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In unpublished work (P. Reddien and H. R. Horvitz), ced-9(n3377) was isolated from a screen for enhancers of the cell-killing defect mediated by a weak loss-of function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.