

**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.

TABLE OF CONTENTS

Acknowledgments	iii
Abstract	v
Table of Contents	vii
List of Figures	viii
Chapter 1 Developmentally Regulated Programmed Cell Death in <i>C. elegans</i> and <i>Drosophila</i>	1
Chapter 2 Echinus Shows Homology to Deubiquitinating Enzymes and is Required for Retinal Cell Death in <i>Drosophila</i>	17
Chapter 3 Novel Regulators of the <i>C. elegans</i> Caspase CED-3	53
Chapter 4 A Deficiency Screen to Isolate Novel Regulators of DIAP1	70

List of Figures and Tables

<i>Number</i>	<i>Page</i>
Figure 2-1 Genomic region of <i>echinus</i>	24
Figure 2-2 <i>echinus</i> mutants have extra interommatidial cells	25
Table 2-1 Echinus does not act as a deubiquitinating enzyme <i>in vitro</i>	28
Figure 2-3 <i>echinus</i> encodes a protein resembling a deubiquitinating enzyme	29
Figure 2-4 <i>echinus</i> is expressed in the pupal eye at the time of apoptosis	30
Figure 2-5 Structure-function analysis of Echinus	32
Figure 2-6 <i>echinus</i> acts upstream or independently of apoptotic machinery	34
Supplementary Figure Genetic crosses of <i>echinus</i> to signaling pathways involved in <i>Drosophila</i> eye development	35
Figure 3-1 <i>C. elegans</i> genes Y39B6A.12 and T23G11.7b suppress CED-3 in yeast	58
Figure 3-2 CG7967, the <i>Drosophila</i> ortholog of T23G11.7b, suppresses death induced by <i>Debcl</i>	59
Figure 3-3 CG7967 suppresses death induced by the JNK pathway and <i>fat facets</i>	60
Figure 3-4 CG7967 regulates Gal4 gene expression and not apoptosis	61
Figure 3-5 Y39B6A.12 in <i>Drosophila</i> suppresses death induced by <i>Debcl</i>	63
Figure 4-1 <i>Diap1</i> RNAi causes a small eye phenotype in the <i>Drosophila</i> eye	72
Table 4-1 Sequences used to knockdown genes in the 90F chromosomal region	74

	ix
Table 4-2 Deficiencies isolated as modifiers of the <i>diap1</i> RNAi mutant	75
Figure 4-2 Genomic region of Df(2L)BSC17	76
Figure 4-3 RNAi of the ribosomal gene <i>RpL13</i> causes a small eye phenotype	77
Figure 4-4 Modifying deficiencies identified in the 90F chromosomal region	78
Figure 4-5 Genomic region of Df(3R)DG2	79
Figure 4-6 Candidates from 90F tested showed no change in <i>diap1</i> RNAi phenotype	79