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A Program of Cell Death

In Chapter 15 and throughout this whole book, you have learned about many different ways to make more cells, grow tissues, and move different cell types around to shape the whole organism. What about shaping embryos through targeted and timed cell reductions? The process of cell death is a vitally important developmental mechanism used by both animals and plants to achieve scale and form. Our nervous system, from brain to synapse, employs the process of programmed cells death or apoptosis to achieve desired morphologies. Consider apoptosis(-ing) some of your study time to further develop your understanding of programmed cell death in development.

"To be, or not to be: that is the question." One of the most puzzling phenomena in the development of the nervous system is neuronal cell death. In many parts of the vertebrate central and peripheral nervous systems, more than half the neurons die during the normal course of development. Moreover, there does not seem to be much conservation of apoptosis patterns across species. For example, about 80 percent of a cat's retinal ganglion cells die, whereas in the chick retina this figure is only 40 percent. In fish and amphibian retinas, no ganglion cells appear to die (Patterson 1992). *What causes this programmed cell death?*

Although we all are constantly poised over life-or-death decisions, this existential dichotomy is exceptionally stark for embryonic cells. Programmed cell death, or apoptosis (both *p*s are pronounced) is a normal part of development (see Fuchs and Steller 2011); the term comes from the Greek word for the natural process of leaves falling from trees or petals falling from flowers. Apoptosis is an active process and is subject to evolutionary selection. (A second type of cell death, necrosis, is a pathological death caused by external factors such as inflammation or toxic injury.)

In the nematode *C. elegans*, in which we can count the number of cells as the animal develops, exactly 131 cells die under the normal developmental pattern. All the cells of *C. elegans* are programmed to die unless they are actively told not to undergo apoptosis. In an adult human, as many as 10^{11} cells die each day and are replaced by other cells. (Indeed, the mass of cells we lose each year through normal cell death is close to our entire body weight!) During embryonic development, we were constantly making and destroying cells, and we generated about three times as many neurons as we eventually ended up with when we were born. Lewis Thomas (1992) wisely noted:

> *By the time I was born, more of me had died than survived. It was no wonder I cannot remember; during that time I went through brain after brain for nine months, finally contriving the one model that could be human, equipped for language.*

Apoptosis is necessary not only for the proper spacing and orientation of neurons, but also for generating the middle ear space, the vaginal opening in females, and the spaces between our fingers and toes (Saunders and Fallon 1966; Rodriguez et al. 1997; Roberts and Miller 1998). Apoptosis prunes unneeded structures (e.g., frog tails, male mammary tissue), controls the number of cells in particular tissues (neurons in vertebrates and flies), and sculpts complex organs (palate, retina, digits, heart).

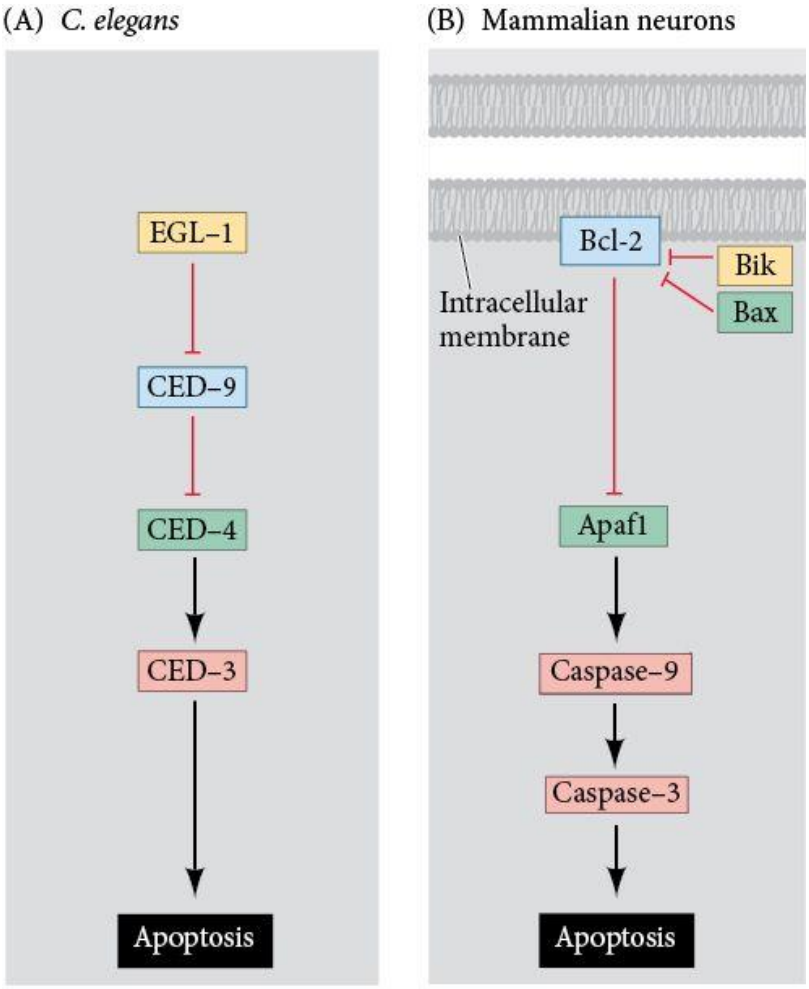
The pathways for apoptosis were delineated primarily through genetic studies of *C. elegans*. Indeed, the importance of these pathways was recognized by awarding a Nobel Prize in Physiology or Medicine to Sydney Brenner, H. Robert Horvitz, and John E. Sulston in 2002. Through their studies,

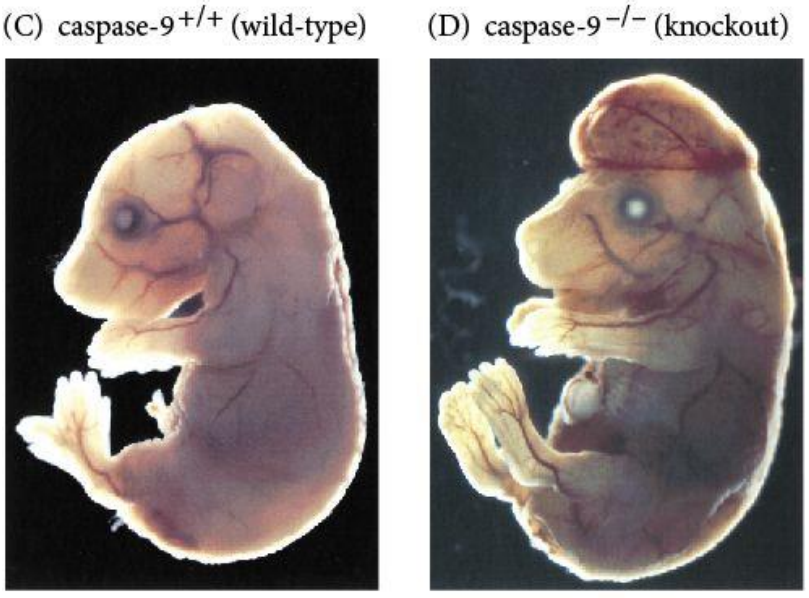
it was found that the proteins encoded by the *ced-3* and *ced-4* genes were essential for apoptosis and that, in the cells that did not undergo apoptosis, those genes were turned off by the product of the *ced-9* gene (Figure 1A; Hengartner et al. 1992). The CED-4 protein is a protease-activating factor that activates the gene for CED-3, a protease that initiates destruction of the cell. CED-9 can bind to and inactivate CED-4. Mutations that inactivate the gene for CED-9 cause numerous cells that would normally survive to activate their *ced-3* and *ced-4* genes and die, leading to the death of the entire embryo. Conversely, gain-of-function mutations in the *ced-9* gene cause its protein to be made in cells that would normally die, resulting in those cells surviving. Thus, the *ced-9* gene appears to be a binary switch that regulates the choice between life and death on the cellular level. It is possible that every cell in the nematode embryo is poised to die, with those cells that survive being rescued by the activation of the *ced-9* gene.

The CED-3 and CED-4 proteins are at the center of the apoptosis pathway that is common to all animals studied. The trigger for apoptosis can be a developmental cue, such as a particular molecule (e.g., BMP4 or glucocorticoids), the loss of adhesion to a matrix, or the lack of sufficient neurotrophic signals. Either type of cue can activate CED-3 or CED-4 proteins or inactivate CED-9 molecules. In mammals, the homologues of the CED-9 protein are members of the Bcl-2 family (which includes Bcl-2, Bcl-X, and similar proteins; Figure 1B). The functional similarities are so strong that if an active human *BCL-2* gene is placed in *C. elegans* embryos, it prevents normally occurring cell death (Vaux et al. 1992).

The mammalian homologue of CED-4 is Apaf1 (*a*poptotic *p*rotease *a*ctivating *f*actor *1*). Apaf1 participates in the cytochrome *c*-dependent activation of the mammalian CED-3 homologues, the proteases caspase-9 and caspase-3 (see Figure 1; Shaham and Horvitz 1996; Cecconi et al. 1998; Yoshida et al. 1998). Activation of the caspase proteins results in a cascade of autodigestion—caspases are strong proteases that digest the cell from within, cleaving cellular proteins and fragmenting DNA.

Although apoptosis-deficient nematodes (i.e., worms deficient for CED-4) are viable despite having 15 percent more cells than wild-type worms, mice with loss-of-function mutations for either caspase-3 or caspase-9 die around the time of birth from massive cell overgrowth in the nervous system (Figure 1C, D; Jacobson et al. 1997; Kuida et al. 1996, 1998). Similarly, mice homozygous for targeted deletions of *Apaf1* have severe craniofacial abnormalities, brain overgrowth, and webbing between their toes.





A and B after J. M. Adams and S. Cory. 1998. *Science* 281: 1322-1326.
C and D from K. Kuida et al. 1998. *Cell* 94: 325-337.

Figure 1 The loss of apoptosis can disrupt normal brain development. (A) In *C. elegans*, the CED-4 protein is a protease-activating factor that can activate CED-3. The CED-3 protease initiates the cell destruction events. CED-9 can inhibit CED-4 (and CED-9 can be inhibited upstream by EGL-1). (B) A similar pathway exists in mammals and appears to function in a similar manner. In this hypothetical scheme for the regulation of apoptosis in mammalian neurons, Bcl-X_L (a member of the Bcl-2 family) binds Apaf1 and prevents it from activating the precursor of caspase-9. The signal for apoptosis allows another protein (here, Bik) to inhibit the binding of Apaf1 to Bcl-X_L. Apaf1 is now able to bind to the caspase-9 precursor and cleave it. Caspase-9 dimerizes and activates caspase-3, initiating apoptosis. The same colors are used to represent homologous proteins. (C,D) In mice in which the genes for caspase-9 have been knocked out, normal neural apoptosis fails to occur, and the overproliferation of brain neurons is obvious. (C) A 6-day embryonic wild-type mouse. (D) A *caspase-9* knockout mouse of the same age. The enlarged brain protrudes above the face, and the limbs are still webbed.

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