Characterization of Detached Cells in Embryos Defective for Programmed Cell Death and Corpse Engulfment

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Programmed cell death plays critical roles in metazoan development and in the elimination of damaged, virus-infected, or cancerous cells. The development of the *C. elegans* hermaphrodite 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. However, a small but detectable number of cells die in animals that completely lack *ced-3* activity, indicating the existence of a *ced-3*-independent death program. In addition, we have observed that *ced-3* embryos, but not wild-type embryos, contain "shed cells" that detach from the developing embryo and remain in the eggshell upon larval hatching. The timing of their appearance suggests that the shed cells normally are fated to die in wild-type embryos and can die -- or at least be eliminated by a shedding mechanism -- even in the absence of *ced-3* activity. We also have observed shed cells in embryos mutant for other genes required for the cell-autonomous execution of programmed cell death (*egl-1*, *ced-9*, and *ced-4*), as well as genes that regulate the timing of the appearance of cell corpses (*ced-8*) and the engulfment of corpses (*ced-1*, *ced-2*, *ced-5*, *ced-6*, *ced-7*, *ced-10*, and *ced-12*). Thus, a defect in cell killing, the temporal control of death onset, or corpse engulfment can result in the appearance of shed cells that are likely to be cells normally fated to die.

To identify additional genes that might regulate the appearance of shed cells, we mutagenized N2 hermaphrodites, screened for F_2 embryos containing shed cells, and isolated three independent mutants from 2000 mutagenized haploid genomes. One isolate carries an allele of *ced-8*. The other two mutations (*n4874* and *n4911*) are allelic and do not appear to be defective in the execution of programmed cell death or the engulfment of cell corpses. Unlike the shed cells of cell death-defective embryos, the detached cell-like bodies observed in *n4874* and *n4911* embryos lack nuclei. These anucleate bodies have irregular sizes and a stippled appearance and are distinguishable from shed cells by Nomarski optics. To characterize the defect underlying the appearance of the anucleate bodies, we plan to map and clone the gene defined by *n4874* and *n4911*.

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