CED-9 AND EGL-1 REGULATE THE SUBCELLULAR LOCALIZATION OF CED-4

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EGL-1, CED-9, CED-4, and CED-3, the core components of the *C. elegans* pathway for programmed cell death, have conserved mammalian counterparts with roles in apoptosis. EGL-1 is a cell-death activating BH3-domain protein that binds the cell-death inhibitor CED-9 and negatively regulates CED-9 activity. CED-9 is a member of the Bcl-2 family of cell-death regulators and negatively regulates CED-4 and CED-3. CED-4 is similar to mammalian Apaf-1, which activates caspases, cysteine proteases that effect cell killing. CED-3 is a caspase. Physical interactions among these components have been demonstrated *in vitro*, in yeast and in mammalian cells.

We generated antibodies against CED-9 and CED-4 and used them to determine the expression patterns and subcellular localizations of these two key cell-death regulators. Endogenous CED-9 and CED-4 are localized to mitochondria in wild-type embryos, in which the majority of cells survive. However, in embryos in which most cells have been induced to die, such as embryos homozygous for loss-of-function mutations in the cell-death inhibitor gene *ced-9* or overexpressing EGL-1, CED-4 is no longer seen at mitochondria but instead localizes to nuclear membranes. CED-4 redistribution induced by EGL-1 overexpression can be blocked by a gain-of-function mutation in *ced-9* but not by a loss-of-function mutation in *ced-3*, suggesting that this redistribution precedes the caspase activation step of programmed cell death. A missense mutation (P23L) within the CED-4 protein disrupts cell killing and CED-4 localization, causing CED-4 to be cytoplasmic both in the presence and absence of CED-9. We are now testing whether targeting this missense form of CED-4 to nuclear membranes is sufficient to rescue the Ced phenotype of this mutant. In addition, we are exploring whether CED-4-interacting proteins identified via two-hybrid screening are important for the localization of CED-4 to nuclear membranes.

Our findings suggest that the subcellular localization of CED-4 correlates with the life-or-death decision of a cell. Cells that survive maintain CED-4 localization at mitochondria, apparently through interaction with CED-9. Cells in which programmed cell death has been induced release CED-4 from mitochondria and relocalize CED-4 to nuclear membranes.