Cell Extrusion: Crowd Pushing and Sticky Neighbours

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Summary

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

Main text

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

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

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

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

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

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

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

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

relocalisation and the convergent cell movements. Similarly, INF2 knockdown also prevented actin relocalisation, convergent movements and impaired Ras^{V12} cell extrusion. Altogether, this suggests that calcium waves facilitate extrusion by inducing actin reorganisation which drives a collective convergent movement toward the extruding cell. This movement initiates apical oncogenic cell constriction most likely through pushing forces and precedes the actomyosin ring formation. Importantly, the authors suggest that this mechanism may be quite universal. Indeed, similar Ca^{2+} waves were preceding and required for Ras^{V12} cell extrusion in zebrafish embryo. Ca^{2+} waves were also observed upon apoptosis induction through Caspase8 expression in MDCK cells or though laser induced apoptosis in zebrafish, with similar actin relocalisation and collective movements. However in these cases the waves are more accessory and only affect the speed of extrusion.

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

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

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

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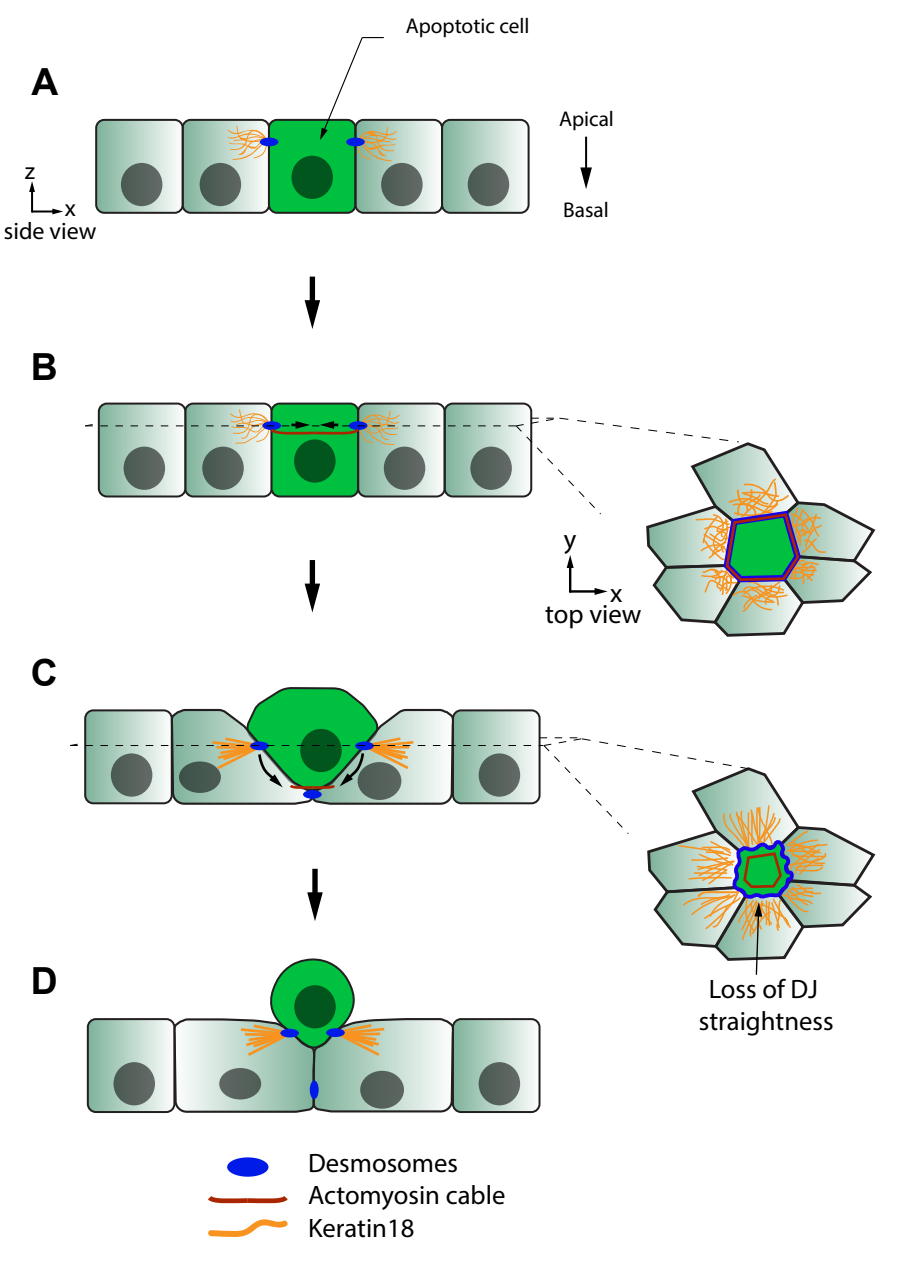
Figures legend

Figure 1: Dynamics of desmosomal junctions during cell extrusion

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

Figure 2: Calcium wave and collective movements during cell extrusion

Adapted from [15]. **A:** Cell extrusion is triggered in MDCK cells or zebrafish either by a transformed Ras^{V12} cells surrounded by WT (EDAC) or through overexpression of Caspase-8. **B:** Calcium (Ca^{2+}, green) level increases in the extruding cell and propagates to the neighbours (green arrow). **C:** Ca^{2+} wave stops 3-16 cells away. The wave triggers cytosolic and perinuclear actin relocalisation (in red, side panel). Cells in the wave exhibit polarised vertices movement toward the extruding cell (yellow arrows). **D:** Coordinated vertices movement persist after wave termination. Eventually actomyosin ring is formed around the extruding cell (red) and is followed by the termination of extrusion.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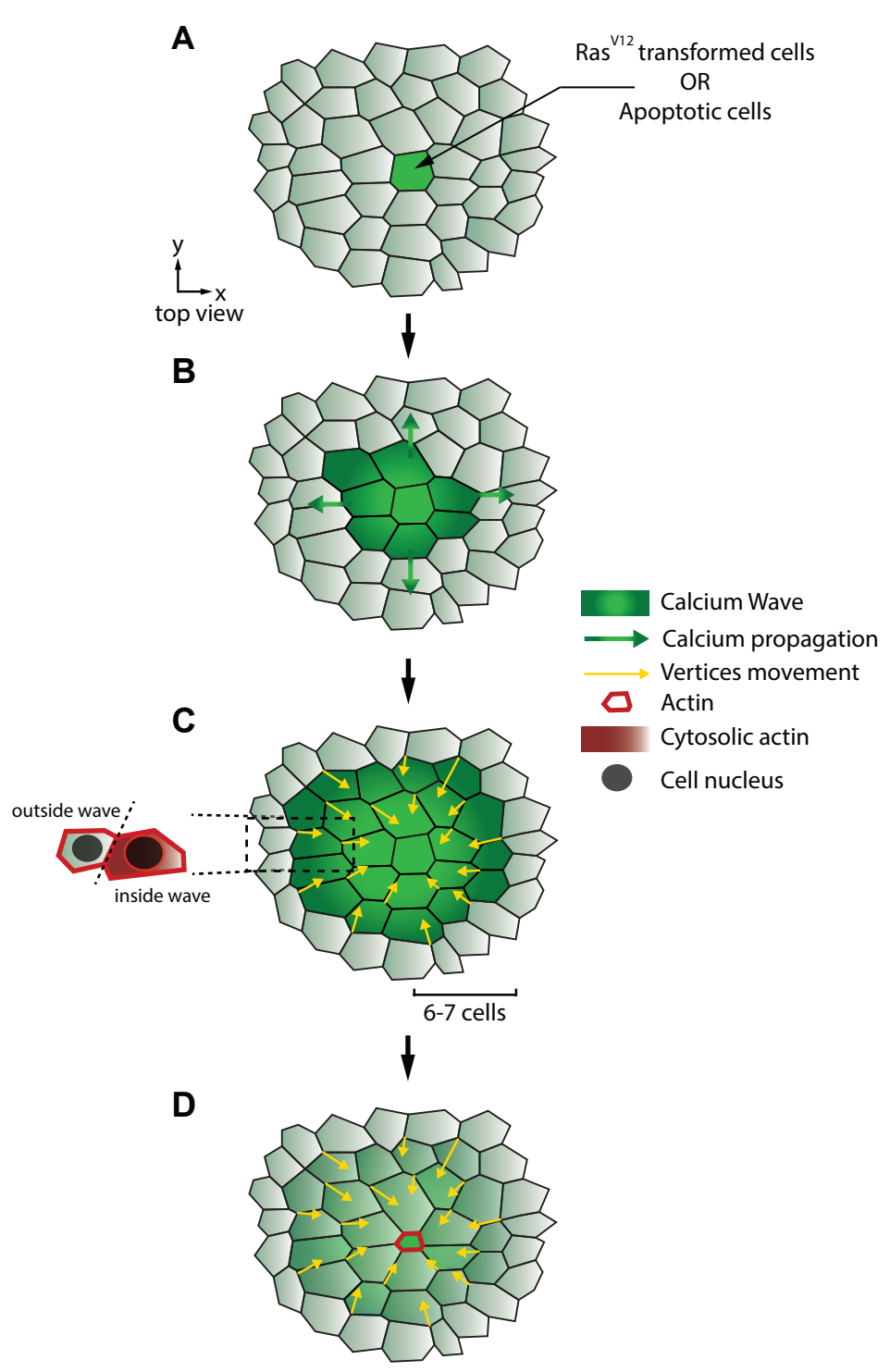
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