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1 **Collective effects in epithelial cell death and cell extrusion**

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7 **Abstract**

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

19 **Keywords:** epithelium, apoptosis, extrusion, self-organisation

20 **Introduction**

21 Tissue size regulation and homeostasis rely on the fine balance between cell death
22 and cell proliferation. While we have a very good understanding of the cellular effectors
23 regulating proliferation and apoptosis, how these processes are fine-tuned and
24 coordinated at the tissue level remains quite enigmatic. Apoptosis is the best known
25 programmed-cell death process. It is characterised by cell shrinkage and chromatin
26 condensation followed by membrane blebbing and fragmentation into apoptotic bodies
27 [1]. Apoptosis is orchestrated by caspases, a conserved family of proteases which
28 control cellular remodeling by cleaving hundreds of proteins [2]. While apoptosis is
29 widespread in tissues during morphogenesis and homeostasis, our understanding of
30 this process is mostly based on isolated cells and was mostly studied in a cell-

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

47 Upstream of cell expulsion, the decision to engage in cell death and/or in extrusion is
48 also a complex cell decision making event that is not purely cell-autonomous. For
49 instance, the modulation of local cell density and pressure can trigger cell extrusion [9-
50 12]. Similarly, cell competition, the context dependent elimination of cells through
51 apoptosis or extrusion, is a perfect illustration of the non-cell autonomous decision to
52 die/extrude [13, 14]. This suggests that the engagement in extrusion/apoptosis not only
53 relies on cell-autonomous parameters but also on collective effects.

54 Here we will review recent literature revealing even more complex cell-cell
55 coordinations at play during cell extrusion and the decision to eliminate a cell. We will
56 first discuss the new roles of neighbouring cells in regulating cell extrusion and the
57 unexpected contribution of distant neighbours. We will then outline recent results
58 illustrating the effects of cell death on the engagement in apoptosis of the neighbouring
59 cells. Altogether, these recent results illustrate how much epithelial cell death is
60 regulated collectively rather than cell-autonomously. While relevant for the topic, this
61 review will not discuss the non-autonomous effects of apoptosis on cell proliferation
62 [15] or morphogenesis [16]. We will not discuss either the concept of cell competition
63 as numerous recent reviews have covered the subject [13, 14, 17].

64 **The new contributions of direct neighbours to cell extrusion**

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

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

97 Cell extrusion not only relies on active remodeling of the neighbouring cells but also
98 on the global mechanical state of the tissue that is more or less extrusion-permissive.
99 For instance, a developmental switch which globally changes E-cad turnover and
100 tissue tension is essential to modulate the rate of larval cell extrusion in the *Drosophila*
101 pupal abdomen (albeit with contrasted contribution) [27, 28]. Similarly, mammalian
102 epithelium also needs to be primed by S1P from the serum to increase
103 mechanosensitive recruitment of p114RhoGEF following initiation of extrusion [20, 21],
104 suggesting that S1P also has a tissue-wide permissive role for extrusion (**Figure 1B**).
105 Extrusion is also inhibited by high tensile prestress of the tissue and in extruding cell
106 neighbours [29, 30]. For instance, increased tension through Caveolin1 depletion
107 throughout the tissue or in the extruding cell neighbours prevents the extrusion of
108 oncogenic cells [30]. This illustrates the tug-of-war at play between the contractile
109 extruding cell and the resistance generated by tissue-wide tension. One way to
110 overcome this mechanical constraint is through local relaxation of tension in
111 neighbouring cell junctions which are not shared with the extruding cell (**Figure 1 F-I**).
112 Accordingly, an early release of tension in the apoptotic cell leads to immediate and
113 transient relaxation of the neighbours, which leads to SFK (Src Family protein tyrosine
114 Kinases) activation and MyoII inhibition in the junctions orthogonal to the dying cell
115 [29]. This mechanism may restrict the range of action of the forces exerted by the
116 extruding cell, thus preventing the stress to build-up and tissue tearing.

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

123 **The contribution of larger collective movements to cell extrusion**

124 Beside the active coordination with close neighbours, recent results revealed larger
125 collective effects also involved in the regulation of cell extrusion. Most of them rely on
126 polarised movements of cells and collective migration toward the extruding cell (**Figure**
127 **2 A-D**). For instance, apoptosis initiation or oncogene activation in MDCK cells and
128 zebrafish embryo triggers calcium waves [31]. These waves propagate from the
129 extruding cells over 3-15 rows of cells through a trigger-wave mechanism combining

130 intracellular calcium storage release, cation channels and gap junctions. They induce
131 actin remodelling in the neighbours which promotes convergent collective cell
132 movements toward the extruding cell and its expulsion (**Figure 2 A-D**). Interestingly,
133 these waves share many similarities with recently described ERK waves induced near
134 oncogenic cells and apoptotic cells [32]. Sustained ERK activation in MCF10A cells
135 (through B-Raf^{V600E}) activates the metalloprotease ADAM17 which releases EGFR
136 ligands and activates ERK over several cell rows. These waves also drive directed cell
137 migration towards the transformed and/or apoptotic cells and promote their extrusion
138 [32] (**Figure 2 A-D**).

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

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

155 **Extrusion feedbacks on cell death in the neighbouring cells**

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

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

162 of cells. Their propagation is dependent on ADAM17-dependent cleavage of pro-EGF
163 [37] (**Figure 2 E,F'**), similar to ERK waves near oncogenic cells [32]. Likewise, we
164 showed in the *Drosophila* pupal notum (a single layer epithelium) that ERK pulses
165 occur in the direct neighbours of extruding cells [38] (**Figure 2 E,F**). Although the range
166 of propagation of these ERK pulses are different in the two cases, both decrease the
167 probability of death in the vicinity of the extruding cell through transient caspase
168 inhibition. Transient caspase inhibition is most likely mediated by the
169 downregulation/inhibition of the pro-apoptotic gene *hid* in the pupal notum [39]
170 (transcriptionally and post-transcriptionally [40, 41]), while the pro-survival early
171 transcriptional targets of ERK remained to be identified in MCF10A cells [37]. The
172 transient nature of this inhibition is related to the finite duration of ERK pulses combined
173 with tissue-wide persistent stress (serum/growth factor starvation and cytotoxic
174 treatment in MCF10A cells, expression of *hid* in the posterior region of the notum).
175 These feedbacks have two essential roles for epithelial integrity. On the one hand, they
176 reduce the global rate of apoptosis hence preventing tissue rupture in situation of acute
177 stress [37] (**Figure 2 G'**). On the other hand, they also alter the spatiotemporal
178 distribution of cell death by locally and transiently inhibiting caspases, hence scattering
179 cell elimination and preventing the extrusion of cells in clusters [38] (**Figure 2 G**), a
180 very detrimental situation for tissue sealing (**Figure 2 H,I**). These two studies suggest
181 that the rate of cell death/cell extrusion and their distribution in space and time are not
182 purely cell-autonomous, but rather emerging properties of local feedbacks driven by
183 cell extrusion/apoptosis.

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

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

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

209 **Conclusions**

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

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

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237 **Declaration of interests**

238 The authors declare no competing interest

239 **References and recommended reading**

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

242 • of special interest

243 •• of outstanding interest

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403

404 **Figure legends**

405 **Figure 1: Cell extrusion and coordination with direct neighbours**

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

431

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

434 **(A)** Top view of an epithelium showing a cell initiating apoptosis or a transformed cell
435 (in green). **(B)** Initiation of apoptosis or oncogene activation both triggers calcium
436 waves (right side of dashed line in green) or ERK waves (left side of dashed line in
437 blue). Arrows show radial propagation of these waves. **(C)** These waves travel to
438 several cell rows albeit through different mechanisms (see main text). Both types of
439 waves trigger collective cell movement/migration (yellow arrows) toward the extruding
440 cell. **(D)** These collective movements promote the extrusion of the apoptotic or
441 transformed cells. **(E)** High rates of cell death (green cells) can arise for instance in
442 condition of acute stress or during development. **(F)** In *Drosophila*, the extruding cell
443 triggers ERK pulses in the direct neighbours (blue). This locally and transiently inhibits
444 caspases and cell extrusion. **(G)** This mechanism alters the spatiotemporal distribution
445 of cell death therefore dispersing cell extrusion in space and time. As a result it
446 maintains epithelial integrity. **(F')** In MCF10A cell culture, the extruding cell triggers
447 ERK waves travelling up to roughly 3 cell rows (blue). **(G')** This protective mechanism
448 decreases globally the rate of apoptosis (red curve, top) therefore decreasing the
449 speed of cell elimination (bottom graph, black line). These two mechanisms **(F-G')**
450 ensure the maintenance of epithelial integrity. **(H-I)** Perturbed scenario without ERK
451 feedbacks. **(H)** Local clusters of cell death can arise, which prevent proper extrusion
452 and impair transiently epithelial sealing **(I)**. Alternatively, the global increase of the
453 speed of cell elimination may lead to tissue tearing.

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