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CED-9-CED-4 Interaction is Likely Required for the Non-Canonical Pro-Apoptotic Function of CED-9

In *Caenorhabditis elegans* apoptosis is inhibited by CED-9. It has been proposed based on protein interaction and localization studies that CED-9 prevents cell death by physically interacting with and sequestering the pro-apoptotic protein CED-4 to mitochondria. Canonically, in cells fated to die EGL-1 (a BH3-only protein) binds CED-9 causing a conformational change in CED-9 that results in the release of CED-4, which then localizes to the perinuclear membrane where it activates the caspase CED-3. A strong *ced-9* loss-of-function mutation leads to maternal-effect lethality, presumably caused by excessive apoptosis since this phenotype can be suppressed by a loss-of-function mutation in *ced-4* or *ced-3,* both proapoptotic genes. In addition to its anti-apoptotic function, *ced-9* has a poorly understood pro-apoptotic function. For example, *ced-9(lf)* mutations can enhance the cell-death defect observed in animals with a weak loss-of-function mutation in *ced-3*.

In unpublished work (P. Reddien and H. R. Horvitz), *ced-9(n3377)* was isolated from a screen for enhancers of the cell-killing defect mediated by a weak loss-of function allele of *ced-3*. *n3377* carries an E74K missense mutation in the presumptive CED-4 binding pocket of CED-9, based on a crystal structure of a CED-9/CED-4 complex (Yan *et al*. 2005). In a wild-type background, *n3377* causes a cell-killing defect but not maternal-effect lethality suggesting that *ced-9(n3377)* retains *ced-9*'s anti-apoptotic function but is mutant in *ced-9*'s pro-apoptotic function.

Using CRISPR-Cas9 I have isolated seven additional alleles of *ced-9* carrying distinct mutations in the CED-4 binding pocket. Like *ced-9(n3377)* mutants, animals carrying these alleles display ectopic survival of VC-like cells, indicating a defect in *ced-9*'s pro-apoptotic function. In a wild-type background, these mutations do not cause maternal-effect lethality, suggesting that, like *ced-9(n3377),* these mutations do not disrupt the canonical anti-apoptotic function of *ced-9.* These observations suggest that CED-9 interacts with CED-4 to drive its pro-apoptotic function.

If the loss of a CED-9/CED-4 interaction eliminates *ced-9*'s pro-apoptotic but not its anti-apoptotic function, then either *ced-9*'s canonical anti-apoptotic activity does not require a CED-9/CED-4 interaction or CED-9 and CED-4 can interact in two functionally distinct ways, one of which mediates *ced-9*'s anti-apoptotic activity and one of which mediates *ced-9*'s pro-apoptotic activity.