the yeast kinetochore and a foundation for future studies.

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http://dx.doi.org/10.1016/j.cub.2012.08.001

Programmed Cell Death: A New Way Worms Get Rid of Unwanted Cells

The genetics and predictable cell death lineages in *Caenorhabditis elegans* have been critical for identifying a conserved apoptosis pathway. Yet, cells still die in mutants that disrupt this pathway. A recent study shows that this death occurs by cell shedding.

Jody Rosenblatt

The simplified genetics of *Caenorhabditis elegans* was critical for elucidating the programmed cell death pathway conserved throughout most species [1–3]. Because these worms have a streamlined version of the conserved genetic pathway required for apoptosis, it had confused researchers for some time that, in certain instances, the apoptotic pathway is redundant [1]. A recent paper from Denning, Hatch, and Horvitz [4] demonstrates that cell shedding can compensate for the death pathway in a number of cells targeted to die by developmental programmed cell death. A number of *C. elegans* mutants in which apoptosis is inhibited at different stages of the pathway shed cells that then eventually die by a caspase-independent type of apoptosis.

One feature of *C. elegans* development that is critical for defining the apoptotic pathway is the ability to precisely predict which cells die, making it easy to identify those that do not. To investigate what controls cell shedding, the authors screened various mutants engineered to express GFP in cells that consistently shed when cell death is inhibited (i.e. in worms lacking the caspase CED-3). In cases where these GFP-positive cells do not shed, they instead divide, producing two GFP-positive cells. Thus, by screening for mutations that produced two GFP-positive cells, they found that cell shedding requires the genes PIG-1, a serine–threonine kinase related to AMP-activated kinase, and a complex that phosphorylates PIG-1, composed of LKB1, STADα and MO25α. By finding genes required for shedding, they discovered that the apoptotic and shedding pathways act redundantly. GFP-labeled cells would still die by programmed cell death in single *ced-3* or *pig-1* mutants, but in a double mutant lacking both the apoptotic and shedding pathways these cells instead divide and produce two cells of the same fate — in the example they studied, an excretory cell (Figure 1).

Although these findings suggest that shedding can compensate to promote cell death in cases where the apoptotic pathway is blocked, another possibility is that normally these cells can both die and be shed. Because *C. elegans* has highly efficient phagocytosis mechanisms, shed cells that are also targeted for cell death may be engulfed so rapidly that they are not apparent. Indeed, mutations in engulfment genes also produce 'floaters', suggesting that typically cells are shed but become engulfed so rapidly that they are not noticeable. Blocking the apoptotic pathway reveals shed cells because

they persist longer in the embryo without triggering engulfment. It is not clear how the shed cells in *ced-3* mutants eventually die; however, they may use a pathway similar to anoikis — a form of apoptosis triggered solely by loss of survival signaling [5]. This raises the question of whether shedding can compensate for apoptosis pathways in all cells programmed to die in the worm, or whether shedding only promotes death of cells derived from epithelial-like tissues, since anoikis has only been associated with epithelia [5].

The idea that the cells investigated in this study might die as a result of shedding is supported by recent findings that epithelia normally extrude cells prior to their death [6,7]. Eisenhoffer *et al.* [6] found that epithelial cells from a variety of tissues — human colon, developing zebrafish epidermis, and cell culture — all extrude or shed live cells, which later die by anoikis. In all cases observed, extrusions occurred at sites of high cell crowding. Further, experimentally crowding cultured epithelial monolayers in a mechanical device confirmed that crowding alone could induce extrusion of live cells. Live extruded cells, as *in vivo*, will later die but can also survive and proliferate if given a new substratum. Similarly, Marinari *et al.* [7] found that during *Drosophila* pupariation, developmental crowding forces drive live cells to extrude or 'delaminate'. While blocking cell death had no impact on live cell extrusion, blocking cell growth elsewhere in the epithelium blocked extrusion, suggesting that cell proliferation produces the crowding forces that then drive extrusion.

By using the cell crowding device and zebrafish genetics, Eisenhoffer *et al.* [6] identified proteins that mediate live cell extrusion following crowding and determined that extrusion normally drives cell death in epithelia. Although triggering cell death can activate extrusion through the apoptotic pathway [6,8,9], live cell extrusion during homeostasis or following experimental cell crowding requires the stretch-activated channel Piezo-1 [6]. Blocking this channel leads to the formation of cell masses, indicating that live cell extrusion drives epithelial cell death. Denning *et al.* [4] also point out that mutations in genes that

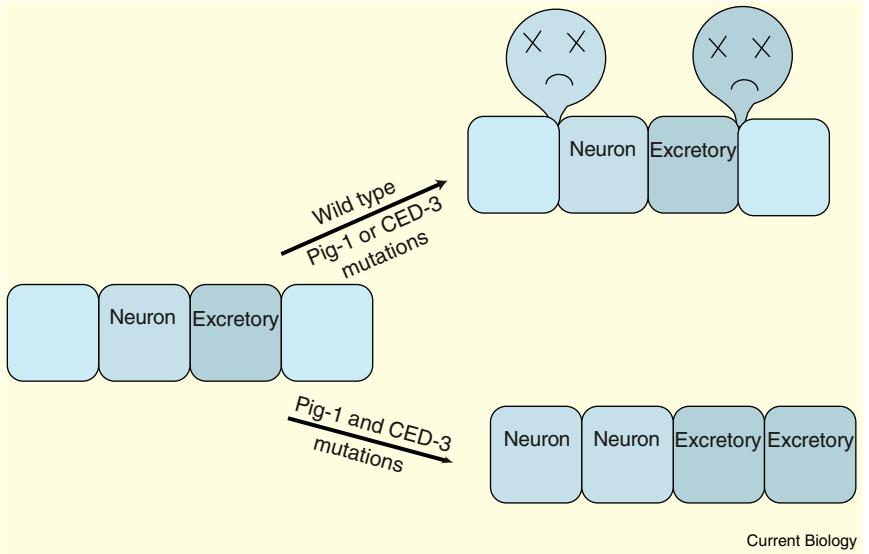

Figure 1. Cell death in developing *C. elegans* can occur through a canonical apoptosis pathway, cell shedding, or both.

During *C. elegans* development, cells targeted for programmed cell death still die in mutants where either apoptosis or shedding is blocked, here CED-3 or PIG-1, respectively (top pathway). However, in mutants in which both pathways are inhibited, these cells instead divide, producing two cells of the same fate (bottom pathway).

they found to be important for shedding results in greater epithelial defects than those arising from mutations in the apoptotic pathway [10–12]. Therefore, cell shedding in *C. elegans* may also promote apoptosis normally.

Does cell shedding in *C. elegans* use the same mechanism described to control epithelial cell extrusion? Epithelial cell extrusion occurs within all epithelia during homeostasis or following apoptotic stimuli. The ability to readily observe extrusion in zebrafish epidermis and tissue culture epithelia has enabled dissection of the cytoskeletal mechanics that drive this process. For extrusion, a cell destined to die produces the lipid sphingosine 1-phosphate, which binds to its receptor in the surrounding epithelial cells, resulting in the formation and contraction of an intercellular actomyosin ring that squeezes the cell out from the epithelium [9,13]. Although the prominent muscles just beneath the epidermis make it difficult to follow actomyosin-based processes in the *C. elegans* epidermis, the Hardin lab has developed tools to follow ventral enclosure of the epidermis during C. *elegans* development [14,15]. Based on the similarity of dorsal closure in *Drosophila melanogaster* to ventral enclosure in *C. elegans*, it is tempting to think that the cells that are shed from the ventral pocket during ventral enclosure in the worm also extrude or delaminate, as they do from the amnioserosa during dorsal closure in *Drosophila* (Figure 2A).

On the other hand, alternative mechanisms may drive cell shedding in *C. elegans*. While it is not clear whether all shed cells in *C. elegans* arise from epithelial-like cells, in the cases reported here, cells that are adjacent to shed cells appear to have epithelial adhesion proteins. However, cells shedding from the ventral pocket lack the cell adhesion proteins seen in their neighboring cells. This suggests that shedding occurs by a different mechanism from epithelial cell extrusion because extruding epithelial cells maintain contacts with their neighbors throughout the extrusion process (Figure 2A). Instead, a cell-sorting mechanism, similar to that seen when either oncogenic Ras or Src is induced within a monolayer, may drive cell shedding in *C. elegans* [16,17]. In these situations, cells with altered adhesion appear to exclude themselves from surrounding wild-type cells. The importance of the endocytic ARF GTPases in shedding [4,18] may indicate that shedding requires endocytosis of adhesion proteins. Loss of adhesion

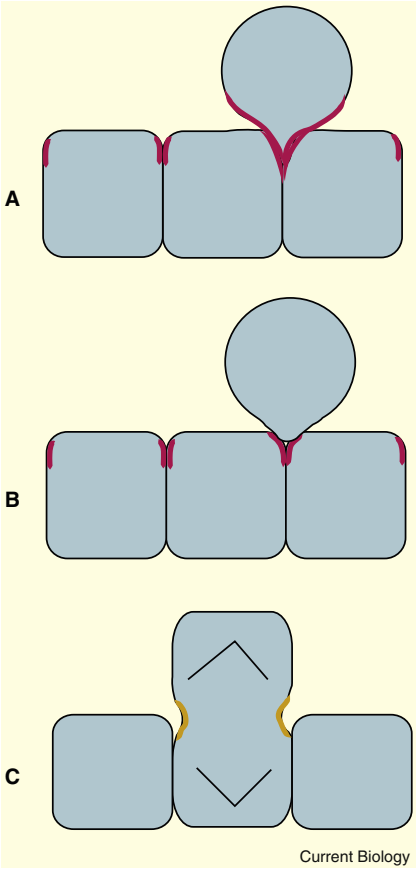

Figure 2. Possible mechanisms governing cell shedding in *C. elegans*.

(A) Shedding may occur by a mechanism similar to epithelial cell extrusion, where cell–cell contacts are maintained as a cell is squeezed out by an intercellular actomyosin ring in neighboring cells. (B) Loss of cell adhesion proteins in one cell may cause it to become excluded from its neighboring cells that maintain adhesion with each other. (C) Asymmetric cell division could produce one daughter cell that no longer maintains adhesive contacts to the matrix and dies by loss of survival signaling. (For all panels, red represents actomyosin and cell–cell adhesions, and yellow represents actin alone.)

proteins in one cell could act to exclude it from surrounding cells to promote its shedding during *C. elegans* development (Figure 2B).

Alternatively, cell shedding could occur by asymmetric cell division. PIG-1 is a kinase that is required cell autonomously for many asymmetric neuroblast cell divisions in *C. elegans* [19,20]. One possibility is that cell shedding in these cases could result from asymmetric divisions during differentiation. The daughter cell that has divided and no longer maintains contacts with the surrounding epidermis could die from lack of attachment to the matrix or other cells (Figure 2C).

Future studies may determine the mechanism by which these cells in developing *C. elegans* shed and die. Yet, the findings by Denning *et al.* [4] suggest that a variety of species have evolved ways of removing unwanted cells that can substitute for programmed apoptotic pathways and may even work in concert with them.

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http://dx.doi.org/10.1016/j.cub.2012.08.013

Nuclear Transport: Shifting Gears in Fungal Nuclear and Cytoplasmic Organization

In fungi, nuclear pore complexes are free to move through the nuclear envelope; however, little is known about how movement is regulated. New evidence reveals roles for molecular motors and potential impacts on genomic organization.

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In eukaryotic cells, mechanisms that modulate nuclear envelope function are critical for linking cytoplasmic events with nuclear gene expression, and vice versa. At the crux of this regulation are the nuclear pore complexes (NPCs), the large