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## **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, are conserved in mammalian apoptosis. EGL-1, a BH3-domain protein that may act by negatively regulating CED-9 activity, has been shown to bind CED-9 protein. CED-9 is a member of the Bcl-2 family of cell-death regulators and negatively regulates the activities of CED-4 and CED-3. CED-4 is similar to mammalian Apaf-1, which activates caspases, cysteine proteases that effect cell killing. CED-3 encodes a caspase. Various physical interactions among these components have been demonstrated *in vitro*, in yeast and in mammalian cells.

We generated polyclonal antibodies against CED-9 and CED-4 and used them to determine the expression patterns and subcellular localizations of these two key cell-death proteins. Endogenous CED-9 and CED-4 proteins 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 *ced-9* or overexpressing the cell-death activator EGL-1, CED-4 is released from mitochondria and assumes a perinuclear localization. CED-4 redistribution induced by EGL-1 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 it precedes cell-death execution. Missense mutations within the CED-4 protein itself also can disrupt CED-4 localization.

These findings suggest that the subcellular localization of CED-4 may correlate with the life-or-death decision of a cell. Cells that survive maintain CED-4 localization at mitochondria, possibly through interaction with CED-9. Cells in which programmed cell death has been activated release CED-4 to redistribute to nuclear membranes.