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Analysis of downstream events in the pathway for programmed cell death

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We are genetically and molecularly analyzing genes that may be involved in the downstream events of programmed cell death, such as the adoption of cell-corpse morphology and the engulfment and degradation of cell corpses. To facilitate phenotypic analyses of cell-death mutants, we are interested in developing markers that identify different stages in the cell-death process. Toward this end, we have developed methods for performing TUNEL (TdT-mediated dUTP Nick End Labeling) assays on *C. elegans*. Our analyses of wild-type, DNA degradation-defective and engulfment-defective embryos indicate that there are at least three steps of DNA degradation in cell corpses: an initiation step in which TUNEL-reactive ends are generated, a *nuc-1*-mediated step in which TUNEL-reactive ends are degraded (or masked) and an engulfing cell-mediated step (Hedgecock et al., *Science* 220, 1277-9, 1983) in which DNA degradation is completed.

ced-8 and *ced-11* are candidate downstream cell-death genes. In *ced-8* mutant embryos, refractile cell corpses appear later than in wild-type embryos. However, TUNEL detects more dying cells at early embryonic stages in *ced-8* embryos than are visible using Nomarski microscopy, suggesting that DNA degradation is less delayed than is the onset of refractility. Thus, *ced-8* may couple cell killing to morphological changes in the dying cell. In *ced-11* embryos, dying cells do not assume the refractile, electron-dense characteristic of normal cell corpses. TUNEL assays indicate that *ced-11* embryos have slightly increased numbers of TUNEL-reactive nuclei relative to the wild type. Thus, mutations in *ced-11* might result in slowed DNA degradation in addition to altered cell-corpse morphology.