Characterization of *ced-3*-independent Programmed Cell Death

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The development of *Caenorhabditis elegans* involves the elimination of 131 somatic cells by programmed cell death. Most programmed cell deaths require the caspase *ced-3*, a cysteine protease that presumably promotes death via the proteolysis of downstream substrates. Although most programmed cell deaths fail to occur in *ced-3* mutants, a small but detectable number of cells die in animals that completely lack *ced-3* activity, indicating that cells can activate a *ced-3*-independent death program (P. Reddien, F. Xie and H. R. Horvitz, unpublished results). We are beginning to investigate the morphological and genetic characteristics of these *ced-3*-independent cell deaths.

To analyze the physical characteristics of *ced-3*-independent cell deaths, we will use electron microscopy for ultrastructural characterizations as well as TUNEL and ANNEXIN V staining experiments to test for apoptosis-associated DNA fragmentation and cell surface markers, respectively. Furthermore, we will test the influence of mutations in other cell-death genes on *ced-3*-independent killing, including genes of the core cell-autonomous execution pathway (*ced-4, ced-9*, and *egl-1*), genes that function in the engulfment of dying cells (*ced-1*, *ced-2, ced-5, ced-6, ced-7, ced-10,* and *ced-12*), and genes that encode homologs of other proteases potentially involved in cell death, such as cathepsin and calpain. We will also examine the role of other caspases in the *ced-3*-independent cell deaths. The *C. elegans* genome contains three additional predicted caspase genes (*csp-1*, *csp-2* and *csp-3*) with no known functions. We are isolating deletion alleles of these *csp* genes to test for possible functions in promoting programmed cell death in the absence of *ced-3* activity.

Through our studies, it may be possible to elucidate a genetic pathway that does not require *ced-3* activity – or perhaps caspase activity in general – for the activation of cell death in *C. elegans*. Programmed cell death has critical roles in the development of higher metazoans and in the elimination of damaged, virus-infected, or cancerous cells. Some vertebrate cells appear to execute caspase-independent programmed cell deaths. Our analysis of a *ced-3*-independent death program may define a pathway in *C. elegans* that will facilitate the characterization of a similar genetic pathway in vertebrates.

Poster
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