The Putative TRP Channel CED-11 Functions to Increase Nuclear Membrane Permeability in *C. elegans* Apoptosis

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Programmed cell death is a fundamental process required for proper development and tissue homeostasis in many organisms. Genetic analyses of programmed cell death in *C. elegans* led to the discovery of an evolutionarily conserved genetic pathway that regulates the activation of apoptosis. In *C. elegans,* a cell dying by apoptosis undergoes a series of morphological changes resulting in the appearance of a round refractile cell corpse as visualized by Nomarski optics. The mechanism underlying these morphological changes remains poorly understood. We identified *ced-11,* a putative TRP channel, in a screen for mutants that modify the refractile appearance of cell corpses. The cell corpses in *ced-11* mutant embryos have a non-refractile disc-like appearance.

We examined the morphology of engulfed wild-type and *ced-11* apoptotic-cell corpses in embryos by electron microscopy and found that wild-type corpses stain darkly by electron microscopy and have lost much of their subcellular definition, whereas *ced-11* corpses do not stain darkly and look more similar to living cells than to dying cells. In addition, we discovered that *ced-11* corpses take an average of 17 minutes longer to degrade than wild-type corpses (48 min vs. 65 min), even though *ced-11* corpses are engulfed at a normal rate. Thus, *ced-11* is required for the normal degradation but not the engulfment of apoptotic-cell corpses.

We found that while mutations in *ced-11* do not prevent cells from dying, they can enhance the ventral-cord cell-death defect of engulfment mutants. These data indicate that *ced-11* functions in parallel to engulfment to facilitate cell killing.

It has previously been shown in mammals that nuclear permeability is increased during apoptosis and postulated that this change might occur through an increase in the nuclear pore passive diffusion limit. This increase in nuclear permeability might be how caspase-3 enters the nucleus during apoptosis. While using fluorescent proteins to analyze *ced-11* corpses, we found that while small proteins such as GFP (27 kDa) were seen in both the cytoplasm and nucleus of wild-type and *ced-11* corpses, larger proteins that are normally cytosolic in living cells (GCaMP3, 50 kDa and GCaMP3::mCherry, 77 kDa) diffused into the nucleus in wild-type but not *ced-11* corpses. This observation suggests that *ced-11* is required for an increase in nuclear permeability during apoptosis and that this increase might drive subsequent events in apoptosis. Further studies of *ced-11* should further elucidate the mechanism and function of increased nuclear permeability during apoptosis.

Talk
Topic: Cell Biology – Cell polarity and cell death
No. characters (counting spaces): 2840