Collective effects in epithelial cell death and cell extrusion

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Abstract

Programmed cell death, notably apoptosis, is an essential guardian of tissue homeostasis and an active contributor of organ shaping. While the regulation of apoptosis has been mostly analysed in the framework of a cell autonomous process, recent works highlighted important collective effects which can tune cell elimination. This is particularly relevant for epithelial cell death, which requires fine coordination with the neighbours in order to maintain tissue sealing during cell expulsion. In this review, we will focus on the recent advances which outline the complex multicellular communications at play during epithelial cell death and cell extrusion. We will first focus on the new unanticipated functions of neighbouring cells during extrusion, discuss the contribution of distant neighbours, and finally highlight the complex feedbacks generated by cell elimination on neighbouring cell death.

Keywords: epithelium, apoptosis, extrusion, self-organisation

Introduction

Tissue size regulation and homeostasis rely on the fine balance between cell death and cell proliferation. While we have a very good understanding of the cellular effectors regulating proliferation and apoptosis, how these processes are fine-tuned and coordinated at the tissue level remains quite enigmatic. Apoptosis is the best known programmed-cell death process. It is characterised by cell shrinkage and chromatin condensation followed by membrane blebbing and fragmentation into apoptotic bodies [1]. Apoptosis is orchestrated by caspases, a conserved family of proteases which control cellular remodeling by cleaving hundreds of proteins [2]. While apoptosis is widespread in tissues during morphogenesis and homeostasis, our understanding of this process is mostly based on isolated cells and was mostly studied in a cell-

autonomous framework. However, cell elimination from within a tissue imposes certain constraints and requires a minimal level of coordination. This is particularly true for epithelia, made of tightly adhesive cells forming mechanical and chemical barriers, where tissue sealing must be constantly maintained despite the elimination of cells. This is normally achieved by cell extrusion: a succession of remodeling steps leading to cell expulsion while maintaining tissue sealing [3-5]. Studies over the last twenty years, mostly using MDCK (Madin-Darby Canine Kydney) cells, have provided a good understanding of cell extrusion. It is initiated by the formation of a contractile actomyosin ring in the extruding cell [3, 6], followed by the formation of supracellular ring in the neighbours through secretion from the extruding cell of sphingosine-1-phosphate (S1P) and binding to its S1P2 receptor in the neighbours [7], leading to microtubules reorganisation, Rho activation [8] and the formation of a contractile purse string. Eventually the ring slides basally and expels the extruding cell out of the epithelium while bringing neighbours together [6] (**Figure 1 A-E**). These previous studies outline the concerted actions of the dying cell and its neighbours and demonstrate that cell extrusion is like a miniature morphogenetic process.

Upstream of cell expulsion, the decision to engage in cell death and/or in extrusion is also a complex cell decision making event that is not purely cell-autonomous. For instance, the modulation of local cell density and pressure can trigger cell extrusion [9-12]. Similarly, cell competition, the context dependent elimination of cells through apoptosis or extrusion, is a perfect illustration of the non-cell autonomous decision to die/extrude [13, 14]. This suggests that the engagement in extrusion/apoptosis not only relies on cell-autonomous parameters but also on collective effects.

Here we will review recent literature revealing even more complex cell-cell coordinations at play during cell extrusion and the decision to eliminate a cell. We will first discuss the new roles of neighbouring cells in regulating cell extrusion and the unexpected contribution of distant neighbours. We will then outline recent results illustrating the effects of cell death on the engagement in apoptosis of the neighbouring cells. Altogether, these recent results illustrate how much epithelial cell death is regulated collectively rather than cell-autonomously. While relevant for the topic, this review will not discuss the non-autonomous effects of apoptosis on cell proliferation [15] or morphogenesis [16]. We will not discuss either the concept of cell competition as numerous recent reviews have covered the subject [13, 14, 17].

The new contributions of direct neighbours to cell extrusion

The removal of epithelial cells through extrusion requires a fine coordination between cell constriction, junction disassembly and formation of new junctions between neighbouring cells. Yet strong mechanical coupling between the extruding and the neighbouring cells is required to maintain tissue cohesion and coordinate cell deformations. Accordingly, E-cad depletion induces extrusion failure and perturb epithelial integrity [18]. Mechanical coupling through E-cad with direct neighbours was previously shown to reorganise actomyosin during extrusion through tension-dependent Coronin1B recruitment and actin reorganisation [19]. A new layer of coordination was recently revealed in MCF7 extruding cells, where E-cad dependent force transmission triggers the tension-dependent recruitment of MyoVI, p114/115 RhoGEF relocalisation and RhoA activation in the neighbours [20, 21] (**Figure 1 B,C**). E-cad coupling not only regulates the actomyosin ring, but also the balance with other active contributors of extrusion. Previously it was shown that basal lamellipodia from the extruding cell neighbours also contribute to cell expulsion, especially in conditions of low cell density [22]. More recently, it was shown that basal protrusions coexist systematically with heterogeneous actomyosin rings and are required to orient the ring and ensure basal sealing [23] (**Figure 1 D,E**). The robustness and efficiency of extrusion is achieved by the coupling between these two mechanisms (basal protrusions and purse-string) that can compensate each other. The strength of mechanical coupling with the neighbours through E-cad and substrate adhesion modulate the relative contribution of these two actors [23].

Eventually adherens junctions need to be disassembled between the extruding cell and its neighbours [24, 25]. Yet to avoid tissue rupture, cell-cell adhesion must be maintained throughout extrusion. This apparent contradiction may be solved by Desmosomal Junctions (DJs), which were recently shown to be maintained throughout apoptotic extrusion of MDCK cells until new DJs are formed between neighbouring cells [26] (**Figure 1 A-E**). In addition, DJs are also coupled to the actomyosin ring through their binding to keratin-18. Depleting Desmoplakin (a component of DJs) leads to a reduction of tissue tension, local tissue tearing and extrusion failure, suggesting that DJs maintenance is essential for extrusion and tissue cohesion [26]. These results argue again for the need of a constant mechanical coupling between the dying cell and its neighbours which relies on diverse adhesive components.

Cell extrusion not only relies on active remodeling of the neighbouring cells but also on the global mechanical state of the tissue that is more or less extrusion-permissive. For instance, a developmental switch which globally changes E-cad turnover and tissue tension is essential to modulate the rate of larval cell extrusion in the *Drosophila* pupal abdomen (albeit with contrasted contribution) [27, 28]. Similarly, mammalian epithelium also needs to be primed by S1P from the serum to increase mechanosensitive recruitment of p114RhoGEF following initiation of extrusion [20, 21], suggesting that S1P also has a tissue-wide permissive role for extrusion (**Figure 1B**). Extrusion is also inhibited by high tensile prestress of the tissue and in extruding cell neighbours [29, 30]. For instance, increased tension through Caveolin1 depletion throughout the tissue or in the extruding cell neighbours prevents the extrusion of oncogenic cells [30]. This illustrates the tug-of-war at play between the contractile extruding cell and the resistance generated by tissue-wide tension. One way to overcome this mechanical constraint is through local relaxation of tension in neighbouring cell junctions which are not shared with the extruding cell (**Figure 1 F-I**). Accordingly, an early release of tension in the apoptotic cell leads to immediate and transient relaxation of the neighbours, which leads to SFK (Src Family protein tyrosine Kinases) activation and MyoII inhibition in the junctions orthogonal to the dying cell [29]. This mechanism may restrict the range of action of the forces exerted by the extruding cell, thus preventing the stress to build-up and tissue tearing.

To conclude, these recent results illustrate the complex coupling at play between the extruding cell, its neighbours and the tissue. Constriction and cell protrusions are coordinated though mechanotransduction and mechanical coupling which is maintained through different junctional components (E-cad, DJs). The multiple layers of regulation of tension are at the basis of the self-organised properties of cell extrusion which ensure its robustness and efficiency.

The contribution of larger collective movements to cell extrusion

Beside the active coordination with close neighbours, recent results revealed larger collective effects also involved in the regulation of cell extrusion. Most of them rely on polarised movements of cells and collective migration toward the extruding cell (**Figure 2 A-D**). For instance, apoptosis initiation or oncogene activation in MDCK cells and zebrafish embryo triggers calcium waves [31]. Theses waves propagate from the extruding cells over 3-15 rows of cells through a trigger-wave mechanism combining

intracellular calcium storage release, cation channels and gap junctions. They induce actin remodelling in the neighbours which promotes convergent collective cell movements toward the extruding cell and its expulsion (**Figure 2 A-D**). Interestingly, these waves share many similarities with recently described ERK waves induced near oncogenic cells and apoptotic cells [32]. Sustained ERK activation in MCF10A cells (through B-Raf^{V600E}) activates the metalloprotease ADAM17 which releases EGFR ligands and activates ERK over several cell rows. These waves also drive directed cell migration towards the transformed and/or apoptotic cells and promote their extrusion [32] (**Figure 2 A-D**).

How then can such collective movements promote cell extrusion and cell death? Convergent flow could trigger local topological defects and local increase of pressure which promote cell death and cell extrusion [9-12]. Accordingly, these movements are reminiscent of the convergent movements of the Wild Type (WT) cells toward Scribble mutant cells during mechanical cell competition [14, 33, 34]. This promotes *Scrib^{KD}* cell elimination through their compaction. In this case the collective movements are driven by the secretion of the chemoattractant FGF21 by the *Scrib^{KD}* cells [35]. Interestingly, similar movements are also required to expel epithelial cells infected by *L. monocytogenes* and are driven by the activation of NF-κB and the relative reduction of stiffness of the infected cells [36].

Taken together, these data highlight unexpected large scale non-autonomous collective effects regulating cell extrusion. These collective movements are observed in a wide range of situations (apoptosis, oncogenic cells, infected cells, mutant cells) and are regulated by different signalling pathways, suggesting that different contexts converged to the same cellular mechanism to expel cells. This unanticipated contribution of distant neighbours to cell extrusion highlights its multiscale regulation.

Extrusion feedbacks on cell death in the neighbouring cells

So far, we discussed how surrounding cells participate in cell extrusion and regulate apoptosis. Reciprocally, recent studies highlight mechanisms by which cell extrusion/cell death can also feedback on cell death probability of the neighbours.

Recent studies from the group of Olivier Pertz and our group described unanticipated anti-apoptotic effects of cell death on neighbouring cells [37, 38]. In MCF10A cells, apoptotic cells trigger ERK/AKT waves which radially propagate over roughly 3 rows

of cells. Their propagation is dependent on ADAM17-dependent cleavage of pro-EGF [37] (**Figure 2 E,F'**), similar to ERK waves near oncogenic cells [32]. Likewise, we showed in the *Drosophila* pupal notum (a single layer epithelium) that ERK pulses occur in the direct neighbours of extruding cells [38] (**Figure 2 E,F**). Although the range of propagation of these ERK pulses are different in the two cases, both decrease the probability of death in the vicinity of the extruding cell through transient caspase inhibition. Transient caspase inhibition is most likely mediated by the downregulation/inhibition of the pro-apoptotic gene *hid* in the pupal notum [39] (transcriptionally and post-transcriptionally [40, 41]), while the pro-survival early transcriptional targets of ERK remained to be identified in MCF10A cells [37]. The transient nature of this inhibition is related to the finite duration of ERK pulses combined with tissue-wide persistent stress (serum/growth factor starvation and cytotoxic treatment in MCF10A cells, expression of *hid* in the posterior region of the notum). These feedbacks have two essential roles for epithelial integrity. On the one hand, they reduce the global rate of apoptosis hence preventing tissue rupture in situation of acute stress [37] (**Figure 2 G'**). On the other hand, they also alter the spatiotemporal distribution of cell death by locally and transiently inhibiting caspases, hence scattering cell elimination and preventing the extrusion of cells in clusters [38] (**Figure 2 G**), a very detrimental situation for tissue sealing (**Figure 2 H,I**). These two studies suggest that the rate of cell death/cell extrusion and their distribution is space and time are not purely cell-autonomous, but rather emerging properties of local feedbacks driven by cell extrusion/apoptosis.

Beside these inhibitory feedbacks, apoptosis was also shown to enhance cell death in the surrounding tissue in other situations. For instance caspase activation following acute tissue damages increases globally S1P levels in the zebrafish epidermis and promotes cell extrusion through pulsatile MyoII contractions and reduced tissue tension [42]. Since S1P is also secreted actively by the extruding cell [7], the same mechanism may generate a local positive feedback on extrusion. Alternatively, massive induction of apoptosis in the posterior compartment of the *Drosophila* larval wing disc is sufficient to trigger ectopic cell death in the other compartment [43]. This is driven by the secretion of the TNF orthologue Eiger from the posterior cells which leads to JNK pathway activation across the compartment and ectopic apoptosis. Interestingly, the same TNF dependent mechanism seems to coordinate hair follicle

destruction in mice, suggesting that it is conserved in mammals [43]. Recently, similar propagation effects were observed for alternative modes of cell death in non-epithelial cells [44]. For instance, ferroptosis can propagate in waves in U937 cells through extracellular factors acting upstream of cell swelling [45]. These studies highlight potential positive feedbacks of cell death and extrusion on their neighbours, however they were described only in relatively artificial conditions of acute stress, and it remains unclear whether similar positive feedbacks exist in more physiological contexts.

In summary, these recent studies demonstrate that the probability of cell extrusion/cell death and their distribution are emerging properties of the tissue which are based on non-cell autonomous positive and negative feedbacks on caspases. The negative feedbacks are essential to maintain epithelial sealing in conditions of high rates of elimination by buffering the global rate of elimination and scattering cell death in space and time. Further studies would be required to understand in which conditions positive feedbacks start to dominate and further promote cell elimination.

Conclusions

While apoptosis was long viewed as a cell-autonomous process, we tried in this review to emphasize the complex multicellular coordinations at play during epithelial extrusion and the regulation of apoptosis itself. Recent studies highlight the diverse contributions of the direct neighbours of extruding cells which allow concerted deformations and maintenance of tissue sealing. The plasticity of the extrusion process reveals self-organised properties which most likely rely on multiple mechanical feedbacks (increase of tension in neighbouring junctions, local relaxation of tension in orthogonal junctions, contribution of basal protrusions). Similarly, global mechanical properties of the tissue and long-range collective movements play respectively a permissive and instructive role in cell extrusion. Upstream of cell expulsion, the activation of caspases also relies on multicellular feedbacks which include local negative feedbacks near extruding cells, and positive feedbacks upon massive cell death induction. Overall, the probability of a cell to extrude is a complex decision-making process that integrates cell-autonomous factors (e.g.: mechanical properties, caspases activity) as well as multiple feedbacks acting on different space-scales, time-scales and layers of regulation. While there is still a long way to go before we will be able to predict when and where an epithelial cell will extrude, the combination of multiplex imaging (including morphological parameters

and live-sensors of signalling pathways) with Bayesian approaches will surely help to evaluate the relative contribution of each layer of regulation to the engagement in cell extrusion.

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Declaration of interests

The authors declare no competing interest

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Papers of particular interest published within the period of review have been highlighted as:

● of special interest

●● of outstanding interest

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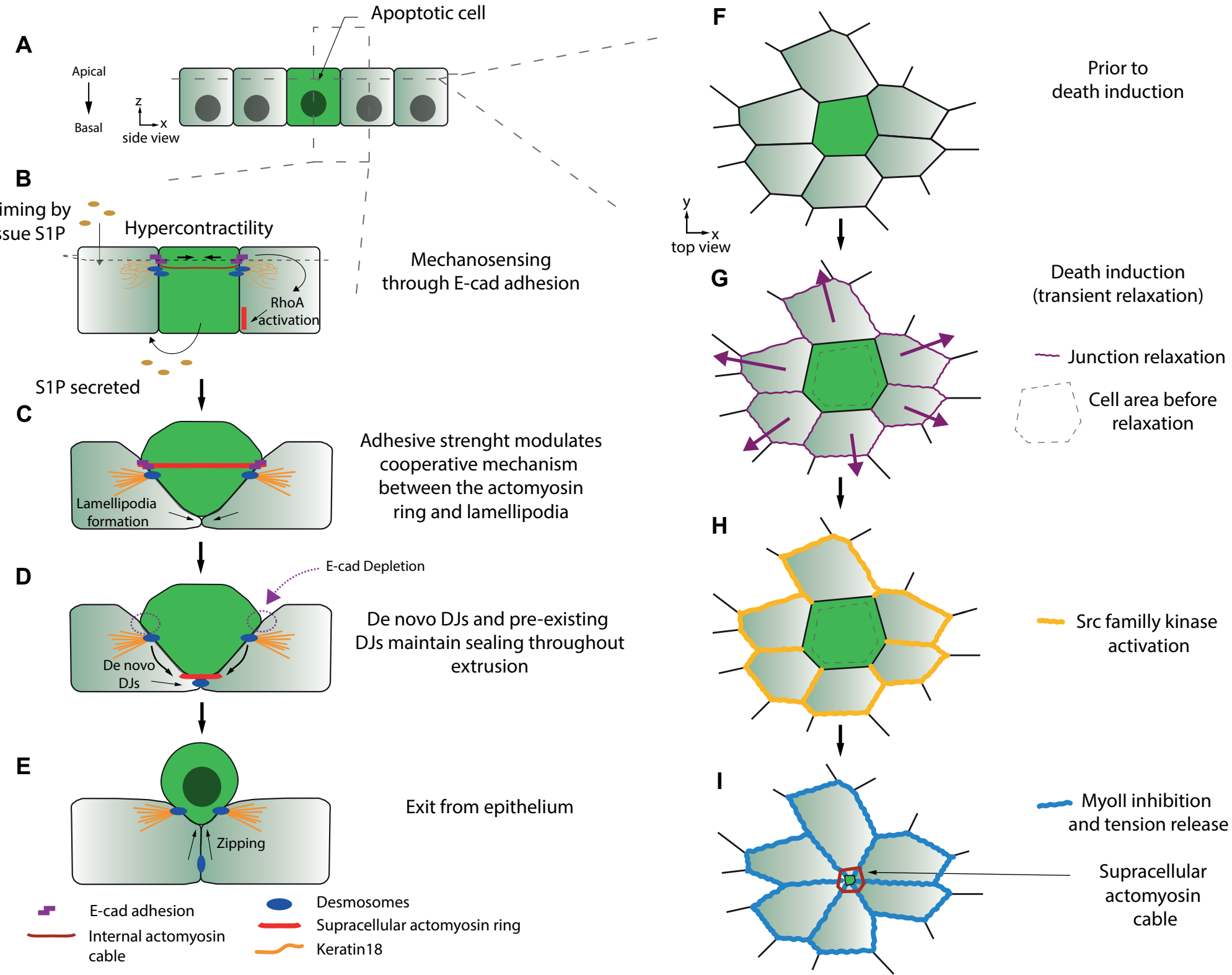
Figure legends

Figure 1: Cell extrusion and coordination with direct neighbours

(A) Side view of an epithelial tissue. Apical side is on top and basal side on the bottom. The green cell represents an apoptotic cell activating caspases. **(B)** Lateral zoom view of the interface of the apoptotic cell with its neighbours. Once it has become apoptotic this cell will become hyper contractile through the formation of an internal actomyosin ring (red line shows the ring, black arrow toward cell centre shows contractility). This will generate forces transmitted to the neighbours by E-cad (purple). This triggers a mechanotransduction pathway in neighbours leading to RhoA activation which requires priming by S1P (brown ellipses). **(C)** Later the neighbours form a supracellular purse string (red line) concomitantly to lamellipodia formation. The two mechanisms contribute to cell extrusion and the balance between both is modulated by adhesion strength (E-cad in purple). Desmosomal Junctions (DJs in blue) remain intact throughout extrusion. They bear forces leading to keratin-18 alignment toward the extruding cells (orange lines). **(D)** The supracellular actomyosin cable is sliding basally while constricting (red line). Newly DJs (blue) are formed at lamellipodia to provide continuous sealing while E-cad is depleted to allow cell expulsion (purple dashed lines). **(E)** The extruding cell is expelled from the epithelium while neighbours are brought together. **(F)** Top view of the epithelium throughout extrusion. **(G)** Following apoptosis induction the extruding cell (green) transiently relaxes (black line=current shape vs dashed line=previous shape). Purple arrows show direction of relaxation. This is concomitant to orthogonal junction transient relaxation (purple curved lines). **(H)** The transient relaxation leads to Src family kinase (SFK) activation at these junctions (yellow curved lines). **(I)** SFK downregulates MyoII specifically at orthogonal junctions further relaxing these junctions. This provides a mechanism to overcome mechanical constraints exerted by the neighbours restricting the constriction of the extruding cell.

Figure 2: Large scale coordinations between the extruding cell and the neighbouring tissue.

(A) Top view of an epithelium showing a cell initiating apoptosis or a transformed cell (in green). **(B)** Initiation of apoptosis or oncogene activation both triggers calcium waves (right side of dashed line in green) or ERK waves (left side of dashed line in blue). Arrows show radial propagation of these waves. **(C)** These waves travel to several cell rows albeit through different mechanisms (see main text). Both types of waves trigger collective cell movement/migration (yellow arrows) toward the extruding cell. **(D)** These collective movements promote the extrusion of the apoptotic or transformed cells. **(E)** High rates of cell death (green cells) can arise for instance in condition of acute stress or during development. **(F)** In *Drosophila*, the extruding cell triggers ERK pulses in the direct neighbours (blue). This locally and transiently inhibits caspases and cell extrusion. **(G)** This mechanism alters the spatiotemporal distribution of cell death therefore dispersing cell extrusion in space and time. As a result it maintains epithelial integrity. **(F')** In MCF10A cell culture, the extruding cell triggers ERK waves travelling up to roughly 3 cell rows (blue). **(G')** This protective mechanism decreases globally the rate of apoptosis (red curve, top) therefore decreasing the speed of cell elimination (bottom graph, black line). These two mechanisms (**F-G'**) ensure the maintenance of epithelial integrity. **(H-I)** Perturbed scenario without ERK feedbacks. **(H)** Local clusters of cell death can arise, which prevent proper extrusion and impair transiently epithelial sealing **(I)**. Alternatively, the global increase of the speed of cell elimination may lead to tissue tearing.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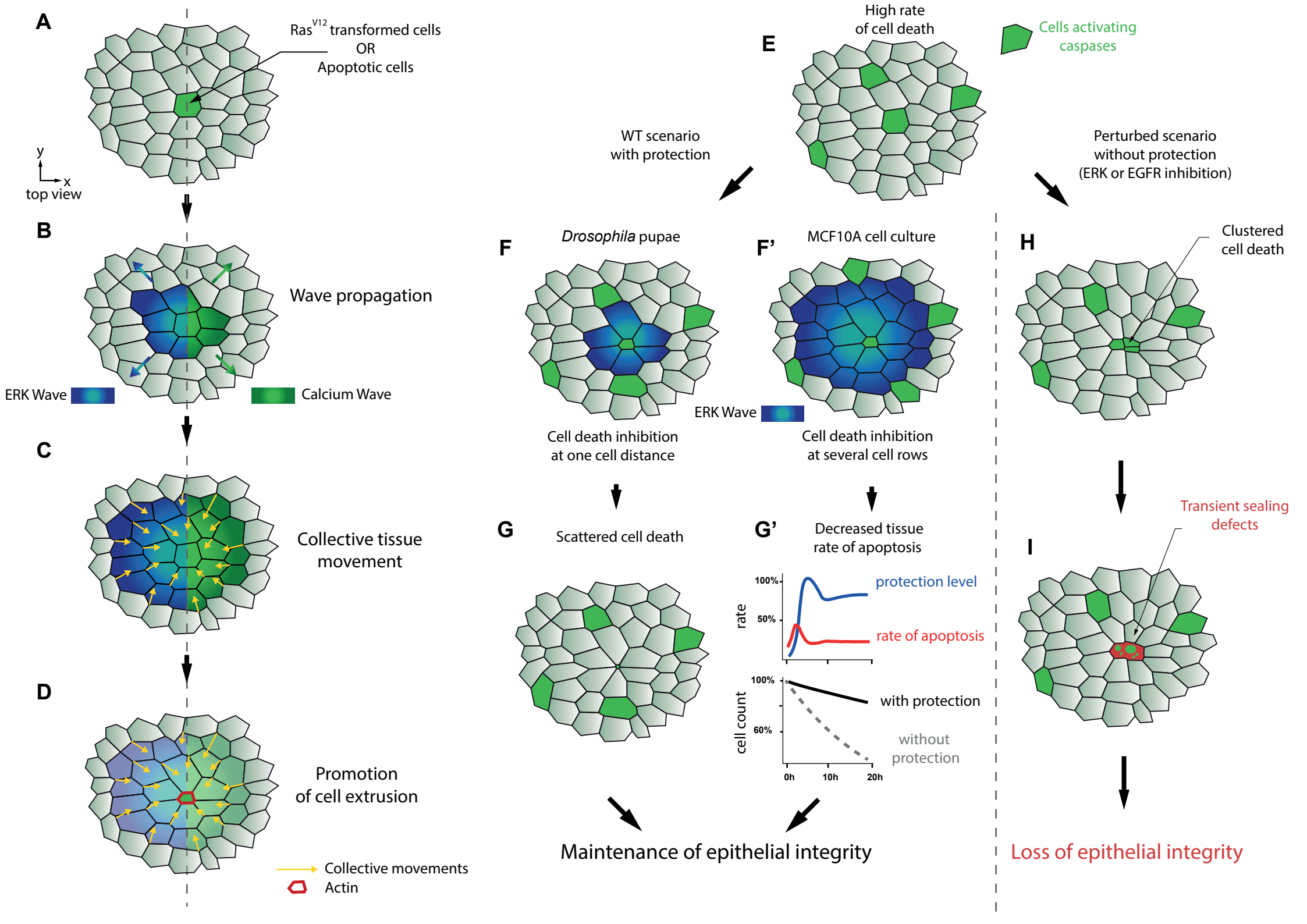
RhoA activation
Mechanosensing through E-cad adhesion
S1P secreted
C
Lamellipodia formation
Adhesive strenght modulates cooperative mechanism between the actomyosin ring and lamellipodia
D
E-cad Depletion
De novo DJs
De novo DJs and pre-existing DJs maintain sealing throughout extrusion
E
Zipping
Exit from epithelium
E-cad adhesion
Internal actomyosin cable
Desmosomes
Supracellular actomyosin ring
Keratin18
F
y
x
top view
Prior to death induction
G
Death induction (transient relaxation)
Junction relaxation
Cell area before relaxation
H
Src familly kinase activation
I
Myoll inhibition and tension release
Supracellular actomyosin cable


A
Ras V12 transformed cells
OR
Apoptotic cells
y
x
top view
B
Wave propagation
ERK Wave
Calcium Wave
C
Collective tissue
movement
D
Promotion
of cell extrusion
Collective movements
Actin
High rate
of cell death
E
Cells activating
caspases
WT scenario
with protection
Perturbed scenario
without protection
(ERK or EGFR inhibition)
F
Drosophila pupae
F'
MCF10A cell culture
ERK Wave
Cell death inhibition
at one cell distance
Cell death inhibition
at several cell rows
G
Scattered cell death
G'
Decreased tissue
rate of apoptosis
100%
50%
rate
protection level
rate of apoptosis
100%
60%
cell count
with protection
without
protection
0h
10h
20h
Maintenance of epithelial integrity
H
Clustered
cell death
Transient sealing
defects
I
Loss of epithelial integrity