



**HAL**  
open science

## Dying under pressure: cellular characterisation and in vivo functions of cell death induced by compaction

Léo Valon, Romain Levayer

► **To cite this version:**

Léo Valon, Romain Levayer. Dying under pressure: cellular characterisation and in vivo functions of cell death induced by compaction. *Biology of the Cell*, 2019, 111 (3), pp.51-66. 10.1111/boc.201800075 . hal-02644671

**HAL Id: hal-02644671**

**<https://hal.science/hal-02644671>**

Submitted on 28 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Dying under pressure: cellular characterisation and *in vivo* functions of cell death induced by compaction

Léo Valon<sup>1</sup> and Romain Levayer<sup>1\*</sup>

1. Institut Pasteur, Department of Developmental and Stem Cell Biology, 25 rue du Dr. Roux, 75015 Paris, France

\*: Correspondence to: [romain.levayer@pasteur.fr](mailto:romain.levayer@pasteur.fr)

## Abstract

Cells and tissues are exposed to multiple mechanical stresses during development, tissue homeostasis and diseases. While we start to have an extensive understanding of the influence of mechanics on cell differentiation and proliferation, how excessive mechanical stresses can also lead to cell death and may be associated with pathologies has been much less explored so far. Recently, the development of new perturbative approaches allowing modulation of pressure and deformation of tissues has demonstrated that compaction (the reduction of tissue size or volume) can lead to cell elimination. Here we discuss the relevant type of stress and the parameters that could be causal to cell death from single cell to multicellular systems. We then compare the pathways and mechanisms that have been proposed to influence cell survival upon compaction. We eventually describe the relevance of compaction-induced death *in vivo*, and its functions in morphogenesis, size regulation, tissue homeostasis, and cancer progression.

## Introduction

Living tissues are constantly exposed to mechanical stresses during development and adulthood. As a result, they are optimised to resist the mechanical stresses that they are most likely to be exposed to during their life. This “mechanical fitness” was already described by D’Arcy Thompson in his chapter “on form and mechanical efficiency” in his seminal book “on Growth and Form” using the morphology and architecture of Vertebrate bones as a case study (Thompson, 1917). While this optimisation is the product of a long process of natural selection, it can also be explained by the capacity of tissue to sense mechanical stresses and to adjust their behavior to those. This ability, so-called mechanosensitivity, has now become an intensive field of research and deals with the way cells and tissues probe and adapt their behavior to local and tissue scale mechanical stresses (Vogel and Sheetz, 2006). Three types of mechanical stresses have been studied in cell culture and *in vivo*: tension, compression and shear stresses. Their modulation can trigger cell and tissue deformation, including stretching, compaction and bending (**Figure 1A-C**).

While a large number of studies has focused on how mechanical stresses modify cell proliferation and cell differentiation (Irvine and Shraiman, 2017, Panciera et al., 2017), much less work has addressed how they induce cell elimination. Here, we propose to focus on the effect of negative strains (compaction) on cell death, in other words any process leading to a decrease of cell length/area/volume affecting cell survival. We first examine the amplitude, timescale and direction of negative strains which can lead to cell death by reviewing studies imposing compressive forces on isolated cells *in vitro*, on 2D and 3D cell cultures and *in vivo*. We review then the putative mechanisms/pathways that sense negative strains and trigger cell elimination. Eventually, we discuss the *in vivo* relevance of those processes in physiological and pathological situations.

### **Perturbative approaches that elicit compaction-driven cell death**

The effect of cell/tissue compaction on cell survival were mostly studied through perturbative approaches allowing modulation of pressure or the modification of tissue strain in one or several dimensions. We will first review the different technics used to compact cells and tissues starting from single cell studies, single cell layer compaction and eventually 3D cell aggregate deformations.

#### *Single cell compaction*

Cell response to compaction was initially studied by subjecting cells to high hydrostatic pressures. Using pistons, pressures ranging from few mega-Pascal (MPa) to hundreds of MPa have been applied to various cell lines (**Figure 1D**, 1 MPa is equal to 10 times the atmospheric pressure). The resulting effect on cell survival varied a lot depending on the cell type: while a 10 min exposure at 0.5-0.7 MPa was sufficient to trigger ganglion neurons cell death (Quan et al., 2014), a 30 min exposure to 85-200 MPa was necessary to induce apoptosis 8 hours later in lymphoblasts (Takano et al., 1997). Those approaches demonstrated that high pressure could trigger cell death and also outlined the high variability of susceptibility to pressure depending on the cell type. However, they are limited by the pleiotropic perturbations induced by high pressure, from modulation of membrane composition, cytoplasmic crowding, cytoskeleton modification to DNA shearing (Zimmerman, 1970). Moreover, the values of pressures applied are usually not relevant for a wide range of physiological and pathological conditions: for instance, osteoblasts are exposed to pressure ranging from 100 to 300 kPa (Thompson et al., 2012), from 1 to 3 order of magnitudes lower than what was performed *in vitro*. More physiological conditions can be obtained by reducing cell volume through osmotic stress. For instance, using culture media of high osmolarity, it was shown that a 30% reduction of human glioma cell volume for 8 hours is

necessary and sufficient to induce apoptosis and maintain caspase activation (Ernest et al., 2008) (**Figure 1E**).

#### *Strain reduction of 2D cell layers*

While the previous experiments tested the effects of an isotropic increase of pressure, several experiments have used perturbations and/or devices to reduce strain along a single dimension. This is particularly relevant when studying polarised cells, as cells are supposed to have different mechanical and biological responses depending on the axis of deformation. It has been established for a long time that cell density and spreading on the substrate can affect a wide range of behavior, from cell migration, cell proliferation to cell survival (Vogel and Sheetz, 2006, Ladoux and Mege, 2017, Vining and Mooney, 2017). However, the direct effect of lateral compaction on cell survival has only been addressed recently. In a seminal work, Chen S. and colleagues have modified the adhesion pattern of human endothelial cells and have shown that below a minimal spreading area, cells undergo apoptosis (Chen et al., 1997). This effect was not depending on the absolute number of integrins bound to ECM, as spacing the adhesive spots in the patterned substrate while keeping the total adhesive area constant was sufficient to downregulate cell death (**Figure 1F**).

Cell death triggered by mechanical perturbations is also observed in culture of epithelial layer for both local and global deformations (**Figure 1G and 1H**). Lateral compaction of epithelial layers was performed using stretchable PDMS substrates (Eisenhoffer et al., 2012). MDCK cells were seeded on a pre-stretched substrate which was then released after confluency. The 30% reduction of epithelial area induced a transient increase of cell density (from 110 to 150 cells per  $100 \mu\text{m}^2$ ) which went back to its initial density after 6 hours. This was driven by a rapid increase of the cell extrusion rate (the removal of cells from the epithelial layer, see below) and cell death (**Figure 1H**). Compaction-driven death can also be driven by spontaneous local deformations in the epithelium. Accordingly, migration of MDCK cells can lead to local aberrations in the alignment of cells which result in a local increase of compression and cell elimination (Saw et al., 2017). In this condition, the epithelium movement is similar to a nematic liquid crystal with long range alignment of cells. Comet-like nematic defects (so called +1/2 defects) correlate with local cell compaction and precede cell extrusion for up to 100 min (**Figure 1G**). The rate of cell elimination was also elegantly controlled by plating cells on star shape patterns which increased the probability of topological defects in the tip and locally increased cell elimination.

#### *In vivo compaction of epithelia*

Similarly local epithelial deformations associated with cell death were also described *in vivo* during morphogenesis of the *Drosophila* pupal thorax (the notum). In this case, tissue flows trigger a local densification of the tissue in the midline region, which induces the extrusion of around 30% of the cells within 8 hours (Marinari et al., 2012, Levayer et al., 2016). Those events of 2D lateral compaction can also be triggered ectopically *in vivo* and *in vitro* through genetic perturbations. Induction of ectopic growth in clones through the activation of some oncogenes in a subset of cells (active Ras, active Yorkie) (Levayer et al., 2016, Tsuboi et al., 2018) can lead to the reduction of the apical area and the elimination of the neighbouring WT (Wild Type) cells in the *Drosophila* pupal notum. In this situation, WT cell area can be reduced by a factor of 2 to 3 within 13 hours concomitant with the elimination of up to 20% of the neighbouring WT cells (**Figure 1I**) (Levayer et al., 2016). A similar process was observed in MDCK cells when *scribble* mutant cells (a component of the apico-basal polarity) were surrounded by WT cells (Wagstaff et al., 2016). Here, the active corralling of the mutant cells by the WT cells increases their density by 4 folds and will eventually lead to full elimination of the mutant population.

#### *Compaction of 3D cell aggregates*

Lastly, the capacity of cells to sense negative strain has also been widely studied in the case of 3D cell aggregates (so called spheroids). Cell aggregates were developed early on to simulate the effect of solid stress on tumour, which was suggested to affect their growth (Butcher et al., 2009). Different strategies have been developed to modulate pressure within the aggregate: contention of the spheroid through the modulation of the elastic modulus of the external cellular environment (e.g.: by changing agar concentration) (**Figure 1J**) (Helmlinger et al., 1997, Cheng et al., 2009), modifying the osmolarity of the external media (Delarue et al., 2014), or encapsulating cells in elastic microspheres (Alessandri et al., 2013). Those studies better reproduced the range of pressures experienced by cells in physiological and pathological conditions (from 1 kPa to 100 kPa) and clearly outlined the link between pressure and downregulation of cell proliferation (Helmlinger et al., 1997, Cheng et al., 2009, Montel et al., 2011, Alessandri et al., 2013, Delarue et al., 2014). However, the effect of pressure on cell survival was very variable from one condition to the other. While the effect of pressure on cell death was neglectable in human colon adenocarcinoma cells for pressures ranging from 7 to 13 kPa (Helmlinger et al., 1997, Delarue et al., 2014), a significant increase of apoptosis and necrosis was described in murine mammary carcinoma cells exposed to 4 kPa in the spheroid, with a positive correlation between pressure levels and the proportion of dying cells observed 3 days after compression (Cheng et al., 2009).

In conclusion, isotropic and planar compaction can lead to cell death both in single cell, 2D and 3D cultures as well as *in vivo*. However the timescales and amplitudes of deformations associated with cell elimination vary extensively between cell types. This may either reflect differences in the cell and tissue mechanical properties which could modify their capacity to be deformed for similar stresses, or alternatively may be based on the genetic backgrounds that modulate their sensitivity to cell death/apoptosis. This opens the question of which mechanisms trigger cell elimination in those perturbative conditions.

### ***Necrosis, apoptosis and cell extrusion: different modes of compaction-driven cell elimination***

Compaction-driven cell elimination has been associated with different modes of cell elimination, including necrosis (uncontrolled destruction of cell integrity) or the induction of apoptosis: the regulated destruction of cell integrity orchestrated by the cysteine proteases called caspases (reviewed in (Fuchs and Steller, 2011)). Moreover, elimination of epithelial cells requires an additional layer of regulation: the concerted remodeling of cell-cell contact and cell-ECM adhesion leading to its exclusion from the epithelial layer (so called “cell extrusion” (Rosenblatt et al., 2001, Teng et al., 2017)). The induction of apoptosis in epithelial cells can trigger extrusion (Rosenblatt et al., 2001, Teng et al., 2017). Moreover cell-cell contacts are required to maintain cell survival (Paoli et al., 2013). As such, epithelial cell death can be either a cause or a consequence of cell extrusion. It is therefore essential to understand whether compaction regulates cell extrusion or apoptosis induction. We will review in this section which processes are the main drivers of cell elimination during tissue/cell compaction.

Hydrostatic pressure-induced death can be both driven by apoptosis and necrosis at very high pressures (Takano et al., 1997) and in milder pressures (Quan et al., 2014). While necrosis is probably driven by direct destruction of cell integrity through membrane rupture, apoptotic cell elimination suggests that cells have the capacity to “sense” pressure through dedicated pathway(s). In glioma cells, a 30% reduction of cell volume for more than 8 hours is sufficient to activate Caspase 9, 8 and 3 (Ernest et al., 2008). In the centre of cell aggregates, both necrotic and apoptotic cells (positive for cleaved Caspase 3, the effector caspase) can be found (Cheng et al., 2009). Similarly, compression-induced damage in the cartilage is primarily driven by chondrocyte apoptosis (Chubinskaya and Wimmer, 2013). Interestingly, apoptosis was significantly reduced in cell aggregates by Bcl-2 overexpression (B cell lymphoma 2, a negative regulator of apoptosis which prevents release of the Cytochrome-c from the mitochondria (Fuchs and Steller, 2011)) suggesting that the mitochondrial apoptosis pathway is required for

compaction-driven death (Cheng et al., 2009). Similarly, mitochondrial functions are impaired in compressed intervertebral disc cells (Ding et al., 2012). Altogether, those studies suggest that active mechanisms can sense cell compression/deformation and activate the intrinsic apoptotic pathway.

The situation is more complex in epithelium, where cell death could be either a cause or a consequence of cell extrusion. The initial observation of crowding-induced elimination suggested that tissue compaction induces live cell extrusion independently of caspase activation both in MDCK cells and *Drosophila* pupal epithelium (Eisenhoffer et al., 2012, Marinari et al., 2012). Cell death was thought to be a consequence of extrusion and anoikis (the loss of cell-cell contact and cell-ECM adhesion). This was supported by the fact that MDCK extruded cells could survive if they were plated again on normal culture conditions. Moreover Bcl-2 overexpression in MDCK cells or *diap1* (a physiological inhibitor of caspase) overexpression in the pupal notum only induced a mild reduction of the rate of compaction-driven extrusion. Finally, very few cells were positive for active Caspase 3 both in crowded MDCK cells, in the human colon and in the zebrafish fin. In this framework, the putative sensor of cell deformation will affect the stability of cells in the epithelial layer and trigger cell extrusion, however the apoptotic pathway per se would not be influenced by mechanics. Yet, the contribution of caspases to cell elimination is controversial as other studies suggested that compaction-driven extrusion requires caspase activation (Levayer et al., 2016, Wagstaff et al., 2016, Saw et al., 2017, Moreno et al., 2018). Spontaneous elimination of MDCK cells driven by topological aberrations can be blocked by a caspase inhibitor (Z-VAD-FMK) and was preceded by caspase activation (visualised with a live marker of caspase activity) (Saw et al., 2017). Similarly, cell elimination in the *Drosophila* pupal notum can be blocked through the genetic inhibition of caspase activation both in clones and in the whole tissue, and caspase activation preceded systematically cell extrusion (Levayer et al., 2016). While those studies do not exclude that cells extrude “live” (in other words, they can still revert back to normal), they clearly show that caspase activation is required for compaction-driven extrusion. Similarly, the relationship between caspases, apoptosis and cell extrusion in the gut villi are still actively debated (Patterson and Watson, 2017).

Altogether, those studies show that multiple mechanisms may drive cell elimination upon tissue compaction. The discrepancy regarding the contribution of caspases may reflect the high complexity of caspase dynamics and its multiple functions. It is nowadays clear that caspases can undergo transient activation (Tang et al., 2012, Ding et al., 2016, Levayer et al., 2016, Teng et al., 2017) and have multiple functions unrelated to apoptosis (Nakajima and Kuranaga, 2017).

As such, transient and low levels of caspases may be required for cell extrusion through apoptosis-independent functions. Extrusion would then lead to definitive engagement in apoptosis. The contribution of caspases to compaction-driven death remains an important question as it will change the predictions regarding the mechanisms sensing cell deformation and triggering cell elimination (discussed below).

### ***From mechanosensation to modulation of morphology: multiple parameters and multiple pathways affecting cell survival upon compaction***

The diversity of mechanisms leading to compaction-driven death already suggests that multiple molecular machineries may be responsible for sensing compaction and triggering cell elimination. Three main classes of sensing have been proposed to participate to compaction sensing and death induction (**Figure 2**):

- 1) Variation of the extracellular environment that affects the processing/concentration of extracellular pro-survival factors or “killing” factors (e.g.: variation of the interstitial volume)
- 2) Variation of the cell geometry that affects the accessibility to extracellular pro-survival factors or cell signaling.
- 3) Direct sensing of cell mechanical parameters (e.g.: membrane/cortex tension).

We will discuss here the relevance of those three classes of sensing.

#### *Sensing through modifications of the extracellular environment*

The reduction of tissue volume can be driven by the reduction of cell size and/or a reduction of the interstitial volume. Accordingly compression of airway cells along apico-basal (AB) axis promotes cell survival and cell proliferation through the reduction of the interstitial volume (a 10% reduction of the AB length leads to a 80% reduction of the intercellular volume) (Tschumperlin et al., 2004). This is mediated by the increased concentration of the prosurvival factor EGF in the interstitial volume (Tschumperlin et al., 2004) (**Figure 2A**), which promotes growth and survival through the EGFR/ERK pathway. The molecular effector of compression sensing in spheroid was not yet identified. Yet, the fact that volume reduction in spheroid is mostly driven by interstitial volume decrease (Delarue et al., 2014) is compatible with a sensing based on the modulation of concentration of an extracellular factor.

#### *Sensing through modification of cell geometry*

In other contexts, direct modification of the cell geometry will be responsible for cell death. For instance, a 30% reduction of cell volume is both necessary and sufficient to activate caspases in glioma cells (Ernest et al., 2008), suggesting that compressive forces could activate caspases through the increased concentration of some unknown cytoplasmic components. Epithelium lateral compaction will also lead to a change in the cell aspect ratio which may modify its capacity to bind/access extracellular pro-survival factors (**Figure 2B,C**). For instance, TGF- $\beta$  signaling can be altered by cell density through the relocalisation of its receptor from apical to basal side during cell polarisation, hence reducing responsiveness to apically-localized TGF- $\beta$  (Nallet-Staub et al., 2015). Similarly, lateral compaction can lead to a reduction of basal surface which could reduce the pro-survival signaling coming from the ECM. ECM binding and activation of Integrins upregulate several pro-survival pathways, including Focal Adhesion Kinase (FAK), Sarc kinase (Src), Integrin Related Kinases (IRK), PI3K and ERK (Paoli et al., 2013). Accordingly, local variations of cell density correlate with local variations of FAK activity (Frechin et al., 2015). Hippo pathway is a key regulator of cell survival and cell proliferation which acts through the exclusion of the transcription factors YAP/TAZ (Yes Associated Protein), Yorkie (Yki) in *Drosophila*, from the nucleus through their phosphorylation by Lats1/2 kinases (Warts in *Drosophila*), regulated by the upstream kinases MST1/2 and Hippo in *Drosophila* (Harvey and Hariharan, 2012). Cell detachment from ECM and cytoskeleton reorganisation can trigger cell death through the activation of Lats1/2 kinases (Zhao et al., 2012), the two central kinases of the Hippo pathway. Moreover high cell density also downregulates YAP phosphorylation (Zhao et al., 2007). Thus, absolute cell density (and therefore cell shape) could directly modulate cell survival through FAK and/or the Hippo pathway. Finally, tissue crowding was also shown to induce cell death through the upregulation of the pro-apoptotic protein p53. Co-culture of *scribble* mutants and WT MDCK cells leads to the corralling of the mutant cells and their elimination (Wagstaff et al., 2016). The competitive elimination is driven by the compaction of *scribble* cells which die through p53 upregulation. This upregulation is mediated by the stress activated kinase p38 and the MyosinII regulator ROCK (Wagstaff et al., 2016). Interestingly, *scribble* mutant cells are more sensitive to crowding because of the high basal levels of p53, suggesting that p53 levels of expression may both relay information about density and tune the sensitivity of cells to crowding. Finally changes in nuclei geometry may also provide information about tissue density. Accordingly extreme cell confinement during cell migration can trigger nucleus deformation which can induce DNA damage and cell death in absence of efficient repair of the nuclear envelop (Raab et al., 2016). Similarly,

the deformation of the nuclear envelope driven by changes of the substrate rigidity can also directly modulate the import/export of YAP in the nucleus (Elosegui-Artola et al., 2017).

#### *Direct sensing of mechanical forces*

Tissue compaction can also directly modify the mechanical properties of cells which will affect cell survival through mechanosensitive pathways. In those situations, conformation changes of membrane or cortical proteins triggered by mechanical forces will modulate pathways affecting cell survival/apoptosis (Vogel and Sheetz, 2006) (**Figure 2D**). Accordingly, crowding-induced extrusion in MDCK cells and in zebrafish was shown to rely on the  $\text{Ca}^{2+}$  stretch-activated channel Piezo (Eisenhoffer et al., 2012). Chemical inhibition of stretched-activated channels (through Gadolinium,  $\text{Gd}^{3+}$ ) was sufficient to prevent crowding-induced extrusion of MDCK cells upon relaxation of pre-stretched substrates. Downregulation of *piezo1* by photo-activable morpholinos also prevented cell extrusion in zebrafish embryo epidermis and generated aberrant mass of epithelial cells in the tail. While those results are quite appealing, the mechanism remained unclear as Piezo would be activated upon cell compaction while it is normally activated by an increase of membrane tension (Geng et al., 2017). Lateral compaction of cells may still lead to a local expansion and stretching of the cytoplasmic membrane. The displacement of the cytoplasm toward the apical and basal side may lead to membrane stretching in the apical and basal sides of the cells. In this framework, it would be essential to evaluate the subcellular localisation of Piezo and membrane shape upon compaction. Importantly, the same authors recently showed that  $\text{Ca}^{2+}$  signaling was not sufficient to trigger cell extrusion in absence of crowding (Gudipaty et al., 2017). This suggests that other features/signalings are required to elicit cell elimination upon compaction.

Other mechanosensitive pathways have also been involved in caspase-dependent compaction-driven death. Topological aberrations in MDCK cells was shown to activate the Hippo pathway and reduce nuclear YAP concentration which drives caspase activation (Saw et al., 2017). In this case, the Hippo pathway is modulated by the local increase of compressive forces measured by traction force microscopy, and poorly correlates with local tissue density, in agreement with the multiple reports showing that Hippo is sensitive to tension/forces (Pancieria et al., 2017). Cell compression may elicit Hippo activation and YAP nuclear exclusion through a reduction of tension at adherens junctions. For instance, decrease of junction tension in *Drosophila* wing disc and in mammalian cells release from the cortex the Ajuba proteins (LIMD1 in Mammals, Jub in *Drosophila*) and their interactors Lats1/2, which activate the Hippo pathway (Rauskolb et al.,

2014, Ibar et al., 2018). This release is mediated by the tension-dependent changes of  $\alpha$ -catenin conformation (Yonemura et al., 2010) and the modulation of its interactions with the Jub family proteins (Rauskolb et al., 2014, Ibar et al., 2018). Alternatively, compression may drive Hippo activation through the modulation of forces applied at focal adhesions (Dupont, 2016). Accordingly, forces exerted at the basal junctions can modify several pro-survival signals emanating from the ECM. As described above, the reduction of endothelial cell surface by decreasing the distance between printed ECM islands is sufficient to trigger cell death despite the conservation of the total number of integrins (Chen et al., 1997). This may be driven by the change of the stress applied on each focal adhesion spot. This is in agreement with the multiple components of the focal adhesion/ECM which are sensitive to mechanical stress, including the Src kinase substrate p130cas (Sawada et al., 2006), Talin (Austen et al., 2015), Vinculin (Grashoff et al., 2010) and Fibronectin (Smith et al., 2007).

Similarly, the local rate of cell death in the *Drosophila* pupal notum correlates with the local rate of compaction (measured by Particle Image Velocimetry) rather than absolute tissue density, suggesting that cell survival is modulated by strain rate and/or forces (Levayer et al., 2016). The sensing of deformations is triggered through the modulation of ERK activity which will impact cell survival via the inhibition of the pro-apoptotic protein Hid (Moreno et al., 2018). Those results are in agreement with the previous reports of ERK activation by cellular tension in cell culture (Tschumperlin et al., 2004, Aoki et al., 2013, Hirata et al., 2015, Kawabata and Matsuda, 2016, Aoki et al., 2017) and *in vivo* (Sano et al., 2016). Finally, even if they have not been formally associated with compaction-driven death yet, other pathways which regulate cell survival and can be modulated by mechanical forces may also be relevant for compaction-driven cell death. Those include the stress-sensing pathway JNK (Pereira et al., 2011) and the modulation of Wnt/Wg pathway by the relocalisation of  $\beta$ -catenin (Farge, 2003, Roper et al., 2018).

To conclude, the diversity of sensing mechanisms may reflect the diversity of mechanical and geometrical inputs leading to cell death upon compaction. While the topology of cells and tissue will modify the type of relay that can be used for efficient mechano-sensing, the characteristic time of the stress will also strongly impact which type of sensors can efficiently probe compaction. For instance, while Hippo/YAP-TAZ pathway and transcription-driven responses may be potent sensors of long term stress (several hours), stretched activated channels and phosphorylation based signaling may be more adapted for short time scale variations of cell and tissue geometry (<1hour). The diversity of molecular mechanisms associated with compaction-driven death also reflects the ambiguity regarding the key parameters

modulating cell survival in all those contexts. While some mechanisms seem to rely only on the modification of the cell and tissue geometry, other are based on real mechano-transduction events (protein structure or protein-protein interactions which are modulated by forces). All the perturbative approaches used to trigger compaction will modulate concomitantly several of those parameters (e.g.: cell tension and/or cell aspect ratio and/or tissue density) making the interpretation of the sensing-mechanism very difficult.

### ***Physiological functions of compaction-driven cell death***

So far, we have described different ways to trigger artificially cell and tissue compaction mostly *in vitro*. As already mentioned above, cell compaction can also occur spontaneously *in vivo* and will be driven by different forces generated within the tissue or at tissue boundaries. This includes deformations triggered by collective movements of cells through convergent flows (Levayer et al., 2016, Saw et al., 2017), compaction driven by cell division and cell growth in a confined environment (Basan et al., 2009) and compaction driven by contractile and/or pushing forces coming from the boundaries (Bielmeier et al., 2016). Yet, cell elimination driven by deformations was only clearly observed and demonstrated in a handful of *in vivo* systems. This includes the elimination of epithelial cells in the *drosophila* pupal midline (Marinari et al., 2012, Levayer et al., 2016) and the elimination of epidermal cells at the margin of the zebrafish tail fin (Eisenhoffer et al., 2012). In both conditions, inhibition of cell death perturbed normal morphogenesis leading respectively to a widening of the midline region and an accumulation of cell mass at tail margins. Crowding-induced extrusion was suggested to take place in the gut villi (Eisenhoffer et al., 2012), but so far the contribution of cell density/deformation to cell elimination was not tested experimentally in the gut. Despite the lack of experimental validations, we hypothesise that compaction-driven death could have several physiological roles (**Figure 3**), including:

- 1) A role in the regulation of tissue size and morphology.
- 2) A role in tissue homeostasis.
- 3) A role for the coordination of cell elimination during tissue clearance.

We will discuss here those three putative functions

#### *Regulation of tissue size and shape*

Despite our very good knowledge of the pathways regulating cell proliferation, cell growth and cell survival, the mechanism regulating final tissue size *in vivo* remains very debated. This also applies to one of the best characterised tissue in flies, the wing imaginal disc (the epithelial sacs in the larvae that will form adult wing) (Hariharan, 2015). Wing disc growth was previously proposed to be regulated by a mechanical feedback where compressive forces would inhibit further cell proliferation (Shraiman, 2005, Hufnagel et al., 2007). In this scenario, the higher growth rate of a centrally located population will lead to the accumulation of compressive forces and eventually inhibit further expansion. This feedback was experimentally validated by triggering clone overgrowth which led to upregulation of the Hippo pathway in the fast growing clones and inhibition of further cell proliferation (Pan et al., 2016). Hippo upregulation is driven by the downregulation of actin, MyoII and tension in the clones, which was hypothesized to be driven by compression and/or the reduction of cell stretching in the fast growing clones (Pan et al., 2016). The same feedback may occur during wing imaginal disc development, where the relative higher rate of cell proliferation in the central part of the wing pouch leads to cell compaction in the centre and cell stretching at the periphery (Legoff et al., 2013, Mao et al., 2013) (**Fig. 3A**). Accordingly, Yki activity decreases over time in the wing pouch (Pan et al., 2018). While the main focus of those studies was the modulation of proliferation, similar rules may apply to cell death. Compaction-driven death may contribute to the fine tuning of the final tissue shape and size (**Fig. 3A**). Although apoptosis was shown to play a negligible role in absolute wing size regulation (Milan et al., 1997), inhibition of apoptosis increases the variability of wing size (de la Cova et al., 2004) which could be compatible with a buffering function of compaction-driven death. However, there is up to now no direct evidence that apoptosis can be modulated by mechanical inputs in the wing disc. Similarly, apoptosis is required to maintain robust segment size despite alteration of growth rate in the *Drosophila* embryo (Parker, 2006), and was hypothesised to be driven by competition for limiting extracellular pro-survival factors as well as cell compaction (Kursawe et al., 2015).

### *Tissue homeostasis*

The fine adjustment of cell proliferation and cell death is also required to maintain tissue homeostasis. In this context, mechanical forces could be an efficient relay which could adjust proliferation and death to tissue wide information. The contribution of compressive forces to tissue homeostasis is well described in the concept of homeostatic pressure (Basan et al., 2009): the pressure at which a tissue reaches a steady state where cell proliferation is perfectly compensated by cell death (**Fig. 3B**). In this framework, any perturbation increasing proliferation

will lead to an increase of the internal pressure which will both increase the rate of cell elimination and reduce the rate of proliferation. Consequently the number of cell will decrease until reaching back the homeostatic pressure. While this concept is very appealing, most of the studies performed *in vivo* rather focused on the coordination of apoptosis and proliferation in homeostatic conditions through competition for limitant diffusive factors (Parker, 2006), or through the secretion of promitotic factors by dying cells (e.g.:Dpp, Wingless, Unpaired, EGF) (Perez-Garijo et al., 2004, Ryoo et al., 2004, Martin et al., 2009, Perez-Garijo et al., 2009, Kashio et al., 2014, Santabarbara-Ruiz et al., 2015, Liang et al., 2017). More recently, crowding-driven apoptosis was suggested to restore the normal density of enterocytes in the fly midgut after a hyperproliferative phase driven by pathogens (Loudhaief et al., 2017). This late wave of apoptosis is controlled by the upregulation of Hippo pathway which was suggested to be driven by the increased cell density. However the link between Hippo and cell density/mechanics was not formally demonstrated in this tissue.

#### *Coordination of cell elimination*

Finally, crowding-induced death may also help to coordinate cell elimination in time and space during development. Large tissue domains are completely cleared during embryogenesis through programmed cell death, as illustrated by the elimination of interstitial tissue between vertebrate digits (Montero and Hurlle, 2010). Similarly several epithelia are used as transient scaffolds and will completely disappear during embryogenesis or during metamorphosis in insects (e.g.: amnioserosa cells during dorsal closure (Toyama et al., 2008), larval accessory cells during pupal abdomen morphogenesis (Ninov et al., 2007)). While intensive efforts have identified molecular cues that will drive apoptosis in those tissues (in other words, the patterning), the coordination in time and space of cell elimination was never really assessed. Yet a fine adjustment of the speed of cell elimination is probably required to maintain the epithelial architecture and to prevent the tissue collapse that would occur if too many cells die simultaneously (**Fig. 3C**). An adjustment of cell death to tissue tension/pressure could be a simple mechanism to fine tune the speed of cell elimination and maintain coherent tissue, however this was never tested experimentally.

#### ***Compaction-driven cell death and pathologies***

Compaction-driven death was also suggested to play a role in perturbed/pathological conditions. Tumoural cells have the capacity to overgrow and can affect the mechanics of their environment (Butcher et al., 2009). Differential growth can generate compressive stresses that could drive cell elimination provided that cells are sensitive enough to this mechanical stress (Shraiman, 2005).

As such compressive forces were proposed to contribute to cell elimination during competition between pretumoural cells and the neighbouring WT cells (Levayer and Moreno, 2013, Vincent et al., 2013) and to promote tumour expansion through the elimination of the neighbouring healthy cells (**Fig. 4A**). However compaction-driven death will give a competitive advantage to the fast growing cells provided that they are less sensitive to compression compared to the neighbouring cells. In the context of a caspase-dependent cell elimination, many oncogenes could provide such resistance by reducing the sensitivity to apoptosis while promoting proliferation/growth. This differential sensitivity was elegantly shown for the elimination of *scribble* mutant MDCK cells (Wagstaff et al., 2016) which have increased sensitivity to crowding because of their high levels of p53. Similarly, activation of Ras in clones in the *Drosophila* pupal notum can trigger compaction and elimination of the neighbouring cells (Levayer et al., 2016) which promotes Ras clone expansion (Moreno et al., 2018). Here differential sensitivity to stress is driven by the strong anti-apoptotic activity of Ras (Bergmann et al., 1998, Kurada and White, 1998). Similarly intestinal stem cell derived tumours in adult flies (driven by Notch depletion) can trigger elimination of the neighbouring enterocytes through their displacement and detachment from ECM (Patel et al., 2015). In this context, the tumour resistance to anoikis is probably driven by the activation of the EGFR pathway in the tumour (although not formally tested). Further work would be required to test whether compaction-driven death occurs in vicinity of tumours in Mammals and whether this contributes to tumour expansion and field cancerisation (Curtius et al., 2018) (the silent expansion of pretumoural cells in healthy tissue).

Alternatively, compaction-driven death may also be required for the elimination of aberrant cells during embryogenesis. Misspecification of a subset of cells in the wing imaginal disc (e.g: modifications of epigenetics markers, modulation of Dpp, modulation of Wingless, activation of JAK/STAT, activation of Ras) can trigger the formation of cyst and/or cell elimination (Gibson and Perrimon, 2005, Shen and Dahmann, 2005, Widmann and Dahmann, 2009, Bielmeier et al., 2016). This shape modification is regulated by interfacial contractility and the accumulation of acto-myosin at the clone boundaries (**Fig. 4B**) (Bielmeier et al., 2016). High interfacial tension should lead to an increase of pressure within the clones, especially for small ones (according to Laplace law:  $\Delta P \approx \frac{2\gamma}{R}$ , with P: pressure,  $\gamma$ : surface tension and R: radius of the clone). This increase of pressure may be responsible for the induction of apoptosis/extrusion of the misspecified cells (Adachi-Yamada et al., 1999, Bielmeier et al., 2016). In this framework, compaction-driven death would also have an anti-tumoural function as it could eliminate cells with high tumourigenic capacities (such as activation of JAK/STAT, activation of Ras (Bielmeier et al.,

2016)). Finally, compaction-induced apoptosis may be relevant to other pathologies such as mechanical-induced death of chondrocyte and arthritis (Patwari et al., 2004) or muscular and neuronal pressure-induced injury (Teng et al., 2011, Ye et al., 2012).

### ***Conclusions and perspectives***

Compaction of cells can trigger a variety of responses depending on the cell type, the amplitude of deformations as well as the direction of the forces applied. While it is nowadays clear that cell compaction will elicit dedicated cell responses that can trigger apoptosis and/or cell extrusion, the exact mechanism(s) of tissue compaction sensing remains quite often unclear. Moreover, while the amplitude of deformations has been analysed in most of the cases, the speed of deformation (strain rate) may be equally relevant for inducing cell elimination. Studies allowing a better identification of the single cell parameter(s) leading to cell elimination will be absolutely necessary to clarify the molecular mechanism driving cell elimination. This will include approaches that can dissociate the relative contribution of tension, strain rate, apical cell geometry, ECM binding and/or extracellular space geometry.

The few studies that clearly identified the molecular mechanism of compaction-sensing already outlined the diversity of sensing mechanisms and illustrate the great versatility of cells when responding to their mechanical environment. Accordingly modulating the subcellular localisation of receptors/channels is sufficient to completely modify the cell response to a similar stress (Gudipaty et al., 2017). This may explain why the same mechanical stress will trigger very different responses in different tissues or at different development times.

While the characterisation of compaction-driven death has been mostly performed in cell culture, the physiological functions of this process remained mostly hypothetical as there is no easy way to block specifically the process *in vivo* without affecting other cellular functions. New *in toto* imaging approaches combined with both measurements of mechanical stress (e.g.: force inference, laser nanosurgery, magnetic bead) and perturbative approaches (e.g.: laser ablations, optogenetic perturbations) could provide systematic correlations between cell elimination and local mechanical inputs during development and in adult tissues. This would allow to evaluate the proportion of cell elimination that is triggered/modulated by compaction. The molecular characterisation of crowding-induced death will eventually help to better understand how cell elimination is fine tuned in time and space and how this contributes to robust development and tissue size control. It may also lead to new therapeutic opportunities that could slow-down the

expansion of pretumoural cells through the modulation of WT cell elimination driven by compaction.

## Acknowledgements

We would like to warmly thank the Société de Biologie Cellulaire Française (SBCF) for its support. We also thank all the members of RL lab for critical reading of the manuscript. LV is supported by a Post-doctoral grant “Aide au Retour en France” from the FRM (Fondation pour la Recherche Médicale, ARF20170938651) and by a Marie Skłodowska-Curie Fellowship . Work in RL lab is supported by the Institut Pasteur (G5 starting package) and the ERC starting grant CoSpaDD (Competition for Space in Development and Disease, grant number 758457).

## Competing interest

The authors declare no competing interest

## Figure legends

### Figure 1: Different technics to trigger compaction

**A-C:** Different sort of stresses and their effect on material deformation. **A:** Resulting forces pointing toward the centre of the cell (compaction, strain  $< 0$ ). **B:** Resulting forces pointing out of the centre (stretching, strain  $> 0$ ). **C:** Forces tangential to the cell periphery (shear stress, strain  $\sim 0$ ). **D-H:** Different modes of compaction *in vitro*. Apoptotic cells are in orange **D:** Single cell piston-driven hydrostatic pressure from 500 kPa to 20,000 kPa (Takano et al., 1997, Quan et al., 2014). **E:** Reduction of cell volume ( $\sim 30\%$ ) through the increased osmolarity of the media (Ernest et al., 2008). **F:** Modulation of cell spreading on the substrate through different spacing of ECM islands (adapted from (Chen et al., 1997)). **G:** MDCK cells migration on 2D pattern (red arrow, velocity vectors) triggers nematic  $+1/2$  defects (comet like structure) leading to cell compression, apoptosis and extrusion (adapted from (Saw et al., 2017)). **H:** Densification of an epithelial layer (red arrows) through the relaxation of a laterally stretched substrate leading to cell extrusion (adapted from (Eisenhoffer et al., 2012)). **I:** Clones of cells with high rate of proliferation and resistance to cell death/extrusion (green) surrounded by wild type population (blue). Over-proliferation leads to neighbouring cell compaction (red arrows) and cell elimination *in vivo* (adapted from (Levayer et al., 2016, Wagstaff et al., 2016)). **J:** Cell aggregates embedded in a 3D gel with controlled stiffness. Compaction forces are induced by internal proliferation (Cheng

et al., 2009) and/or osmotic pressure modified using high concentration of Dextran (Montel et al., 2011). Red arrows indicate the compaction at the periphery.

### **Figure 2: Mechanisms of crowding sensing**

**A:** Epithelial compaction can modify the extracellular volume. Compaction along apico-basal axis (red arrow) will reduce interstitial volume (red) leading to a higher concentration of prosurvival and/or pro-apoptotic ligands. **B:** Lateral compaction of the epithelial layer (red arrows) will modify cell aspect ratio (elongation along the AB axis, reduction of the apical and/or basal area). **C:** Reduction of the apical area may reduce the total pool of apical receptors (purple) sensing an extracellular pro-survival factor. Similarly, reduction of the basal area may reduce the number of activated integrins (green) hence leading to apoptosis induction. In both situations, the turnover of those components has to be faster than the characteristic time of deformation. **D:** Lateral compaction can also reduce the tension applied to the ECM and basal junctions. This will trigger conformation changes in the components of the focal adhesion (e.g.: Paxilin, p130Cas) which will reduce the levels of pro-survival pathways.

### **Figure 3: Putative physiological functions of crowding-induced death**

**A:** Compaction-driven death and fine tuning of tissue morphogenesis. Schematic of a drosophila wing imaginal disc (A: anterior, P: posterior, D: dorsal, V: ventral). The relative increase of cell proliferation in the central part of the wing pouch (orange arrows) leads to cell compaction in the centre and cell stretching at the periphery (red arrows). This may modulate the rate of cell elimination in the wing pouch which will help to adjust tissue size to changes in the environment or local changes of proliferation. Absence of compaction-driven death may increase the variability of wing size and/or wing shape (right scheme). **B:** Homeostatic pressure ( $P_{\text{homeo}}$ ) helps to maintain constant cell density. An increase of cell proliferation increases cell density and the pressure ( $P$ ) experienced by the tissue (red arrows), hence reducing cell proliferation and increasing compaction-driven death. The reduction of cell number will reduce  $P$  until it reaches back  $P_{\text{homeo}}$ . The brown circle symbolises an elastic tissue boundary, the red double-headed arrows thickness is proportional to tension forces. **C:** Coordination of cell clearance during development. The orange cells are fated to death (based on patterning). The sensitivity of cell extrusion to cell shape/tension could produce a transient negative feedback on the neighbouring cells during extrusion. This will evenly distribute in time and space cell elimination, hence maintaining tissue coherence. In absence of feedbacks, several neighbouring cells may be eliminated concomitantly which could impair tissue sealing.

## Figure 4: Compaction-driven death, tumour progression and elimination of misspecified cells

**A:** Clones of pretumoural cells (green) with an increased rate of proliferation and resistance to cell death/extrusion. Over-proliferation will lead to neighbouring cell compaction (middle) and compaction-driven cell elimination (red cells). The WT cells (blue) will be preferentially eliminated as they are more sensitive to compaction. Eventually, the pretumoural cells could completely replace the WT cells (right) leading to field cancerisation. **B:** Appearance of misspecified cells (green) in the wing imaginal disc leads to an accumulation of actomyosin at the clone boundary along the lateral side of cells (bottom lateral view). The actomyosin belt increases pressure within the clone, hence leading to compaction-driven death and clearance of the aberrant cells.

## References

- Adachi-Yamada, T., K. Fujimura-Kamada, Y. Nishida and K. Matsumoto (1999). "Distortion of proximodistal information causes JNK-dependent apoptosis in *Drosophila* wing." *Nature* **400**(6740): 166-169.
- Alessandri, K., B. R. Sarangi, V. V. Gurchenkov, B. Sinha, T. R. Kiessling, L. Fetler, F. Rico, S. Scheuring, C. Lamaze, A. Simon, S. Geraldo, D. Vignjevic, H. Domejean, L. Rolland, A. Funfak, J. Bibette, N. Bremond and P. Nassoy (2013). "Cellular capsules as a tool for multicellular spheroid production and for investigating the mechanics of tumor progression in vitro." *Proc Natl Acad Sci U S A* **110**(37): 14843-14848.
- Aoki, K., Y. Kondo, H. Naoki, T. Hiratsuka, R. E. Itoh and M. Matsuda (2017). "Propagating Wave of ERK Activation Orients Collective Cell Migration." *Dev Cell* **43**(3): 305-317 e305.
- Aoki, K., Y. Kumagai, A. Sakurai, N. Komatsu, Y. Fujita, C. Shionyu and M. Matsuda (2013). "Stochastic ERK activation induced by noise and cell-to-cell propagation regulates cell density-dependent proliferation." *Mol Cell* **52**(4): 529-540.
- Austen, K., P. Ringer, A. Mehlich, A. Chrostek-Grashoff, C. Kluger, C. Klingner, B. Sabass, R. Zent, M. Rief and C. Grashoff (2015). "Extracellular rigidity sensing by talin isoform-specific mechanical linkages." *Nat Cell Biol* **17**(12): 1597-1606.
- Basan, M., T. Risler, J. F. Joanny, X. Sastre-Garau and J. Prost (2009). "Homeostatic competition drives tumor growth and metastasis nucleation." *HFSP J* **3**(4): 265-272.
- Bergmann, A., J. Agapite, K. McCall and H. Steller (1998). "The *Drosophila* gene *hid* is a direct molecular target of Ras-dependent survival signaling." *Cell* **95**(3): 331-341.
- Bielmeier, C., S. Alt, V. Weichselberger, M. La Fortezza, H. Harz, F. Julicher, G. Salbreux and A. K. Classen (2016). "Interface Contractility between Differently Fated Cells Drives Cell Elimination and Cyst Formation." *Curr Biol* **26**(5): 563-574.
- Butcher, D. T., T. Alliston and V. M. Weaver (2009). "A tense situation: forcing tumour progression." *Nat Rev Cancer* **9**(2): 108-122.
- Chen, C. S., M. Mrksich, S. Huang, G. M. Whitesides and D. E. Ingber (1997). "Geometric control of cell life and death." *Science* **276**(5317): 1425-1428.
- Cheng, G., J. Tse, R. K. Jain and L. L. Munn (2009). "Micro-environmental mechanical stress controls tumor spheroid size and morphology by suppressing proliferation and inducing apoptosis in cancer cells." *PLoS One* **4**(2): e4632.

Chubinskaya, S. and M. A. Wimmer (2013). "Key Pathways to Prevent Posttraumatic Arthritis for Future Molecule-Based Therapy." Cartilage **4**(3 Suppl): 13S-21S.

Curtius, K., N. A. Wright and T. A. Graham (2018). "An evolutionary perspective on field cancerization." Nat Rev Cancer **18**(1): 19-32.

de la Cova, C., M. Abril, P. Bellosta, P. Gallant and L. A. Johnston (2004). "Drosophila myc regulates organ size by inducing cell competition." Cell **117**(1): 107-116.

Delarue, M., F. Montel, D. Vignjevic, J. Prost, J. F. Joanny and G. Cappello (2014). "Compressive stress inhibits proliferation in tumor spheroids through a volume limitation." Biophys J **107**(8): 1821-1828.

Ding, A. X., G. Sun, Y. G. Argaw, J. O. Wong, S. Easwaran and D. J. Montell (2016). "CasExpress reveals widespread and diverse patterns of cell survival of caspase-3 activation during development in vivo." Elife **5**.

Ding, F., Z. W. Shao, S. H. Yang, Q. Wu, F. Gao and L. M. Xiong (2012). "Role of mitochondrial pathway in compression-induced apoptosis of nucleus pulposus cells." Apoptosis **17**(6): 579-590.

Dupont, S. (2016). "Role of YAP/TAZ in cell-matrix adhesion-mediated signalling and mechanotransduction." Exp Cell Res **343**(1): 42-53.

Eisenhoffer, G. T., P. D. Loftus, M. Yoshigi, H. Otsuna, C. B. Chien, P. A. Morcos and J. Rosenblatt (2012). "Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia." Nature **484**(7395): 546-549.

Elosegui-Artola, A., I. Andreu, A. E. M. Beedle, A. Lezamiz, M. Uroz, A. J. Kosmalska, R. Oria, J. Z. Kechagia, P. Rico-Lastres, A. L. Le Roux, C. M. Shanahan, X. Trepas, D. Navajas, S. Garcia-Manyès and P. Roca-Cusachs (2017). "Force Triggers YAP Nuclear Entry by Regulating Transport across Nuclear Pores." Cell **171**(6): 1397-1410 e1314.

Ernest, N. J., C. W. Habela and H. Sontheimer (2008). "Cytoplasmic condensation is both necessary and sufficient to induce apoptotic cell death." J Cell Sci **121**(Pt 3): 290-297.

Farge, E. (2003). "Mechanical induction of Twist in the Drosophila foregut/stomodaeal primordium." Curr Biol **13**(16): 1365-1377.

Frechin, M., T. Stoeger, S. Daetwyler, C. Gehin, N. Battich, E. M. Damm, L. Stergiou, H. Riezman and L. Pelkmans (2015). "Cell-intrinsic adaptation of lipid composition to local crowding drives social behaviour." Nature **523**(7558): 88-91.

Fuchs, Y. and H. Steller (2011). "Programmed cell death in animal development and disease." Cell **147**(4): 742-758.

Geng, J., Q. Zhao, T. Zhang and B. Xiao (2017). "In Touch With the Mechanosensitive Piezo Channels: Structure, Ion Permeation, and Mechanotransduction." Curr Top Membr **79**: 159-195.

Gibson, M. C. and N. Perrimon (2005). "Extrusion and death of DPP/BMP-compromised epithelial cells in the developing Drosophila wing." Science **307**(5716): 1785-1789.

Grashoff, C., B. D. Hoffman, M. D. Brenner, R. Zhou, M. Parsons, M. T. Yang, M. A. McLean, S. G. Sligar, C. S. Chen, T. Ha and M. A. Schwartz (2010). "Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics." Nature **466**(7303): 263-266.

Gudipaty, S. A., J. Lindblom, P. D. Loftus, M. J. Redd, K. Edes, C. F. Davey, V. Krishnegowda and J. Rosenblatt (2017). "Mechanical stretch triggers rapid epithelial cell division through Piezo1." Nature **543**(7643): 118-121.

Hariharan, I. K. (2015). "Organ Size Control: Lessons from Drosophila." Dev Cell **34**(3): 255-265.

Harvey, K. F. and I. K. Hariharan (2012). "The hippo pathway." Cold Spring Harb Perspect Biol **4**(8): a011288.

Helmlinger, G., P. A. Netti, H. C. Lichtenbeld, R. J. Melder and R. K. Jain (1997). "Solid stress inhibits the growth of multicellular tumor spheroids." Nat Biotechnol **15**(8): 778-783.

Hirata, H., M. Gupta, S. R. Vedula, C. T. Lim, B. Ladoux and M. Sokabe (2015). "Actomyosin bundles serve as a tension sensor and a platform for ERK activation." EMBO Rep **16**(2): 250-257.

Hufnagel, L., A. A. Teleman, H. Rouault, S. M. Cohen and B. I. Shraiman (2007). "On the mechanism of wing size determination in fly development." Proc Natl Acad Sci U S A **104**(10): 3835-3840.

Ibar, C., E. Kirichenko, B. Keepers, E. Enners, K. Fleisch and K. D. Irvine (2018). "Tension-dependent regulation of mammalian Hippo signaling through LIMD1." J Cell Sci **131**(5).

Irvine, K. D. and B. I. Shraiman (2017). "Mechanical control of growth: ideas, facts and challenges." Development **144**(23): 4238-4248.

Kashio, S., F. Obata and M. Miura (2014). "Interplay of cell proliferation and cell death in Drosophila tissue regeneration." Dev Growth Differ **56**(5): 368-375.

Kawabata, N. and M. Matsuda (2016). "Cell Density-Dependent Increase in Tyrosine-Monophosphorylated ERK2 in MDCK Cells Expressing Active Ras or Raf." PLoS One **11**(12): e0167940.

Kurada, P. and K. White (1998). "Ras promotes cell survival in Drosophila by downregulating hid expression." Cell **95**(3): 319-329.

Kursawe, J., P. A. Brodskiy, J. J. Zartman, R. E. Baker and A. G. Fletcher (2015). "Capabilities and Limitations of Tissue Size Control through Passive Mechanical Forces." PLoS Comput Biol **11**(12): e1004679.

Ladoux, B. and R. M. Mege (2017). "Mechanobiology of collective cell behaviours." Nat Rev Mol Cell Biol **18**(12): 743-757.

Legoff, L., H. Rouault and T. Lecuit (2013). "A global pattern of mechanical stress polarizes cell divisions and cell shape in the growing Drosophila wing disc." Development **140**(19): 4051-4059.

Levayer, R., C. Dupont and E. Moreno (2016). "Tissue Crowding Induces Caspase-Dependent Competition for Space." Curr Biol **26**(5): 670-677.

Levayer, R. and E. Moreno (2013). "Mechanisms of cell competition: themes and variations." J Cell Biol **200**(6): 689-698.

Liang, J., S. Balachandra, S. Ngo and L. E. O'Brien (2017). "Feedback regulation of steady-state epithelial turnover and organ size." Nature **548**(7669): 588-591.

Loudhaief, R., A. Brun-Barale, O. Benguetat, M. P. Nawrot-Esposito, D. Pauron, M. Amichot and A. Gallet (2017). "Apoptosis restores cellular density by eliminating a physiologically or genetically induced excess of enterocytes in the Drosophila midgut." Development **144**(5): 808-819.

Mao, Y., A. L. Tournier, A. Hoppe, L. Kester, B. J. Thompson and N. Tapon (2013). "Differential proliferation rates generate patterns of mechanical tension that orient tissue growth." EMBO J **32**(21): 2790-2803.

Marinari, E., A. Mehonic, S. Curran, J. Gale, T. Duke and B. Baum (2012). "Live-cell delamination counterbalances epithelial growth to limit tissue overcrowding." Nature **484**(7395): 542-545.

Martin, F. A., A. Perez-Garijo and G. Morata (2009). "Apoptosis in Drosophila: compensatory proliferation and undead cells." Int J Dev Biol **53**(8-10): 1341-1347.

Milan, M., S. Campuzano and A. Garcia-Bellido (1997). "Developmental parameters of cell death in the wing disc of Drosophila." Proc Natl Acad Sci U S A **94**(11): 5691-5696.

Montel, F., M. Delarue, J. Elgeti, L. Malaquin, M. Basan, T. Risler, B. Cabane, D. Vignjevic, J. Prost, G. Cappello and J. F. Joanny (2011). "Stress clamp experiments on multicellular tumor spheroids." Phys Rev Lett **107**(18): 188102.

Montero, J. A. and J. M. Hurlle (2010). "Sculpturing digit shape by cell death." Apoptosis **15**(3): 365-375.

Moreno, E., L. Valon, F. Levillayer and R. Levayer (2018). "Competition for Space Induces Cell Elimination Through Compaction-Driven ERK Downregulation." Current Biology **29**(1-12).

Nakajima, Y. I. and E. Kuranaga (2017). "Caspase-dependent non-apoptotic processes in development." Cell Death Differ **24**(8): 1422-1430.

Nallet-Staub, F., X. Yin, C. Gilbert, V. Marsaud, S. Ben Mimoun, D. Javelaud, E. B. Leof and A. Mauviel (2015). "Cell density sensing alters TGF-beta signaling in a cell-type-specific manner, independent from Hippo pathway activation." Dev Cell **32**(5): 640-651.

Ninov, N., D. A. Chiarelli and E. Martin-Blanco (2007). "Extrinsic and intrinsic mechanisms directing epithelial cell sheet replacement during *Drosophila* metamorphosis." *Development* **134**(2): 367-379.

Pan, Y., H. Alegot, C. Rauskolb and K. D. Irvine (2018). "The dynamics of hippo signaling during *Drosophila* wing development." *Development*.

Pan, Y., I. Heemskerk, C. Ibar, B. I. Shraiman and K. D. Irvine (2016). "Differential growth triggers mechanical feedback that elevates Hippo signaling." *Proc Natl Acad Sci U S A*.

Panciera, T., L. Azzolin, M. Cordenonsi and S. Piccolo (2017). "Mechanobiology of YAP and TAZ in physiology and disease." *Nat Rev Mol Cell Biol* **18**(12): 758-770.

Paoli, P., E. Giannoni and P. Chiarugi (2013). "Anoikis molecular pathways and its role in cancer progression." *Biochim Biophys Acta* **1833**(12): 3481-3498.

Parker, J. (2006). "Control of compartment size by an EGF ligand from neighboring cells." *Curr Biol* **16**(20): 2058-2065.

Patel, P. H., D. Dutta and B. A. Edgar (2015). "Niche appropriation by *Drosophila* intestinal stem cell tumours." *Nat Cell Biol* **17**(9): 1182-1192.

Patterson, A. M. and A. J. M. Watson (2017). "Deciphering the Complex Signaling Systems That Regulate Intestinal Epithelial Cell Death Processes and Shedding." *Front Immunol* **8**: 841.

Patwari, P., V. Gaschen, I. E. James, E. Berger, S. M. Blake, M. W. Lark, A. J. Grodzinsky and E. B. Hunziker (2004). "Ultrastructural quantification of cell death after injurious compression of bovine calf articular cartilage." *Osteoarthritis Cartilage* **12**(3): 245-252.

Pereira, A. M., C. Tudor, J. S. Kanger, V. Subramaniam and E. Martin-Blanco (2011). "Integrin-dependent activation of the JNK signaling pathway by mechanical stress." *PLoS One* **6**(12): e26182.

Perez-Garijo, A., F. A. Martin and G. Morata (2004). "Caspase inhibition during apoptosis causes abnormal signalling and developmental aberrations in *Drosophila*." *Development* **131**(22): 5591-5598.

Perez-Garijo, A., E. Shlevkov and G. Morata (2009). "The role of Dpp and Wg in compensatory proliferation and in the formation of hyperplastic overgrowths caused by apoptotic cells in the *Drosophila* wing disc." *Development* **136**(7): 1169-1177.

Quan, X., K. Guo, Y. Wang, L. Huang, B. Chen, Z. Ye and Z. Luo (2014). "Mechanical compression insults induce nanoscale changes of membrane-skeleton arrangement which could cause apoptosis and necrosis in dorsal root ganglion neurons." *Biosci Biotechnol Biochem* **78**(10): 1631-1639.

Raab, M., M. Gentili, H. de Belly, H. R. Thiam, P. Vargas, A. J. Jimenez, F. Lautenschlaeger, R. Voituriez, A. M. Lennon-Dumenil, N. Manel and M. Piel (2016). "ESCRT III repairs nuclear envelope ruptures during cell migration to limit DNA damage and cell death." *Science* **352**(6283): 359-362.

Rauskolb, C., S. Sun, G. Sun, Y. Pan and K. D. Irvine (2014). "Cytoskeletal tension inhibits Hippo signaling through an Ajuba-Warts complex." *Cell* **158**(1): 143-156.

Roper, J. C., D. Mitrossilis, G. Stirnemann, F. Waharte, I. Brito, M. E. Fernandez-Sanchez, M. Baaden, J. Salamero and E. Farge (2018). "The major beta-catenin/E-cadherin junctional binding site is a primary molecular mechano-transducer of differentiation in vivo." *Elife* **7**.

Rosenblatt, J., M. C. Raff and L. P. Cramer (2001). "An epithelial cell destined for apoptosis signals its neighbors to extrude it by an actin- and myosin-dependent mechanism." *Curr Biol* **11**(23): 1847-1857.

Ryoo, H. D., T. Gorenc and H. Steller (2004). "Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways." *Dev Cell* **7**(4): 491-501.

Sano, T., T. Kobayashi, H. Negoro, A. Sengiku, T. Hiratsuka, Y. Kamioka, L. S. Liou, O. Ogawa and M. Matsuda (2016). "Intravital imaging of mouse urothelium reveals activation of extracellular signal-regulated kinase by stretch-induced intravesical release of ATP." *Physiol Rep* **4**(21).

Santabarbara-Ruiz, P., M. Lopez-Santillan, I. Martinez-Rodriguez, A. Binagui-Casas, L. Perez, M. Milan, M. Corominas and F. Serras (2015). "ROS-Induced JNK and p38 Signaling Is Required for Unpaired Cytokine Activation during *Drosophila* Regeneration." *PLoS Genet* **11**(10): e1005595.

Saw, T. B., A. Doostmohammadi, V. Nier, L. Kocgozlu, S. Thampi, Y. Toyama, P. Marcq, C. T. Lim, J. M. Yeomans and B. Ladoux (2017). "Topological defects in epithelia govern cell death and extrusion." Nature **544**(7649): 212-216.

Sawada, Y., M. Tamada, B. J. Dubin-Thaler, O. Cherniavskaya, R. Sakai, S. Tanaka and M. P. Sheetz (2006). "Force sensing by mechanical extension of the Src family kinase substrate p130Cas." Cell **127**(5): 1015-1026.

Shen, J. and C. Dahmann (2005). "Extrusion of cells with inappropriate Dpp signaling from Drosophila wing disc epithelia." Science **307**(5716): 1789-1790.

Shraiman, B. I. (2005). "Mechanical feedback as a possible regulator of tissue growth." Proc Natl Acad Sci U S A **102**(9): 3318-3323.

Smith, M. L., D. Gourdon, W. C. Little, K. E. Kubow, R. A. Eguiluz, S. Luna-Morris and V. Vogel (2007). "Force-induced unfolding of fibronectin in the extracellular matrix of living cells." PLoS Biol **5**(10): e268.

Takano, K. J., T. Takano, Y. Yamanouchi and T. Satou (1997). "Pressure-induced apoptosis in human lymphoblasts." Exp Cell Res **235**(1): 155-160.

Tang, H. L., H. M. Tang, K. H. Mak, S. Hu, S. S. Wang, K. M. Wong, C. S. Wong, H. Y. Wu, H. T. Law, K. Liu, C. C. Talbot, Jr., W. K. Lau, D. J. Montell and M. C. Fung (2012). "Cell survival, DNA damage, and oncogenic transformation after a transient and reversible apoptotic response." Mol Biol Cell **23**(12): 2240-2252.

Teng, B. T., E. W. Tam, I. F. Benzie and P. M. Siu (2011). "Protective effect of caspase inhibition on compression-induced muscle damage." J Physiol **589**(Pt 13): 3349-3369.

Teng, X., L. Qin, R. Le Borgne and Y. Toyama (2017). "Remodeling of adhesion and modulation of mechanical tensile forces during apoptosis in Drosophila epithelium." Development **144**(1): 95-105.

Thompson, D. A. W. (1917). On growth and form. Cambridge Eng., University press.

Thompson, W. R., C. T. Rubin and J. Rubin (2012). "Mechanical regulation of signaling pathways in bone." Gene **503**(2): 179-193.

Toyama, Y., X. G. Peralta, A. R. Wells, D. P. Kiehart and G. S. Edwards (2008). "Apoptotic force and tissue dynamics during Drosophila embryogenesis." Science **321**(5896): 1683-1686.

Tschumperlin, D. J., G. Dai, I. V. Maly, T. Kikuchi, L. H. Laiho, A. K. McVittie, K. J. Haley, C. M. Lilly, P. T. So, D. A. Lauffenburger, R. D. Kamm and J. M. Drazen (2004). "Mechanotransduction through growth-factor shedding into the extracellular space." Nature **429**(6987): 83-86.

Tsuboi, A., S. Ohsawa, D. Umetsu, Y. Sando, E. Kuranaga, T. Igaki and K. Fujimoto (2018). "Competition for space is controlled by apoptosis-induced change of local epithelial topology." Current Biology ( ).

Vincent, J. P., A. G. Fletcher and L. A. Baena-Lopez (2013). "Mechanisms and mechanics of cell competition in epithelia." Nat Rev Mol Cell Biol **14**(9): 581-591.

Vining, K. H. and D. J. Mooney (2017). "Mechanical forces direct stem cell behaviour in development and regeneration." Nat Rev Mol Cell Biol **18**(12): 728-742.

Vogel, V. and M. Sheetz (2006). "Local force and geometry sensing regulate cell functions." Nat Rev Mol Cell Biol **7**(4): 265-275.

Wagstaff, L., M. Goschorska, K. Kozyrska, G. Duclos, I. Kucinski, A. Chessel, L. Hampton-O'Neil, C. R. Bradshaw, G. E. Allen, E. L. Rawlins, P. Silberzan, R. E. Carazo Salas and E. Piddini (2016). "Mechanical cell competition kills cells via induction of lethal p53 levels." Nat Commun **7**: 11373.

Widmann, T. J. and C. Dahmann (2009). "Wingless signaling and the control of cell shape in Drosophila wing imaginal discs." Dev Biol **334**(1): 161-173.

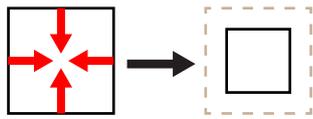
Ye, Z., Y. Wang, X. Quan, J. Li, X. Hu, J. Huang and Z. Luo (2012). "Effects of mechanical force on cytoskeleton structure and calpain-induced apoptosis in rat dorsal root ganglion neurons in vitro." PLoS One **7**(12): e52183.

Yonemura, S., Y. Wada, T. Watanabe, A. Nagafuchi and M. Shibata (2010). "alpha-Catenin as a tension transducer that induces adherens junction development." Nat Cell Biol **12**(6): 533-542.

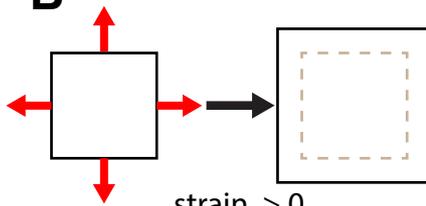
Zhao, B., L. Li, L. Wang, C. Y. Wang, J. Yu and K. L. Guan (2012). "Cell detachment activates the Hippo pathway via cytoskeleton reorganization to induce anoikis." Genes Dev **26**(1): 54-68.

Zhao, B., X. Wei, W. Li, R. S. Udan, Q. Yang, J. Kim, J. Xie, T. Ikenoue, J. Yu, L. Li, P. Zheng, K. Ye, A. Chinnaiyan, G. Halder, Z. C. Lai and K. L. Guan (2007). "Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control." Genes Dev **21**(21): 2747-2761.

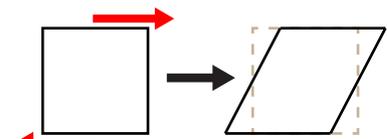
Zimmerman, A. M. (1970). High pressure effects on cellular processes. New York,, Academic Press.

**A**

strain  $< 0$   
compaction

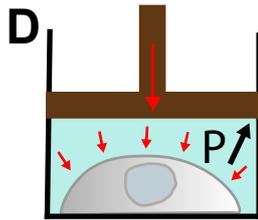
**B**

strain  $> 0$   
stretching

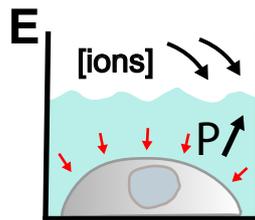
**C**

strain  $\sim 0$   
shear

isotropic change in pressure

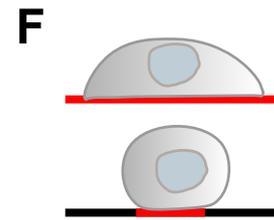


mechanical piston



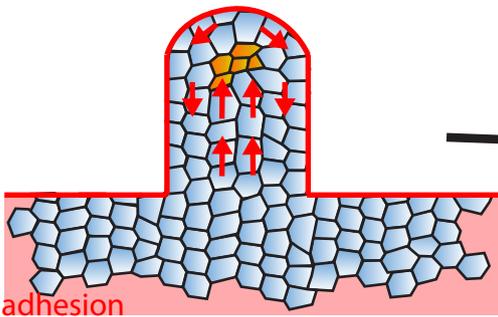
osmotic shock

substrate modification



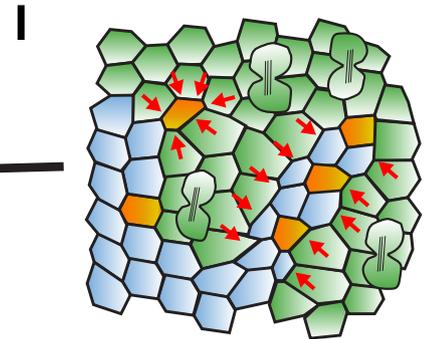
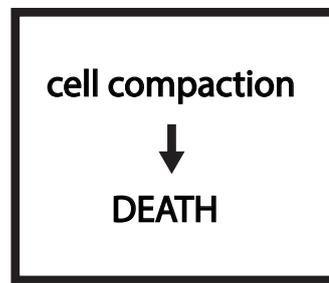
adhesion

single cell change in volume

**G**

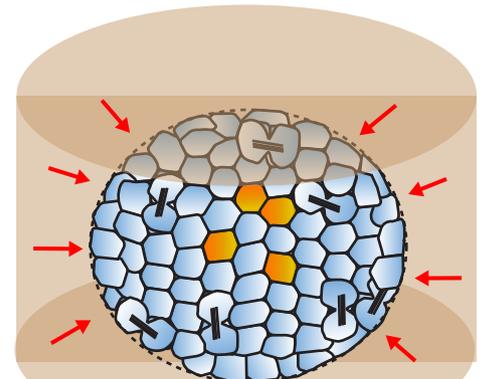
adhesion

2D cell monolayer local deformation



in vivo clone compaction

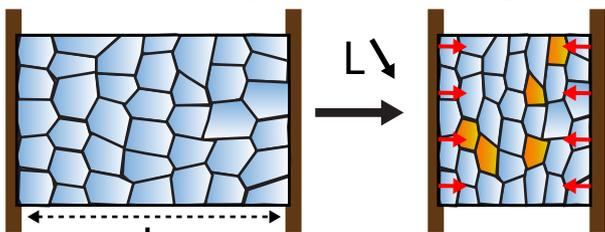
3D growing spheroids compaction



constrained micro-environment  
mechano-osmotic control of pressure

**H**

2D cell monolayer uniaxial compaction



mechanically controlled substrate

**J**

