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activity, indicating that cells can activate a ced-3-independent death program (P. Reddien, F. Xie and H. R. Horvitz, unpublished results).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.