Identification of Novel Cell Death Regulators in *C. elegans* and *Drosophila*

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ABSTRACT

Apoptosis is a form of cell death executed by a class of cysteine proteases called caspases. Though caspases are well-conserved, the mechanisms by which caspases are regulated vary remarkably. This dissertation addresses three independent aspects of apoptosis and its regulation.

In the developing *Drosophila* eye, apoptosis is activated to remove extra cells that are initially present between ommatidia. Mutants for the gene *echinus* have a disorganized eye structure due to a failure of these cell deaths to occur. We demonstrate that *echinus* resembles a deubiquitinating enzyme, that it is expressed in the pupal eye during the time of cell death, and that *echinus* acts genetically upstream or independently of the death-inducing genes *head involution defective*, *reaper*, and *grim*. Based on *in vitro* assays and the fact that the Echinus enzyme lacks a catalytic cysteine residue, we propose that *echinus* and its orthologs constitute a novel class of inactive deubiquitinating enzymes, perhaps functioning in a dominant-negative manner to inhibit deubiquitination of specific substrates.

In *C. elegans*, the model for caspase inhibition is quite different from that in *Drosophila* and in mammals. To look for genes that directly inhibit the CED-3 caspase, we screened a

C. elegans cDNA library for CED-3 suppressors in the yeast *S. cerevisiae* and found several suppressors. Experiments in yeast suggest that one of these genes, Y39B6A.12, requires the prodomain of CED-3 for suppression, and ectopic expression in the *Drosophila* eye shows that it can suppress apoptosis induced by the Bcl2 family member *Debcl*.

In *Drosophila*, DIAP1 is the focal point in the regulation of apoptosis. To identify novel regulators of DIAP1, deficiency chromosomes spanning the *Drosophila* genome were screened for dominant modifiers of a *diap1* knockdown phenotype. Nine deficiencies were isolated that cover no known regulators, and two modifiers were mapped to small genomic regions. This screen has provided a starting point for identifying some of the many uncharacterized genes that are involved in regulating apoptosis.

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