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1 **Cell Extrusion: Crowd Pushing and Sticky Neighbours**

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7 **Summary**

8 Cell extrusion is a highly coordinated process allowing the removal of an epithelial cell
9 from the tissue layer without impairing its sealing. While previous works already showed
10 that extrusion is driven both by the extruding cell and its neighbours, two new studies in
11 this issue shed a new light on even more complex cell-cell coordination at play during cell
12 extrusion.

13 **Main text**

14 Epithelia are made of tightly connected cells acting as mechanical and chemical barriers.
15 They are also incredibly dynamics during morphogenesis or during homeostasis through
16 rapid cell turnover. Accordingly, epithelia can remove cells either during normal
17 homeostatic process[1] or for the elimination of aberrant cells through cell competition[2,
18 3] . Yet, these removals do not compromise the barrier function of epithelia as they are
19 driven by cell extrusion: a succession of remodelling steps leading to cell expulsion while
20 maintaining epithelium sealing[4].

21 Cell extrusion is a highly coordinated multicellular process involving active contraction of
22 the extruding cell and its neighbours[4-7]. Seminal works in MDCK cells have outlined
23 two steps of contraction. A contractile actomyosin ring is first formed in the extruding
24 cell[5]. This will pull cell neighbours and triggers the formation of a supra-cellular
25 actomyosin ring in the neighbouring cells[4, 5, 8]. The ring slides basally and eventually
26 pushes the cell out apically while bringing neighbours together[5]. The formation of the
27 actomyosin ring is driven by juxtacrine communication between the extruding cell and
28 neighbours through Sphingosine-1-phosphate (S1P) and S1P2 receptor[9], leading to

29 microtubule reorganisation and Rho activation[10]. The actomyosin ring also relies on
30 strong mechanical coupling between cells mediated by E-cadherin (E-cad) adhesion[8,
31 11]. Indeed, when E-cad is depleted sealing and extrusion defaults appear[11]. This
32 requirement is counter-intuitive as adherens junctions eventually need to be
33 disassembled to allow cell detachment[12]. Accordingly a gradual reduction of E-cad
34 levels at the interface between the extruding cell and its neighbours was observed in
35 several systems[6, 13]. How the tissue maintains then mechanical coupling and prevents
36 tearing despite the increased contractility remained so far mysterious.

37 In this issue, two new studies outline the complex cell-cell coordination at play during cell
38 extrusion. In the first study, Thomas and colleagues addressed the question of adhesion
39 maintenance during MDCK cell extrusion and characterised for the first time the
40 behaviour of Desmosomal Junctions (DJs)[14]. Using UV induced apoptosis to trigger cell
41 extrusion, they first show that in contrast to adherens junctions, DJs between the
42 extruding cell and its neighbours are conserved throughout extrusion (**Figure 1 A-D**).
43 Moreover, new DJs are formed at the basal side of neighbouring cells prior to old DJs
44 disassembly (**Figure 1 C**). Thus adhesion between the extruding cell and its neighbours
45 is constantly maintained during extrusion through DJs. DJs remodelling involved constant
46 turnover of DJs components (assessed by FRAP), and inhibiting DJs turnover prevented
47 cell extrusion. To dissect the mechanism of DJs remodelling, the authors focused then
48 on intermediate filaments. Using a photo-convertible Keratin-18, they could show that new
49 Keratin 18 was recruited in the neighbouring cells near the extruding cell interfaces. More
50 importantly, the Keratin filaments progressively realigned toward the extruding cell and
51 bear higher tension (**Figure 1 C,D**). This suggested that DJs anchored forces during
52 extrusion and could be involved in cell-cell mechanical coupling. The contractile
53 actomyosin cable is a key component of extrusion in MDCK cells. Interestingly, the cable
54 is in close vicinity with DJs at the beginning of extrusion and get detached later (**Figure**
55 **1 B,C**). The detachment correlates with a loss of DJs straightness, an indication of tension
56 reduction . This suggested that DJs are mechanically coupled to the actomyosin ring and
57 may be required to transmit contractile forces during extrusion.

58 To test the functional role of DJs during extrusion, the authors either knocked-down
59 Desmoplakin (a component of DJs) by siRNA or prevented keratin linkage to DJs using

60 a dominant negative Desmoplakin. Global downregulation of Desmoplakin reduced actin,
61 Myosin IIA and RhoA junctional levels as well as tissue tension. In this condition, half of
62 the apoptotic cells displayed defective extrusions. Similar defects were observed upon
63 DJs knockdown either in the extruding cell or in the neighbours. More importantly, they
64 could be rescued by RhoA activation, suggesting that extrusion defects were mostly
65 driven by the downregulation of tension upon DJs knockdown. Last but not least,
66 extrusions were also frequently associated with tissue tearing upon DJs knockdown. In
67 conclusion, Thomas and colleagues demonstrated that DJs are essential for extrusion by
68 ensuring mechanical coupling between the extruding cell and its neighbours throughout
69 the process, which is required to maintain cell-cell adhesion and build up tension.

70 In the other study, Takeuchi and colleagues characterised an unexpected contribution of
71 distant cells (3-16 cells) to cell extrusion coordinated by Calcium (Ca^{2+}) waves originating
72 from the extruding cell[15]. Oncogenic cells (including cells expressing an active form of
73 Ras, Ras^{V12}) surrounded by wild type cells are removed from the epithelial layer through
74 apical extrusion (a process called EDAC: Epithelial Defence Against Cancer[2]). By
75 performing live imaging of Ca^{2+} after induction of Ras^{V12} in a subset of MDCKC cells, they
76 observed acute bursts of Ca^{2+} in oncogenic cells which rapidly propagated to neighbours
77 through a so-called trigger wave mechanism[16] (**Figure 2 A-C**). Inhibition of IP3R
78 (Inositol-3-Phosphate Receptor, responsible for ER Ca^{2+} release) or TRPC1 (a Ca^{2+}
79 mechanosensitive channel) suppressed both the initial calcium burst in the Ras^{V12} cells
80 and the waves, while gap junction inhibition only affects their propagation. Interestingly,
81 the waves preceded apical extrusion in 70% of the cases, suggesting that they may
82 promote cell extrusion. Accordingly suppressing the wave using the conditions mentioned
83 above systematically abolished apical extrusion and also prevented the formation of the
84 actomyosin ring normally associated with cell extrusion.

85 To investigate the mechanism by which the wave promotes cell extrusion, the author first
86 looked at neighbouring cell behaviour. Upon Ca^{2+} increase, neighbouring cells exhibited
87 polarised vertices movement toward the extruding cell (**Figure 2 C,D**). The polarised
88 movements are concomitant in space and time with a cytosolic and a perinuclear
89 accumulation of actin, which is mediated by the inverted formin 2 (INF2) (**Figure 2 C**).
90 Inhibition of the calcium wave through TRPC1 knockdown was sufficient to prevent actin

91 relocalisation and the convergent cell movements. Similarly, INF2 knockdown also
92 prevented actin relocalisation, convergent movements and impaired Ras^{V12} cell extrusion.
93 Altogether, this suggests that calcium waves facilitate extrusion by inducing actin
94 reorganisation which drives a collective convergent movement toward the extruding cell.
95 This movement initiates apical oncogenic cell constriction most likely through pushing
96 forces and precedes the actomyosin ring formation. Importantly, the authors suggest that
97 this mechanism may be quite universal. Indeed, similar Ca²⁺ waves were preceding and
98 required for Ras^{V12} cell extrusion in zebrafish embryo. Ca²⁺ waves were also observed
99 upon apoptosis induction through Caspase8 expression in MDCK cells or though laser
100 induced apoptosis in zebrafish, with similar actin relocalisation and collective movements.
101 However in these cases the waves are more accessory and only affect the speed of
102 extrusion.

103 To conclude, these two studies illustrate in very different ways the complexity of cell-cell
104 coordination during cell extrusion. The work of Thomas M. and colleagues demonstrate
105 that components from other adhesive complexes, here desmosomal junctions, are also
106 essential for extrusion. Their late maintenance and the overlap with *de novo* DJs
107 formation is essential to maintain epithelial sealing and to build-up the tension required
108 for cell constriction. This may solve the apparent contradiction between early E-cad
109 depletion and the building-up of contractility in the extruding cell. So far, most of the
110 studies of extrusion have focused on the role of actomyosin and E-cad. This study nicely
111 illustrates the need to explore the role and the dynamics of other cytoskeletal and
112 adhesive components during extrusion (such as extracellular matrix adhesion [17] or tight
113 junctions).

114 In the other manuscript, Takeuchi and colleagues highlight an unexpected long range
115 contribution of the surrounding cells to extrusion through collective convergent
116 movements driven by a calcium wave. The wave and its contribution to extrusion is
117 conserved from mammalian cells to zebrafish and is observed both during EDAC and
118 apoptotic cell elimination. It is striking that two very different mediators of extrusion
119 (caspases or EDAC) can trigger a similar molecular response. Since the wave is not
120 preceded by any visible morphological changes, what initiates the first calcium burst
121 remains quite mysterious. The requirement of stretch sensitive channel also for the first

122 calcium burst suggests that membrane tension may increase very early on in apoptotic
123 cells or Ras^{V12} cells (without obvious changes of cell shape). The recent development of
124 live membrane tension sensor may help to explore this hypothesis[18]. Moreover, it is
125 very difficult at this stage to connect the perinuclear relocalisation of actin with the
126 convergent movement of cells. Interestingly, these waves share many similarities with a
127 recent description of ERK waves observed in vicinity of apoptotic cells and BRAF
128 transformed cells[19]. ERK waves also trigger collective movements toward the
129 transformed cells which drive their apical extrusion. Finally, very similar calcium waves
130 and collective movements were observed in various models of epithelial wound
131 healing[20]. After all, cell extrusion may not be so different from a regular wound healing
132 process.

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186

187 **Figures legend**

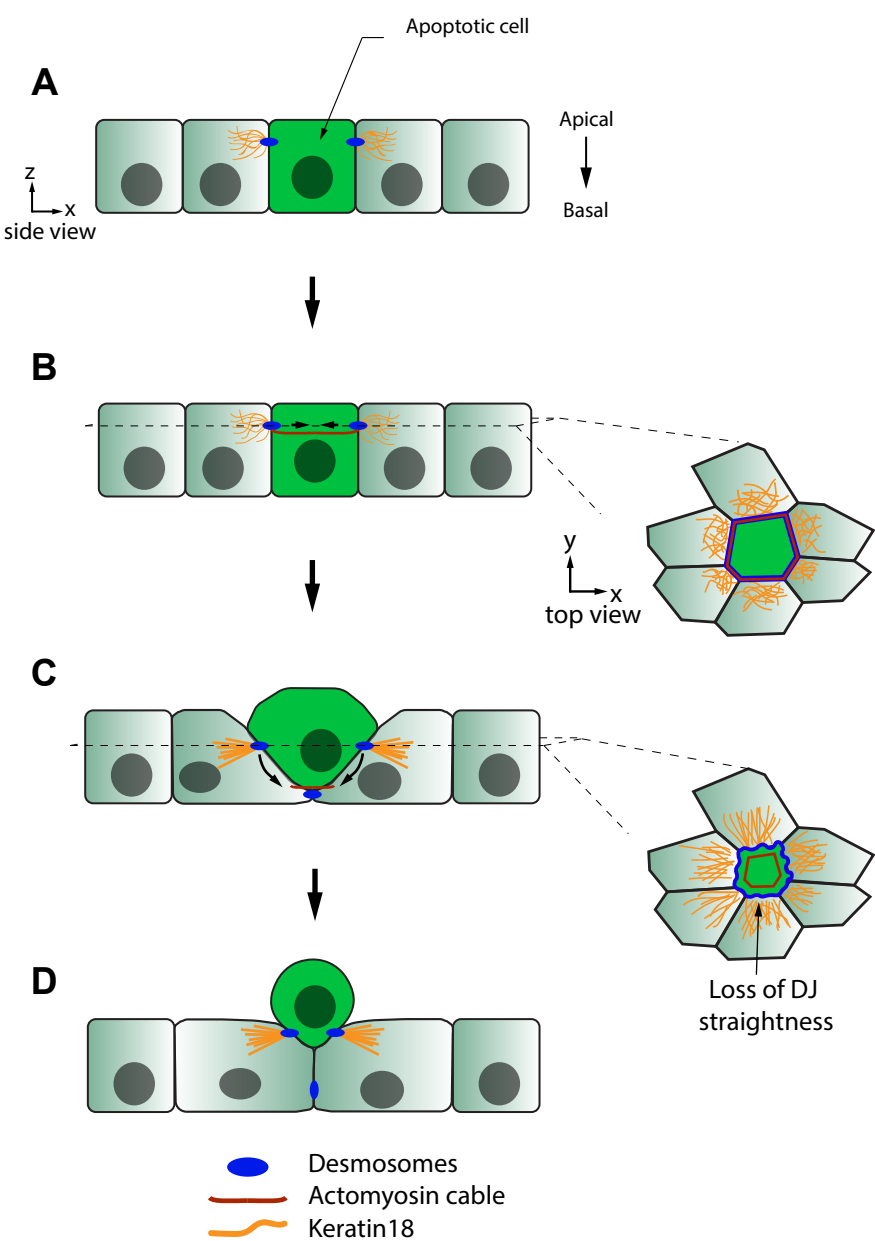
188 **Figure 1: Dynamics of desmosomal junctions during cell extrusion**

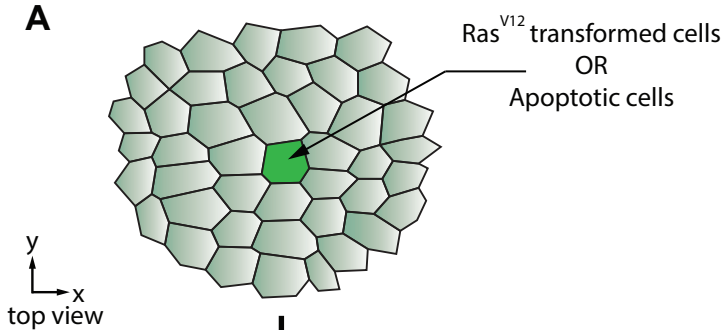
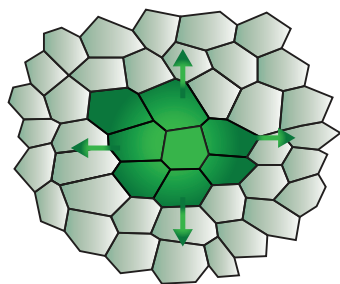
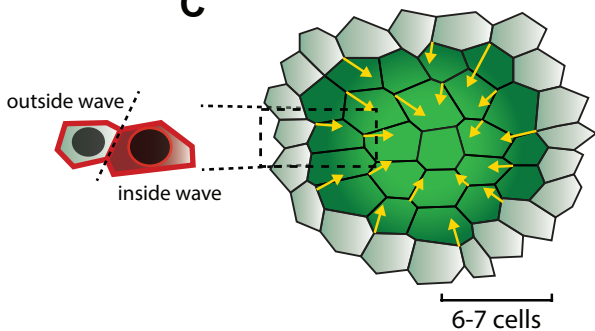
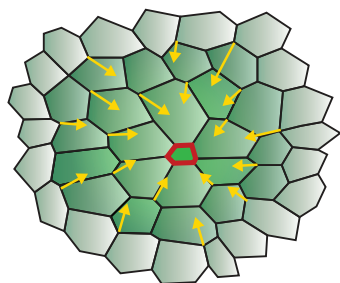
189 Adapted from [14]. **A:** UV-induced apoptotic cell extrusion in MDCK cells. **B:** Desmosomal
190 Junctions (DJs, blue) are dynamics and stay intact during extrusion. An actomyosin ring
191 forms in the vicinity of desmosomes (red). **C:** The actomyosin ring detaches from
192 desmosomes and is displaced basally (black arrows). This correlates with loss of DJ
193 straightness (side panel, apical view) and triggers reorientation and accumulation of
194 Keratin18 filaments in neighbours (yellow lines). New desmosomes are formed between
195 neighbouring cells beneath the extruding cells. **D:** The newly formed desmosomes mature
196 while the cell is being expelled from the layer. Axis show the direction of the view : xy =
197 top/apical view, zx = side view.







198 **Figure 2: Calcium wave and collective movements during cell extrusion**

199 Adapted from [15]. **A:** Cell extrusion is triggered in MDCK cells or zebrafish either by a
200 transformed Ras^{V12} cells surrounded by WT (EDAC) or through overexpression of
201 Caspase-8. **B:** Calcium (Ca²⁺, green) level increases in the extruding cell and propagates
202 to the neighbours (green arrow). **C:** Ca²⁺ wave stops 3-16 cells away. The wave triggers
203 cytosolic and perinuclear actin relocalisation (in red, side panel). Cells in the wave exhibit
204 polarised vertices movement toward the extruding cell (yellow arrows). **D:** Coordinated
205 vertices movement persist after wave termination. Eventually actomyosin ring is formed
206 around the extruding cell (red) and is followed by the termination of extrusion.

207



A**B****C****D**

-  Calcium Wave
-  Calcium propagation
-  Vertices movement
-  Actin
-  Cytosolic actin
-  Cell nucleus