

TWO NEW CELL-DEATH GENES AND CELL-CORPSE ENGULFMENT GENES BOTH CONTRIBUTE TO CELL KILLING

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The gene *ced-3*, which is the most downstream known component in the cell-death execution pathway, encodes a cysteine protease or caspase. How the CED-3 protease kills cells is unknown. Mutations in genes controlling parallel cell-killing activities downstream of *ced-3* might not have been previously identified because of their redundant nature. Animals with strong loss-of-function mutations in *ced-3* lack most if not all programmed cell deaths. However, there are weaker *ced-3* mutants that lack only a small percentage of programmed cell deaths. We reasoned that mutations in genes controlling cell-killing activities downstream of CED-3 might enhance a cell-killing defect conferred by a partial loss-of-function mutation in *ced-3*.

We found that weak *ced-3* mutations are enhanced by mutations in genes involved in cell-corpse engulfment (*ced-1*, *-2*, *-5*, *-6*, *-7*, and *-10*). Because cell corpses are generated in engulfment-defective mutants, the proposed function of engulfment has long been solely the removal of unwanted apoptotic cell bodies. We have discovered, however, that in addition to functioning in cell-corpse removal, engulfment assists in the killing of dying cells. Specifically, mutations in engulfment genes result in a low-penetrance survival of some cells that normally die in the ventral cord. Lineage analysis shows that cells that fail to die initially show some morphological characteristics of programmed cell death but ultimately appear morphologically indistinguishable, using Nomarski optics, from living cells. Surviving Pn.aap cells in the ventral cord lineages express a VC cell-type specific reporter *lin-11::gfp*, suggesting they can differentiate. We conclude that a block in engulfment can result in the survival and differentiation of cells programmed to die.

We have performed a screen for enhancers of a partial *ced-3* loss-of-function allele and isolated 37 mutations. Of these, at least three are mutations in *ced-9*, two in *ced-4*, and six in *ced-3*. Nine mutations confer defects in cell-corpse engulfment. On the basis of complementation tests and map positions, we have defined at least two new cell-killing genes. We will discuss our characterization of these genes.