local perturbations of polarity cues can also lead to cell elimination. We will first describe the key remodelling steps associated with cell extrusion and outline the diversity and versatility of this process. We will then provide an overview of the parameters that can modulate the direction of cell extrusion and describe interplays between apico-basal polarity and cell extrusion. In the second part, we will describe the consequences of local perturbations of polarity and how this can lead to cell elimination or cell exclusion (exit of the epithelial layer not followed by cell death). We will more specifically describe the direct causality between polarity defects and the context-dependent elimination of cells.

2. Cell extrusion, a polarized remodeling process

[inset somewhere in this section Figure 1, full page in total]

Most of our knowledge of cell extrusion initially came from the studies of MDCK (Madin-Darby Canine Kidney) cell extrusion upon UV damage. The expulsion of the dying cells is initiated by the increase of contractility driven by actomyosin accumulation in the dying cells (Kuipers et al., 2014), which is then followed by the formation of a supracellular cable of actomyosin in the neighbors (Kuipers et al., 2014; Rosenblatt et al., 2001) that slides basally while constricting (**Figure 1A**). Eventually, this will expel the cell on the apical side. The formation of the actomyosin cable is regulated by the secretion of the bioactive lipid Sphingosin 1 phosphate (S1P) by the dying cell which binds the S1P2 receptor in the neighbors and activates Rho GTPase (Gu et al., 2011), although recent results suggest that S1P may just have a global permissive role rather than an instructive role for local Rho activation (Atieh et al., 2021; Duszyc et al., 2021). Alternatively, the activation of Rho is also promoted by the transmission of forces to

the neighbors through the tension dependent recruitment of Coronin1B which reorganizes actin in bundles and promotes the formation of the actomyosin ring (Michael et al., 2016). Force transmission relies on the mechanical coupling between cells thanks to adherens junctions and E-cadherin. Accordingly, E-cad depletion impairs actomyosin ring formation and slows down cell extrusion in MDCK and MCF7 cells (Lubkov and Bar-Sagi, 2014; Michael et al., 2016). Mechanotransduction is also driven by the tension-dependent recruitment of MyosinVI in the neighbors which stimulates Rho activity through the recruitment of the p114-RhoGEF at adherens junctions (Acharya et al., 2018; Duszyc et al., 2021). Meanwhile, basal protrusions from the neighboring cells also contribute to cell extrusion by promoting the basal detachment of the dying cell (Duszyc et al., 2021; Kocgozlu et al., 2016; Le et al., 2021). The contribution of these basal protrusions is particularly central at low cell density (Kocgozlu et al., 2016) or in conditions of low cell-cell adhesion (Le et al., 2021). These contractile events have also to be coordinated with cell-cell junctions remodeling, which on the one hand need to be maintained for sealing and mechanical coupling, while being eventually disassembled to terminate extrusion. In this regard, the dynamics of desmosomes during extrusion is particularly interesting (**Figure 1A**). Desmosomes between the dying cell and its neighbors in MDCK cells are maintained throughout cell extrusion while Keratin 18 undergo progressive remodeling coupled to the formation of the actomyosin ring (Thomas et al., 2020). More importantly, *de novo* formation of desmosomes between the neighboring cells occurs on the basal side prior to the termination of extrusion and the disassembly of old desmosomes. As such, mechanical coupling between cells is maintained throughout the process.

This brief description of the central remodeling steps of extrusion already outlines a number of key events that rely on the polarized localization of cellular components, including adherens junctions, Rho activity and actomyosin organization, ECM binding and desmosomes junctions. Like other epithelial morphogenetic events, the subcellular regulation of contractility and the local activation of Rho seem to play a key role in setting the direction of expulsion. In that regard, it seems essential to understand how the initial constriction mostly located at the level of junctions will then give rise to a ring constricting on the basal side. We will discuss this point in the following sections.

2.1 The versatility and diversity of cell extrusion

The apparent uniform picture of extrusion that we provided so far is rapidly challenged when compared with extrusion in other epithelia and organisms. While the majority of extrusions occurs on the apical side in Vertebrates, most of the extrusions in *Drosophila* epithelia (sometimes also called delamination or cell ingression) drive cell expulsion on the basal side (**Figure 1B**). For instance, cell extrusions during dorsal closure in the fly embryo expel the cells on the basal side (Toyama et al., 2008). This also applies in more pseudostratified epithelia like the wing imaginal disc or the joint region of the pupal leg (Manjon et al., 2007; Monier et al., 2015). Apoptotic cells in pupal epithelia are also expelled on the basal side. This includes epithelial cells in the notum (Levayer et al., 2016; Marinari et al., 2012; Valon et al., 2021; Villars et al., 2022) (the future fly thorax) or the larval epidermal cells in the pupal abdomen (Teng et al., 2017). One notable exception is the expulsion of enterocytes in the fly gut which are extruded apically (Buchon et al., 2010; Liang et al., 2017; Martin et al., 2018) like mammalian gut enterocytes (Bullen et al., 2006; Eisenhoffer et al., 2012). Atypical basal extrusion also occurs for thin hematopoietic stem cells in the zebrafish aorta floor, which relies on the self-closing of cells on the apical side and the wrapping of an apical lumen before detachment (Lancino et al., 2018). Some atypical extrusions can even bypass the expulsion on one side of the tissue layer. For instance, the extrusion of the polar follicle cells in the fly egg chamber, so called monosis, promotes simultaneously apical and basal detachment of the dying cells which is followed by the encapsulation of the apoptotic debris in-between the neighbors (Torres et al., 2017).

This versatility of the direction and types of deformations comes along a diversity of cellular processes initiating and driving extrusion. While the early extrusion of epidermal larval accessory cells also relies on cell autonomous contractility, E-cad disassembly and formation of a supracellular actomyosin cable in the neighbors (like in MDCK cells) (Teng et al., 2017) (**Figure 1B**), the late elimination of the midline epidermal cells is mostly triggered by a pulsatile medio-apical mesh of actomyosin (Michel and Dahmann, 2020). Similarly, the delamination of neuroblasts from the *Drosophila* embryo ectoderm is driven by a relative increase of the amplitude and frequency of medio-apical pulses of actomyosin compared to neighboring cells (An et al., 2017; Simoes et al., 2017). Medio-apical actomyosin pulses were also described in zebrafish larval epidermis following acute stress and are permissive for the later formation of the actomyosin purse-string and cell extrusion (Atieh et al., 2021).

Alternatively, basal pulsatile actomyosin rings were described in extruding *Drosophila* gut enterocytes (Martin et al., 2018), albeit with much longer period than in other epithelial cells (30-60 min compared to few minutes (An et al., 2017; Michel and Dahmann, 2020; Simoes et al., 2017)). The versatility of actomyosin dynamics and subcellular localization is also accompanied by a diversity of dynamics of junctional disassembly, which can either occur early on during extrusion, like in the larval epidermal cells (Teng et al., 2017) or enterocyte shedding in the *Drosophila* gut (Liang et al., 2017; Martin et al., 2018), or show no sign of disassembly prior to full apical closure like in the *Drosophila* pupal notum (Villars et al., 2022) or in the pupal leg epithelium (Monier et al., 2015). The requirement of actomyosin upregulation can also be context dependent. Accordingly, we recently revealed that the early phase of apical constriction during cell extrusion in the pupal notum is not associated with any significant changes of actomyosin or Rho activity/dynamics, but is driven instead by a global disassembly of microtubules by caspases, which increases cell deformability (Villars et al., 2022). This initial phase is then followed by the formation of a supracellular cable of actomyosin which promotes late cell rounding. Interestingly, the mode of constriction can be very plastic. While ROCK inhibition (a central regulator of Myosin II, MyoII, activity) has only a minor impact on the speed of extrusion, it did change the mode of deformation, where late cell constriction is associated with a significant reduction of cell circularity (a good read-out of line tension). Alternatively, microtubule stabilization drastically slowed-down extrusion, which was now associated with a progressive accumulation of actomyosin and a constant increase of cell circularity.

This plasticity of extrusion is most likely at the basis of the great robustness of the process, which can adjust the mode of cell deformation depending on the mechanical state of the tissue (Atieh et al., 2021; Michel and Dahmann, 2020; Teo et al., 2020a; Teo et al., 2020b). This ensures the maintenance of epithelial sealing associated with dying/differentiating cell expulsion even in very perturbed conditions. The mode of extrusion mostly depends on the global mechanical properties of the epithelial layer, which at low tension/high cell density seems to promote purse string constriction, while high tension/low density are rather associated with extrusion driven by basal protrusion and/or medio-apical pulsatile MyoII (Kocgozlu et al., 2016; Le et al., 2021; Michel and Dahmann, 2020). Although this was never formally explored, the large variety of cell

aspect-ratios (from highly columnar to lamellar) may also explain the different timing of junction remodeling and localization of actomyosin, which imposes different constrains on the duration of apical/basal constriction relative to the duration of apical or basal sliding.

Despite the diversity of the remodeling processes, the directionality of cell extrusion remains very robust in a large range of perturbative conditions in all these contexts. What are then the main cellular factors setting the direction of expulsion and what are the consequences of the modification of this direction?

2.2 Mechanical parameters setting the direction of extrusion

The direction of cell expulsion correlates with the polarized shape remodeling of cells which is either dominated by apical or basal constriction. As such, it is essential to recapitulate what are the dominant forces setting the epithelial cell shape in 3D to understand what sets the directionality of extrusion. The three-dimensional shape of epithelial cells is dominated by the balance between apical line tension (set by the balance between cell-cell adhesion and junctional contractility), lateral tension (the balance of lateral membrane tension and lateral junction complexes such as desmosomes, or septate junctions in invertebrates) and the basal area elasticity (the balance between ECM adhesion and basal contractility) (Hannezo et al., 2014) (**Figure 2A**). High apical line tension and/or high lateral adhesion promote columnar shape while high lateral contractility and/or high substrate adhesion promote squamous shape. From this, one can extrapolate that the direction of extrusion and the dominant constriction side can be set by the ratio of apical line tension versus basal area elasticity (**Figure 2B,C**). This was indeed confirmed by simulations of extrusion using 3D vertex models (Okuda and Fujimoto, 2020), where it was also shown that extrusion can spontaneously occur due to local mechanical instabilities promoted by low number of cell neighbors and high tissue density. Interestingly, while this was not explored theoretically, a local modulation of tension either apically or basally in the neigboring cells may also influence the direction of cell deformation (**Figure 2**). Accordingly, a local reduction of tension in the neighboring cells driven by Src activation is permissive for MCF7 cell extrusion (Teo et al., 2020b).

[inset somewhere in this section Figure 2, full page in total]

Based on these theoretical grounds, can we now better understand what are the main cellular parameters setting the direction of extrusion? Apical extrusion in MDCK cells is indeed associated with the sliding of a supracellular contractile ring from the apical to the basal side (Kuipers et al., 2014), while neighboring cell basal protrusions should lead to a progressive reduction of extruding cell binding to the ECM (Kocgozlu et al., 2016). These two mechanisms should lead to a relative increase of the energy penalty associated with the maintenance of basal area hence promoting basal constriction relative to apical constriction. Alternatively, basal extrusion in *Drosophila* is almost systematically associated with an apical enrichment of actomyosin either at the junction or in the medio-apical pool (An et al., 2017; Michel and Dahmann, 2020; Simoes et al., 2017; Teng et al., 2017). While ECM binding dynamics was poorly characterized during *Drosophil*a extrusion, Talin - a component of focal adhesion - is clearly maintained during basal extrusion of columnar cells in the pupal leg (Ambrosini et al., 2019). However, induction of the pro-EMT (Epithelial Mesenchymal Transition) transcription factor Snail in a subset of cells triggers either basal or apical extrusion for respectively large or small group of cells, which can be reproduced by increased contractility combined with a downregulation of cell matrix adhesion (Wee et al., 2020). This suggests that these two parameters (ECM binding and contractility) are not sufficient to predict the direction of extrusion. The relative deformation of the apical and basal side should also rely on the relative timing of adherens junction, focal adhesion and lateral junction disassembly, and the timing of the formation of new junctions between neighboring cells. For instance, early formation of new adherens junctions between neighboring cells above the extruding cell should promote basal extrusion. Along this line, the disassembly of E-cad junctions and the formation of new adherens junctions between neighors precede the basal exclusion of larval epidermal cells (Teng et al., 2017) (**Figure 1B**), while the formation of new desmosomes between neigboring MDCK cells below the dying cell precedes the remodeling of adherens junctions and the formation of new adherens junctions between neighbors (Thomas et al., 2020) (**Figure 1A**). In this view, the direction of exclusion would also be dictated by the relative capacity of neighboring cells to seal new junction either apically or basally. This view is however contrasted by the effect of extracellular cleavage of E-cad by TEV which triggers MDCK cell apical extrusion despite early disassembly of adherens junctions (Grieve and Rabouille, 2014).

What are then the main regulators of the polarized contractility and the regulators of the timing of junction remodeling? Subcellular contractility is mostly regulated by the subcellular localization of active Rho (Munjal et al., 2015; Vasquez et al., 2014) (a key regulator of actin polymerization and MyoII activity (Levayer and Lecuit, 2012)) which is set by the subcellular localization of RhoGAP and RhoGEF (Garcia De Las Bayonas et al., 2019; Simoes et al., 2006). Microtubules have previously been shown to participate to the local delivery of RhoGEF for apical constriction in the fly mesoderm (Rogers et al., 2004) or for the regulation of cytokinetic ring formation (Basant and Glotzer, 2018). Similarly, microtubules have been involved in the basal delivery of the p115-RhoGEF in the neighbors of the extruding cells hence promoting the formation of the basal supracellular actomyosin cable and apical extrusion (Slattum et al., 2009). Accordingly, microtubule depletion through Nocodazole or stabilization through Taxol both lead to a reduction of the proportion of apical extrusion relative to basal extrusion in MDCK cells and zebrafish larval epidermis, which is phenocopied by p115-RhoGEF depletion in MDCK cells (Slattum et al., 2009). Alternatively, polarized changes of cell deformability may also lead to polarized contraction. Accordingly, while depletion of microtubules occurs throughout the cell during pupal notum cell extrusion, the strong initial enrichment of microtubules on the apical side should lead to a much more significant increase of cell deformability on the apical side (Villars et al., 2022). While local regulation of contractility is rather well described, the regulation of the timing of junction remodeling during cell extrusion remains poorly understood. Several components could contribute to junction disassembly, including the direct cleavage of junctional components by caspases (Chandraratna et al., 2007; Kessler and Muller, 2009) or the disassembly of junctions promoted by the progressive increase of tension and shear stress (Cavanaugh et al., 2020; Kale et al., 2018; Levayer et al., 2011; Staddon et al., 2019). However, what regulates the precise timing of this disassembly in different extrusion contexts remains unknown. Similarly, the relatively static view of contractility regulation that we described so far fails to explain the coupling between the requirement in mammalian cells of initial apical constriction, adherens junction reorganization and mechanotransduction (Duszyc et al., 2021), which is then followed by the dominant basal constriction(Kuipers et al., 2014). Part of the answer could come from the tight mechanical coupling between the apical and basal side. Accordingly, tension also builds up along the apico-basal axis during extrusion (Le et al., 2021; Monier et al., 2015) and contributes to the coupling between cell-cell adhesion, ECM

and the formation of the supracellular ring (Le et al., 2021). Similarly, in the fly pupal leg long actomyosin cables couple the apical and basal side of the cells through direct anchorage from adherens junctions to the nucleus, while the nucleus is connected to focal adhesions through basal accumulation of actin (Ambrosini et al., 2019). While this apico-basal tension was not studied for its contribution to the direction of extrusion, it reveals a strong mechanical coupling that could help to coordinate constriction on the apical, basal and lateral side (**Figure 2B,C**).

Finally, the existence of poorly compliant external boundaries may also affect the direction of extrusion, provided there is a minimal mechanical coupling with the epithelial layer. For instance, most of the conditions associated with basal extrusion in *Drosophila* correlate with the existence of a rigid boundary on the apical side, the cuticle (at the pupal stage) or the vitelline membrane (in the embryo), while gut cells are mostly constrained by the visceral muscle layer on the basal side (Aghajanian et al., 2016). Alternatively, most of the investigations of cell extrusion in cell culture were performed on rigid substrates. Accordingly, MDCK cells extrude on the basal side in curved area of alginate tubes covered with Matrigel (mostly on the inner side of the bends), which are quite compliant (Maechler et al., 2019), while MDCK cells extrude apically from the valley region of curved PDMS microprinted substrates (Huang et al., 2022). Yet, this constrain is unlikely to be the main driver of the direction since MDCK cells also extrude apically in 3D cyst embedded in Matrigel (Ganier et al., 2018), and there is no specific polarized constrains that could *a priori* explain extrusion on the basal side in the wing imaginal disc of *Drosophila* (Tripathi and Irvine, 2022). Alternatively, differential pressure of the liquid compartment in the apical or basal side may also influence the direction of expulsion. Accordingly, blood flow is required for basal extrusion of hematopoietic stem cell in the zebrafish aorta (Lancino et al., 2018) while polarized fluid flow driven by osmotic gradient provokes the basal detachment of MDCK cells from valley regions of curved substrates hence promoting apical extrusion (Huang et al., 2022).

2.3 The role of apico-basal core regulators in cell extrusion

Despite the highly polarized nature of the extrusion process, we actually know very little about the link between the core apico-basal polarity complexes and cell extrusion. For instance, there is to our knowledge no quantitative description of the dynamics of core apico-basal complexes during apoptosis-related cell extrusion. Yet, some of these

components are directly targeted by caspases including the apical marker Bazooka (*Drosophila* Par3) (Chandraratna et al., 2007), or the lateral protein Dlg (Crawford et al., 2012; Valon et al., 2021), which suggests that they might be actively remodeled during apoptotic-driven extrusion. The contribution of core apico-basal components has been better described in the context of collective cell delamination or single cell delamination and differentiation. Recently, the fine tuning of the apical determinant Crumbs (Tepass et al., 1990) was described during neuroblast delamination in the *Drosophila* embryo (Simoes et al., 2022). Crumbs depletion by endocytosis during the late phase of constriction is permissive for apical constriction and delamination, however its early maintenance is associated with more reproducible constriction rates. This transition is regulated by the E3 ubiquitin ligase Neuralized which deactivates the Crumbs interactor Stardust (Perez-Mockus et al., 2017; Simoes et al., 2022). This “break” function is in good agreement with the role of Crumbs depletion during collective cell delamination and EMT. For instance, the formation of neural stem cells in the fly optic lobes is driven by the spatially controlled EMT of the neuroepithelium, set by the local downregulation of Crumbs by Neuralized (Shard et al., 2020). The impact of polarity proteins on constriction can be decomposed in a cell-autonomous and non-cell autonomous contribution. On the one hand, Crumbs and Par3 can directly recruit the RhoGEF Cysts, the orthologue of the mammalian p114-RhoGEF, at the apico-lateral membrane in the fly embryo (Silver et al., 2019), while the PAR complexes and aPKC can directly modulate the dynamics of medio apical MyoII (David et al., 2010). Alternatively, polarity complexes can tune the stability of adherens junction by regulating E-cadherin endocytosis (Georgiou et al., 2008; Leibfried et al., 2008). On the other hand, spatial differences of the concentration of polarity complexes between neighboring cells can directly regulate MyoII. For instance, the anisotropy of Crumbs complexes near the EMT boundary of the optic lobe results in the accumulation of junctional Myosin which contributes to cell ingression (Shard et al., 2020). The impact of anisotropic localization of Crumbs was well described during the invagination of the salivary gland in the fly embryo (Roper, 2012) where anisotropic membrane distribution of Crumbs reduces the dissociation rate of the MyoII regulator Rok hence promoting its local enrichment (Sidor et al., 2020). Alternatively, aPKCi overexpression in mouse mammary gland luminal cells or the human breast cancer cells MCF10A also promotes basal extrusion through the accumulation of MyoII at overexpressing cell interfaces (Villeneuve et al., 2019). This is related to the decrease of Vinculin at cell-cell junction

and its relocalization to focal adhesion (Villeneuve et al., 2019). Since many polarity components are targeted by caspases (see above), the same anisotropy may emerge in dying cells and contribute to the formation of the actomyosin cable in the neighboring cells. Overall core polarity components may help to orchestrate cell extrusion either through cell-autonomous direct regulation of adhesive components and the contractile machinery, or through the impact of cell-to-cell heterogeneity which can also participate to the local enrichment of MyoII in the neighboring cells. Further work will be required to describe their dynamics and functions during apoptosis-related extrusion.

2.4 Switching the direction of cell extrusion and pathological consequences

So far, we have been focusing on the mechanisms regulating the direction of cell extrusion for wild type cells. Yet, interesting insights can also come from the regulation of abnormal/mutant cell extrusion. Moreover, the redirection of extrusion to the basal side may lead to invasive behaviors if combined with resistance to anoikis (Gu et al., 2015). Accordingly, single basal cell extrusion of K-Ras cells from primary cell masses has been recently correlated with the formation of secondary internal masses in zebrafish larvae (Fadul et al., 2021). Thus, characterizing the mutations associated with a redirection of extrusion may be very relevant for cancer progression and metastasis formation.

The perturbation of core regulators of cell extrusion in MDCK cells are usually associated with a relative increase of basal extrusion, including the stabilization or depolymerization of microtubules (Slattum et al., 2009) or the depletion of S1P2 receptor (Gu et al., 2011; Gu et al., 2015). However, the impact on the absolute number of basal extrusions remains unclear in most of these conditions and it is still possible that the downregulation of apical extrusion is not necessarily associated with an absolute increase of basal extrusion numbers. Interestingly, the loss of tumor suppressor genes or the activation of oncogenes is frequently associated with an abnormal direction of cell extrusion. For instance, APC (Adenomatous Polyposis Coli) depletion redirects cell extrusion to the basal side in MDCK cells and zebrafish larval epidermis, most likely through the absence of microtubule reorganization in the extruding cell neighbors (Marshall et al., 2011). Alternatively, the oncogene K-Ras prevents normal apical extrusion through the degradation of S1P by autophagy

(Slattum et al., 2014), while high H-Ras^{V12} active cells are extruded basally from MCF10A acini when surrounded by WT cells (Liu et al., 2012).

These results are in sharp contrast with the activation of H-Ras in single cells which mostly promotes apical extrusion (Kajita and Fujita, 2015). The apical elimination of active Ras cell was proposed to be an active process of defense against oncogenic cells (so called EDAC: Epithelial Defense Against Cancer (Kajita and Fujita, 2015)) since it leads to the exclusion of oncogenic cells into the lumen. EDAC involves the modification of cell boundary mechanics through the local enrichment of the actin binding proteins EPLIN (Ohoka et al., 2015), Filamin (Kajita et al., 2014), or Ephrin (Porazinski et al., 2016) and the convergent collective migration of the neighboring cells toward the active Ras cells (Aikin et al., 2020; Mori et al., 2022; Takeuchi et al., 2020). In certain conditions, this extrusion can be redirected to the basal side, for instance upon depletion of E-cad (Hogan et al., 2009), depletion of Vimentin in the neighboring cells (Kajita et al., 2014) or by blocking the metabolic switch (upregulation of glycolysis and downregulation of the TCA cycle) occurring in Ras^{V12} cells surrounded by WT cells (Kon et al., 2017).

Mutant backgrounds have also been associated with a redirection of cell extrusion from the basal to the apical side in the *Drosophila* wing disc. For instance, the transcription factor Ets21c promotes apical cell extrusion upon Hippo pathway upregulation in the wing disc (where normally extrusion leads to basal cell expulsion) (Ai et al., 2020). Depletion of apico-basal polarity components in clones (by depleting Scrib or Lgl, two core lateral components) leads to aberrant apical extrusion and the formation of apical cell masses in regions of the wing disc associated with higher basal JAK-STAT activity (Tamori et al., 2016a). The same aberrant extrusions can be triggered ectopically by downregulating the Rho activator RhoGEF2 combined with JAK-STAT activation (Tamori et al., 2016b).

Overall, mutations of several tumor-suppresor genes or activation of oncogenes are often associated with the perturbation of the direction of extrusion. While this redirection is likely to be multifactorial and context-dependent, this would have important consequences for the formation of secondary tumors, as suggested by recent results in zebrafish (Fadul et al., 2021). However, it remains unclear in many contexts whether these perturbations lead to an absolute increase of the probability of aberrant basal extrusion, which would significantly change the consequences on the

probability of tumor dissemination. Interestingly, these perturbed conditions also include the mutation of core regulators of apico-basal polarity in clones, which can directly impact the survival and extrusion of cells. In the next part, we will describe more thoroughly how local modulation of polarity can also be a driving force for cell death and cell extrusion.

3. Polarity perturbations as a driving force for cell extrusion and death induction

Cell polarity proteins can be altered in pathological conditions, such as cancer (Humbert et al., 2008; Wodarz, 2000; Wodarz and Näthke, 2007). Loss of cell polarity and cell adhesion is commonly observed in advances tumors, where it correlates with local tissue invasion and metastatic dissemination. Historically, the *scrib*, *lgl* and *dlg* tumor suppressors were identified as genes in which zygotic loss of function mutations caused cancerous overgrowths in imaginal discs in *Drosophila* larval tissues (Bilder, 2004; Bilder et al., 2000a; Gateff, 1978; Gateff and Schneiderman, 1974; Stewart et al., 1972; Woods and Bryant, 1989, 1991). Tissues mutant for these tumor suppressors show increased proliferation, altered differentiation and tissue architecture associated with polarity defects (Bilder, 2004). These phenotypes are reminiscent of malignant tumors in vertebrates. These tumor suppressors first identified in *Drosophila* are also found to be mutated in human cancers and can be associated with invasive phenotypes. Indeed, Dlg, Scrib and Lgl proteins are downregulated and misslocalized in tumors in mice and human patients (Fuja et al., 2004; Gardiol et al., 2006; Huang et al., 2003; Kuphal et al., 2005; Schimanski et al., 2005; Zhan et al., 2008). Loss of Dlg correlates with the invasive and poorly differentiated phenotype in breast and colorectal tumors in human patients (Fuja et al., 2004; Gardiol et al., 2006). Moreover, multiple somatic mutations in the *dlg* gene are present in human breast cancer samples (Fuja et al., 2004), and Dlg, as well as Scrib, can be directly targeted for degradation by oncoproteins such as the HPV E6 protein in cervical cancer (Massimi et al., 2004; Nakagawa and Huibregtse, 2000).

Mechanistic insights in the role of polarity proteins in tumorigenesis have been provided in later studies (Brumby and Richardson, 2003; Doggett et al., 2011; Froldi et al., 2010; Igaki et al., 2006; Pagliarini and Xu, 2003; Wu et al., 2010; Zhan et al., 2008). Cooperation of polarity proteins with oncogenes in tumor progression has first been shown in *Drosophila* (Brumby and Richardson, 2003; Igaki et al., 2006; Pagliarini and Xu, 2003). For instance combination of the expression of an activated Ras^{V12} or

activated Notch and loss of either one of the polarity proteins Scrib, Lgl or Dlg in the larval eye disc lead to (i) the overgrowth of an amorphous mass (Brumby and Richardson, 2003; Pagliarini and Xu, 2003), (ii) the degradation of the basement membrane (Pagliarini and Xu, 2003; Srivastava et al., 2007) (a hallmark of local tumor invasion in vertebrates), (iii) a downregulation of E-cadherin, (iv) cell migration and metastasis in distant organs, such as the ventral nerve cord, leg discs and trachea (Pagliarini and Xu, 2003). In *Ras^{V12}/Scrib* clones, JNK mediates the expression of matrix metalloproteases that degrade the basement membrane, thus allowing for tumor cell delamination and dissemination (Pagliarini and Xu, 2003; Srivastava et al., 2007). JNK activation and downregulation of E-cadherin in *Ras^{V12}/Scrib* clones is directly triggered by the loss of cell polarity (Igaki et al., 2006; Srivastava et al., 2007).

Scrib does collaborate with oncogenes in vertebrate models as well (Zhan et al., 2008). Zhan and colleagues have found that loss of Scrib cooperates with the oncogene c-myc to drive the transformation of mammary epithelial cells (Zhan et al., 2008). Indeed, the loss of Scrib inhibits c-myc-induced apoptosis, which results in an increased tumor burden in a mouse model of mammary tumorigenesis (Zhan et al., 2008).

Interestingly, even though zygotic mutations in polarity genes lead to cancerous overgrowths in *Drosophila,* when mutations in polarity proteins arise in isolated cells in the *Drosophila* epithelium these cells are outcompeted and eliminated from the tissue (Brumby and Richardson, 2003; Igaki et al., 2006; Igaki et al., 2009; Uhlirova et al., 2005). Thus, when they are not coupled to cooperative mutations such as mutations in oncogenes, polarity deficient cells are removed from the epithelium. We will now focus on the mechanisms driving elimination of polarity mutants.

3.1 How to eliminate polarity-deficient cells?

We will discuss the cell-autonomous and non-cell-autonomous mechanisms leading to the elimination of polarity-deficient cells. When surrounded by WT cells, polarity-deficient cells are eliminated through cell competition (Brumby and Richardson, 2003; Igaki et al., 2006; Igaki et al., 2009; Tamori et al., 2010; Uhlirova et al., 2005), a context-dependent elimination of aberrant cells when confronted to a WT cell population. We have previously mentioned that JNK signaling is activated in cells with loss-of-function mutations in polarity proteins (Igaki et al., 2006). In vertebrates and *Drosophila*, JNK induces a stress signaling leading to cell death (Davis, 2000), and accordingly JNK

has been shown to induce death of *scrib* mutant cells in the *Drosophila* eye disc (Brumby and Richardson, 2003; Uhlirova et al., 2005). But how is JNK activated in polarity-deficient cells? A mechanism linking Eiger (the *Drosophila* TNF ligand orthologue) to JNK activation in polarity-deficient cells has been described by Igaki and co-authors for the elimination of *scrib* and *dlg* mutant clones in antenna and wing discs (Igaki et al., 2009). JNK activation is triggered by the upregulation of Eiger endocytosis and its accumulation in endosomes in polarity deficient clones (Igaki et al., 2009). Accordingly, endocytosis blockage using $Rab5^{DN}$ prevents JNK activation and rescues *scrib* clones. The receptor of Eiger, named Grindelwald, was later identified and shown to be required for the elimination of *scrib* mutant cells (Andersen et al., 2015). The role of JNK-dependent cell death for polarity mutant elimination seems to be quite general. Indeed, cells harboring a null mutation in *lgl* in a WT wing disc are basally extruded and undergo as well JNK-dependent apoptosis (Tamori et al., 2010). Interestingly, overexpression of the polarity protein Mahjong (a binding partner of Lgl) is able to rescue cell death in Lgl-deficient clones, indicating that Mahjong acts downstream of Lgl in cell competition and basal cell extrusion (Tamori et al., 2010). However this view has been challenged by recent results showing that Mahjong-mediated cell competition is driven by a mechanism different from *lgl* mutant. Contrary to *lgl*, *mahjong* mutant elimination requires the transcription factor Xrp1, similar to the elimination of Minute/ribosomal proteins mutants, a classic context of cell competition (Kumar and Baker, 2022).

Apart from the cell-autonomous role of JNK in the apoptosis of polarity-deficient cells, JNK also plays a role in neighboring cells, in a non-cell-autonomous manner (Ohsawa et al., 2011). WT cells surrounding polarity-deficient cells activate nonapoptotic JNK signaling, and this activation promotes the elimination of polarity mutant cells. JNK activation leads to the upregulation of PVR (the Drosophila PDGF/VEGF receptor), which in turn activates the ELMO/Mbc-mediated phagocytic machinery, leading to the engulfment of polarity-deficient neighbors and their elimination (Ohsawa et al., 2011). A later study suggested however that engulfment has only a passive and secondary role in mutant clone elimination (Lolo et al., 2012). Alternatively, STAT is also activated in neighboring cells and required to eliminate *scrib* mutant cells (Schroeder et al., 2013). In the absence of STAT in neighboring WT cells, *scrib* mutant cells activate the transcription activator Yorkie (YAP in vertebrates) and overproliferate, thus becoming

tumorigenic. Thus, the local environment of the polarity-deficient cells determines whether they will be eliminated or retained into the tissue, which is a defining feature of cell competition.

We have described the cell-autonomous and non-cell-autonomous signaling pathways at play in the death of polarity-deficient cells. However, what are the signals sensed by polarity-deficient cells or WT cells driving loser cell elimination? To which extent are these eliminations directly related to the perturbations of polarity? We propose to focus now on mechanisms that could directly be related to polarity defects. This includes mechanisms based on ligand/receptor binding, and the mechanisms based on the modulation of sensitivity to mechanical stress or modulation of the mechanical environment. We will then also briefly discuss the impact of local defects in the other type of polarity, namely planar cell polarity (PCP), on cell elimination and cell extrusion.

3.2 Cell elimination driven by ligand-receptor misslocalization

[inset somewhere in this section Figure 3, half page in total]

In cell competition, polarity-deficient cells are eliminated from the tissue when surrounded by WT cells. However, the mechanism of recognition of polarity-deficient cells by WT cells prior to their elimination has remained elusive for a long time. Part of the answer recently emerged thanks to a genetic screen for ligand-based factors undertaken by Yamamoto and colleagues (Yamamoto et al., 2017). By screening for genes required in WT cells for the elimination of polarity-deficient neighbors in the *Drosophila* eye disc, they identified non-sense mutations in the *sas* gene, encoding a cell-surface ligand protein. The Sas protein in neighboring WT cells is required for the elimination of *scrib*$^{-/-}$ (or *dlg*$^{-/-}$) clones. Sas is normally localized at the apical surface of epithelial cells, however it relocalizes to the lateral cell surface at the interface between polarity-deficient and WT cells (**Figure 3A**). The authors then searched for the receptor of Sas on the polarity-deficient cells. A known interactor of Sas is PTP10D, a tyrosine protein phosphatase, which is relocalized by loser cells at their interfaces with WT cells. The depletion of PTP10D in polarity-deficient cells abolishes their elimination. In *scrib* loser cells, PTP10D restrains EGFR signaling, which enables JNK signaling to drive cell elimination. In the absence of PTP10D, EGFR/Ras cooperates with JNK to decrease Hippo signaling, thus increasing Yorkie pro-proliferative activity and suppressing the elimination of loser cells. Interestingly, the Sas-PTP10D pathway does

not drive elimination of other types of loser cells, such as Minute, *myc*, *mahjong* or *yorkie* mutant cells (Yamamoto et al., 2017), indicating that this mechanism is specific to the elimination of polarity mutant cells. This elegant model explains how polarity defects can lead to ectopic ligand-receptor interactions thanks to the extension of the apical domain (**Figure 3A**). However in principle this should occur throughout the mutant clone and it remains unclear why Sas and PTP10D are specifically enriched at the clone/WT cell interfaces. Yet, this lateral enrichment of apical proteins at the clone interfaces seems quite universal since other apical proteins such as Bazooka, Patj and aPKC are also enriched at mutant clone boundaries (Yamamoto et al., 2017). It should be noted however that recent results have questioned the requirement of PTP10D for the elimination of polarity-deficient cells (Gerlach et al., 2022), leaving open the question of its systematic requirement for polarity mutant cell elimination.

Recently, receptor misslocalization in polarity deficient cells has also been proposed to autonomously promote cell death independently of cell competition mechanisms (de Vreede et al., 2022) (**Figure 3B**). Accordingly, soluble Eiger binds preferentially to Dlg (or Scrib) depleted cells compared to WT cells in the fly wing disc. Surprisingly, bound Eiger is produced by a distant tissue, the fat body (an endocrine organ), and secreted into the haemolymph, the circulatory fluid that bathes the wing disc basolateral side. Preferential binding of Eiger in mutant clones is driven by the ectopic localization of its receptor Grindelwald on the basolateral side of polarity deficient cells, while the apical localization of the receptor in WT cells normally prevents interaction with the baso-laterally circulating Eiger (de Vreede et al., 2022). Thus, the perturbation of the spatial segregation of the TNF death receptor and its ligand causes JNK activation and elimination of polarity mutants. Importantly, this mechanism does not require the presence of a neighboring WT cell and occurs also in the context of fully mutant wing disc. What explains then the growth of fully mutant tissue compared to the elimination of mutant clones? This could be explained by different levels of activation of JNK, which might be higher in the context of competition thanks to the other mechanisms of activation described above, hence leading to more cell death. Alternatively, cell proliferation might be differentially affected in homotypic and mosaic conditions.

3.3 Cell elimination driven by changes of mechanical properties

3.3.1 Elimination driven by differential homeostatic density

[inset somewhere in this section Figure 4, half page in total]

Recently, mechanical stress has also been shown to participate to cell competition (Bove et al., 2017; Levayer et al., 2016; Matamoro-Vidal and Levayer, 2019; Moreno et al., 2019; Wagstaff et al., 2016b). This process relies on the generation of mechanical stress and on the differential sensitivity of the two cell populations to this stress (Matamoro-Vidal and Levayer, 2019).This concept was experimentally validated for the elimination of polarity-deficient cells in epithelial cell culture. Mechanical cell competition has mainly been studied using Mammalian MDCK cells. MDCK cells knocked down for Scrib ($Scrib^{KD}$) are eliminated through cell death when cultured in presence of WT MDCK cells, while they survive in pure cultures (Norman et al., 2012). It was later shown that $Scrib^{KD}$ cells are eliminated by compaction driven by WT MDCK cells (Wagstaff et al., 2016b). Two components participate to the preferential elimination of polarity deficient cells: their compaction driven by the active migration of WT cells towards $Scrib^{KD}$ cells upon contact (Ogawa et al., 2021; Wagstaff et al., 2016b), and their hypersensitivity to compaction driven by elevated p53 levels (Bove et al., 2017; Wagstaff et al., 2016b) (**Figure 4A**). p53 further increases upon compaction through activation of ROCK and p38 hence leading to rapid $Scrib^{KD}$ cell elimination (Wagstaff et al., 2016b). However, it is not clear at this stage why $Scrib^{KD}$ cells have higher basal p53 levels compared to WT cells. These results validate the concept of homeostatic pressure which was proposed in a seminal theoretical study by Basan and colleagues (Basan et al., 2009). Assuming that a cell population behaves like a fluid and that pressure will negatively affect proliferation while promoting apoptosis, they found that each population should grow until reaching a homeostatic pressure (HP) at which proliferation is balanced by cell death. Importantly, the mixing of two populations with different homeostatic pressures will systematically lead to the full elimination of the population with lower homeostatic pressure (HP^{low}). Intuitively, once the mixed population reaches HP^{low}, the loser population stops expanding while the population with the higher homeostatic pressure (HP^{high}) continues to proliferate and drives the pressure above HP^{low}. As a result, the apoptosis rate of the population with the lower HP becomes larger than its proliferation rate, which drives its gradual elimination. In the context of $Scrib^{KD}$ competition, the lower homeostatic density of $Scrib^{KD}$ cells is regulated by their higher p53 levels (**Figure 4A**). Accordingly, high p53

levels are sufficient to reduce MDCK cell homeostatic density and drive competitive elimination (Wagstaff et al., 2016b).

What is then the relative contribution of homeostatic density differences and the active compaction driven by migration of WT cells to Scrib^{KD} cell elimination? On the one hand, Wagstaff and colleagues found that contact between WT and Scrib^{KD} cells initiates a collective and directional migration with Scrib^{KD} cells at the migration front. This migration promotes Scrib^{KD} compaction and cell elimination, but is not strictly required for cell elimination, since E-cad knockdown abolishes this collective migration but does not prevent Scrib^{KD} cell elimination (Wagstaff et al., 2016b). The collective migration is driven by the secretion of the chemoattractant FGF21 by the polarity deficient cells, which promotes WT cell motility and accelerates Scrib^{KD} cell elimination (Ogawa et al., 2021). Thus, collective migration promotes but is not strictly required for Scrib^{KD} cell elimination. To better sort the contribution of migration and density, systematic cell tracking and cell-based modeling were used to predict to which extend differential homeostatic density is sufficient to recapitulate Scrib^{KD} cell elimination (Bove et al., 2017; Gradeci et al., 2021). Using deep learning-based segmentation and tracking of thousands of cells, the authors first confirmed that local density is one of the best predictors of cell elimination (Bove et al., 2017; Gradeci et al., 2021). They then simulated Scrib^{KD} cell competition in a cellular potts model and confirmed that differential homeostatic density was sufficient to recapitulate quantitatively the dynamics of WT cells growth and Scrib^{KD} cell elimination (Gradeci et al., 2021). Interestingly, the lower stiffness/higher deformability of the loser population also emerged as central regulator of the outcome of cell competition. However, to our knowledge the difference of stiffness between Scrib^{KD} and WT cells has never been measured experimentally.

These studies show that mechanical stress could contribute to polarity-deficient cell elimination. However, it is not clear how polarity disorganization could directly affect the homeostatic density of cells. In the next section, we will discuss how polarity may directly tune cell survival though its epistatic link with well-known pro-survival pathways including the Hippo pathway and YAP/Yki and the EGFR/ERK pathway.

3.3.2 How polarity defects may affect density sensing and homeostatic density

The higher sensitivity of Scrib^{KD} cells to compaction points to a mechanism linking tissue density to cell death/fate. While density sensing was related to p53 in MDCK cells, this does not seem to hold in *Drosophila* tissue (Wagstaff et al., 2016b). Could there be then other pathways connecting cell survival, density and polarity defects? In principle, any pathway sensitizing cells to apoptosis could play this role. In this regard, the ubiquitous increase of JNK pathway in polarity mutants could be sufficient to increase the rate of cell death at a given density and decrease their homeostatic pressure (de Vreede et al., 2022; Kucinski et al., 2017). An alternative candidate for this coupling could be Hippo, a central regulator of Yki-YAP/TAZ which interacts with apico-basal polarity proteins and is well known for its role in density sensing (Dupont, 2016). Indeed, apical proteins such as Crumbs can activate Hippo, thus blocking Yki/YAP nuclear import and leading to a downregulation of cell proliferation and cell survival (Chen et al., 2010; Genevet and Tapon, 2011). Accordingly, Crumbs was suggested to directly participate to density sensing in cell cultures, where low Crumbs concentration at low density is permissive for YAP activation and nuclear translocation, while at high density cells exhibit high levels of Crumbs which inactivates YAP and further inhibits cell division (Thompson et al., 2013; Varelas et al., 2010). Mechanistically, Crumbs binds Expanded (Ex) via its FERM-binding domain, and this binding is required for Ex apical localization and the activation of the Hippo pathway, phosphorylation of Yorkie by Warts and its exclusion from the nucleus (Grzeschik et al., 2010; Ling et al., 2010; Robinson et al., 2010). Thus, depletion of lateral proteins such as Scrib could lead to an expansion of the apical domain and trigger Hippo activation through Crumbs upregulation. However, this view is contrasted by numerous results in *Drosophila* showing that overexpression of the apical protein aPKC or Crumbs rather lead to Yki target activation and hyperproliferation (Chen et al., 2010; Grzeschik et al., 2010; Robinson et al., 2010), either through Ex segregation (Chen et al., 2010; Grzeschik et al., 2010) or through the misslocalization and colocalization of Hippo with its negative regulator RASSF (Grzeschik et al., 2010). Accordingly, *lgl*$^{-/-}$ clones activate Yki targets in the larval and pupal eye disc (Grzeschik et al., 2010). How to explain then this discrepancy with the apparent higher sensitivity to cell death of the polarity mutants? Part of the answer could come from the context dependent effect on Hippo/Yki in homotypic mutants and mosaic conditions. Accordingly, clonal induction of *scrib*$^{-/-}$ mutant is rather associated with a suppression of Yki activity which is required for their competitive elimination (Chen et al., 2012). Similarly, clonal

overexpression of Crumbs rather leads to clone elimination (Hafezi et al., 2012). This opposite effect may be driven by JNK activation which can directly antagonize Yki (Enomoto et al., 2015). To conclude, the perturbation of apico-basal polarity can directly modulate cell survival through genetic and physical interactions with components of the Hippo pathway. While the effects can be very contrasted depending on the tissue, the level of depletion/overexpression and the mosaic context, it is most likely one essential factor explaining the impact of polarity defects on survival and growth. Finally, EGFR/ERK pathway could be an alternative pathway connecting density sensing and cell elimination. Downregulation of ERK by crowding was recently associated with mechanical cell competition and cell elimination (Moreno et al., 2019) and ERK was also shown to respond to cell density in mammalian cells (Aoki et al., 2013). Since *scrib*$^{-/-}$ clones were previously associated with downregulation of ERK (Yamamoto et al., 2017), it could also sensitize polarity mutants to mechanical stress.

3.3.3 Modulation of interfacial contractility and cell elimination

We previously discussed the impact of local anisotropy of the apical protein Crumbs on the relocalization of actomyosin and activation of Rho (Roper, 2012; Shard et al., 2020; Sidor et al., 2020). Although this was not formally explored for other polarity mutants, it suggests that local differences of polarity between neighboring cells could lead to an increase of interfacial contractility between the mutant clones and the WT cells. Interfacial contractility was previously shown to trigger the segregation of misspecified cells that will either form cyst (Bielmeier et al., 2016; Gibson and Perrimon, 2005; Klipa and Hamaratoglu, 2019; Shen and Dahmann, 2005; Shen et al., 2010; Widmann and Dahmann, 2009), or be eliminated when below a threshold size (Bielmeier et al., 2016). This size-dependent effect could be easily explained by the Young-Laplace law, which stipulates that the pressure exerted at the interface between two fluids will be proportional to the line tension divided by the curvature radius (Lecuit and Lenne, 2007). As a result, smaller clones should experience higher pressure which may promote their elimination (Bielmeier et al., 2016; Matamoro-Vidal and Levayer, 2019). However, there is so far no formal demonstration that cell elimination in this context is indeed driven by pressure, and recent results also outlined the contribution JNK activation at the interfaces with the clone and its role in cell elimination (Prasad et al., 2022). Whether interfacial tension is indeed generally modulated near apico-basal

mutant clones, and whether this can contribute to cell extrusion and cell death remain so far an open question.

3.4 Planar polarity defects and cell elimination

In this chapter, we have been focusing on the relationship between cell extrusion and the most evident form of polarity of epithelial cells, namely apico-basal polarity. However, epithelia also have an in-plane polarity which allows them to align locally and globally across the epithelial sheet. This so called planar cell polarity (PCP) plays an essential role for the orientation of cilia, hairs and the orientation of cell deformations and junction remodeling during morphogenesis (Lavalou and Lecuit, 2022). PCP is regulated by the core PCP pathway involving the transmembrane protein Flamingo, Frizzled and Van Gogh, as well as alternative PCP pathways such as Fat/Dachsous or more recently Toll8 /Cirl. In all these contexts, the asymmetric localization of these proteins within the cell biases the distribution of a number of cellular effectors and will also influence the localization of partner proteins in the neighboring cells. As such, local depletion of PCP regulators will have strong non-autonomous effects which can affect the mechanics at the boundary. This is exactly what happens with the Toll8 asymmetric localization in the early fly embryo which polarizes the distribution of MyoII in the ectoderm (Pare et al., 2014). Accordingly, ectopic expression or depletion of Toll8 or its GPCR effector Cirl in wing disc clones is sufficient to increase interfacial tension through the circumferential recruitment of MyoII (Lavalou et al., 2021). In principle, this could also trigger clone elimination through increased pressure, just like misspecified clones (Bielmeier et al., 2016), but this has never been formally investigated. Further work will help to establish a putative link between local planar polarity defects and cell elimination.

Another potential link between planar polarity and cell extrusion was outlined in MDCK cells. Collective migration can generate local discontinuities in the alignment of the cell long axis which are reminiscent of the topological defects normally described in liquid crystals (Doostmohammadi and Ladoux, 2022). One particular type of defect, the so-called comet-like +1/2 defects (**Figure 4B**), correlates with caspase activation and MDCK cell extrusion (Saw et al., 2017). Importantly, the spatial distribution of these defects can be biased by changing the shape of the 2D substrate boundary, which will also lead to a local increase of cell extrusion and apoptosis. +1/2 defects are associated with a local increase of pressure, which most likely triggers cell death

through the exclusion of YAP from the nucleus. While topological defects were not associated with the core PCP pathway, they are *de facto* a manifestation of local discontinuities of the planar polarity of cells which cause caspase activation and apoptosis. The impact of these spontaneous and stochastic misalignments on apoptosis reveals once again the impressive capacity of cells and tissues to detect and correct local discontinuities.

4. Conclusions and perspectives

Since polarity is a defining feature of epithelia, it is not surprising to find that the control of polarity is central for the process of cell death and cell extrusion. Whereas there has been significant advances in the understanding of the regulation of cell extrusion, we still know very little about the link between core polarity complexes and extrusion. Similarly, we still do not know which parameters are predictive of the direction of extrusion. This is likely to remain an important line of research in the coming years since the directionality of cell extrusion can completely change its impact on tumor progression, from a tumor suppressor mechanism when excluding aberrant cells into the lumen, to facilitating invasive behaviors by promoting oncogenic cell exclusion on the basal side. A better integrative view of this morphogenetic process, including precise characterization of the mechanical parameters of the extruding cell in 3D, its neighbors, the ECM and the apical and basal boundaries, will be required to fully understand this simple and yet highly orchestrated remodeling process. Similarly, the quantitative phenomenology of polarity complexes, adherens and septate junctions and other essential morphological regulators during apoptotic extrusion remains to be explored. As illustrated in the second part of this review, epithelia have developed various mechanisms to detect and eliminate local distortions of polarity. Part of these processes are cell autonomous and rely on the misslocalization of ligands and receptors, while others seem more subtle and rely on the combination of cell autonomous changes of survival combined with global mechanical stress or mechanical stress generated at the boundary between WT and polarity-deficient cells. This outlines the capacity of epithelia to detect even small local distortions of cell properties, hence ensuring robustness of epithelial organization. Interestingly, work on polarity-deficient cell elimination has been largely focused on the regulation of apoptosis, however the direct impact of polarity defects on cell extrusion itself has been poorly explored so far. This could constitute another leverage for mutant clone

elimination. Further work will be required to disentangle the contribution of caspase regulation by local polarity defects and the effect of cell polarity on cell mechanical properties that could directly impact cell extrusion.

Acknowledgements

We thank members of RL lab for critical reading of the manuscript. RS is supported by an ARC postdoctoral grant Aide individuelle (PDF20191209565), work in RL lab is supported by the Institut Pasteur, the ERC starting grant CoSpaDD (Competition for Space in Development and Disease, grant number 758457), the CNRS (UMR 3738) and the ANR PRC CoECECa.

Competing interests

The authors declare no competing interests.

References

Acharya, B.R., Nestor-Bergmann, A., Liang, X., Gupta, S., Duszyc, K., Gauquelin, E., Gomez, G.A., Budnar, S., Marcq, P., Jensen, O.E., Bryant, Z., Yap, A.S., 2018. A Mechanosensitive RhoA Pathway that Protects Epithelia against Acute Tensile Stress. Dev Cell 47, 439-452 e436.

Aghajanian, P., Takashima, S., Paul, M., Younossi-Hartenstein, A., Hartenstein, V., 2016. Metamorphosis of the Drosophila visceral musculature and its role in intestinal morphogenesis and stem cell formation. Dev Biol 420, 43-59.

Ai, X., Wang, D., Zhang, J., Shen, J., 2020. Hippo signaling promotes Ets21c-dependent apical cell extrusion in the Drosophila wing disc. Development 147.

Aikin, T.J., Peterson, A.F., Pokrass, M.J., Clark, H.R., Regot, S., 2020. MAPK activity dynamics regulate non-cell autonomous effects of oncogene expression. Elife 9.

Ambrosini, A., Rayer, M., Monier, B., Suzanne, M., 2019. Mechanical Function of the Nucleus in Force Generation during Epithelial Morphogenesis. Dev Cell 50, 197-211 e195.

An, Y., Xue, G., Shaobo, Y., Mingxi, D., Zhou, X., Yu, W., Ishibashi, T., Zhang, L., Yan, Y., 2017. Apical constriction is driven by a pulsatile apical myosin network in delaminating Drosophila neuroblasts. Development 144, 2153-2164.

Andersen, D.S., Colombani, J., Palmerini, V., Chakrabandhu, K., Boone, E., Röthlisberger, M., Toggweiler, J., Basler, K., Mapelli, M., Hueber, A.O., Léopold, P., 2015. The Drosophila TNF receptor Grindelwald couples loss of cell polarity and neoplastic growth. Nature 2015 522:7557 522, 482-486.

Aoki, K., Kumagai, Y., Sakurai, A., Komatsu, N., Fujita, Y., Shionyu, C., Matsuda, M., 2013. Stochastic ERK activation induced by noise and cell-to-cell propagation regulates cell density-dependent proliferation. Mol Cell 52, 529-540.

Atieh, Y., Wyatt, T., Zaske, A.M., Eisenhoffer, G.T., 2021. Pulsatile contractions promote apoptotic cell extrusion in epithelial tissues. Curr Biol 31, 1129-1140 e1124.

Baker, N.E., 2020. Emerging mechanisms of cell competition. Nat Rev Genet 21, 683-697.

Basan, M., Risler, T., Joanny, J.F., Sastre-Garau, X., Prost, J., 2009. Homeostatic competition drives tumor growth and metastasis nucleation. HFSP J 3, 265-272.

Basant, A., Glotzer, M., 2018. Spatiotemporal Regulation of RhoA during Cytokinesis. Curr Biol 28, R570-R580.

Bielmeier, C., Alt, S., Weichselberger, V., La Fortezza, M., Harz, H., Julicher, F., Salbreux, G., Classen, A.K., 2016. Interface Contractility between Differently Fated Cells Drives Cell Elimination and Cyst Formation. Curr Biol 26, 563-574.
Bilder, D., 2004. Epithelial polarity and proliferation control: links from the Drosophila neoplastic tumor suppressors. Genes Dev 18, 1909-1925.
Bilder, D., Li, M., Perrimon, N., 2000a. Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. Science 289, 113-116.
Bilder, D., Li, M., Perrimon, N., 2000b. Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. Science 289, 113-116.
Bove, A., Gradeci, D., Fujita, Y., Banerjee, S., Charras, G., Lowe, A.R., 2017. Local cellular neighborhood controls proliferation in cell competition. Mol Biol Cell 28, 3215-3228.
Brumby, A.M., Richardson, H.E., 2003. scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. EMBO J 22, 5769-5779.
Buchon, N., Broderick, N.A., Kuraishi, T., Lemaitre, B., 2010. Drosophila EGFR pathway coordinates stem cell proliferation and gut remodeling following infection. BMC Biol 8, 152.
Buckley, C.E., St Johnston, D., 2022. Apical-basal polarity and the control of epithelial form and function. Nat Rev Mol Cell Biol 23, 559-577.
Bullen, T.F., Forrest, S., Campbell, F., Dodson, A.R., Hershman, M.J., Pritchard, D.M., Turner, J.R., Montrose, M.H., Watson, A.J., 2006. Characterization of epithelial cell shedding from human small intestine. Lab Invest 86, 1052-1063.
Cavanaugh, K.E., Staddon, M.F., Munro, E., Banerjee, S., Gardel, M.L., 2020. RhoA Mediates Epithelial Cell Shape Changes via Mechanosensitive Endocytosis. Dev Cell 52, 152-166 e155.
Chandraratna, D., Lawrence, N., Welchman, D.P., Sanson, B., 2007. An in vivo model of apoptosis: linking cell behaviours and caspase substrates in embryos lacking DIAP1. J Cell Sci 120, 2594-2608.
Chen, C.L., Gajewski, K.M., Hamaratoglu, F., Bossuyt, W., Sansores-Garcia, L., Tao, C., Halder, G., 2010. The apical-basal cell polarity determinant Crumbs regulates Hippo signaling in Drosophila. Proc Natl Acad Sci U S A 107, 15810-15815.
Chen, C.L., Schroeder, M.C., Kango-Singh, M., Tao, C., Halder, G., 2012. Tumor suppression by cell competition through regulation of the Hippo pathway. Proc Natl Acad Sci U S A 109, 484-489.
Crawford, E.D., Seaman, J.E., Barber, A.E., 2nd, David, D.C., Babbitt, P.C., Burlingame, A.L., Wells, J.A., 2012. Conservation of caspase substrates across metazoans suggests hierarchical importance of signaling pathways over specific targets and cleavage site motifs in apoptosis. Cell Death Differ 19, 2040-2048.
David, D.J., Tishkina, A., Harris, T.J., 2010. The PAR complex regulates pulsed actomyosin contractions during amnioserosa apical constriction in Drosophila. Development 137, 1645-1655.
Davis, R.J., 2000. Signal Transduction by the JNK Group of MAP Kinases. Cell 103, 239-252.
de Vreede, G., Gerlach, S.U., Bilder, D., 2022. Epithelial monitoring through ligand-receptor segregation ensures malignant cell elimination. Science 376, 297-301.
Doggett, K., Grusche, F.A., Richardson, H.E., Brumby, A.M., 2011. Loss of the Drosophila cell polarity regulator Scribbled promotes epithelial tissue overgrowth and cooperation with oncogenic Ras-Raf through impaired Hippo pathway signaling. BMC Developmental Biology 11, 1-20.
Doostmohammadi, A., Ladoux, B., 2022. Physics of liquid crystals in cell biology: (Trends in Cell Biology 32, 140-150, 2022). Trends Cell Biol 32, 365.
Dupont, S., 2016. Role of YAP/TAZ in cell-matrix adhesion-mediated signalling and mechanotransduction. Exp Cell Res 343, 42-53.
Duszyc, K., Gomez, G.A., Lagendijk, A.K., Yau, M.K., Nanavati, B.N., Gliddon, B.L., Hall, T.E., Verma, S., Hogan, B.M., Pitson, S.M., Fairlie, D.P., Parton, R.G., Yap, A.S., 2021. Mechanotransduction activates RhoA in the neighbors of apoptotic epithelial cells to engage apical extrusion. Curr Biol 31, 1326-1336 e1325.
Eisenhoffer, G.T., Loftus, P.D., Yoshigi, M., Otsuna, H., Chien, C.B., Morcos, P.A., Rosenblatt, J., 2012. Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. Nature 484, 546-549.

Enomoto, M., Kizawa, D., Ohsawa, S., Igaki, T., 2015. JNK signaling is converted from anti- to pro-tumor pathway by Ras-mediated switch of Warts activity. Dev Biol 403, 162-171.
Fadul, J., Zulueta-Coarasa, T., Slattum, G.M., Redd, N.M., Jin, M.F., Redd, M.J., Daetwyler, S., Hedeen, D., Huisken, J., Rosenblatt, J., 2021. KRas-transformed epithelia cells invade and partially dedifferentiate by basal cell extrusion. Nat Commun 12, 7180.
Flores-Benitez, D., Knust, E., 2016. Dynamics of epithelial cell polarity in Drosophila: how to regulate the regulators? Curr Opin Cell Biol 42, 13-21.
Froldi, F., Ziosi, M., Garoia, F., Pession, A., Grzeschik, N.A., Bellosta, P., Strand, D., Richardson, H.E., Pession, A., Grifoni, D., 2010. The lethal giant larvae tumour suppressor mutation requires dMyc oncoprotein to promote clonal malignancy. BMC Biol 8, 33.
Fuja, T.J., Lin, F., Osann, K.E., Bryant, P.J., 2004. Somatic Mutations and Altered Expression of the Candidate Tumor Suppressors CSNK1ε, DLG1, and EDD/hHYD in Mammary Ductal Carcinoma. Cancer Research 64, 942-951.
Ganier, O., Schnerch, D., Nigg, E.A., 2018. Structural centrosome aberrations sensitize polarized epithelia to basal cell extrusion. Open Biol 8.
Garcia De Las Bayonas, A., Philippe, J.M., Lellouch, A.C., Lecuit, T., 2019. Distinct RhoGEFs Activate Apical and Junctional Contractility under Control of G Proteins during Epithelial Morphogenesis. Curr Biol 29, 3370-3385 e3377.
Gardiol, D., Zacchi, A., Petrera, F., Stanta, G., Banks, L., 2006. Human discs large and scrib are localized at the same regions in colon mucosa and changes in their expression patterns are correlated with loss of tissue architecture during malignant progression. International Journal of Cancer 119, 1285-1290.
Gateff, E., 1978. Malignant neoplasms of genetic origin in Drosophila melanogaster. Science 200, 1448-1459.
Gateff, E., Schneiderman, H.A., 1974. Developmental capacities of benign and malignant neoplasms ofDrosophila. Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen 1974 176:1 176, 23-65.
Genevet, A., Tapon, N., 2011. The Hippo pathway and apico-basal cell polarity. Biochem J 436, 213-224.
Georgiou, M., Marinari, E., Burden, J., Baum, B., 2008. Cdc42, Par6, and aPKC regulate Arp2/3-mediated endocytosis to control local adherens junction stability. Curr Biol 18, 1631-1638.
Gerlach, S.U., de Vreede, G., Bilder, D., 2022. PTP10D-mediated cell competition is not obligately required for elimination of polarity-deficient clones. Biol Open 11.
Gibson, M.C., Perrimon, N., 2005. Extrusion and death of DPP/BMP-compromised epithelial cells in the developing Drosophila wing. Science 307, 1785-1789.
Gradeci, D., Bove, A., Vallardi, G., Lowe, A.R., Banerjee, S., Charras, G., 2021. Cell-scale biophysical determinants of cell competition in epithelia. Elife 10.
Grieve, A.G., Rabouille, C., 2014. Extracellular cleavage of E-cadherin promotes epithelial cell extrusion. J Cell Sci 127, 3331-3346.
Grzeschik, N.A., Parsons, L.M., Allott, M.L., Harvey, K.F., Richardson, H.E., 2010. Lgl, aPKC, and Crumbs Regulate the Salvador/Warts/Hippo Pathway through Two Distinct Mechanisms. Current Biology 20, 573-581.
Gu, Y., Forostyan, T., Sabbadini, R., Rosenblatt, J., 2011. Epithelial cell extrusion requires the sphingosine-1-phosphate receptor 2 pathway. J Cell Biol 193, 667-676.
Gu, Y., Shea, J., Slattum, G., Firpo, M.A., Alexander, M., Mulvihill, S.J., Golubovskaya, V.M., Rosenblatt, J., 2015. Defective apical extrusion signaling contributes to aggressive tumor hallmarks. Elife 4, e04069.
Guirao, B., Bellaiche, Y., 2017. Biomechanics of cell rearrangements in Drosophila. Curr Opin Cell Biol 48, 113-124.
Hafezi, Y., Bosch, J.A., Hariharan, I.K., 2012. Differences in levels of the transmembrane protein Crumbs can influence cell survival at clonal boundaries. Dev Biol 368, 358-369.

Hannezo, E., Prost, J., Joanny, J.F., 2014. Theory of epithelial sheet morphology in three dimensions. Proc Natl Acad Sci U S A 111, 27-32.
Hogan, C., Dupre-Crochet, S., Norman, M., Kajita, M., Zimmermann, C., Pelling, A.E., Piddini, E., Baena-Lopez, L.A., Vincent, J.P., Itoh, Y., Hosoya, H., Pichaud, F., Fujita, Y., 2009. Characterization of the interface between normal and transformed epithelial cells. Nat Cell Biol 11, 460-467.
Huang, C.-K., Yong, X., She, D.T., Lim, C.T., 2022. Surface curvature and basal hydraulic stress induce spatial bias in cell extrusion. bioRxiv, 2022.2004.2001.486717.
Huang, J.H.Y., Rajkovic, A., Szafranski, P., Ochsner, S., Richards, J.A., Goode, S., 2003. Expression of Drosophila neoplastic tumor suppressor genes discslarge, scribble, and lethal giant larvae in the mammalian ovary. Gene Expression Patterns 3, 3-11.
Humbert, P.O., Grzeschik, N.A., Brumby, A.M., Galea, R., Elsum, I., Richardson, H.E., 2008. Control of tumourigenesis by the Scribble/Dlg/Lgl polarity module, Oncogene. Nature Publishing Group, pp. 6888-6907.
Igaki, T., Pagliarini, R.A., Xu, T., 2006. Loss of cell polarity drives tumor growth and invasion through JNK activation in Drosophila. Curr Biol 16, 1139-1146.
Igaki, T., Pastor-Pareja, J.C., Aonuma, H., Miura, M., Xu, T., 2009. Intrinsic tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in Drosophila. Dev Cell 16, 458-465.
Kajita, M., Fujita, Y., 2015. EDAC: Epithelial defence against cancer-cell competition between normal and transformed epithelial cells in mammals. J Biochem 158, 15-23.
Kajita, M., Sugimura, K., Ohoka, A., Burden, J., Suganuma, H., Ikegawa, M., Shimada, T., Kitamura, T., Shindoh, M., Ishikawa, S., Yamamoto, S., Saitoh, S., Yako, Y., Takahashi, R., Okajima, T., Kikuta, J., Maijima, Y., Ishii, M., Tada, M., Fujita, Y., 2014. Filamin acts as a key regulator in epithelial defence against transformed cells. Nat Commun 5, 4428.
Kale, G.R., Yang, X., Philippe, J.M., Mani, M., Lenne, P.F., Lecuit, T., 2018. Distinct contributions of tensile and shear stress on E-cadherin levels during morphogenesis. Nat Commun 9, 5021.
Katsukawa, M., Ohsawa, S., Zhang, L., Yan, Y., Igaki, T., 2018. Serpin Facilitates Tumor-Suppressive Cell Competition by Blocking Toll-Mediated Yki Activation in Drosophila. Curr Biol 28, 1756-1767 e1756.
Kessler, T., Muller, H.A., 2009. Cleavage of Armadillo/beta-catenin by the caspase DrICE in Drosophila apoptotic epithelial cells. BMC Dev Biol 9, 15.
Klipa, O., Hamaratoglu, F., 2019. Cell elimination strategies upon identity switch via modulation of apterous in Drosophila wing disc. PLoS Genet 15, e1008573.
Kocgozlu, L., Saw, T.B., Le, A.P., Yow, I., Shagirov, M., Wong, E., Mege, R.M., Lim, C.T., Toyama, Y., Ladoux, B., 2016. Epithelial Cell Packing Induces Distinct Modes of Cell Extrusions. Curr Biol 26, 2942-2950.
Kon, S., Ishibashi, K., Katoh, H., Kitamoto, S., Shirai, T., Tanaka, S., Kajita, M., Ishikawa, S., Yamauchi, H., Yako, Y., Kamasaki, T., Matsumoto, T., Watanabe, H., Egami, R., Sasaki, A., Nishikawa, A., Kameda, I., Maruyama, T., Narumi, R., Morita, T., Sasaki, Y., Enoki, R., Honma, S., Imamura, H., Oshima, M., Soga, T., Miyazaki, J.I., Duchen, M.R., Nam, J.M., Onodera, Y., Yoshioka, S., Kikuta, J., Ishii, M., Imajo, M., Nishida, E., Fujioka, Y., Ohba, Y., Sato, T., Fujita, Y., 2017. Cell competition with normal epithelial cells promotes apical extrusion of transformed cells through metabolic changes. Nat Cell Biol 19, 530-541.
Kucinski, I., Dinan, M., Kolahgar, G., Piddini, E., 2017. Chronic activation of JNK JAK/STAT and oxidative stress signalling causes the loser cell status. Nat Commun 8, 136.
Kuipers, D., Mehonic, A., Kajita, M., Peter, L., Fujita, Y., Duke, T., Charras, G., Gale, J.E., 2014. Epithelial repair is a two-stage process driven first by dying cells and then by their neighbours. J Cell Sci 127, 1229-1241.
Kumar, A., Baker, N.E., 2022. The CRL4 E3 ligase Mahjong/DCAF1 controls cell competition through the transcription factor Xrp1, independently of polarity genes. Development 149.

Kuphal, S., Wallner, S., Schimanski, C.C., Bataille, F., Hofer, P., Strand, S., Strand, D., Bosserhoff, A.K., 2005. Expression of Hugl-1 is strongly reduced in malignant melanoma. Oncogene 2006 25:1 25, 103-110.
Lancino, M., Majello, S., Herbert, S., De Chaumont, F., Tinevez, J.Y., Olivo-Marin, J.C., Herbomel, P., Schmidt, A., 2018. Anisotropic organization of circumferential actomyosin characterizes hematopoietic stem cells emergence in the zebrafish. Elife 7.
Lavalou, J., Lecuit, T., 2022. In search of conserved principles of planar cell polarization. Curr Opin Genet Dev 72, 69-81.
Lavalou, J., Mao, Q., Harmansa, S., Kerridge, S., Lellouch, A.C., Philippe, J.M., Audebert, S., Camoin, L., Lecuit, T., 2021. Formation of polarized contractile interfaces by self-organized Toll-8/Cirl GPCR asymmetry. Developmental Cell 56, 1574-1588.e1577.
Le, A.P., Rupprecht, J.F., Mege, R.M., Toyama, Y., Lim, C.T., Ladoux, B., 2021. Adhesion-mediated heterogeneous actin organization governs apoptotic cell extrusion. Nat Commun 12, 397.
Lecuit, T., Lenne, P.F., 2007. Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. Nat Rev Mol Cell Biol 8, 633-644.
Leibfried, A., Fricke, R., Morgan, M.J., Bogdan, S., Bellaiche, Y., 2008. Drosophila Cip4 and WASp define a branch of the Cdc42-Par6-aPKC pathway regulating E-cadherin endocytosis. Curr Biol 18, 1639-1648.
Levayer, R., Dupont, C., Moreno, E., 2016. Tissue Crowding Induces Caspase-Dependent Competition for Space. Curr Biol 26, 670-677.
Levayer, R., Lecuit, T., 2012. Biomechanical regulation of contractility: spatial control and dynamics. Trends Cell Biol 22, 61-81.
Levayer, R., Pelissier-Monier, A., Lecuit, T., 2011. Spatial regulation of Dia and Myosin-II by RhoGEF2 controls initiation of E-cadherin endocytosis during epithelial morphogenesis. Nat Cell Biol 13, 529-540.
Liang, J., Balachandra, S., Ngo, S., O'Brien, L.E., 2017. Feedback regulation of steady-state epithelial turnover and organ size. Nature 548, 588-591.
Ling, C., Zheng, Y., Yin, F., Yu, J., Huang, J., Hong, Y., Wu, S., Pan, D., 2010. The apical transmembrane protein Crumbs functions as a tumor suppressor that regulates Hippo signaling by binding to expanded. Proceedings of the National Academy of Sciences of the United States of America 107, 10532-10537.
Liu, J.S., Farlow, J.T., Paulson, A.K., Labarge, M.A., Gartner, Z.J., 2012. Programmed cell-to-cell variability in Ras activity triggers emergent behaviors during mammary epithelial morphogenesis. Cell Rep 2, 1461-1470.
Lolo, F.N., Casas-Tinto, S., Moreno, E., 2012. Cell competition time line: winners kill losers, which are extruded and engulfed by hemocytes. Cell Rep 2, 526-539.
Lubkov, V., Bar-Sagi, D., 2014. E-cadherin-mediated cell coupling is required for apoptotic cell extrusion. Curr Biol 24, 868-874.
Maechler, F.A., Allier, C., Roux, A., Tomba, C., 2019. Curvature-dependent constraints drive remodeling of epithelia. J Cell Sci 132.
Manjon, C., Sanchez-Herrero, E., Suzanne, M., 2007. Sharp boundaries of Dpp signalling trigger local cell death required for Drosophila leg morphogenesis. Nat Cell Biol 9, 57-63.
Marinari, E., Mehonic, A., Curran, S., Gale, J., Duke, T., Baum, B., 2012. Live-cell delamination counterbalances epithelial growth to limit tissue overcrowding. Nature 484, 542-545.
Marshall, T.W., Lloyd, I.E., Delalande, J.M., Nathke, I., Rosenblatt, J., 2011. The tumor suppressor adenomatous polyposis coli controls the direction in which a cell extrudes from an epithelium. Mol Biol Cell 22, 3962-3970.
Martin, J.L., Sanders, E.N., Moreno-Roman, P., Jaramillo Koyama, L.A., Balachandra, S., Du, X., O'Brien, L.E., 2018. Long-term live imaging of the Drosophila adult midgut reveals real-time dynamics of division, differentiation and loss. Elife 7.

Massimi, P., Gammoh, N., Thomas, M., Banks, L., 2004. HPV E6 specifically targets different cellular pools of its PDZ domain-containing tumour suppressor substrates for proteasome-mediated degradation. Oncogene 2004 23:49 23, 8033-8039.
Matamoro-Vidal, A., Levayer, R., 2019. Multiple Influences of Mechanical Forces on Cell Competition. Curr Biol 29, R762-R774.
Michael, M., Meiring, J.C.M., Acharya, B.R., Matthews, D.R., Verma, S., Han, S.P., Hill, M.M., Parton, R.G., Gomez, G.A., Yap, A.S., 2016. Coronin 1B Reorganizes the Architecture of F-Actin Networks for Contractility at Steady-State and Apoptotic Adherens Junctions. Dev Cell 37, 58-71.
Michel, M., Dahmann, C., 2020. Tissue mechanical properties modulate cell extrusion in the Drosophila abdominal epidermis. Development 147.
Miroshnikova, Y.A., Le, H.Q., Schneider, D., Thalheim, T., Rubsam, M., Bremicker, N., Polleux, J., Kamprad, N., Tarantola, M., Wang, I., Balland, M., Niessen, C.M., Galle, J., Wickstrom, S.A., 2018. Adhesion forces and cortical tension couple cell proliferation and differentiation to drive epidermal stratification. Nat Cell Biol 20, 69-80.
Monier, B., Gettings, M., Gay, G., Mangeat, T., Schott, S., Guarner, A., Suzanne, M., 2015. Apico-basal forces exerted by apoptotic cells drive epithelium folding. Nature 518, 245-248.
Moreno, E., Valon, L., Levillayer, F., Levayer, R., 2019. Competition for Space Induces Cell Elimination through Compaction-Driven ERK Downregulation. Curr Biol 29, 23-34 e28.
Mori, Y., Shiratsuchi, N., Sato, N., Chaya, A., Tanimura, N., Ishikawa, S., Kato, M., Kameda, I., Kon, S., Haraoka, Y., Ishitani, T., Fujita, Y., 2022. Extracellular ATP facilitates cell extrusion from epithelial layers mediated by cell competition or apoptosis. Curr Biol 32, 2144-2159 e2145.
Munjal, A., Philippe, J.M., Munro, E., Lecuit, T., 2015. A self-organized biomechanical network drives shape changes during tissue morphogenesis. Nature 524, 351-355.
Nakagawa, S., Huibregtse, J.M., 2000. Human Scribble (Vartul) Is Targeted for Ubiquitin-Mediated Degradation by the High-Risk Papillomavirus E6 Proteins and the E6AP Ubiquitin-Protein Ligase. Molecular and Cellular Biology 20, 8244-8244.
Norman, M., Wisniewska, K.A., Lawrenson, K., Garcia-Miranda, P., Tada, M., Kajita, M., Mano, H., Ishikawa, S., Ikegawa, M., Shimada, T., Fujita, Y., 2012. Loss of Scribble causes cell competition in mammalian cells. J Cell Sci 125, 59-66.
Ogawa, M., Kawarazaki, Y., Fujita, Y., Naguro, I., Ichijo, H., 2021. FGF21 Induced by the ASK1-p38 Pathway Promotes Mechanical Cell Competition by Attracting Cells. Curr Biol 31, 1048-1057 e1045.
Ohoka, A., Kajita, M., Ikenouchi, J., Yako, Y., Kitamoto, S., Kon, S., Ikegawa, M., Shimada, T., Ishikawa, S., Fujita, Y., 2015. EPLIN is a crucial regulator for extrusion of RasV12-transformed cells. J Cell Sci 128, 781-789.
Ohsawa, S., Sugimura, K., Takino, K., Xu, T., Miyawaki, A., Igaki, T., 2011. Elimination of oncogenic neighbors by JNK-mediated engulfment in Drosophila. Dev Cell 20, 315-328.
Ohsawa, S., Vaughen, J., Igaki, T., 2018. Cell Extrusion: A Stress-Responsive Force for Good or Evil in Epithelial Homeostasis. Dev Cell 44, 532.
Okuda, S., Fujimoto, K., 2020. A Mechanical Instability in Planar Epithelial Monolayers Leads to Cell Extrusion. Biophys J 118, 2549-2560.
Osswald, M., Morais-de-Sa, E., 2019. Dealing with apical-basal polarity and intercellular junctions: a multidimensional challenge for epithelial cell division. Curr Opin Cell Biol 60, 75-83.
Pagliarini, R.A., Xu, T., 2003. A Genetic Screen in Drosophila for Metastatic Behavior. Science 302, 1227-1231.
Pare, A.C., Vichas, A., Fincher, C.T., Mirman, Z., Farrell, D.L., Mainieri, A., Zallen, J.A., 2014. A positional Toll receptor code directs convergent extension in Drosophila. Nature 515, 523-527.
Patterson, A.M., Watson, A.J.M., 2017. Deciphering the Complex Signaling Systems That Regulate Intestinal Epithelial Cell Death Processes and Shedding. Front Immunol 8, 841.
Perez-Mockus, G., Roca, V., Mazouni, K., Schweisguth, F., 2017. Neuralized regulates Crumbs endocytosis and epithelium morphogenesis via specific Stardust isoforms. J Cell Biol 216, 1405-1420.

Porazinski, S., de Navascues, J., Yako, Y., Hill, W., Jones, M.R., Maddison, R., Fujita, Y., Hogan, C., 2016. EphA2 Drives the Segregation of Ras-Transformed Epithelial Cells from Normal Neighbors. Curr Biol 26, 3220-3229.
Prasad, D., Illek, K., Fischer, F., Holstein, K., Classen, A.-K., 2022. Bilateral JNK activation is a hallmark of interface contractility and promotes elimination of aberrant cells. bioRxiv, 2022.2006.2020.496791.
Robinson, B.S., Huang, J., Hong, Y., Moberg, K.H., 2010. Crumbs Regulates Salvador/Warts/Hippo Signaling in Drosophila via the FERM-Domain Protein Expanded. Current Biology 20, 582-590.
Rogers, S.L., Wiedemann, U., Hacker, U., Turck, C., Vale, R.D., 2004. Drosophila RhoGEF2 associates with microtubule plus ends in an EB1-dependent manner. Curr Biol 14, 1827-1833.
Roper, K., 2012. Anisotropy of Crumbs and aPKC drives myosin cable assembly during tube formation. Dev Cell 23, 939-953.
Rosenblatt, J., Raff, M.C., Cramer, L.P., 2001. An epithelial cell destined for apoptosis signals its neighbors to extrude it by an actin- and myosin-dependent mechanism. Curr Biol 11, 1847-1857.
Saw, T.B., Doostmohammadi, A., Nier, V., Kocgozlu, L., Thampi, S., Toyama, Y., Marcq, P., Lim, C.T., Yeomans, J.M., Ladoux, B., 2017. Topological defects in epithelia govern cell death and extrusion. Nature 544, 212-216.
Schimanski, C.C., Schmitz, G., Kashyap, A., Bosserhoff, A.K., Bataille, F., Schäfer, S.C., Lehr, H.A., Berger, M.R., Galle, P.R., Strand, S., Strand, D., 2005. Reduced expression of Hugl-1, the human homologue of Drosophila tumour suppressor gene lgl, contributes to progression of colorectal cancer. Oncogene 2005 24:19 24, 3100-3109.
Schroeder, M.C., Chen, C.L., Gajewski, K., Halder, G., 2013. A non-cell-autonomous tumor suppressor role for Stat in eliminating oncogenic scribble cells. Oncogene 32, 4471-4479.
Shard, C., Luna-Escalante, J., Schweisguth, F., 2020. Tissue-wide coordination of epithelium-to-neural stem cell transition in the Drosophila optic lobe requires Neuralized. J Cell Biol 219.
Shen, J., Dahmann, C., 2005. Extrusion of cells with inappropriate Dpp signaling from Drosophila wing disc epithelia. Science 307, 1789-1790.
Shen, J., Dahmann, C., Pflugfelder, G.O., 2010. Spatial discontinuity of optomotor-blind expression in the Drosophila wing imaginal disc disrupts epithelial architecture and promotes cell sorting. BMC Dev Biol 10, 23.
Sidor, C., Stevens, T.J., Jin, L., Boulanger, J., Roper, K., 2020. Rho-Kinase Planar Polarization at Tissue Boundaries Depends on Phospho-regulation of Membrane Residence Time. Dev Cell 52, 364-378 e367.
Silver, J.T., Wirtz-Peitz, F., Simoes, S., Pellikka, M., Yan, D., Binari, R., Nishimura, T., Li, Y., Harris, T.J.C., Perrimon, N., Tepass, U., 2019. Apical polarity proteins recruit the RhoGEF Cysts to promote junctional myosin assembly. J Cell Biol 218, 3397-3414.
Simoes, S., Denholm, B., Azevedo, D., Sotillos, S., Martin, P., Skaer, H., Hombria, J.C., Jacinto, A., 2006. Compartmentalisation of Rho regulators directs cell invagination during tissue morphogenesis. Development 133, 4257-4267.
Simoes, S., Lerchbaumer, G., Pellikka, M., Giannatou, P., Lam, T., Kim, D., Yu, J., Ter Stal, D., Al Kakouni, K., Fernandez-Gonzalez, R., Tepass, U., 2022. Crumbs complex-directed apical membrane dynamics in epithelial cell ingression. J Cell Biol 221.
Simoes, S., Oh, Y., Wang, M.F.Z., Fernandez-Gonzalez, R., Tepass, U., 2017. Myosin II promotes the anisotropic loss of the apical domain during Drosophila neuroblast ingression. J Cell Biol 216, 1387-1404.
Slattum, G., Gu, Y., Sabbadini, R., Rosenblatt, J., 2014. Autophagy in oncogenic K-Ras promotes basal extrusion of epithelial cells by degrading S1P. Curr Biol 24, 19-28.
Slattum, G., McGee, K.M., Rosenblatt, J., 2009. P115 RhoGEF and microtubules decide the direction apoptotic cells extrude from an epithelium. J Cell Biol 186, 693-702.
Srivastava, A., Pastor-Pareja, J.C., Igaki, T., Pagliarini, R., Xu, T., 2007. Basement membrane remodeling is essential for Drosophila disc eversion and tumor invasion. Proceedings of the National Academy of Sciences of the United States of America 104, 2721-2726.

Staddon, M.F., Cavanaugh, K.E., Munro, E.M., Gardel, M.L., Banerjee, S., 2019. Mechanosensitive Junction Remodeling Promotes Robust Epithelial Morphogenesis. Biophys J 117, 1739-1750.
Stewart, M., Murphy, C., Fristrom, J.W., 1972. The recovery and preliminary characterization of X chromosome mutants affecting imaginal discs ofDrosophila melanogaster. Developmental Biology 27, 71-83.
Takeuchi, Y., Narumi, R., Akiyama, R., Vitiello, E., Shirai, T., Tanimura, N., Kuromiya, K., Ishikawa, S., Kajita, M., Tada, M., Haraoka, Y., Akieda, Y., Ishitani, T., Fujioka, Y., Ohba, Y., Yamada, S., Hosokawa, Y., Toyama, Y., Matsui, T., Fujita, Y., 2020. Calcium Wave Promotes Cell Extrusion. Curr Biol.
Tamori, Y., Bialucha, C.U., Tian, A.G., Kajita, M., Huang, Y.C., Norman, M., Harrison, N., Poulton, J., Ivanovitch, K., Disch, L., Liu, T., Deng, W.M., Fujita, Y., 2010. Involvement of Lgl and Mahjong/VprBP in cell competition. PLoS Biol 8, e1000422.
Tamori, Y., Suzuki, E., Deng, W.-M., 2016a. Epithelial Tumors Originate in Tumor Hotspots, a Tissue-Intrinsic Microenvironment. PLOS Biology 14, e1002537-e1002537.
Tamori, Y., Suzuki, E., Deng, W.M., 2016b. Epithelial Tumors Originate in Tumor Hotspots, a Tissue-Intrinsic Microenvironment. PLoS Biol 14, e1002537.
Teng, X., Qin, L., Le Borgne, R., Toyama, Y., 2017. Remodeling of adhesion and modulation of mechanical tensile forces during apoptosis in Drosophila epithelium. Development 144, 95-105.
Teo, J.L., Gomez, G.A., Weeratunga, S., Davies, E.M., Noordstra, I., Budnar, S., Katsuno-Kambe, H., McGrath, M.J., Verma, S., Tomatis, V., Acharya, B.R., Balasubramaniam, L., Templin, R.M., McMahon, K.A., Lee, Y.S., Ju, R.J., Stebhens, S.J., Ladoux, B., Mitchell, C.A., Collins, B.M., Parton, R.G., Yap, A.S., 2020a. Caveolae Control Contractile Tension for Epithelia to Eliminate Tumor Cells. Dev Cell 54, 75-91 e77.
Teo, J.L., Tomatis, V.M., Coburn, L., Lagendijk, A.K., Schouwenaar, I.M., Budnar, S., Hall, T.E., Verma, S., McLachlan, R.W., Hogan, B.M., Parton, R.G., Yap, A.S., Gomez, G.A., 2020b. Src kinases relax adherens junctions between the neighbors of apoptotic cells to permit apical extrusion. Mol Biol Cell 31, 2557-2569.
Tepass, U., Theres, C., Knust, E., 1990. crumbs encodes an EGF-like protein expressed on apical membranes of Drosophila epithelial cells and required for organization of epithelia. Cell 61, 787-799.
Thomas, M., Ladoux, B., Toyama, Y., 2020. Desmosomal Junctions Govern Tissue Integrity and Actomyosin Contractility in Apoptotic Cell Extrusion. Curr Biol 30, 682-690 e685.
Thompson, B.J., Pichaud, F., Röper, K., 2013. Sticking together the Crumbs — an unexpected function for an old friend. Nature Reviews Molecular Cell Biology 2013 14:5 14, 307-314.
Torres, A.Y., Malartre, M., Pret, A.M., Agnes, F., 2017. JAK/STAT signaling is necessary for cell monosis prior to epithelial cell apoptotic extrusion. Cell Death Dis 8, e2814.
Toyama, Y., Peralta, X.G., Wells, A.R., Kiehart, D.P., Edwards, G.S., 2008. Apoptotic force and tissue dynamics during Drosophila embryogenesis. Science 321, 1683-1686.
Tripathi, B.K., Irvine, K.D., 2022. The wing imaginal disc. Genetics.
Uhlirova, M., Jasper, H., Bohmann, D., 2005. Non-cell-autonomous induction of tissue overgrowth by JNK/Ras cooperation in a Drosophila tumor model. Proceedings of the National Academy of Sciences of the United States of America 102, 13123-13128.
Valon, L., Davidović, A., Levillayer, F., Villars, A., Chouly, M., Cerqueira-Campos, F., Levayer, R., 2021. Robustness of epithelial sealing is an emerging property of local ERK feedback driven by cell elimination. Developmental Cell 56, 1700-1711.e1708.
Varelas, X., Samavarchi-Tehrani, P., Narimatsu, M., Weiss, A., Cockburn, K., Larsen, B.G., Rossant, J., Wrana, J.L., 2010. The Crumbs Complex Couples Cell Density Sensing to Hippo-Dependent Control of the TGF-β-SMAD Pathway. Developmental Cell 19, 831-844.
Vasquez, C.G., Tworoger, M., Martin, A.C., 2014. Dynamic myosin phosphorylation regulates contractile pulses and tissue integrity during epithelial morphogenesis. J Cell Biol 206, 435-450.
Villars, A., Matamoro-Vidal, A., Levillayer, F., Levayer, R., 2022. Microtubule disassembly by caspases is an important rate-limiting step of cell extrusion. Nat Commun 13, 3632.

Villeneuve, C., Lagoutte, E., de Plater, L., Mathieu, S., Manneville, J.B., Maitre, J.L., Chavrier, P., Rosse, C., 2019. aPKCi triggers basal extrusion of luminal mammary epithelial cells by tuning contractility and vinculin localization at cell junctions. Proc Natl Acad Sci U S A 116, 24108-24114.
Wagstaff, L., Goschorska, M., Kozyrska, K., Duclos, G., Kucinski, I., Chessel, A., Hampton-O'Neil, L., Bradshaw, C.R., Allen, G.E., Rawlins, E.L., Silberzan, P., Carazo Salas, R.E., Piddini, E., 2016a. Mechanical cell competition kills cells via induction of lethal p53 levels. Nat Commun 7, 11373.
Wagstaff, L., Goschorska, M., Kozyrska, K., Duclos, G., Kucinski, I., Chessel, A., Hampton-O'Neil, L., Bradshaw, C.R., Allen, G.E., Rawlins, E.L., Silberzan, P., Carazo Salas, R.E., Piddini, E., 2016b. Mechanical cell competition kills cells via induction of lethal p53 levels. Nature Communications 7, 11373-11373.
Wee, K., Hediyeh-Zadeh, S., Duszyc, K., Verma, S., B, N.N., Khare, S., Varma, A., Daly, R.J., Yap, A.S., Davis, M.J., Budnar, S., 2020. Snail induces epithelial cell extrusion by regulating RhoA contractile signalling and cell-matrix adhesion. J Cell Sci 133.
Widmann, T.J., Dahmann, C., 2009. Wingless signaling and the control of cell shape in Drosophila wing imaginal discs. Dev Biol 334, 161-173.
Wodarz, A., 2000. Tumor suppressors: linking cell polarity and growth control. Curr Biol 10, R624-626.
Wodarz, A., Näthke, I., 2007. Cell polarity in development and cancer. Nature Cell Biology 2007 9:9 9, 1016-1024.
Woods, D.F., Bryant, P.J., 1989. Molecular cloning of the lethal(1)discs large-1 oncogene of Drosophila. Developmental Biology 134, 222-235.
Woods, D.F., Bryant, P.J., 1991. The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions. Cell 66, 451-464.
Wu, M., Pastor-Pareja, J.C., Xu, T., 2010. Interaction between Ras(V12) and scribbled clones induces tumour growth and invasion. Nature 463, 545-548.
Yamamoto, M., Ohsawa, S., Kunimasa, K., Igaki, T., 2017. The ligand Sas and its receptor PTP10D drive tumour-suppressive cell competition. Nature 542, 246-250.
Zhan, L., Rosenberg, A., Bergami, K.C., Yu, M., Xuan, Z., Jaffe, A.B., Allred, C., Muthuswamy, S.K., 2008. Deregulation of Scribble Promotes Mammary Tumorigenesis and Reveals a Role for Cell Polarity in Carcinoma. Cell 135, 865-878.

Figure Legends

Figure1: Illustration of the steps of apical extrusion in a MDCK cell and basal extrusion in a larval epidermal cell in *Drosophila* pupae

A Apical extrusion of a dying MDCK cell (green). Cell autonomous constriction drives the reorganisation of actomyosin in the neighbouring cells through E-cad-dependent force transmission, Corinin1B, MyoVI and p114RhoGEF. In parallel, S1P is permissive for the upregulation of tension in the neighbours through its receptor S1P2. A supracellular actomyosin ring is formed in the neighbours and slides basally. This is reinforced by the basal release of p115RhoGEF by microtubules. In the meantime, basal protrusions progressively detach the basal side of the extruding cell. Desmosomes (purple) are maintained throughout the process and new desmosomes are formed basally between the neigbhoring cells. Eventually new adherens junctions and new desmosomes junctions are formed while the extruding cell terminates

apoptosis on the apical side. Question marks show hypothetical remodelling which have not yet been clearly described or characterised. Legends are shown below.

B Basal extrusion of a dying larval epidermal cell from the *Drosophila* pupal abdomen (green), note that these cells are squamous. Cell autonomous constriction is first initiated, followed by disassembly of adherens junctions. This triggers transient stress-release followed by the formation of a supracellular actomyosin cable in the neighbouring cells, which terminates the apical constriction (the top view is shown on the right). Septate junctions (orange dots) are maintained throughout the process. Question mark shows hypothetical remodelling which has not yet been clearly described or characterised. Note that integrins are also located on the apical side and can interact with the apical ECM and the cuticle. Legends are shown below.

Figure2: Mechanical parameters influencing the direction of extrusion

A Three-dimensional view of an extruding cell (green) and its neighbours. The main mechanical parameters controlling the 3D shape are all indicated in the legend. Surface energy penalises a given surface and reduces it. This energy increases with contractility and is reduced by adhesive forces.

B Apical or basal constrictions will lead to basal or apical extrusion. The parameters indicated next to the arrows are the one favouring one direction or the other. Increase of lateral surface energy of the extruding cells relative to the surface energy of the other cells should favour equally the two topologies.

C Reduction of the apico-basal length of the extruding cell is favoured by high lateral surface energy (either through a reduction of lateral adhesion or an increase of tension along apico-basal axis).

Figure 3: Receptor misslocalization can drive elimination of polarity deficient cells

A Left: Wing disc entirely composed of polarity-deficient cells (eg. *scribble*, *dlg*, or *lgl* mutants) exhibits unregulated growth and forms a tumor-like hyperplastic mass. Right: Clones mutant for polarity genes are eliminated through cell competition.

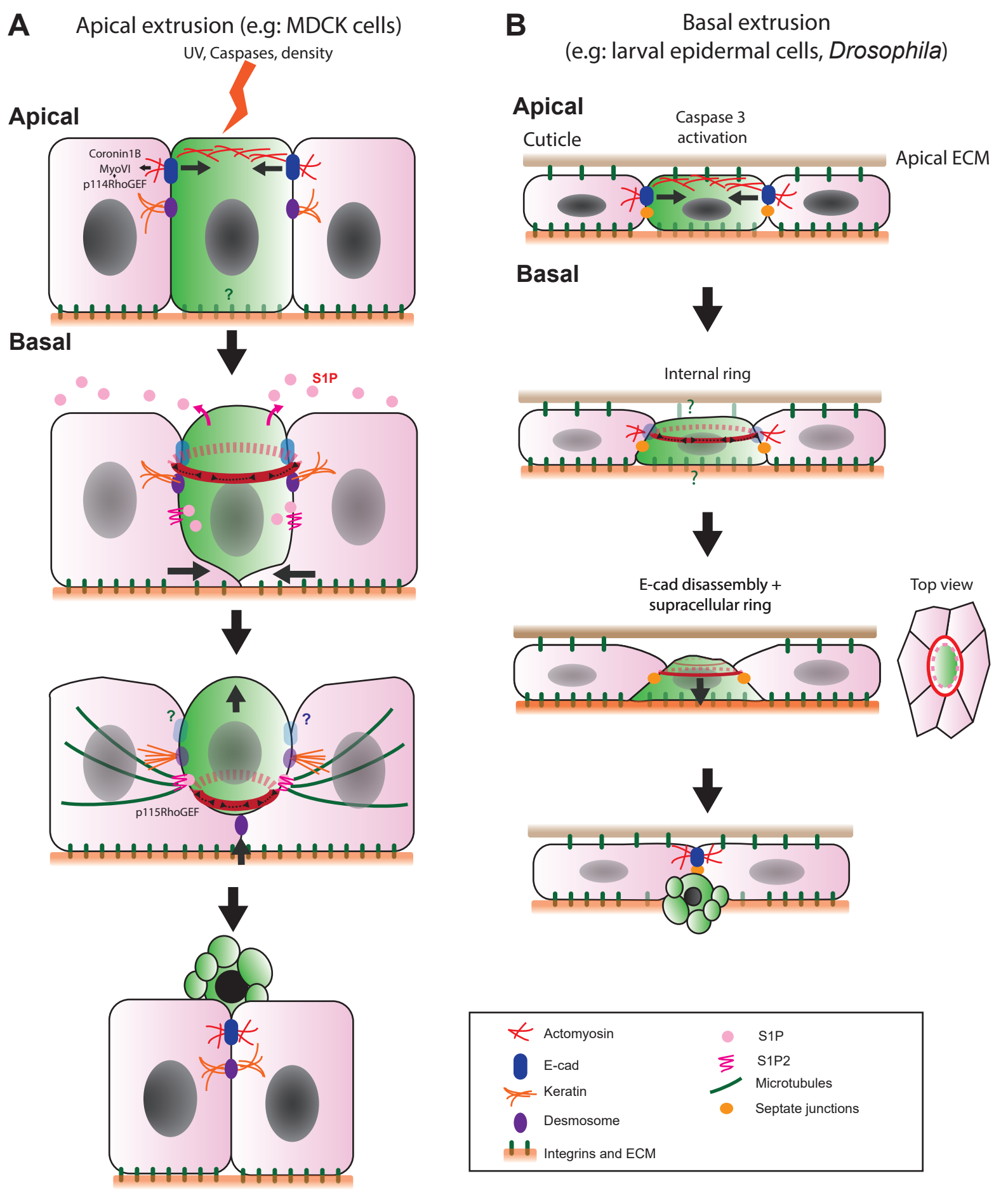
B Misslocalization of the SAS transmembrane protein and the PTP10D receptor for SAS at the interface between WT and in *scrib*$^{-/-}$ cells leads to *scrib*$^{-/-}$ cell elimination through decreased EGFR signaling and elevated JNK (Yamamoto et al., 2017).

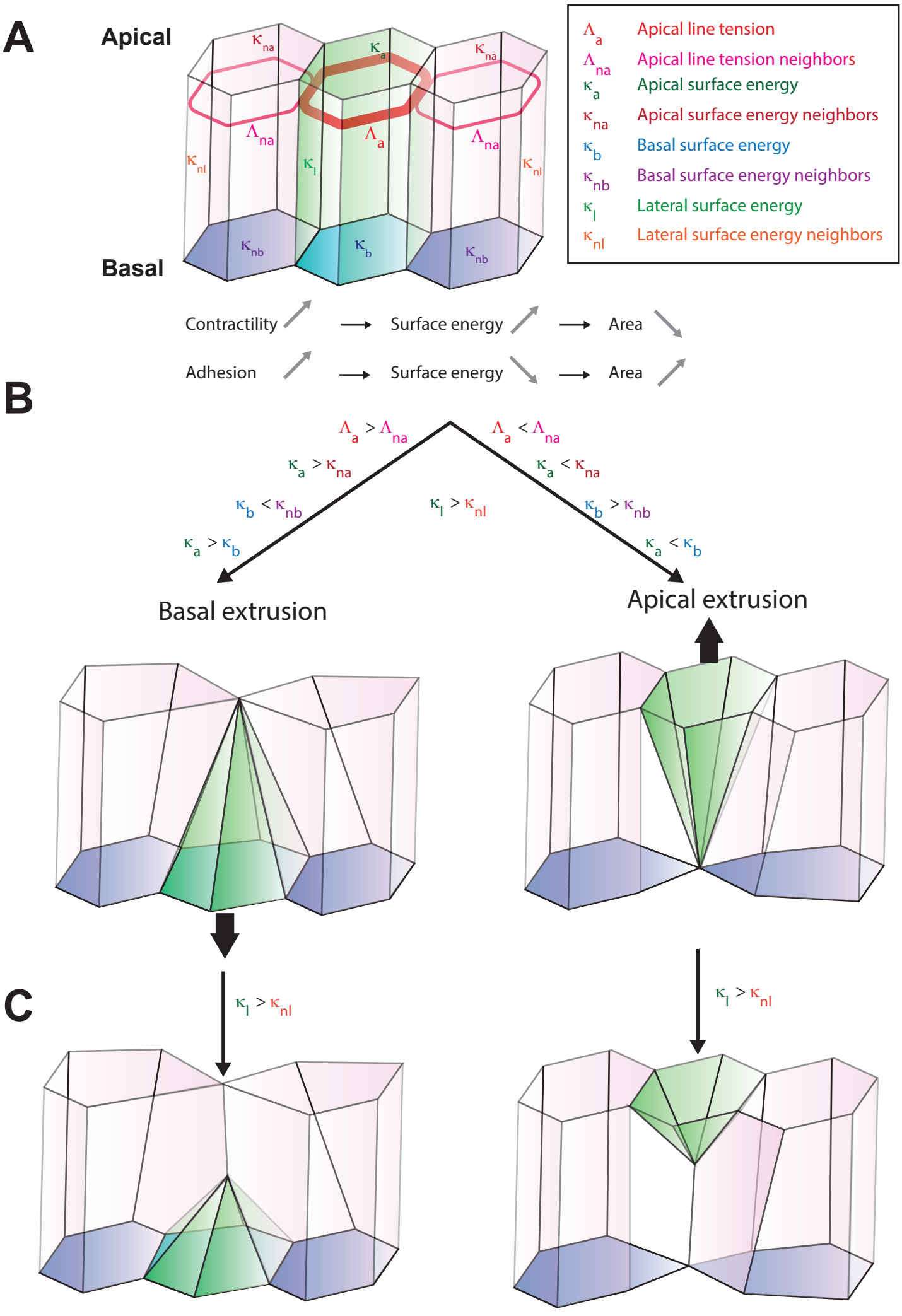
C Basolateral relocalization of Grindelwald, a receptor for Eiger (the *Drosophila* Tumor necrosis factor) in *scrib*$^{-/-}$ cells allows the binding of Eiger present in the basolateral circulating hemolymph, which leads to cell death through JNK signaling in *scrib*$^{-/-}$ cells (de Vreede et al., 2022).

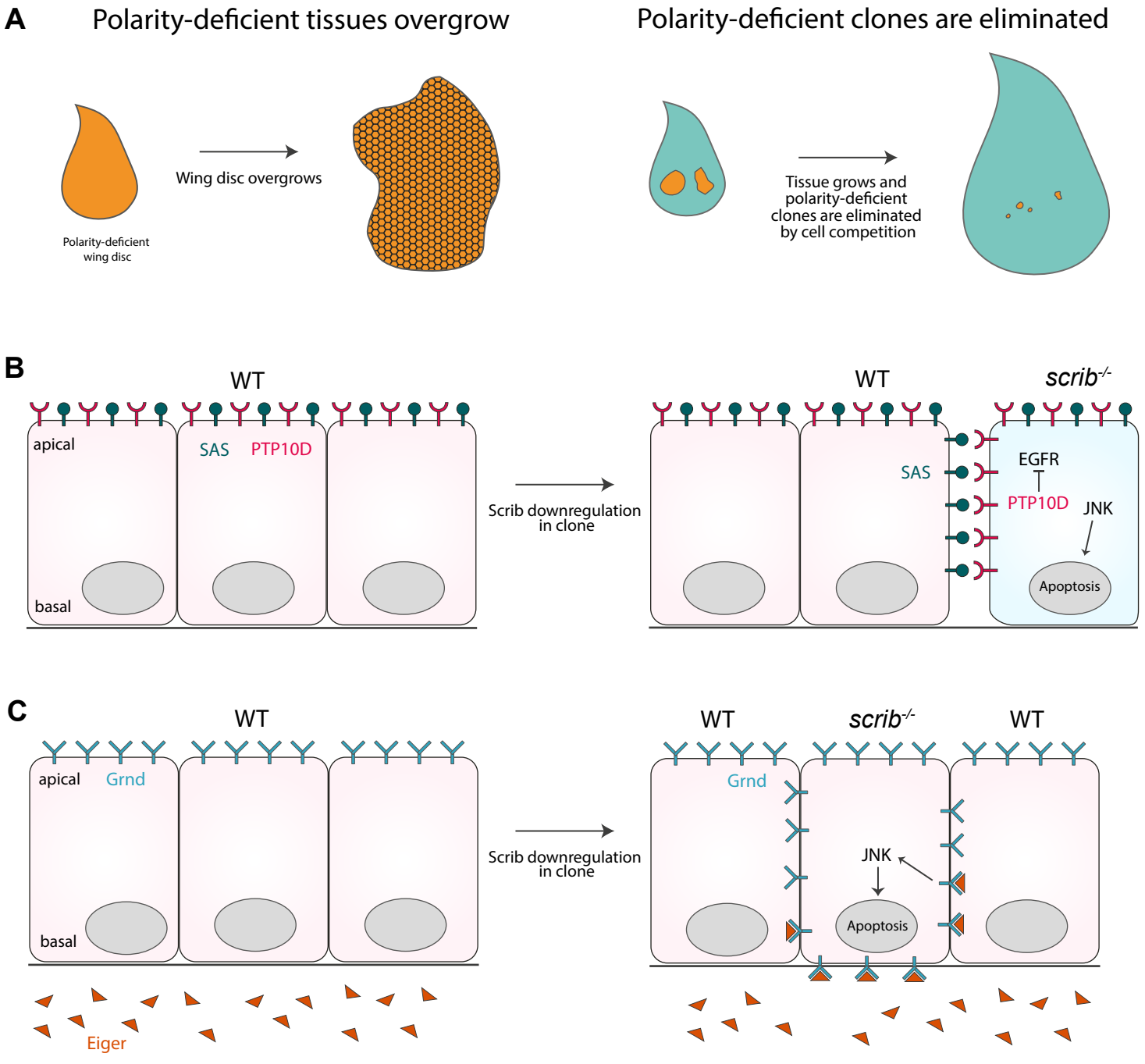
Figure 4: Polarity defects and mechanical driven elimination

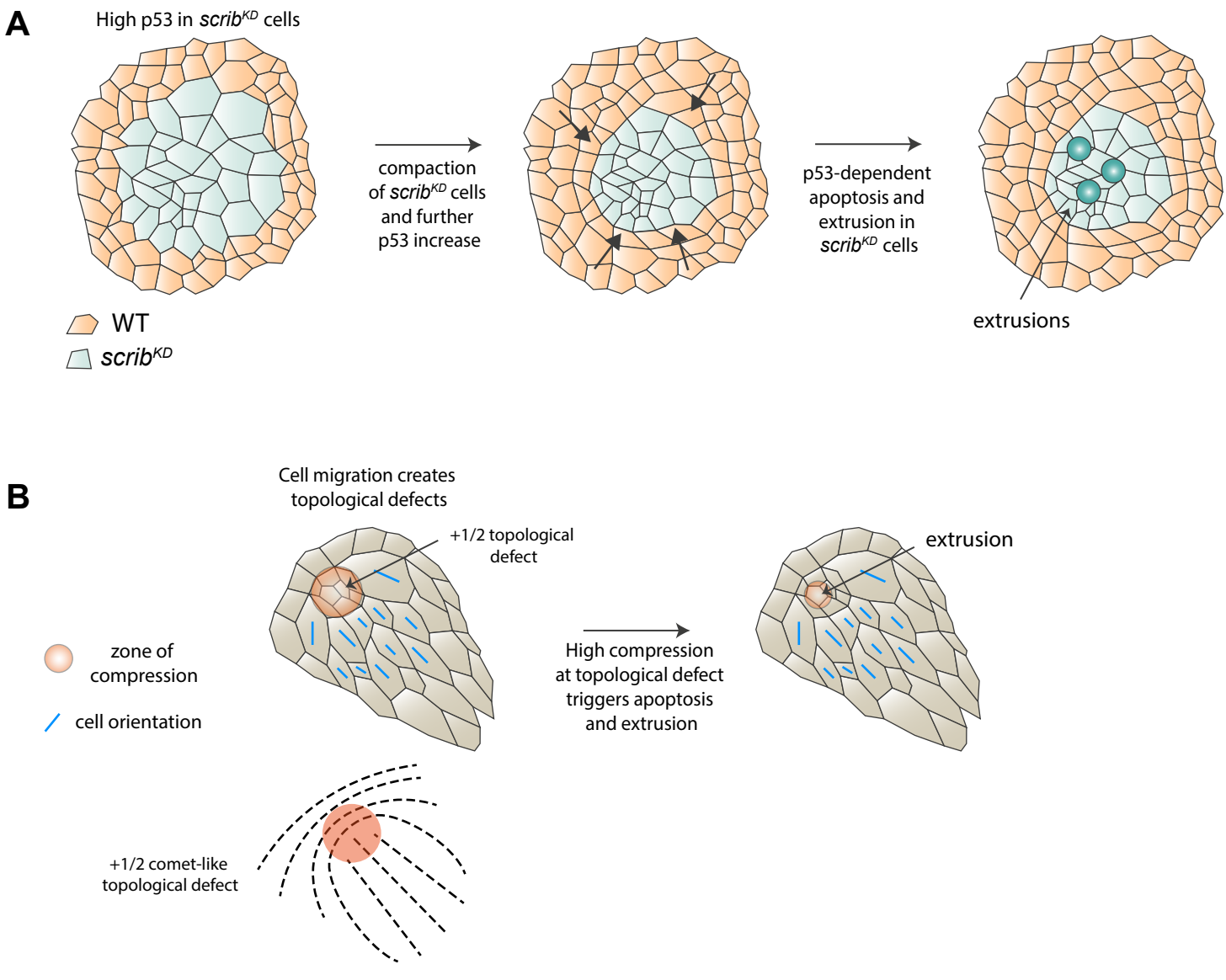
A Scrib^{KD} cells exhibiting high p53 levels are compressed by WT MDCK cells, which drives further p53 increase through a ROCK-p38 axis, cell death and extrusion (Wagstaff et al., 2016a).

B MCDK cell migration generates local topological defects (defects in cell orientation, yellow lines) in 2D cell cultures. At comet-like defects ("+1/2 defects", see the orientation of cells in blue and the typical alignment, dashed line below), cells experience compressive forces which drive apoptotic cell death and extrusion associated with cytoplasmic relocalization of YAP (Saw et al., 2017).


A
Apical extrusion (e.g: MDCK cells)
UV, Caspases, density
Apical
Coronin1B
MyoVI
p114RhoGEF
?
Basal
S1P
?
?
p115RhoGEF
B
Basal extrusion
(e.g: larval epidermal cells, Drosophila)
Apical
Caspase 3
activation
Cuticle
Apical ECM
Basal
Internal ring
?
?
E-cad disassembly +
supracellular ring
Top view
Actomyosin
E-cad
Keratin
Desmosome
Integrins and ECM
S1P
S1P2
Microtubules
Septate junctions


A
Apical
Basal
κ_{na}
κ_a
κ_{na}
Λ_{na}
Λ_a
Λ_{na}
κ_{nl}
κ_l
κ_{nl}
κ_{nb}
κ_b
κ_{nb}
Λ_a Apical line tension
Λ_{na} Apical line tension neighbors
κ_a Apical surface energy
κ_{na} Apical surface energy neighbors
κ_b Basal surface energy
κ_{nb} Basal surface energy neighbors
κ_l Lateral surface energy
κ_{nl} Lateral surface energy neighbors
Contractility → Surface energy → Area
Adhesion → Surface energy → Area
B
$\Lambda_a > \Lambda_{na}$
$\kappa_a > \kappa_{na}$
$\kappa_b < \kappa_{nb}$
$\kappa_a > \kappa_b$
$\kappa_l > \kappa_{nl}$
$\Lambda_a < \Lambda_{na}$
$\kappa_a < \kappa_{na}$
$\kappa_b > \kappa_{nb}$
$\kappa_a < \kappa_b$
Basal extrusion
Apical extrusion
C
$\kappa_l > \kappa_{nl}$
$\kappa_l > \kappa_{nl}$


A
Polarity-deficient tissues overgrow
Polarity-deficient wing disc
Wing disc overgrows
Polarity-deficient clones are eliminated
Tissue grows and polarity-deficient clones are eliminated by cell competition
B
WT
apical
basal
SAS
PTP10D
Scrib downregulation in clone
WT
scrib-/-
SAS
EGFR
PTP10D
JNK
Apoptosis
C
WT
apical
Grnd
basal
Eiger
Scrib downregulation in clone
WT
scrib-/-
WT
Grnd
JNK
Apoptosis


A
High p53 in scribKD cells
compaction of scribKD cells and further p53 increase
p53-dependent apoptosis and extrusion in scribKD cells
extrusions
WT
scribKD
B
Cell migration creates topological defects
+1/2 topological defect
extrusion
zone of compression
cell orientation
High compression at topological defect triggers apoptosis and extrusion
+1/2 comet-like topological defect