

Minireview

Letting go: modification of cell adhesion during apoptosis

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Abstract

Apoptosis appears to be a carefully orchestrated process for the ordered dismantling of cells. A recent paper in *BMC Developmental Biology* shows that the disassembly of adherens junctions during apoptosis in *Drosophila* is progressive and requires the amino-terminal cleavage of the β -catenin Armadillo by the apoptotic effector caspase DrICE.

Apoptosis, a morphologically and mechanistically distinct form of programmed cell death, is essential for normal animal development and tissue homeostasis. The key executioners in apoptosis are caspases (cysteine aspartases), a family of proteases that have been conserved through much of animal evolution. Caspases are present as inactive precursor proteins in virtually all cells and are specifically activated by proteolytic cleavage. Their activation is regulated by both activators, which promote the conversion of the weakly active precursor caspase to the mature protease, and inhibitors, which prevent unwanted caspase activity and cell death [1]. One important family of caspase inhibitors comprises the inhibitor of apoptosis proteins (IAPs), which can directly bind to and inhibit caspases. In *Drosophila*, Diap1 is required to prevent inappropriate caspase activation and ubiquitous apoptosis. In response to death-inducing stimuli, antagonists of IAPs such as Reaper, Hid and Grim are produced to inactivate Diap1 and thereby remove the 'brakes on death'. Although caspases are often viewed as general destroyers of cellular components during apoptosis, there are now many studies showing that they can act with a great degree of local specificity to remove unwanted cellular compartments [2-4].

Cleavage by caspases can either activate or inactivate their substrates; for example, cleavage activates the Rho-associated kinase ROCK1, which promotes membrane blebbing [5,6], whereas proteolysis by a caspase inhibits the DNase inhibitor iCAD and unleashes DNA fragmentation by the CAD nuclease [7,8]. Among the very large number of caspase substrates identified so far, only a few have been linked to a specific apoptotic function. In a recent paper in *BMC Developmental Biology*, Kessler and Muller [9] describe one such example. They show that cleavage of the β -catenin homolog Armadillo (Arm) by the effector caspase DrICE in *Drosophila* is essential to regulate the adhesive properties of apoptotic cells.

Destabilizing adherens junctions

The protein β -catenin has two crucial functions in epithelial cells. It can act as a transcriptional coactivator in the Wnt signaling pathway (Wingless in *Drosophila*). It is also essential for maintaining the adherens junctions that link epithelial cells together; these contain multiprotein adhesion complexes composed of the adhesion molecule E-cadherin, β -catenin and α -catenin. E-cadherins on

adjacent cells initiate the assembly of an adhesion complex by homophilic binding of their extracellular domains. β -Catenin binds to the cytoplasmic portion of E-cadherin and connects it, via α -catenin, to the actin cytoskeleton. The linkage of cadherin to the cytoskeleton by β - and α -catenins is essential both for establishing cell-cell contacts and organizing the cytoskeleton.

To study the morphological changes in *Drosophila* apoptotic cells *in vivo*, Kessler and Muller used embryos genetically deficient in Diap1, in which apoptosis is activated in virtually all cells [9]. They define, morphologically and molecularly, two separate steps in the apoptotic process, revealing a progressive destruction of the adherens junction and shining new light on the mechanism by which the adhesive complexes are destabilized. During early apoptosis, Arm is cleaved and the amounts of E-cadherin at the cell surface greatly reduced, whereas α -catenin remains stable. α -Catenin is only affected in a second step, defined as late-stage apoptosis, when E-cadherin and Arm have disappeared completely.

The authors show that Arm is cleaved in its amino-terminal region *in vivo* and that the cleavage can be reproduced *in vitro* by DrICE (a *Drosophila* homolog of mammalian caspase-3). Cleavage occurs at the DQVD88 motif, as demonstrated *in vivo* by the cleavage resistance of Arm with an aspartate (D) to alanine (A) mutation in the DQVD88 motif (Arm^{D88A}). When Arm^{D88A} is overexpressed in Diap1-lacking embryos, E-cadherin and Arm^{D88A} are maintained at the membrane until late apoptosis, whereas endogenous Arm is removed, showing that Arm cleavage is required for the removal of these two junctional components from the membrane.

Cleaved catenins

Notably, the cleaved form of Arm is stable *in vivo* and co-localizes with α -catenin in the periphery of the cell. This stability suggests a specific role for the truncated Arm during apoptosis. Given this co-localization, truncated Arm may ensure the sequential dissociation of the adherens junction, permitting the dying cell to first detach from its neighbors (loss of E-cadherin), and then shrink (loss of α -catenin, cleaved Arm and retraction of actin microfilaments). Hence, the work of Kessler and Muller [9] constitutes an important step in defining the function of a cleaved caspase substrate in the morphological progression of apoptosis. Arm is probably not a unique case as, in contrast to the widespread notion that caspase substrates are rapidly degraded, a number of caspase-cleavage products can persist [2]. This suggests that caspases can generate truncated proteins with new activities. Now that numerous caspase substrates have

been identified [2,3], one of the big challenges will be to understand how the selective cleavages they catalyze lead to a sequential and organized degradation of the cell.

An exciting prospect will be to elucidate the precise mechanism of adherens junction destabilization by cleaved Arm, as the truncated protein retains binding sites for both E-cadherin and α -catenin. One model proposed by Kessler and Muller [9] is that the amino-terminal truncation of Arm may inhibit its association with E-cadherin, as shown for β -catenin in mammals. However, Arm cleavage does not seem to completely abolish adherens junction formation, as suggested by an experiment in which an *arm* mutant can be at least partially rescued by amino-terminally truncated Arm. An alternative is that modifications of other components of the adherens junction complex (cleavage of E-cadherin has been reported in mammals [10]) contribute to the sequential dissociation of the junction.

β -Catenin was already a known substrate of caspase-3 in mammals, and its cleavage there coincides with the destabilization of adherens junctions. However, the physiological significance of this cleavage remains to be tested, and it is not yet known whether the separation of the adherens junctions is progressive, as it is in *Drosophila* (Figure 1). It has been shown in mammalian cells that the truncated β -catenin loses its ability to bind α -catenin, thus releasing α -catenin from the junction and leading to the retraction of the microfilament system [11]. However, these data are controversial [12], and loss of α -catenin-binding capacity by cleaved β -catenin might depend on the cell type. Also, there are some differences in behavior between Arm and β -catenin during apoptosis. Arm is only cleaved once by DrICE, and this cleavage does not remove the α -catenin-binding domain, and does not prevent truncated Arm from binding α -catenin *in vivo*. Nevertheless, like β -catenin, Arm is cleaved near the amino terminus at a conserved position (DQVD88 in *Drosophila*, ADID83 in mammals), suggesting that the global mechanism of adherens junction degradation during apoptosis could be partly conserved between insects and mammals.

The progressive degradation of adherens junctions might serve to coordinate the elimination of dying cells with morphological changes in the surrounding tissue that are aimed at restoring epithelial organization. This leads to the question of how an apoptotic cell interacts with its neighbors. Apoptosis not only serves to eliminate cells in an ordered manner, but it also plays an important role in morphogenesis. For example, apoptosis alters the shape of surrounding cells during leg-joint development in *Drosophila* [13], and apoptotic cells can stimulate the proliferation of progenitors to promote the regeneration of damaged

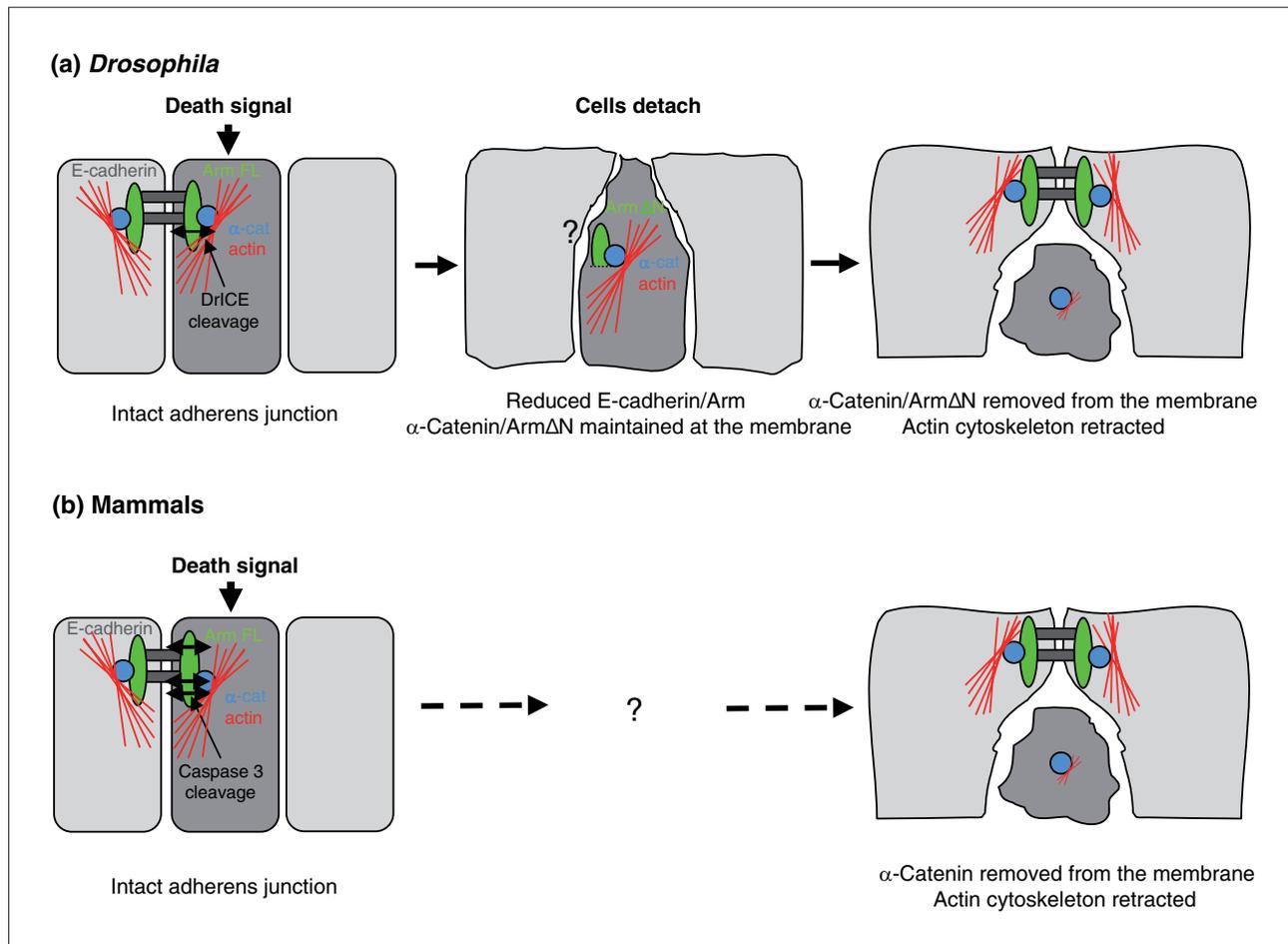


Figure 1
 Caspase-mediated cleavage of β -catenin promotes changes in cell adhesion and cell shape **(a) *Drosophila***; **(b) mammals**. Adherens junctions are composed of adhesion complexes of E-cadherin (gray bars), β -catenin (Armadillo (Arm); green ovals) and α -catenin (α -cat; blue circles), which link to the actin cytoskeleton. When apoptosis is induced, DrICE in *Drosophila* or its homolog caspase-3 in mammals are activated in the apoptotic cell (dark gray). DrICE cleaves Armadillo near the amino terminus (Arm Δ N), whereas mammalian caspase-3 cleaves β -catenin near both the amino and carboxyl termini. In *Drosophila*, an early stage of apoptosis has been described in which the cleaved form of Armadillo remains at the membrane linked to α -catenin, whereas E-cadherin is removed from the membrane by an unknown mechanism. In mammals, nothing is known so far about an intermediate step in adherens junction degradation in response to induction of apoptosis. At a later stage of apoptosis, all adherens junction components are removed from the membrane and the actin cytoskeleton retracts. Meanwhile, neighboring cells form new adherens junctions with each other and close the gap created by the retraction of the dying cell.

tissues [14]. This implies that a dying cell can send signals to its neighbors to coordinate morphological events. In these and many other cases, it seems likely that modifications of adhesive contacts between dying cells and their surviving neighbors are carefully regulated.

Finally, whereas the study by Kessler and Muller [9] focuses on the regulation of cell adhesion by caspases, changes in cell adhesion are also known to regulate caspases. Loss of cellular attachment often leads to a form of apoptosis termed ‘anoikis’, which is an important mechanism for

preventing detached cells surviving in inappropriate places and growing dysplastically. It will be interesting to examine what happens to adherens junctions during anoikis, and to determine how the event of cellular detachment is transmitted to the core apoptotic machinery.

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