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1 **Toward a predictive understanding of epithelial cell death**

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11 **Abstract**

12
13 Epithelial cell death is highly prevalent during development and tissue homeostasis.
14 While we have a rather good understanding of the molecular regulators of programmed
15 cell death, especially for apoptosis, we still fail to predict when, where, how many and
16 which specific cells will die in a tissue. This likely relies on the much more complex
17 picture of apoptosis regulation in a tissular and epithelial context, which entails cell
18 autonomous but also non-cell autonomous factors, diverse feedback and multiple
19 layers of regulation of the commitment to apoptosis. In this review, we illustrate this
20 complexity of epithelial apoptosis regulation by describing these different layers of
21 control, all demonstrating that local cell death probability is a complex emerging
22 feature. We first focus on non-cell autonomous factors that can locally modulate the
23 rate of cell death, including cell competition, mechanical input and geometry as well as
24 systemic effects. We then describe the multiple feedback mechanisms generated by
25 cell death itself. We also outline the multiple layers of regulation of epithelial cell death,
26 including the coordination of extrusion and regulation occurring downstream of effector
27 caspases. Eventually, we propose a roadmap to reach a more predictive
28 understanding of cell death regulation in an epithelial context.

29
30 **Keywords:** apoptosis, epithelium, cell competition, mechanics, caspases, cell
31 extrusion, modelling, system biology

32
33 **Approximate words number:** 12,500

37 **Introduction**

38

39 Epithelia, the most common tissues in Metazoans, are the first line of defence against
40 the environment and are instrumental in compartmentalising physiological functions in
41 multicellular organisms. They are composed of tightly adherent cells forming a
42 mechanically resistant and impermeable boundary thanks to the polarised organisation
43 of cell-cell junctions. Yet, this barrier function must accommodate a certain level of
44 plasticity required during organ morphogenesis, growth, tissue turnover or
45 regeneration. This involves a high level of cell elimination through apoptosis, the most
46 common form of programmed cell death. Accordingly, apoptosis is highly prevalent
47 during embryogenesis and is essential for growth and morphogenesis through the
48 generation of pulling forces to fuse[1] or bend tissues[2], to locally modulate tissue
49 growth[3], or to promote the disassembly of transient scaffolding tissues[1, 4].
50 Furthermore, epithelial apoptosis is also essential to buffer developmental
51 fluctuations[5, 6] and correct putative developmental errors[7-9]. In adult tissues,
52 epithelial cell death can occur at very high rates in organs undergoing fast turnover,
53 such as the gut where billions of enterocytes die on a daily basis[10]. Alternatively,
54 biased wild type cell elimination can promote cancer progression by promoting the
55 expansion of cancerous clones[5, 11-14].

56 All these contexts require tight regulation of the number and the localisation of dying
57 cells. Moreover, which specific cell will die in a region will directly influence clonal
58 evolution, and as such the genetic make-up of the final tissue and putative pathological
59 evolution. Thus, understanding what regulates epithelial morphogenesis, homeostasis
60 and clonal evolution directly relies on our capacity to build a quantitative and predictive
61 understanding of epithelial cell death. What is then regulating the precise localisation,
62 number, and selection of specific cells which will die in a tissue?

63 Work over the last thirty years has provided a wealth of data regarding the molecular
64 regulation of apoptosis. Since the identification of Caspases in *C. elegans*[15], the
65 pathway regulating programmed cell death and more specifically apoptosis has been
66 intensively studied in cell culture and *in vivo*, illustrating the conservation of the
67 caspases as core regulators[16] (**Figure 1**). Caspase proteases exist in the form of
68 inactive pro-proteins, which upon cleavage become active and cleave hundreds of
69 proteins in the cell, triggering the characteristic morphological features of apoptosis
70 and engagement in cell death[17]. Several routes can lead to caspase activation,

71 especially in mammals, including the intrinsic and extrinsic pathways (**Figure 1**). The
72 intrinsic pathway, which is more widely conserved, centres on mitochondrial outer
73 membrane permeabilization (MOMP)[18], the occurrence of which is controlled by the
74 balance of pro- and anti-apoptotic BCL-2 family proteins. Pro-apoptotic BCL-2 proteins
75 stimulate oligomerisation of BAK and BAX at the mitochondrial membrane[19],
76 promoting the formation of pores in the outer membrane which release cytochrome c
77 and SMAC/DIABLO proteins[20]. Cytochrome c combines with APAF-1 to form the
78 apoptosome complex[21], recruiting and cleaving Caspase-9, which in turn cleaves
79 and activates effector caspases (Caspase-3 and 7)[22]. In parallel, SMAC/DIABLO
80 proteins released from mitochondria inhibit IAP (Inhibitor of Apoptosis)[20], enhancing
81 caspase activation by releasing the inhibition of Caspase-9 and effector caspases.
82 Alternatively, the extrinsic pathway is driven by the activation of death receptors at the
83 plasma membrane by death-inducing ligands (TNF: Tumor Necrosis Factor, TRAIL:
84 Tumor necrosis factor-related apoptosis-inducing ligand)[23]. This promotes the
85 processing of Caspase-8/10, which will then activate effector caspases while
86 promoting MOMP through the activation of BID[24, 25]. In *Drosophila*, stress or
87 developmentally regulated signals leading to tissue patterning can locally upregulate
88 the pro-apoptotic genes *hid* (head involution defective), *reaper*, or *grim*, which promote
89 the degradation and inhibition of the apoptosis inhibitor Diap1[26], hence leading to
90 activation of Dronc (*Drosophila* Caspase-9)[27] and effector caspase activation
91 (primarily Drice and Dcp1)[16, 28, 29] (**Figure 1**). Further molecular details can be
92 found in many reviews[16, 30].

93 Considering the detailed molecular characterisation and the high relevance for
94 physiology and pathologies, there have been many attempts to dissect in a predictive
95 manner the engagement in apoptosis[31-34]. However, these were mostly performed
96 in cell culture and applied in a single-cell centric manner which may not apply in the
97 context of a tissue or an epithelium. To our knowledge such methodology has not been
98 applied yet *in vivo* or in a tissular context and we are still far from having a complete
99 understanding of the factors that control cell death quantitatively in tissues. What are
100 then the shortcomings and difficulties associated with the prediction of cell death *in*
101 *vivo* and in epithelia?

102 In this review, we would like to illustrate the different layers of complexity that can
103 modulate apoptosis engagement in epithelial cells, especially relative to apoptosis
104 studies in single cells. We present a collection of recent studies illustrating the

105 complexity of cell death regulation in an epithelial context, which all argue that the local
106 rate of cell death in an epithelium is a complex emerging feature of diverse feedback
107 encompassing several epistatic layers of regulation. We will first focus on the multiple
108 non-cell autonomous contributions that can directly modulate the probability of cell
109 elimination, including contact-dependent communication, mechanical stress, tissue
110 geometry and systemic controls. We will then illustrate the numerous feedback
111 mechanisms triggered by apoptosis itself, directly affecting the local rate of elimination
112 at different spatial and temporal scales. We will also discuss novel emerging
113 complexity in the decision step downstream of effector caspase activation as well as
114 the regulation of epithelial cell extrusion. Finally, we will provide a tentative roadmap
115 of the combination of novel approaches which may help to get closer to a predictive
116 understanding of epithelial cell death.

117

118 **1. Collective effects and non-cell autonomous factors**

119 Epithelial cell death relies not only on cell-autonomous factors, but also on the cell
120 microenvironment, which includes the status of neighbouring cells, the mechanical
121 state of the tissue, local 3D shape and systemic factors. In this section we will illustrate
122 the influence of these external factors on epithelial cell death (**Figure 2**).

123

124 *1.1 Cell competition and tissue heterogeneity*

125 One of the best illustrations of non-cell autonomous regulation of cell death is the
126 process of cell competition. Cell competition is a context-dependent elimination leading
127 to the expulsion/cell death of miss-specified or suboptimal cells from a tissue[35-40].
128 Cell competition was initially described in *Drosophila* using *Minute*[41], a group of
129 dominant mutations which affect the speed of development, shown later to target
130 various ribosome subunits[42]. While *Minute/+* (heterozygous) tissues have no obvious
131 developmental defects, *Minute/+* clones generated in a WT (wild type) background in
132 the wing imaginal disc (the wing primordium) systematically disappeared from growing
133 tissues and could not be recovered at adult stage[41]. This effect relies on close-range
134 interactions between *Minute* heterozygous mutant and WT cells[41, 43], and
135 specifically on the elimination of *Minute* clones during larval stage by apoptosis of
136 mutant cells contacting WT cells[44, 45]. Later, a similar mechanism was shown to
137 promote the elimination of WT cells when neighbouring cells with additional copies of

138 the oncogene Myc[5, 11], a process named by opposition “supercompetition” (mutant
139 cells outcompeting WT cells). Since then, the number of genetic contexts and tissues
140 in which cell competition was observed has steadily increased[35-40]. Overall, three
141 main categories of cell elimination mechanisms were proposed, some based on direct
142 ligand-receptor interaction at the interface between the two populations, some based
143 on diffusive factors that can directly modulate cell survival, and lastly mechanisms
144 based on the mechanical stress generated by the mixing of two cell populations[38,
145 46].

146 Few transmembrane proteins were associated with cell competition, including Flower,
147 which can directly modulate cell survival at the interface between cells expressing
148 different isoforms both in flies and mice[47, 48]. Alternatively, the ligand and receptor
149 Sas and PTP10D were shown to accumulate specifically at the interface between WT
150 cells and cells mutant for apico-basal polarity genes in *Drosophila* imaginal discs[49],
151 promoting mutant cell elimination by downregulating the EGFR/ERK pathway.
152 Similarly, local misspecification of cells by altering morphogen gradients and position
153 information[8, 44, 50-53] or by modulating key transcription factors or epigenetic
154 factors modulating cell identity[54, 55] triggers cell elimination by local activation of the
155 JNK pathway specifically at the interface between the two populations[51, 52, 55]. In
156 all these mechanisms, the probability of cell elimination should be directly influenced
157 by the surface of contact between the two cell types. This was indeed confirmed by
158 live imaging and calculation of cell death probability in the context of Myc and *Minute*-
159 dependent competition[45, 56], where cells proportionally sharing more contact and for
160 longer time scales with the other cell types are more likely to die. In this framework,
161 the tissue mechanical properties and the local affinity between the two cell populations
162 (regulated by cell-cell adhesion and tension) will directly influence cell death probability
163 mostly through the modification of the contact surface[39, 57].

164 While the examples above are based on direct interactions between the two cell types,
165 other mechanisms were proposed to rely on the context-dependent diffusion of pro-
166 apoptotic factors. This includes the secretion of the protease ModSP by high-Myc cells,
167 which locally processes and activates the Toll ligand Spätzle and promotes low-Myc
168 cell elimination by activating the NF- κ B pathway and pro-apoptotic genes[58, 59].
169 Alternatively, cell elimination was also proposed to rely on the local depletion of pro-
170 survival factors by more competitive cells, such as the morphogen Dpp[44], or the
171 downregulation of Wnt signaling by the secretion of the Wnt inhibitor Notum near Wnt

172 super-activated cells[13, 14, 53] (although Notum rather promotes cell differentiation
173 in mammalian gut[13, 14]). While the exact range of action and diffusion were not
174 necessarily analysed in all these contexts, they all seem to generate a quite local effect
175 (over the diameter of a few cells), which should also be influenced by the shape of the
176 interface and clone size[60].
177 Overall, these studies illustrate that the probability of cell elimination relies not only on
178 cell-autonomous factors, but also on the genotype of the neighbouring cells as well as
179 the surface of contact between the two populations (**Figure 2A**).

180

181 *1.2 Tissue density and cell shape*

182 In a homotypic environment, epithelial cell death will also be directly influenced by the
183 local tissue organisation, including local mechanical properties and cell shape[61]
184 (**Figure 2B**). Cell density has long been known to influence epithelial cell survival
185 through various pathways, including the Hippo/YAP/TAZ [62, 63], TGF- β [64], or
186 EGFR/ERK pathways[65, 66], whose downregulation at high density promote cell
187 death. Similarly, MDCK (Madin-Darby Canine Kidney) cell crowding induced by
188 stretchable PDMS substrate relaxation is sufficient to promote cell extrusion through
189 the stretch-sensitive channel Piezo and calcium release, which then promotes cell
190 death by anoikis[67] (the apoptotic process triggered by detachment from the
191 extracellular matrix[68]). Alternatively, the local increase of cell density through ectopic
192 growth or tissue movement also promotes cell extrusion and caspase activation in the
193 *Drosophila* pupal notum (a single layer epithelium in the pupal thorax)[66, 69, 70].
194 Further discussion on the relevant mechanical/geometrical parameters causative to
195 cell death can be found elsewhere [39, 61].

196 What are then the key parameters that can locally modulate cell density and cell
197 survival? Local cell proliferation will directly influence local density and pressure and
198 modulate cell survival (**Figure 2B bottom left**). Differential growth was proposed to
199 generate local pressure in solid-like tissue[71] and can promote the elimination of WT
200 cells near oncogenic clones (fast-growing and apoptosis-resistant) in the context of
201 mechanical cell competition[66, 70]. Similarly, the relationship between cell
202 density/pressure and proliferation/survival will also directly set the homeostatic density
203 of the tissue, the value where proliferation is perfectly balanced by cell elimination[72].
204 Mixing populations with different homeostatic densities/pressures can have dramatic
205 effects and lead to complete elimination of the population with the lowest homeostatic

206 density[72]. This was very well illustrated in the context of MDCK *scribble* KD cell
207 elimination by WT cells[57, 73, 74], where p53 expression in the mutant cells reduces
208 their homeostatic density[73], leading to their rapid elimination upon progressive
209 compaction driven by WT cell proliferation[57, 73, 74]. Alternatively, cell compaction
210 can be driven by modulation of the mechanical properties of the boundaries (**Figure**
211 **2B top left and right**). Increased boundary tension can lead to a local increase of
212 pressure, as depicted by the Young-Laplace law which describes the relationship
213 between curvature, line tension and local pressure between two fluids [39, 75]. This
214 mechanism helps to segregate misspecified cells in the wing imaginal disc which
215 accumulate actomyosin at their boundaries [54], similar to the segregation of lineages
216 by compartment boundaries [76], and can also trigger cell elimination for small
217 clones[54]. Alternatively, modulation of bulk tension in a subpopulation of cells will also
218 lead to local modulation of cell shape and crowding, either by a relative increase in
219 tension or by releasing tension in the neighbouring cells (**Figure 2B top right**). This
220 occurs for instance upon activation of some oncogenes in clones which directly
221 modulate cell mechanical properties and relative tension[77, 78]. Finally, local cell
222 density/pressure can be directly affected by collective migration and relative cell
223 movements (**Figure 2B bottom right**). The compaction and elimination of *scribble* KD
224 MDCK cells is enhanced by convergent migration of the WT cells toward the mutant
225 cells[73] through the secretion of the chemoattractant FGF21[79]. Similar collective
226 movements are also driven by waves of ERK [80] or calcium [81] near Ras-active cells
227 or apoptotic cells and directly promote their extrusion. In a homotypic context,
228 spontaneous local increases of pressure can occur through topological defects and
229 misalignment of cells [82]. These so-called +1/2 defects (or comet-like defects), initially
230 described in liquid crystals, also appear spontaneously in MDCK cells and promote cell
231 death through the local increase of pressure, YAP nuclear exclusion and caspase
232 activation.

233 Thus, local tissue density/pressure are complex features influenced by the distribution
234 of cell proliferation and growth, relative mechanical properties, boundary conditions
235 and collective movements which will all influence the rate and distribution of epithelial
236 cell death.

237

238 *1.3 3D shape and curvature*

239 We have focused so far on 2D cell shape and local density, however recent studies
240 also outlined a strong influence of tissue curvature on tissue homeostasis (**Figure 2C**).
241 For instance, epithelial cells cultured in curved alginate tubes revealed a high
242 probability of cell extrusion toward the basal side in convex regions[83], while MDCK
243 cells cultured on curved PDMS have a higher thickness and low YAP in valleys[84]
244 (hence potentially sensitising cells to death). Similarly, a higher rate of MDCK cell
245 extrusion and death was also reported in concave regions of curved PDMS substrates,
246 driven by a local increase of basal hydrostatic pressure, cell detachment and
247 downregulation of the pro-survival kinases FAK and Akt[85]. Similar to local tissue
248 density, curvature can be influenced by multiple factors emerging from local and tissue-
249 wide properties, including fold formation driven by local apical tension increase[86],
250 buckling driven by tissue flow or growth[87, 88], folds driven by local modulation of
251 ECM attachment[89, 90], differential stiffness of ECM and tissue[91, 92], and curvature
252 imposed by the rigid external boundaries of the embryo[93, 94], all potentially
253 influencing curvature, extrusion and cell death.

254

255 *1.4 Systemic control of the susceptibility to cell death*

256 As well as local modulators of the spatiotemporal distribution of cell death, systemic
257 factors can also be essential for precise temporal control of cell death or the global
258 modulation of apoptosis rate. Circulating hormones have long been known to directly
259 impact cell survival and apoptosis, especially during metamorphosis in Insects and
260 Amphibians. Holometabola insects dramatically remodel their larval tissue during
261 metamorphosis which usually involves extensive apoptosis. These processes are
262 controlled and timed by pulses of the steroid hormone Ecdysone, secreted by the
263 prothoracic glands (**Figure 2D**), which triggers organ disassembly mostly through the
264 upregulation of the pro-apoptotic genes *hid* and *reaper*[95]. While this time-controlled
265 full depletion of organs is not necessarily the most relevant for the topic of this review,
266 previous work also suggested that subtle variations of ecdysone levels can sensitise
267 cells to apoptosis at late L3 larval stage in *Drosophila*, most likely through the
268 upregulation of core pro-apoptotic regulators such as Dronc (Caspase-9), Dark (Apaf1)
269 and Drice (Caspase-3)[96]. This suggests that global levels of apoptosis and the
270 probability to enter apoptosis following a local stress can be adjusted at the systemic
271 levels. This was also illustrated through other circulating hormones, which can for
272 instance modulate the elimination of clones during cell competition. High insulin levels

273 can prevent the elimination of Scribble mutant cells from the *Drosophila* eye disc by
274 promoting mTor signaling and blocking apoptosis[97]. Similarly, larvae nutrition can
275 directly modulate cell competition [43, 45] which may be related to a global modulation
276 of apoptosis susceptibility. Alternatively, free circulating Eiger (the *Drosophila*
277 orthologue of TNF- α , Tumor Necrosis Factor alpha) secreted from the fat body can
278 trigger JNK activation and elimination of Scribble mutant in the wing disc[98]. This is
279 permitted by the mislocalization of the receptor Grindelwald to the basal side in the
280 Scribble mutant cells, allowing its activation by basally located Eiger, while Eiger
281 binding to Grindelwald is limited in WT cells due to Grindelwald's apical localisation
282 [98] (**Figure 2D**). While this was not yet formally explored, modulation of the basal
283 levels of circulating Eiger may also affect global susceptibility to apoptosis even in WT
284 cells, similar to the impact of TNF- α on local inflammation and local rate of cell
285 death[99].

286 Overall, multiple systemic inputs can link environmental stress, development stage and
287 aging to the global modulation of apoptosis susceptibility. While this will not change
288 the spatial pattern of cell elimination, it is likely to impact the timing as well as the global
289 rate of epithelial cell death.

290

291 **2. The contribution of feedback triggered by cell death**

292 We have considered so far systemic and local effects which modulate cell death.
293 However, cell death events also trigger diverse feedback mechanisms, which operate
294 across different scales in space and time and will influence the rate of cell elimination.

295

296 *2.1 Negative feedback – Dying cells protecting their neighbours*

297 Perhaps the most widely studied and understood responses to cell death events are
298 those promoting survival and proliferation of the neighbouring cells. Such mechanisms
299 are vital for ensuring the sealing and the homeostatic size of epithelia. Apoptosis-
300 induced proliferation (AiP; also called compensatory proliferation) is the phenomenon
301 by which cell proliferation is promoted near dying cells. While this was primarily
302 described in the case of massive cell death and regeneration or upon perturbation of
303 apoptosis completion[100, 101], a more recent report clearly demonstrated a
304 correlation between single apoptotic events and local increases of proliferation[102].

305 However, AiP will not be covered in this section, which rather focusses on how death
306 events influence apoptosis itself.

307 Recent studies have outlined the pro-survival effect of dying cells on their neighbours.
308 These modes of non-autonomous negative feedback include the activation of pro-
309 survival EGFR/ERK signalling in the neighbours of dying cells, which is crucial for the
310 maintenance of epithelial sealing and tissue integrity. Single cell extrusion from the
311 *Drosophila* pupal notum triggers a pulse of ERK signalling in the direct neighbouring
312 cells, which downregulates caspases and reduces their death probability for roughly
313 one hour[103]. Depletion of EGFR abolished these ERK pulses, removing the local
314 death inhibition and allowing for aberrant clustered cell death which compromised
315 tissue sealing. Gagliardi *et al.* similarly described the occurrence of pro-survival waves
316 of ERK and Akt activation propagating from death events in monolayers of cultured
317 epithelial cells (MDCK and MCF10a cells) which buffer down cell death[104]. These
318 waves were dependent on the presence of matrix metalloproteinases (MMPs) in the
319 dying cells, and EGFR in the surviving neighbours, suggesting that dying cells activate
320 enzymes to cleave growth factor pro-ligands which then bind EGFR in the neighbours.
321 In this context, the EGFR-dependent pro-survival pulses spread and persist over a
322 larger scale in space and time (3 cell rows; 3-4 hours) compared to the *Drosophila*
323 pupal notum (1 cell row, 1 hour). Such non-autonomous protection from death has also
324 been described in HeLa cells, in which apoptotic stress triggered secretion of the FGF2
325 growth factor, stimulating MEK-ERK signalling in neighbouring cells which upregulates
326 pro-survival BCL-2 proteins[105]. This upregulation persisted for at least 2 days hence
327 generating longer-term apoptosis protection. Interestingly, apoptotic stress could still
328 activate this protection despite deletion of the MOMP regulators BAK and BAX,
329 suggesting that caspase activation and cell death is not required for this negative
330 feedback. Finally, long-term apoptotic inhibition was also described in the *Drosophila*
331 wing disc. Expression of either pro-apoptotic genes *hid* or *reaper*, or exposure of the
332 whole tissue to ionizing radiation (IR), upregulated the pro-survival microRNA *bantam*
333 non-cell autonomously, protecting cells from apoptosis by further insult with IR[106].
334 This non-autonomous protection was dependent on caspase activation in the dying
335 cells, and propagated through *tie*, the *Drosophila* homolog of the mammalian
336 Angiopoietin receptor. Together, these results indicate that dying cells promote the
337 survival of their neighbours through growth factor receptor signalling in various model

338 systems, with the feedback extending to different spatiotemporal scales depending on
339 the context (**Figure 3**).

340

341 *2.2 Acquired resistance to apoptotic stimuli and memory effect*

342 In addition to non-cell autonomous effects which protect neighbouring cells, previous
343 exposure to apoptotic stimuli can also inhibit later cell death cell-autonomously.
344 Accordingly, the 10-30% of MCF10A cells surviving a first exposure to TRAIL showed
345 transient resistance to a second dose of the ligand 24 hours later[107]. This acquired
346 resistance was also observed after TRAIL treatment combined with death inhibition
347 using a caspase inhibitor, showing that it was not dependent on the release of factors
348 from dying neighbours. Molecularly, this transient phenotype is mediated by the
349 reduced ability of ligand-bound death receptors to form intracellular signalling
350 complexes, impairing downstream MOMP and caspase cleavage. Glioblastoma (GBM)
351 cell lines can also acquire resistance to TRAIL-induced apoptosis after long-term
352 culturing with the ligand through the downregulation of death receptor proteins,
353 upregulation of pro-survival BCL-2 proteins, and alteration of inflammatory
354 signalling[108]. Together these results demonstrate that cells which survive a past
355 extrinsic apoptosis stimulus can acquire resistance to further insult via several
356 transcriptional and signal transduction alterations.

357

358 *2.3 Positive feedback – Cell death spreading and amplifying*

359 In contrast with mechanisms of apoptosis-induced survival, in certain contexts dying
360 cells non-autonomously promote the elimination of others, allowing death to propagate
361 across tissues. Death propagation in waves was mostly described for other types of
362 cell death, including ferroptosis (an iron-dependent mode of intrinsic cell death distinct
363 from apoptosis) and necroptosis (a formed of programmed necrosis) [109-112].
364 However, long distance propagation of apoptosis has also been described in certain
365 conditions. Widespread induction of apoptosis in one compartment of the *Drosophila*
366 wing disc by locally expressing the Ricin toxin, PTEN or a repressor of translation has
367 been associated with ectopic apoptosis in the neighbouring compartment[113, 114].
368 Similarly, expression of the pro-apoptotic genes *hid* or *reaper* in one compartment was
369 accompanied by increased ectopic death in the adjacent compartment, here named
370 apoptosis-induced apoptosis (AiA)[115]. AiA was also observed when *hid* and *reaper*
371 were combined with the caspase inhibitor p35, thus blocking downstream cell death

372 and generating so-called 'undead cells'. In this context, AiA is mediated by the
373 secretion of the TNF orthologue Eiger by the undead cells, which activates JNK and
374 apoptosis at long distance. However, later work did not observe ectopic JNK activation
375 in the adjacent compartment, suggesting that Eiger function in AiA might be
376 indirect[116]. The production of ROS triggered by apoptosis and JNK activation,
377 leading to increased oxidative stress in the neighbouring compartment, could be an
378 alternative regulator of AiA, though this has not yet been formally tested to our
379 knowledge[116]. Similarly, Vascular smooth muscle cells (VSMCs) in culture treated
380 concomitantly with drugs stimulating both intrinsic and extrinsic apoptosis pathways
381 rapidly underwent cell death, and were shown to secrete multiple soluble cytokines
382 promoting further apoptosis in adjacent cells[117]. However, when apoptosis of
383 VSMCs was triggered *in vivo* in the arteries of mice, release of the same key cytokines
384 rather promoted cell proliferation, highlighting the context-dependent effect of this
385 feedback. Overall, these studies outline the existence of several propagation
386 mechanisms that depend on the context and the level of death induction.

387

388 *2.4 Local feedback and emerging rate of cell death*

389 We have described different pro-survival and pro-death feedback mechanisms acting
390 on very different temporal and spatial scales (one cell to a full tissue, one hour to
391 several days), prompting the question of how these different scales impact the final
392 rate and distribution of cell elimination. This can be conceptualised by comparing the
393 intrinsic rate of cell death, a combinatorial effect of various cell-autonomous
394 parameters, and the emerging rate of cell death at the tissue level after integration of
395 feedback[103, 104]. We previously explored theoretically the impact of the duration of
396 negative feedback on cell death rate and demonstrated that the effect is highest in the
397 case of long feedback duration and high intrinsic rates of cell elimination[103] (**Figure**
398 **3**). Intuitively, the spread of feedback in time and space defines a spatiotemporal
399 volume around the dying cell, in which cell death is blocked. This will have a
400 neglectable effect if death events are sufficiently sparse in time and space (low intrinsic
401 rate) and a strong impact if many cell deaths should occur in the refractory volume
402 (high intrinsic rate). While this was never explored theoretically, the integration of
403 various positive and negative feedback modes at different spatial and temporal scales
404 may have the capacity to generate complex spatiotemporal patterns of cell elimination,
405 such as oscillatory eliminations, wave propagations or even Turing-like patterns.

406

407 **3. Commitment to cell death is a multi-layered decision process**

408

409 In addition to the various non-autonomous factors which influence a cell's propensity
410 to die at a given moment, the decision-making process which takes place within the
411 cell is also complex and multi-layered. While engagement in apoptosis can be
412 considered as a simple binary switch between surviving or dying, the point of
413 irreversible commitment remains poorly characterised and may be highly context-
414 dependent. We will outline in this section the relevant layers of decision-making that
415 were characterised in epithelial cells.

416

417 *3.1 Decision-making downstream of caspases*

418 The core apoptosis signalling pathways, both extrinsic and intrinsic, culminate in the
419 activation of the effector caspases, which was for a long time considered a point-of-
420 no-return in the apoptotic cascade[118]. However, this paradigm has been challenged
421 by the discovery that the effector caspases have numerous functions which are
422 independent from their role in triggering death[119] and the widespread survival from
423 transient effector caspase activation during development[70, 103, 120]. Here we will
424 describe the evidence supporting the existence of this new layer of decision-making
425 downstream of caspases.

426

427 *3.1.1 Non-apoptotic functions of caspases*

428 Many members of the caspase family are known to perform functions outside the scope
429 of cell death, and some such as Caspase-1 are generally considered as inflammatory
430 rather than apoptotic[121]. However, the finding that the most downstream apoptotic
431 caspases, often referred to as the executioner caspases (Caspase-3 and Caspase-7
432 in mammals), have functions entirely separate from stimulating death, was more
433 surprising. Early examples of such findings were primarily focussed on differentiation.
434 During the terminal differentiation of rodent lens fibres *in vitro* and human epidermal
435 keratinocytes *ex vivo*, caspase activation is required for de-nucleation of cells but does
436 not result in their elimination[122, 123]. Furthermore, Caspase-3 activation is required
437 for morphological changes during the differentiation of non-epithelial cell types such as
438 erythroids and skeletal muscle cells[124, 125]. Since these discoveries, new non-
439 apoptotic functions of effector caspases are being characterised regularly in diverse

440 systems and processes (more exhaustive details can be found elsewhere [119, 126-
441 128]). These include roles for Caspase-3 in morphogenesis of excretory systems such
442 as the ureter in mouse [129] and the Malpighian tubules in *Drosophila*[130],
443 endocytosis regulation and morphogenesis in *Drosophila* trachea[131], a contribution
444 of low basal levels of effector caspase activity to proliferation regulation in the
445 *Drosophila* wing disc[132], and the inhibition of cell migration and invasive behaviour
446 [133] triggered by irradiation, chromosome instability and long term JNK activation in
447 the *Drosophila* wing disc[134, 135].

448

449 3.1.2 Widespread survival from caspase activation

450 Given that active effector caspases do not always trigger death and can have functions
451 independently of apoptosis, one key question to address is how widespread such non-
452 apoptotic caspase activation is during normal development. The CasExpress tool is
453 activated following cleavage by effector caspases, and persistently labels the cell and
454 its progeny, allowing lineage tracing of all cells which have switched on effector
455 caspase at some point in their lifetime [120, 136]. This tool revealed that the majority
456 of cells which make up the body of the adult *Drosophila* are derived from cells which
457 have activated caspase during their lifetime, with some differences between organs
458 (e.g.: high in oviduct and low in the central brain and nerve cord). These patterns of
459 caspase survival may simply represent the different levels of caspase activity in
460 different tissues, or different levels of tolerance/survival to caspase activation. It is also
461 unclear at this stage whether they reflect systemic and low basal levels of caspase
462 activity, which might be related to non-apoptotic functions, or survival from sporadic
463 and acute caspase activation. Nonetheless, more recent work clearly demonstrated
464 that a large proportion of cells in the wing imaginal disc were marked with CasExpress
465 following transient pro-apoptotic stress (irradiation, heat stress, or apoptotic gene
466 induction), demonstrating that cells can survive acute caspase activation *in vivo*[137].
467 Indeed, reversal of a seemingly late apoptotic state has been observed in HeLa and
468 NIH T3T cells and termed 'anastasis'[138]. A fraction of cells exposed to ethanol can
469 survive after removal of the stress despite clear morphological signs of late-apoptosis
470 stages, including Caspase-3 cleavage, translocation of lipids to the outer leaflet of the
471 plasma membrane, membrane blebbing and DNA damage. These surviving cells
472 displayed some persisting phenotypic and genetic alterations, namely unrepaired DNA
473 damage, as well as transcriptional upregulation of several pro-survival genes. Probing

474 the transcriptional state of anastatic cells revealed that their recovery from a late
475 apoptotic phenotype involves first prioritising re-entry to the cell cycle and proliferation,
476 and then gaining increased migratory capacity[139]. Together these results suggest
477 that cells which return from a late-stage apoptotic phenotype may acquire oncogenic
478 potential.

479

480 *3.2 Identifying decision points downstream of pro-apoptotic signals*

481 The same apoptotic stress will not always trigger death in all cells, leading to a
482 phenomenon named fractional killing[107]. Similarly, cells activating effector caspase
483 will not necessarily die (see previous section). Therefore the question remains: is an
484 individual cell's apoptotic potential determined by its capacity to activate caspase, its
485 likelihood to survive a given level of caspase activity, or a combination of the two?
486 Furthermore, identifying how these thresholds/potentials are established and
487 modulated is key to understanding how a cell makes the decision to die or survive.

488 In mammalian cells, live imaging of cytochrome c release[18] and FRET sensors of
489 effector caspase activity[140] suggested early on that MOMP and caspase activation
490 are fast and all-or nothing processes. This is in sharp contrast with inherent variability
491 of the timing of apoptosis induction between cells upon TRAIL exposure, which can
492 range from 40 minutes to more than 12 hours [140, 141]. The timing of apoptosis is
493 however strongly correlated between daughter cells for a given period of time, which
494 was explained by the similarity of protein concentration following cell division which will
495 progressively fade away due to protein turnover[34, 142]. While the regulation of this
496 timing is most likely multifactorial and hard to predict, it amounts to different speed of
497 activation of Caspase-8, which leads to apoptosis once passing a well-defined
498 threshold of rate of activation and duration[34, 141, 143]. Altogether, these studies
499 suggest that the key death decision point mostly resides upstream of MOMP[31].

500 However, just like effector caspase activation, MOMP can also be survived with
501 oncogenic consequences. Various mammalian cell types treated with apoptosis-
502 inducing actinomycin D or UV irradiation can undergo incomplete MOMP (also referred
503 to as partial or minority MOMP) visualised by the persistence of some intact
504 mitochondria[144]. Minority MOMP leads to sub-lethal effector caspase activity which
505 causes DNA damage through activation of the CAD DNase enzyme, a downstream
506 target of Caspase-3 [145], promoting oncogenic transformation and tumorigenesis.
507 Furthermore, release of sublethal levels of cytochrome c during minority MOMP has

508 been shown to reprogramme translation independently of caspase, promoting a
509 'persister phenotype' in which cells gain heightened metastatic potential and drug
510 resistance[146], hence promoting oncogenic transformation.

511 Widespread MOMP triggers extensive effector caspase activation leading to apoptosis,
512 while minority MOMP causes sub-lethal effector caspase activation and cell
513 survival[145], suggesting the existence of an effector caspase activity threshold at
514 which a cell commits to die. Such a threshold may explain the existence of multiple
515 non-apoptotic functions of effector caspases (see above). Accordingly, mutation of one
516 of the effector caspases in *Drosophila* wing discs can modulate the sensitivity to
517 radiation indicating a threshold effect [147], however this work did not assess caspase
518 threshold and apoptosis engagement at the single cell level. Moreover, recent results
519 questioned the existence of a well-defined threshold of effector caspase activity. Direct
520 optogenetic activation of Caspase-3 in HeLa cells combined with a live sensor of
521 effector caspase activity indicated that neither the rate, peak level nor cumulative
522 amount of total effector caspase activation were sufficient to predict whether cell death
523 will occur[148]. These results suggest that unknown factors can modulate the response
524 to effector caspase activation and that there is no unique caspase threshold associated
525 with apoptosis engagement.

526 To conclude, these studies illustrate that individual cells within a seemingly
527 homogenous population can vary in their likelihood to undergo MOMP, likelihood to
528 activate initiator and effector caspases and sensitivity to a given value of caspase
529 activity, all of which will influence the probability of apoptosis.

530

531 *3.3 Cell death and extrusion*

532 Epithelial cell elimination must be orchestrated to protect the integrity and sealing of
533 the tissue. Thus, commitment to cell death is inextricably linked with extrusion, the
534 series of remodelling steps expelling the cell from the tissue layer[149-151]. Extrusion
535 itself is also related to commitment to apoptosis through the process of anoikis[68],
536 which promotes apoptosis upon ECM detachment by the reduction of FAK activation.
537 As commitment to cell death in epithelia is closely related to engagement in extrusion,
538 it is key to investigate the relationship between caspase activity and cell extrusion.

539 Both intrinsic and extrinsic apoptotic pathways drive cell extrusion in MDCK cells, with
540 caspase activation required to trigger the contractile force which removes the cell from
541 the layer[152]. Actomyosin contractility can also be directly regulated by the cleavage

542 of caspase substrates, including the activation of ROCK by cleavage[153], or the
543 activation of MRCK which is required for actomyosin cable formation and
544 extrusion[154]. Spontaneous extrusion in MDCK cells driven by topological defects is
545 also preceded by caspase activation and is inhibited by a pan-caspase inhibitor[82]. In
546 *Drosophila*, effector caspase activation precedes cell extrusion and their inhibition
547 blocks extrusion in many epithelia, including the amnioserosa cells in the embryo[1],
548 in the pupal leg[2], the pupal notum[70] or the larval epidermal cells in the
549 abdomen[155]. In the pupal notum, effector caspase activation promotes cell extrusion
550 and apical constriction through the depletion of microtubules[156]. Together, these
551 results argue that in these epithelial contexts, cell extrusion is a caspase-dependent
552 event which precedes the completion of apoptosis. Of note, these results do not
553 exclude a contribution of anoikis to apoptosis progression and completion.

554 However, in other systems the series of events linking death with extrusion is not so
555 clear. MDCK cell extrusion driven by crowding does not require caspase activation and
556 is not inhibited by Bcl2 over-expression, and as such was called “live extrusion”[157].
557 Previous reports also suggested that live extrusion could occur in the *Drosophila* pupal
558 notum upon cell compaction[69], however later studies rather suggested that the vast
559 majority of extrusions in the pupal notum are caspase-dependent[66, 70, 103, 156,
560 158, 159]. This however is not exclusive with cells being still alive while extruding.
561 Accordingly, a recent report proposed that ROS and superoxide anions promote
562 caspase activation and cell extrusion in the pupal notum, but that H₂O₂ tends on the
563 contrary to delay the completion of apoptosis and cell fragmentation[159]. Further work
564 will be necessary to better understand the relationship between progression in
565 extrusion, caspases and apoptosis engagement.

566 In the context of live extrusion, cell death is a consequence of extrusion and anoikis.
567 While the role of caspases in intestinal cell shedding was long debated[160], it was
568 recently shown that Caspase-3/7 double knockout in the intestinal epithelial cells of
569 mice does not affect gut homeostasis and cell shedding from the tip of the villus[161],
570 where apoptosis-induced extrusion is compensated by increased live-cell extrusion.
571 Furthermore, cells which are normally eliminated by a caspase-dependent process in
572 *C.elegans* can still be shed in mutants lacking all four caspase genes through
573 downregulation of cell-adhesion molecules[162]. Together, these findings suggest that
574 the dependence on caspase for extrusion is tissue- and context-specific and highly
575 plastic. Yet, the role of caspase in extrusion can dramatically change the identification

576 of the key predicting parameters regulating epithelial cell death. In the context of live
577 extrusion followed by anoikis, it should mostly rely on core cellular and mechanical
578 parameters regulating extrusion. In the context of caspase-dependent extrusion, it will
579 be set by parameters regulating caspase activation and the downstream steps leading
580 to extrusion progression and apoptosis commitment. These opposing results may
581 explain why caspase activity has a varying predictive value for death/survival decisions
582 in different systems, which should be higher in the context of caspase-dependent
583 extrusion.

584

585 To conclude, while we now have a far better understanding of the subtleties in the
586 apoptotic signalling pathways, confidently defining a point-of-no-return in commitment
587 to cell death remains a very challenging question. Multiple commitment points will
588 emerge depending on the cellular and stress context, from MOMP induction to
589 uncharacterised decisions downstream of effector caspases, and engagement in cell
590 extrusion.

591

592 **4. A roadmap to reach a predictive understanding of epithelial cell death**

593

594 We have illustrated so far the multiple layers of regulation at the cellular and tissular
595 scales which complexify our understanding of epithelial cell death. What are then the
596 ongoing methodologies and approaches that could help us to better integrate these
597 layers of regulation and have a more quantitative understanding of epithelial cell
598 death?

599

600 *4.1 Modelling apoptosis at the single cell level*

601 The best quantitative understanding of the cell death decision was obtained at the
602 single cell level using extensive modelling of the apoptotic pathway with Ordinary
603 Differential Equations (ODE)[31, 163-165]. While these approaches integrate a large
604 number of inputs and rates which are not necessarily known experimentally, they
605 helped to define key features of the apoptosis decision and its dynamics. This includes
606 its binary nature and the “all-or-nothing” switch occurring downstream of MOMP [18],
607 as well as the rapid cleavage of targets of effector caspases after their activation [140],
608 all recapitulated in different models [33, 166-168]. Alternatively, combining quantitative
609 live-imaging and modelling has helped to define conditions allowing sublethal

610 activation of effector caspases and partial substrate cleavage. For instance, models of
611 TRAIL-induced apoptosis suggested that high effector caspase turnover through
612 protein degradation combined with low XIAP1 concentration and high Bcl2 could lead
613 to transient and partial cleavage of effector caspase targets, which was confirmed
614 experimentally by perturbation of the proteasome and Bcl2 overexpression[33].
615 Alternatively, other models suggested that high XIAP1 concentrations can promote
616 sublethal effector caspase activation by slowing down the rate of target cleavage,
617 which was also confirmed experimentally in HeLa cells[166]. ODE combined with
618 Bayesian approaches can also help to discriminate between different apoptotic
619 network architecture and the one most likely to explain the experimentally observed
620 dynamics[169]. Finally, probabilistic models integrating random fluctuations of
621 threshold can help to analyse the impact of cell-to-cell heterogeneity and temporal
622 fluctuations on death probability[170].

623

624 *4.2 Integrating collective effects and feedback*

625 We have so far described modelling approaches focusing on the biochemical reactions
626 regulating apoptosis at the single cell level. This signalling complexity must also be
627 integrated with the regulation of cell extrusion and the role of mechanical inputs and
628 shape as well as the multiple layers of feedback. Cell-based modelling has been widely
629 used to describe the impact of mechanics on tissue dynamics, shape and cell
630 behaviour (**Figure 4**). This includes vertex models, which can recapitulate the evolution
631 of epithelial cell shape in 2D or 3D by simulating the displacement over time of vertices
632 (the points of intersection of intercellular junctions). This type of model is generated by
633 minimizing an energy function influenced by line tension (a product of cortical tension
634 and cell-cell adhesion) and cell apical area compressibility, and can also integrate the
635 impact of growth and the local modulation of mechanical parameters[171-173]. Vertex
636 models have been successfully used to study the regulation of cell extrusion or the
637 impact of mechanical stress on cell death[54, 69, 156, 174-176]. While they did not
638 include explicit relationships between the regulation of apoptosis, caspases and cell
639 extrusion, they could be directly included in the model by implementing feedback
640 between cell shape, caspase activity and in return the modulation of key mechanical
641 parameters promoting cell extrusion. Accordingly, hybrid vertex models have been
642 previously developed to integrate the impact of various signalling pathways on cell
643 differentiation, stem cell activation and cell mechanics[177-180]. Alternatively, cellular

644 Potts models were also widely used to describe the evolution of epithelial cell
645 shape[181]. A Cellular Potts model simulates cells using a lattice of pixels whose
646 evolution over time is influenced by the minimisation of an energy function set by the
647 relative affinity between cells and a compressibility term. Similar to vertex models,
648 cellular Potts models have already been combined with the simulation of various
649 biochemical reactions[182-184]. Cellular Potts models have also been used to
650 investigate the impact of mechanics on cell extrusion and tissue multilayering[185],
651 and can successfully recapitulate the dynamics of elimination of *scribble* KD MDCK
652 cells by WT cells during mechanical cell competition, confirming that lower homeostatic
653 density and lower stiffness are sufficient to recapitulate cell elimination[57]. This last
654 example is the perfect illustration of how cell-based models combined with quantitative
655 datasets can reveal emerging properties at the population level that will directly
656 influence the rate of cell death, notably here the role of homeostatic density. Combining
657 these cell-based models with simulations of the dynamics of all -or parts- of the
658 apoptotic pathway as well as relevant spatio-temporal feedback and cell-cell
659 interaction could help in the near future to explore the relationship between local stress,
660 cellular neighbourhood, caspase activity, cell extrusion and emerging rate and
661 distribution of cell elimination (**Figure 4**). This task could be progressively
662 democratised thanks to the numerous libraries allowing user-friendly implementation
663 of Potts and vertex models combined with ODEs[186-188].

664

665 *4.3 Identifying new relevant modes of feedback and predictive parameters*

666 While these approaches outline emerging properties coming from mechanical inputs
667 as well as biochemical reactions, they require prior knowledge on the relevant
668 signalling cascades, relationships between cell shape and signalling as well as
669 relevant spatiotemporal feedback. Identification of novel feedback and non-cell
670 autonomous factors regulating epithelial cell death requires large datasets with
671 extensive characterisation of the number and the distribution of dying cells through
672 long-term live imaging. The detection of cell death events can rapidly become an
673 important bottleneck for this type of analysis. This may rapidly be solved thanks to the
674 recent development of methods to automatise cell death detection through deep
675 learning. These have been successfully applied *in vivo* in *Drosophila* epithelia to detect
676 extrusion through fluorescent live imaging[189], in cell culture using transmission light

677 microscopy [190, 191], and to discriminate between various types of cell death through
678 live imaging and immunostainings [192, 193].

679 Feedback and spatial coordination can then be revealed from the spatio-temporal
680 distributions of cell death using point pattern analysis, which can outline biases in
681 distributions compared to a so-called Poisson process (where every event is
682 independent in time and space). These include local and transient clustering or
683 dispersive effects [103, 180, 194], as well as putative coupling between cellular
684 events[180, 195]. Alternatively, network representation of cell-cell interactions and
685 tissue architecture can help to reduce the dimensionality of the imaging dataset, while
686 encapsulating information about cell geometry and distances[196]. This can be used
687 to apply graph theory to reveal new cell-cell interactions, as previously applied to study
688 the interactions between bacterial populations[197]. The combination of graph theory
689 with machine learning in Graph Neural Networks can also be used to infer new
690 interactions between cells, and was successfully applied to mouse epidermis movies
691 to reveal coupling between cell delamination/differentiation and a local and transient
692 upregulation of cell division, as well as negative feedback between delamination
693 events[198]. More generally, interpretable deep-learning approaches can help to
694 outline new rules and reveal parameters predictive of cell death engagement[199,
695 200]. For instance, unsupervised machine learning applied to movies of cell
696 competition between *scribble* KD and WT MDCK cells outlined again the key role of
697 local cell density in the commitment to cell death[200], in agreement with previous
698 experimental and theoretical works[57, 73, 74]. While the predictive features may be
699 hard to interpret biologically, these approaches can be used to identify critical time-
700 periods at which decisions are starting to be made as well as minimal features sufficient
701 to reach a good level of prediction.

702 Eventually, the predictions from modelling and machine learning can be directly tested
703 by quantitative perturbations using drugs, mutants or RNAi. More recently, the
704 development of optogenetic tools allowing quantitative and spatio-temporal control of
705 caspase activity by blue light exposure opens new possibilities, which will surely be
706 instrumental to dissect the decision steps leading to apoptosis engagement as well as
707 putative spatio-temporal couplings [103, 148, 201, 202] (**Figure 4**).

708

709 **Declaration of interest**

710 The authors declare no competing interest

711

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720

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1216

1217 **Figure legends**

1218

1219 **Figure 1: Simplified apoptotic pathway in mammals and *Drosophila***

1220

1221 **A:** apoptotic pathway in mammals decomposed in intrinsic pathway (left) and extrinsic
1222 pathway (right). For the intrinsic pathway, the balance between pro and anti-apoptotic
1223 Bcl2 family member regulates the oligomerisation of BAX and BAK at the mitochondrial
1224 membrane. Mitochondrial Outer Membrane Permeabilization (MOMP) will then lead to
1225 Cytochrome-c release, which promotes the formation of the apoptosome by Apaf1, and
1226 the activation of Caspase-9. XIAPs inhibition by SMAC/ARTS/HTRA2 proteins permit
1227 the activation of Caspase-9 and effector Caspases. The extrinsic pathway is initiated
1228 by death ligands binding to FADD receptors, leading to Caspase-8 activation which
1229 promotes BAK/BAX activation by BID as well as direct activation of Caspase-3 and 7.

1230 **B:** Apoptotic pathway in *Drosophila*. The colour code gives the correspondences
1231 between fly and mammalian regulators when they exist. Apoptotic stimuli lead to the
1232 upregulation of the apoptotic genes *hid*, *grim* or *reaper*, whose association with
1233 mitochondria is required for the degradation and inhibition of Diap1, an inhibitor of
1234 Dronc (Caspase 9) and effector caspases (mostly Drice and Dcp1). Dronc activation
1235 is also promoted by the activation of Dark and the apoptosome.

1236

1237 **Figure 2: Non-cell autonomous factors modulating epithelial cell death**

1238

1239 **A:** Apoptosis (red cell) promoted by cell competition at the interface between two
1240 populations. Cell death can be triggered either by receptor (dark blue) ligand (orange)
1241 interactions at the interface, or through the local secretion of pro-apoptotic factors (dark
1242 blue circles). **B:** Different mechanisms that can lead to local increase of cell density
1243 and/or pressure and/or reduction of tension, which will locally promote epithelial cell
1244 extrusion and cell death. The dark green cells in the center are the compacted
1245 population, red cells are apoptotic cells. The four black squares on the side illustrate
1246 different modes of compaction (dark green ellipsoids are the compacted portions of the
1247 tissue, light green the non-compacted populations). Local compaction can be driven
1248 by an increase of interfacial tension which applies a local pressure on the dark green
1249 cell population (top left), by a relative decrease of tension in the neighbouring cells (top

1250 right), a relative increase of growth rate at the periphery (through cell division or
1251 changes of cell volume, bottom left), or through collective migration and convergent
1252 movements (bottom right). **C:** Modulation of cell extrusion and cell death by local tissue
1253 curvature. Valley regions (concave) are associated with higher cell height, lower YAP
1254 activity and potentially more extrusions. **D:** Influence of circulating hormones on the
1255 global susceptibility to cell death in the wing disc (center, bottom view is a cross section
1256 with peripodal cells at the top and wing proper at the bottom). The top left organ is the
1257 larval brain, the top right organ is the fat body. Ecdysone secreted from Prothoracic
1258 Glands (PG) near the larval brain can directly bind to its receptor in cell nucleus
1259 (bottom, EcR) and sensitise cells to apoptosis (pink cell). Similarly, Eiger/TNF- α is
1260 secreted by fat body and can promote polarity mutant cell death (dark red cell) through
1261 the binding to the miss localised receptor Grindelwald on the basal side (purple,
1262 bottom).

1263

1264 **Figure 3. Local feedback mechanisms impact the global rate of death.**

1265

1266 Cells dying in an epithelium (red) triggering the transient protection of their neighbours
1267 (orange). An inhibitory signal which diffuses over a short distance in space and time
1268 will transiently protect direct neighbours while allowing death in cells further away or at
1269 later timepoints (left). If the signal spreads far in space and persists in time, more
1270 neighbours will be protected for a longer time (right). Bottom graph: Emerging rate of
1271 cell death (y-axis) as a function of the duration and spatial spread of the inhibitory
1272 feedback (x-axis) when death probability follows a Poisson process with a given rate
1273 (“intrinsic rate of cell death”). The emerging rate of cell death (intrinsic rate + feedback)
1274 exponentially decreases with the duration/spread of the feedback. This inhibitory effect
1275 is much more significant in conditions of high intrinsic rate of cell death (red curve)
1276 compared to moderate (orange curve) and low rates (green curve) where the feedback
1277 has little impact on cell death. Based on simulations performed in [103]

1278

1279 **Figure 4: A roadmap for building a more systematic and predictive**
1280 **understanding of epithelial cell death.**

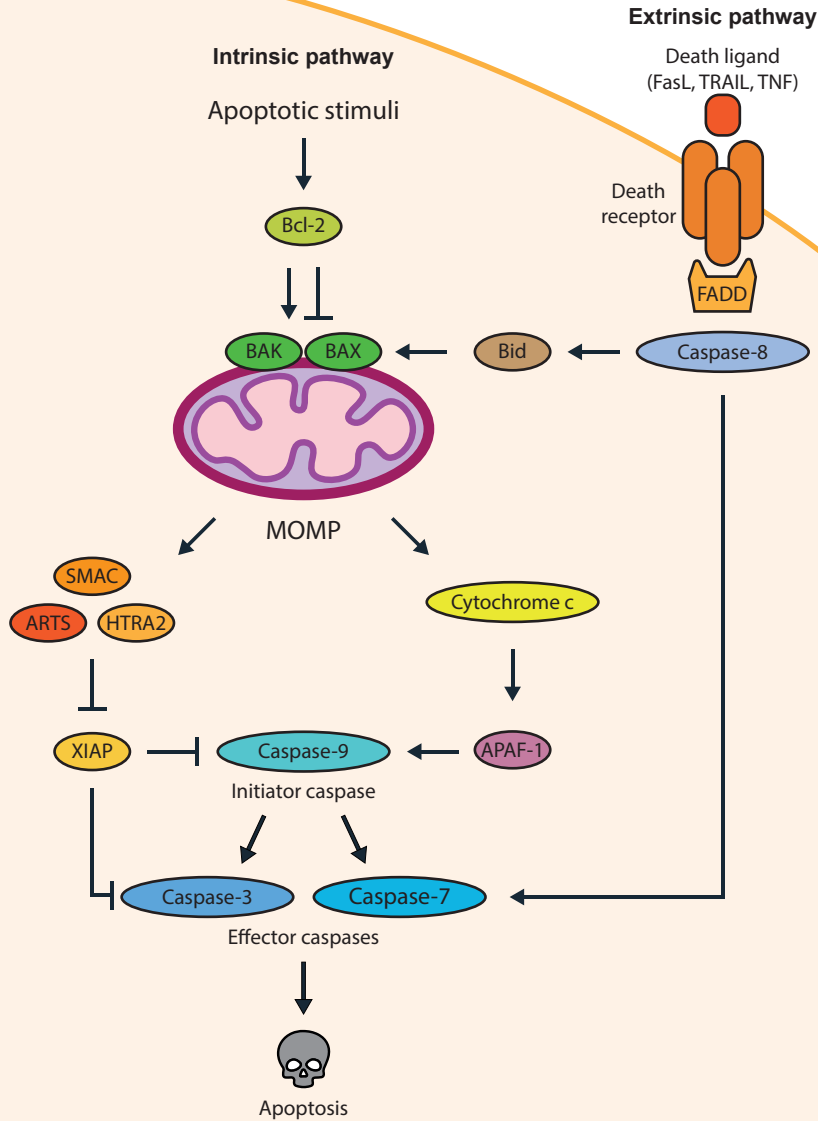
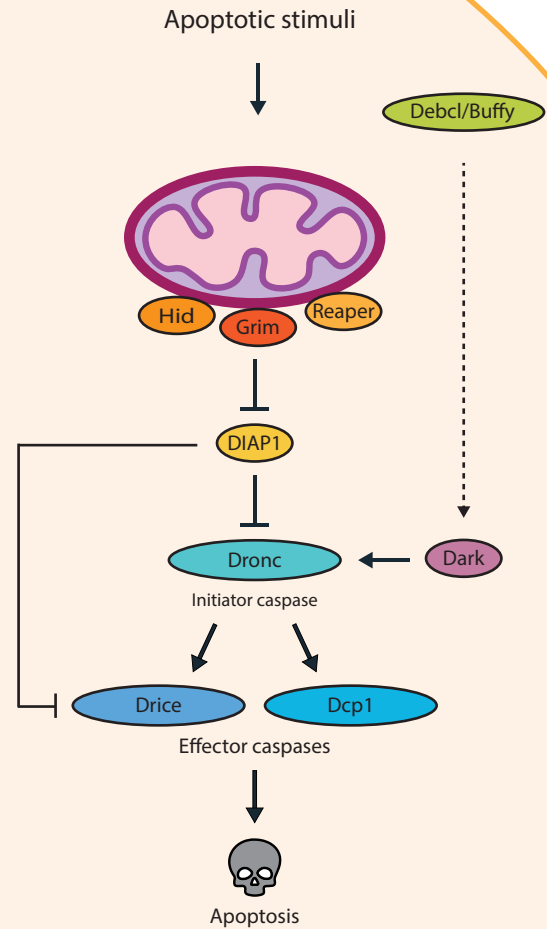
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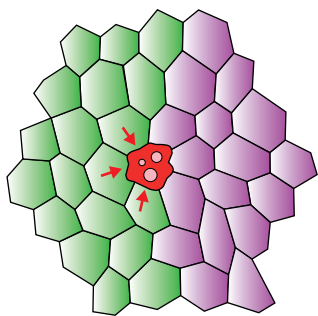
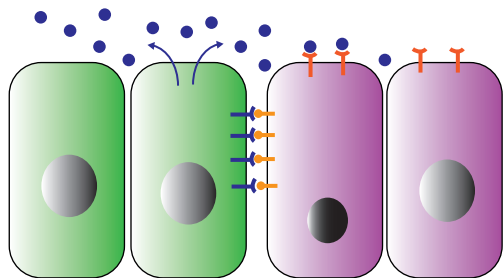
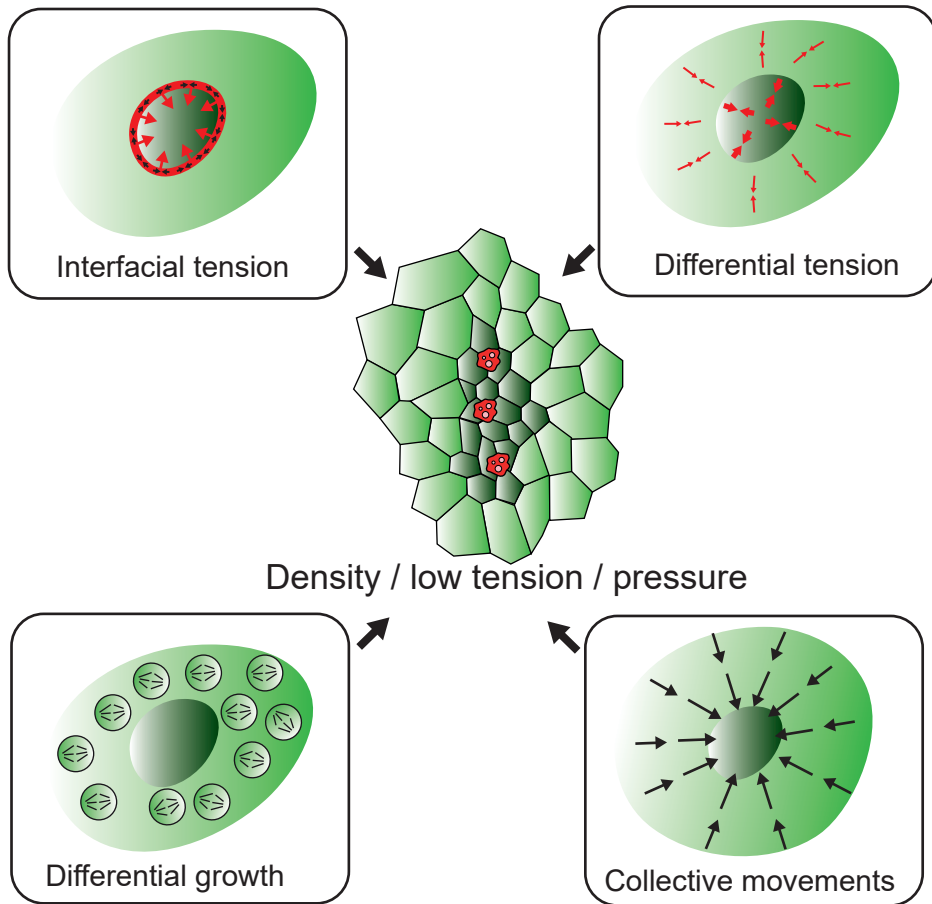
1282 Conceptual framework that could help to reach a more predictive understanding of
1283 epithelial cell death. Top left: snapshot of a pupal notum in *Drosophila* with machine

1284 learning-based detection of cell death (yellow), cell division (magenta) and sensory
1285 organs (blue) (adapted from [189]), and a segmented notum (right) illustrating the need
1286 of computer-assisted event recognition for high throughput analysis of epithelial cell
1287 death. Top right: network representation of the tissue and extraction of new feedbacks
1288 and relevant parameters by deep-learning. Parameters extracted from these two
1289 approaches can be used to feed ODEs of various signaling pathways including
1290 regulators of apoptosis (green), which can be included in cell-based models (vertex or
1291 Potts model). The predictions of the emerging rate and distribution of cell death can be
1292 tested by quantitative perturbations of the system using drug, genetic perturbations or
1293 optogenetics.

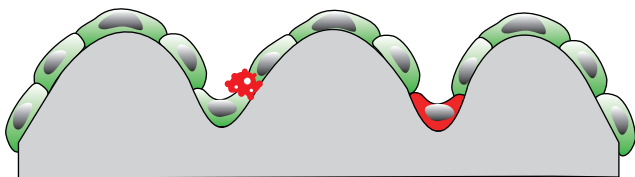
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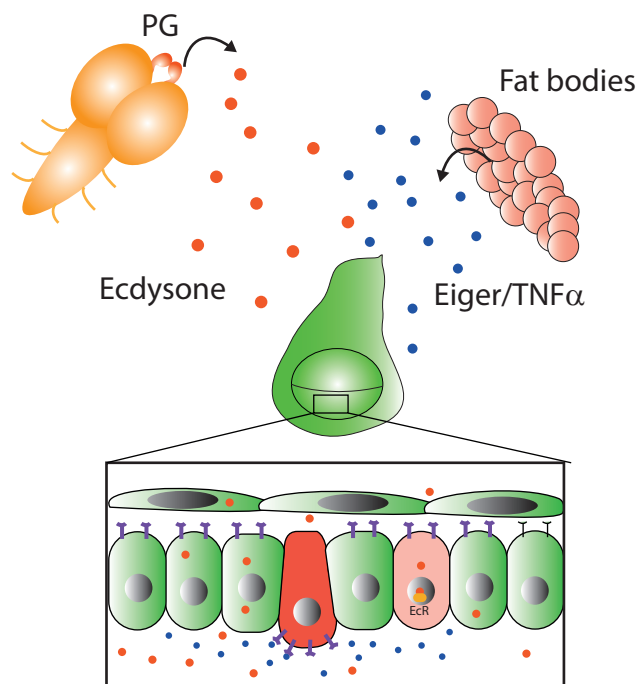
A*Mammals***B***Drosophila*

A**B**

Cell competition:
contact-dependent,
secreted factors

C

3D geometry and curvature

D

Systemic effects

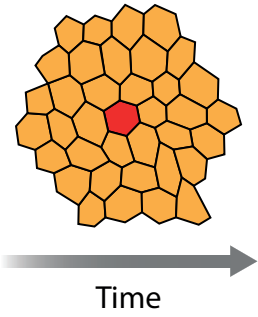
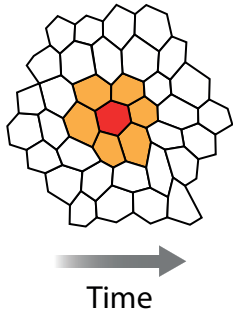
Apoptotic cell

Protected cell

Transient effect
over short distance

Prolonged effect
over long distance

Single
Event



Tissue
Scale

