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New emerging roles for epithelial cell extrusion

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Abstract

Epithelia use a unique process called ‘cell extrusion’ to remove cells from a layer, while preserving their barrier function. Specifically, a cell destined to die triggers formation of an actin- and myosin-ring in the live neighboring epithelial cells surrounding it, which squeeze the dying cell out. During extrusion, the surrounding cells expand toward one another and meet to fill the gap left by the extruded cell. Recent studies have revealed new roles of extrusion in controlling developmental morphogenesis, maintaining homeostatic cell numbers, and how this process is usurped during bacterial pathogenesis. Here, we review recent advances in new processes that require cell extrusion and the signaling pathways controlling it.

Introduction

Epithelia made of one or two cell layers encase and protect organs. The cells comprising epithelia are constantly turning over by cell division and death. Cell death, or apoptosis, could compromise the barrier function of the epithelium, but it does not. Instead, epithelia have devised a process called ‘cell extrusion’ to remove cells from a layer, while preserving their barrier function [1]. Specifically, an epithelial cell destined to die triggers formation of an actin- and myosin-ring in the live neighboring cells surrounding it, which then squeeze it out (Figure 1). Extrusion occurs without disrupting epithelial continuity because the surrounding cells constrict and meet to form new multi-cellular junctions below the extruding cell [1–2]. While extrusion clearly functions to maintain epithelial barrier function, its discovery during different developmental morphogenetic events in *Drosophila* and a variety of vertebrates has led to an understanding that extrusion may be controlling more than just barrier function. While extrusion removes cells apically in most species, cells predominantly extrude basally during *Drosophila* development, where it is typically referred to as delamination (Figure 1). Here, we discuss new emerging roles for cell extrusion in controlling developmental morphogenesis, maintaining homeostatic cell numbers, and how this process is hijacked during bacterial pathogenesis. Because extrusion plays such an important role in many processes, we also discuss new insights into the signaling pathways that control extrusion.

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Live cell extrusion maintains epithelial homeostasis

Maintenance of epithelial function requires that cell death be exquisitely coupled to cell proliferation. Little was known about how these two processes were linked; yet misregulation of this balance could lead to excess cells or too few cells, which could result in tumor formation or barrier function diseases, respectively. Recently, however, two independent studies demonstrated that overcrowding induces extrusion of live cells to maintain epithelial homeostasis [3••–4••]. Both studies found that during cell turnover in colon epithelia, zebrafish epidermis, and fly notum, very few cells undergo apoptosis within the epithelium, but are instead first pushed out by live cell extrusion. Live cell extrusion occurs at the locations with highest cell densities, such as the fin edges of zebrafish, colon surfaces, and the midline of fly notum. Cell crowding forces result from cell proliferating and migrating from other sites within the epithelium. For instance, in the intestinal epithelium, stem cells divide within the crypts, differentiate, and migrate to the tips of the villi, which become 1.8 times more crowded than crypts [4••]. Individual cells then extrude at the crowded villus tips [5–6] at a rate of approximately 10^{11} cells per day in the human small intestine [5]. Marinari *et al.* found that blocking cell growth eliminates the crowding and subsequent delamination, suggesting that crowding forces from proliferation drive delamination [3••].

To test if the overcrowding seen *in vivo* can directly induce epithelial cell extrusion, Eisenhoffer *et al.* cultured MDCK cells to confluence on a stretched silicone membrane and then releasing them from stretch [4••]. Using this device, they found that cell crowding triggers extrusion. This also enabled them to dissect the signaling pathway controlling crowd-induced extrusion (referred to below). In doing so, they were able to block homeostatic extrusion in zebrafish larvae, which resulted in formation of epidermal cell masses, suggesting that extrusion is critical for epithelial cell turnover [4••]. Additionally, they found that normally live extruded cells eventually die through a process termed anoikis, or apoptosis due to loss of survival signaling [7–8] but if replated directly were competent to proliferate into a new monolayer [4••]. Similarly, *Marinari et al.* observed cells dying after their complete removal from the epithelial monolayer in the developing fly. These two studies, demonstrate that cell division and migration within epithelia cause overcrowding, which induce live cells to extrude and die in order to maintain epithelial homeostasis.

Developmental *Drosophila* morphogenesis requires epithelial cell extrusion

Epithelial cell extrusion plays a key role in several epithelial-based morphogenesis events during *Drosophila* development. An important process for shaping of the three-dimensional body plan during development is the dynamic replacement of earlier epithelial cell populations with more mature ones [9]. Apoptotic epithelial cell extrusion has been shown to be a driving force for *Drosophila* epithelial sheet movement and fusion in distinct developmental processes, such as during dorsal closure and development of adult abdominal epidermis [10–11] (Figure 2). Dorsal closure is a process whereby two lateral epithelial cell sheets advance to progressively cover a transient epithelium covering the dorsal side called the amnioserosa (AS). Initially, the lateral epidermis migrates as a sheet to the midline until it forms an eye-shaped opening. As this happens, the whole AS contracts by apically constricting. Simultaneously, cells extrude underneath the epithelium to the interior of the embryo [12]. The combined AS constriction and extrusion helps drive closure of the attached lateral epidermis [11]. AS cell extrusion is estimated to contribute between one third and one half of the net force required to drive dorsal closure [11]. Following dorsal closure, the extruded AS cells die by anoikis and are engulfed by hemocytes, or early macrophages [13].

Later, during metamorphosis from the larval to pupal stage, another type of epithelial cell replacement occurs. Pupal epidermal cells, histoblasts, replace the larval epidermal cells (LECs) that coat the abdomen. Histoblasts are initially dormant and organized in nests localized around the LECs until pupation when they expand rapidly and fuse to replace LECs, which die and extrude basally [10,14]. Cell sheet replacement requires epithelial cell extrusion of the LECs, as inhibition of apical myosin contraction in these cells results in their retention. Blocking LEC apoptosis slows cell replacement and, interestingly, causes the histoblasts to die and extrude instead [10]. Conversely, blocking histoblast cell division by Dacapo overexpression increases LEC survival, suggesting that cell competition rather than programmed cell death drive extrusion and epithelial cell replacement [10]. Ninov *et. al* found that LECs secrete Decapentapalegic (Dpp, a TGF-beta/BMP homolog) that recruits histoblasts and keeps the LECs alive until the histoblasts force them out [15•]. Moreover, artificial boundaries of Dpp signaling in *Drosophila* wing disc epithelia cause live cells to extrude [16–17], as do discrete developmentally induced boundaries of Dpp expression in the fly leg disc [18]. Thus, autocrine and paracrine Dpp signaling may ensure maintenance of a protective barrier as the epidermis undergoes developmental transitions [15•].

When considering these findings on how epithelial cells are replaced, it may be useful to consider that epithelial crowding forces can also control extrusion [3••–4••]. Just as crowding forces control epithelial refinement in the pupal notum by forcing cells to compete for limited space, mechanical forces from movements of new epithelial cell populations during dorsal closure or abdominal cell replacement may also act to extrude the old epithelial cell populations (Figure 2). For instance, when histoblast proliferation is blocked, there is no extrusion of LECs [10], suggesting that normally the new cells similarly force out the old. Additionally, during dorsal closure, higher rates of cell ingression occur closest to the enveloping lateral epidermis, where more force is present [19•] and inhibiting cell death in one tissue can accelerate rates of cell delamination in an adjacent tissue [20]. Both findings support an additional role for force and cell competition controlling epithelial cell replacements during development.

Extrusion facilitates intestinal invasion and dissemination of bacterial pathogens

A variety of enteric pathogens have found ways to hijack extrusion to invade host gut epithelium (Figure 3, left panel). *Salmonella, Vibrio parahaemolyticus*, and enterohemorrhagic *E. coli* (EHEC) can induce epithelial cell extrusion [21••–23•]. Unlike extrusion induced by apoptotic stimuli or crowding, enteropathogens use a variety of factors to trigger extrusion. Salmonella infection triggers caspase-1activation, which activates both IL-18, a cytokine regulator of inflammation, and extrusion, since inhibiting caspase-1 greatly reduces extrusion rates [21••]. On the other hand, expression of the EHEC protein EspM-2 directly activates RhoA to trigger extrusion in the absence of any apparent cell death [23•]. Alternatively, extrusion of pathogen infected epithelial cells could result indirectly from activating proinflammatory cytokines such Tumor Necrosis Factor, which can also activate apoptotic extrusion [24–26]. Release of Tumor Necrosis Factor or other cytotoxic factors may also cause the extensive extrusion that leads to loss of epithelial barrier function, inflammation, and cavities in the small intestinal epithelium associated with *Vibrio parahaemolyticus* and *Salmonella* infections [21••–22•].

Pathogen-induced cell extrusion could benefit the pathogen or the host. Although inducing extrusion could enable the release of pathogen-infected cells into the lumen to promote their spread [21••], it could also help the host eliminate adherent bacteria. It may be for this reason that both EHEC and *Salmonella* produce a virulent factor, OspE that inhibits cell shedding by stabilizing focal adhesion by binding integrin-linked kinase [27]. Therefore,

these pathogens could essentially trigger apoptosis but prevent extrusion as a way to increase epithelial cell permeability and make it more susceptible to invasion (Figure 3, middle panel). Understanding how these different pathogens promote or inhibit extrusion also gives us hints about how factors like Rho, caspase-1, and integrin-linked kinase may normally regulate extrusion.

For *Listeria monocytogenes*, extrusion provides an opportunity for cell entry (Figure 3, right panel) [28]. Pentecost et al. showed that *L. monocytogenes* bind junctions during cell extrusion at the villus tips [28]. *L. monocytogenes* invasion requires binding of its surface protein Internalin A with host E-cadherin. Because E-cadherins are engaged in cell-cell interactions and, therefore, not exposed in a gut epithelium, *L. monocytogenes* bind E-cadherins that are transiently exposed as adherens junctions are remodeled during cell extrusion [28]. Entry of this pathogen then takes advantage of E-cadherins endocytosing to invade the cell and proliferate [29••]. These beautiful studies showing how *L. monocytogenes* can hijack extrusion to gain access to the cell cytoplasm also reveal new information on the mechanism of extrusion. By rapidly recycling adherens junctions through endocytosis, the epithelium can maintain a tight barrier throughout the extrusion process.

Signaling pathways controlling epithelial cell extrusion

Since extrusion is involved in so many important physiological processes, it is essential to understand signaling pathways that drive cell extrusion. Recent studies in vertebrate systems have revealed that the Sphingosine 1-Phosphate (S1P) pathway controls both apoptotic and live epithelial cell extrusion [4,30••]. S1P is a bioactive sphingolipid that regulates diverse cellular processes including survival, proliferation, and cytoskeleton remodeling in a paracrine/autocrine fashion [31]. Most S1P driven processes are mediated through five specific G-protein coupled receptors (designated $S1P_{1–5}$) located on the cell surface. Cells destined to extrude produce and release S1P, which binds to $S1P_2$ on the surface of surrounding cells to trigger formation and contraction of an actin/myosin ring [30••]. p115 RhoGEF, a guanine nucleotide exchange factor (GEF) for Rho GTPase, and Rho, both of which are $S1P_2$ effectors, are also required for epithelial cell extrusion [1,32]. While the S1P-$S1P_2$ pathway is critical for both live during homeostasis and dying cell extrusion in response to apoptotic stimuli, different signaling activates these pathways in either case (Figure 4). Generation of S1P during apoptotic cell extrusion requires apoptotic stimuli [26,30••]. In contrast, crowding-induced live cell extrusion requires the stretch activated channel Piezo 1 [4••], an ion channel activated by mechanical deformation of the plasma membrane [33]. Because S1P is a key regulator of macrophage and T-cell migration and function [34], it will be interesting to determine if pathogen-induced extrusion also uses S1P, which could also activate an immune cell response. Additionally, future studies will also need to determine if the S1P signaling pathway controls basal cell delaminations in Drosophila epithelia.

Signaling controlling the direction a cell extrudes

Because tumors typically upregulate survival signaling, the direction a tumor cell extrudes could be important for its fate. Apical extrusion appears to play a pivotal role in controlling cell numbers by removing excess cells and could eliminate tumor cells, even if their survival signaling was upregulated. On the other hand, basal extrusion could enable tumor cells to invade the tissue and potentially initiate metastasis. Interestingly, loss of the tumor suppressor adenomatous polyposis coli (APC) drives cells to extrude predominantly basally, whereas wild type cells typically extrude apically [35]. Mutations or loss of APC severely misregulate microtubule dynamics, which are critical for controlling the direction a cell extrudes. Dynamic microtubules target the microtubule-associated p115 RhoGEF to activate

contraction at the base of the cell and extrude it apically [35]. The lack of the microtubule-binding sites in *Drosophila* APC [36] could account for the basal extrusion in *Drosophila* epithelia. Future work will need to identify other mechanisms controlling the direction a cell extrudes and if basal extrusion could promote invasion for tumors where APC is mutated.

Concluding remarks

Since its discovery over ten years ago [1], several labs have found new roles for extrusion in driving epithelial cell turnover, morphogenetic changes during development, and bacterial pathogenesis. Future studies should bring new insights into the mechanisms that govern it and how they are misregulated to drive tumor initiation and progression, epithelial barrier function diseases, and enable pathogenic infection.

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•• of outstanding interest

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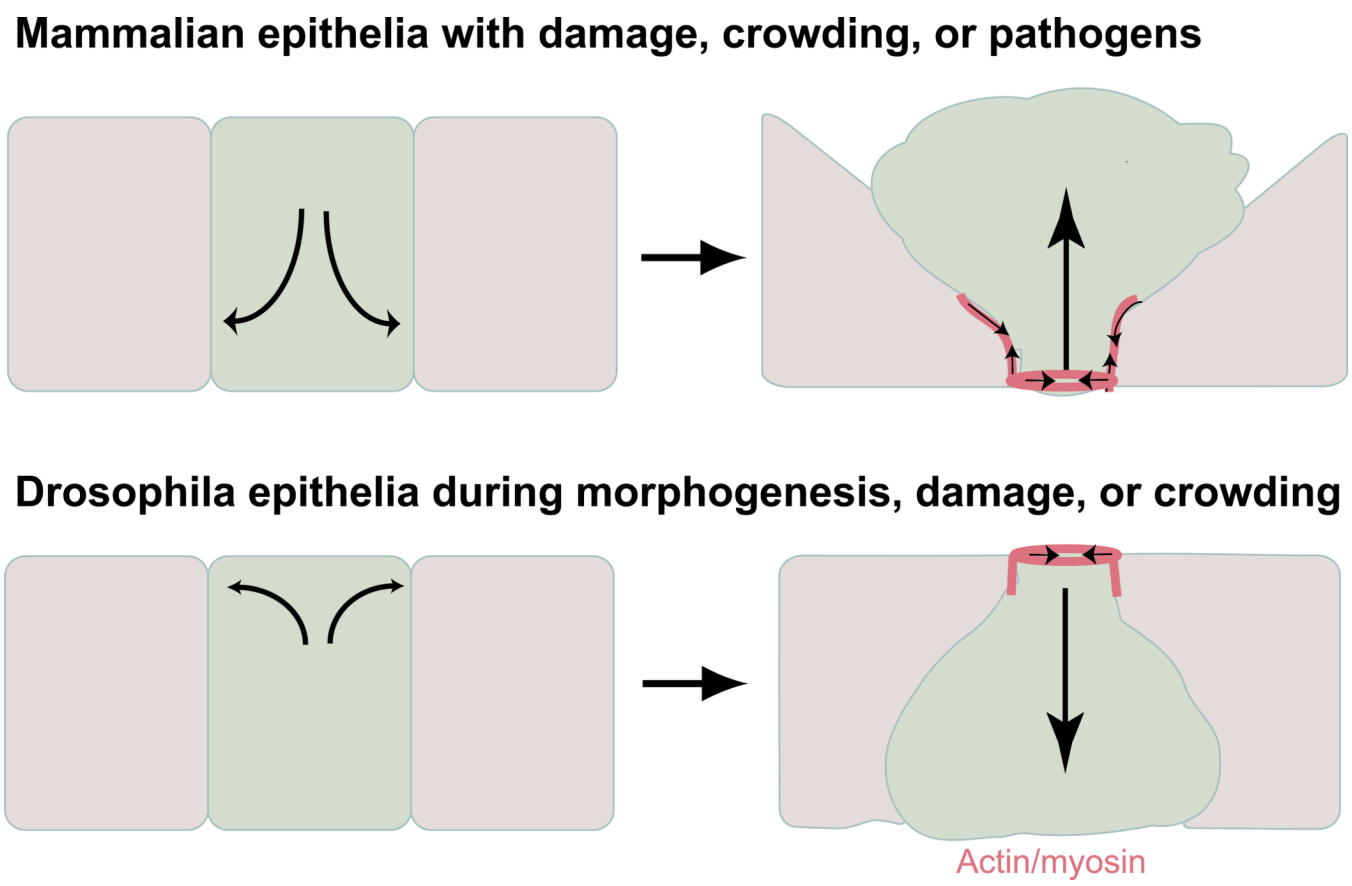

Figure 1.
Model for apical and basal (delamination) extrusion. Cells can be extruded apically (upper panel) or basally (lower panel) depending on the localization of actin/myosin (dark pink) contraction. Situations where these different types of extrusion occur are also indicated.

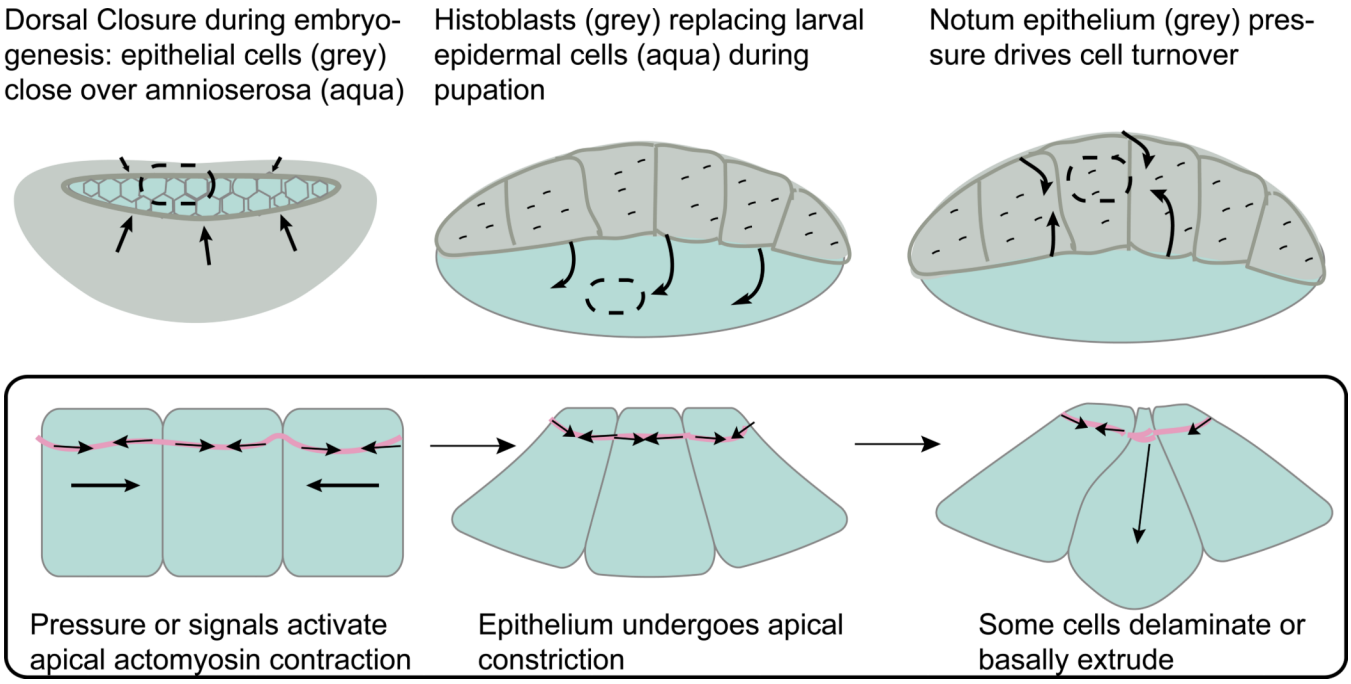

Figure 2.
Extrusion drives epithelia cell replacement during embryogenesis and pupariation. Insets (below) of cells apically constricting and extruding (delaminating) from oval regions.

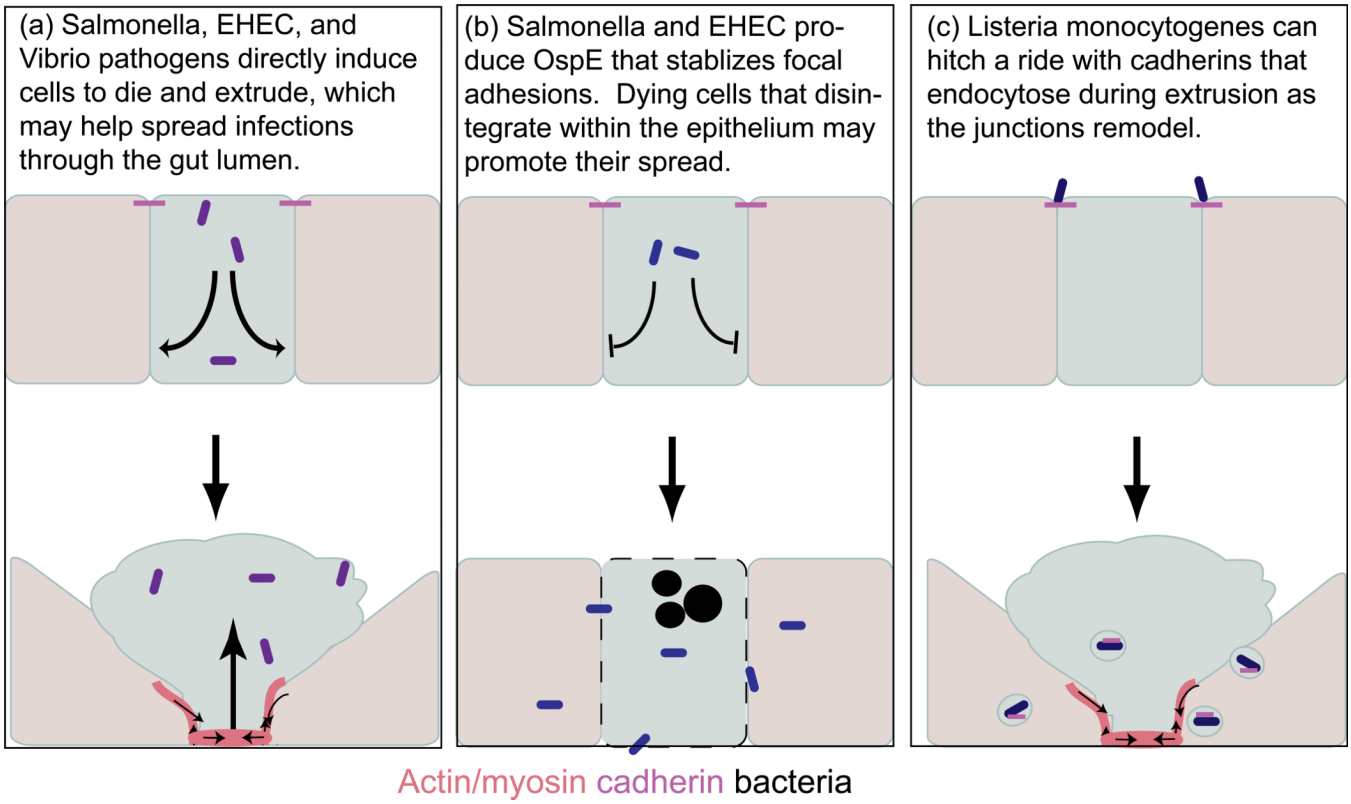

Figure 3.
Models showing how bacterial pathogens can highjack extrusion to invade host cells. Different pathogens can either induce (a) or block (b) extrusion, or take advantage of extrusion to enable invasion.

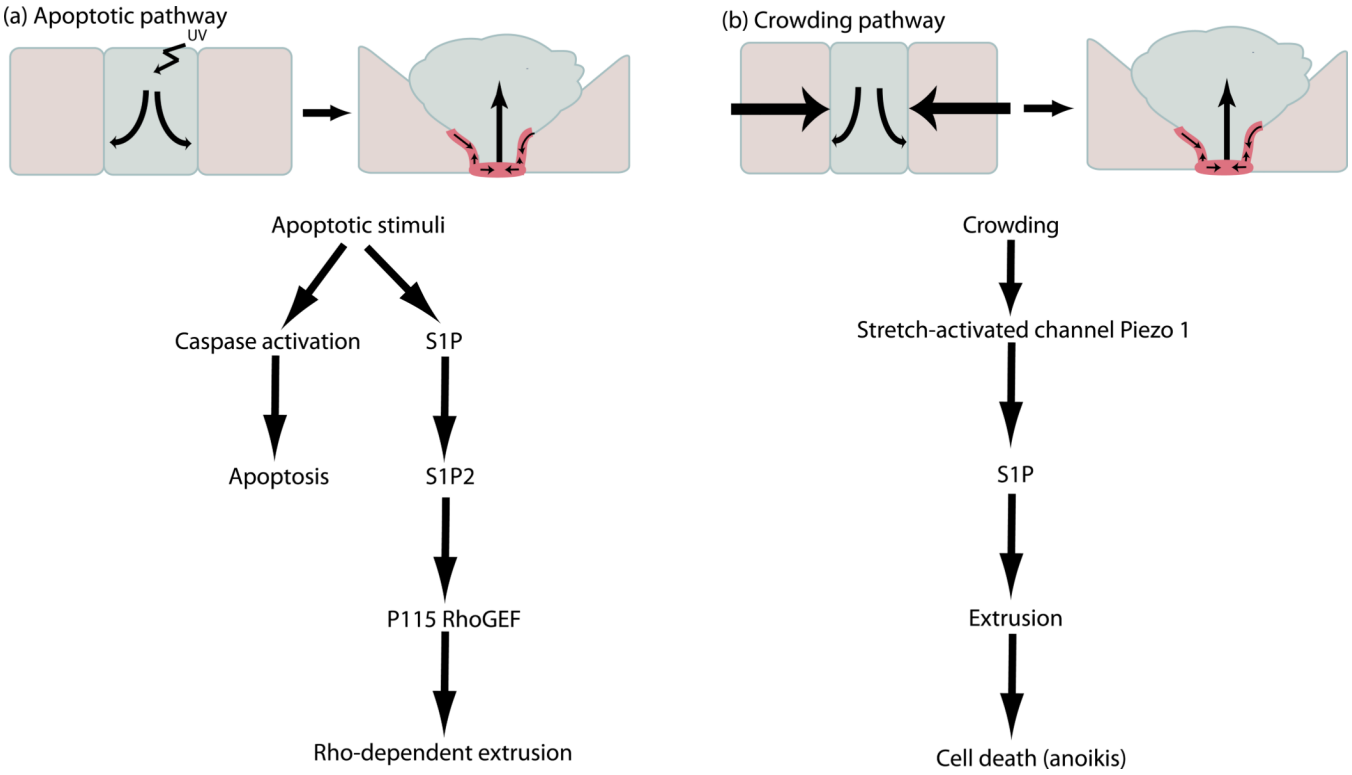

Figure 4.
The signaling pathways that control apoptosis- and crowding-induced extrusion. This schematic overview shows the most recent findings of important factors required for extrusion.