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Review

# Cell death in development: Signaling pathways and core mechanisms

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ABSTRACT

Programmed cell death eliminates unneeded and dangerous cells in a timely and effective manner during development. In this review, we examine the role cell death plays during development in worms, flies and mammals. We discuss signaling pathways that regulate developmental cell death, and describe how they communicate with the core cell death pathways. In most organisms, the majority of developmental cell death is seen in the nervous system. Therefore we focus on what is known about the regulation of developmental cell death in this tissue. Understanding how the cell death is regulated during development may provide insight into how this process can be manipulated in the treatment of disease.



**Contents**



*Abbreviations:* AbdA, abdominal A; AbdB, abdominal B; apaf-1, apoptotic protease-activating factor 1; BMP, bone morphogenetic protein; ced3, cell death protein-3; Ced4, cell death protein 4; Ced9, cell death protein-9; cycE, cyclin E; Cyt-c, cytochrome-c; Dark, Apaf-1 related killer DARK; DIABLO, direct IAP binding protein with low p*I*; DIAP1, Drosophila inhibitor apoptosis protein 1; DP, dimerizing partner protein; Dpp, decapentaplegic; E2F, transcription factor E2f; EGFR, Epidermal Growth Factor Receptor; Egl1, egg-laying defective protein 1; ERK 1, extracellular-signal-regulated kinases1; ERK2, extracellular-signal-regulated kinases2; FGF8, fibroblast growth factor 8; Foxo, forkhead box O; GSK-3, glycogen synthase kinase-3; Hox, homeobox genes; IAPs, inhibitor of apoptosis; JNK, c-Jun N-terminal kinases; LIN-39, abnormal cell LINeage-39; MAPK, mitogen-activated protein kinase 1; Mek1, mitogen-activated protein kinase kinase 1; Mek2, mitogen-activated protein kinase kinase 2; OMI/HTRA2, HtrA serine peptidase 2; PcG, Polycomb-group protein; PI3K, phosphoinositide 3-kinase; Rb, retinoblastoma; RHG, reaper, hid, grim; sce, Sex combs extra; SMAC, second mitochondria-derived activator of caspase; Su(H), Suppressor of Hairless; Wg, wingless; YAP, yes-associated protein; Yki, Yorkie.

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## 1. Introduction

Cells die throughout the lifespan of multicellular organisms, and this physiologic cell death is critical for developmental plasticity and for organismal health [1]. In this review we describe the general functions of developmental cell death, focusing on nervous system development. The core cell death pathways that contribute to the majority of developmental cell death will be introduced, and the upstream regulation of these pathways in the context of the developing organism will be discussed.

## 2. The canonical pathway of cell death

Genes important for apoptosis are highly conserved from worms to man, and include the caspases, and their regulators (Fig. 1). The control of caspase activity is central to the regulation of developmental death. Caspase activity can be controlled by regulating both activation and inhibition. The relative importance of these two apoptosis control strategies varies between species and also between cell types, as well as in response to different apoptotic stimuli.

In *Caenorhabditis elegans*, the activation of the ced3 caspase by the ced4 adapter is inhibited by the bcl2-like ced9 protein [2]. The egl1 protein, a BH3 only family protein, is transcribed in cells fated to die [1]. In the presence of egl1, ced9 is inhibited and ced3 is activated.

In mammalian systems the role of the bcl2 family proteins in regulating caspase activation is more indirect. Upstream signals influence the balance of pro-and anti-apoptotic bcl2 family members, impacting Bax and Bak on the mitochondrial membrane [2]. Bax and Bak induce changes in the mitochondrial membrane, resulting in the release of mitochondrial proteins including Cytochrome-c. Cytochrome-c binds to Apaf-1, forming an apoptosome complex with procaspase-9. Caspase-9 is activated at the apoptosome. Subsequent activation of effector caspases results in cell death.

In flies, a caspase inhibitor, DIAP1, restrains caspase activity in most cells, and cell death is activated when this inhibition is removed [2]. DIAP1 is a member of the Inhibitor of Apoptosis Protein (IAP) family, which can act as direct caspase inhibitors. The RHG proteins, reaper, hid, grim and sickle, bind to DIAP1 and inhibit its anti-apoptotic activity resulting in cell death. The four RHG genes are transcribed in various combinations in cells fated to die [3–7]. Interestingly, the process of cell death in flies is very rapid; cells are eliminated within hours of RHG protein expression [3–5]. The bias in the Drosophila system toward this more poised apoptotic state may reflect the need for rapid apoptosis activation during development.

IAP proteins can also regulate cell death in mammals. There are eight IAP family members in humans [8] (see Fig. 1). In the nervous system, there is a role for IAPs in inhibiting caspase activity in apoptosis and in axonal and dendrite pruning [9–11]. SMAC/DIABLO and OMI/HTRA2 are functional homologs of the fly RHG family. These proteins bind to and negatively regulate IAPs and can kill cells under certain conditions [8,12].

## 3. Functions of cell death in development

Cell death is prevalent during the development of multicellular organisms. The majority of developmental cell death appears to be apoptotic [13], although alternative death pathways such as autophagy and necrosis may also contribute to the elimination of cells. The amount of cell death occurring during development can be underestimated, as phagocytes often eliminate dying cells within an hour of the initiation of death [14–16].

Examination of the distribution of dying cells and genetic disruption of cell death pathways has revealed important functions of cell death during development. These include the removal of unneeded tissues and cells and amelioration of developmental errors [17]. In certain situations isolated cells die, while in other cases, whole tissues are eliminated.

### 3.1. Removal of unneeded tissues

Entire tissues can be removed by programmed cell death as part of the initial shaping of the developing organism, or after the tissue has served its function [18,19]. For example, during the development of the mammalian reproductive system Mullerian ducts are differentially eliminated from males and Wolffian ducts from females, in response to hormonal signaling [13,18]. The Drosophila salivary glands are also removed in response to a steroid hormone pulse after they have served their function [20,21]. Interestingly, the elimination of the salivary gland requires both apoptotic and autophagic pathways [22–24]. Digit separation in vertebrates involves the elimination of inter-digital mesenchymal cells in response to developmental signaling pathways [25,26]. Analysis of mutants in the apoptotic machinery suggests that there are backup non-apoptotic pathways that can also contribute to the elimination of these cells [27]. Other examples of tissue elimination during development include the removal of the pronephric kidney in mammals and loss of the tadpole tail and intestine [17].

### 3.2. Removal of unneeded cells

The death of isolated cells is seen in many developing tissues, but is best described in the developing nervous system. Many more cells are generated during nervous system development than are present in the fully developed tissue. Roughly 10% of cells in the *C. elegans* nervous system are removed by apoptosis [28,29]. In Drosophila and mammals the number of cells eliminated rises to more than 50% [3,28–31]. Programmed cell death in the nervous system is likely to play an important role in developmental plasticity. Production of excess cells and their later elimination facilitates the matching of neurons to their targets [32].

The phenotype of cell death mutants illustrates the importance of apoptosis in nervous system development. Deletion of pro-death genes can result in severely malformed nervous systems in mammals and flies. Mice deficient for Caspase-3, caspase-9 and Apaf-1 show persistent neural precursors and exhibit nervous system patterning defects such as multiple indentations of the cerebrum and periventricular masses [33–38]. Interestingly, these phenotypes may not be caused by the survival of large numbers of cells, but rather may be secondary to the inappropriate survival of an FGF8 signaling center, resulting in defects in neural tube closure and insufficient brain ventricle expansion [39].

Bcl-2 family proteins also play a significant role in developmental cell death. Mice null for Bcl-2 family genes show defects in early nervous system development. For example, mice deficient for the anti-apoptotic Bcl-XL and Mcl-1 proteins die early in development due to massive apoptosis of immature neurons and hematopoietic cells [40,41]. Mice null for Bax, a pro-apoptotic member of the Bcl-2 family, have increased numbers of neurons, which are resistant to apoptosis induced by nerve growth factor deprivation [42]. Pro-apoptotic BH3-only proteins have redundant functions in developmental apoptosis. Individual knockouts of Bid, Bim and Puma result in only minor delays in developmental death of neurons. However, simultaneous deletion of all the three genes results in a strong resistance to stress-induced death in cerebellar granule neurons [43].

In the fly, apoptosis also plays an important role in shaping the developing central nervous system. Apoptosis is required to