Chapter 3: Novel Regulators of the *C. elegans* Caspase CED-3

3.1 Abstract

The hallmark of apoptosis is the presence of activated caspases, a conserved family of cysteine proteases. Proper regulation of caspases is crucial to maintain cell viability. IAPs are a class of proteins known to inhibit caspases in *Drosophila* and mammals, though they do not regulate the *C. elegans* caspase CED-3. To understand how CED-3 is regulated, we have performed a yeast-based screen for CED-3 suppressors and isolated two previously uncharacterized genes, Y39B6A.12 and T23G11.7b. Characterization of these genes in *Drosophila* shows that T23G11.7b and its ortholog CG7967 are regulators of gene expression and not caspases. Y39B6A.12, on the other hand, inhibits the Bcl-2 gene *Debcl*, but fails to regulate the pro-apoptotic genes *Grim*, *Hid*, or *Dronc*. It still remains unclear if Y39B6A.12 is a critical regulator of CED-3 in viable *C. elegans* cells.

3.2 Introduction

Programmed cell death is a regulated process used by an organism for the removal of deleterious cells or developmentally transient cells. Characterization of programmed cell

death began in the nematode *Caenorhabditis elegans* and led to the eventual identification of the genes involved (Ellis and Horvitz, 1986). *ced-3* and *ced-4* were shown to be necessary for cell death while the gene *ced-9* blocks their function (reviewed in Metzstein et al., 1998).

Later studies from other model systems showed that the *ced* genes were highly conserved in apoptosis. *ced-3* belongs to a family of proteases now known as caspases, which are the executioners of programmed cell death (Yuan et al., 1993). The mitochondrial localized CED-4 facilitates the irreversible activation of CED-3 (Seshagiri and Miller, 1997). Subsequent studies show that Apaf-1 is structurally similar to CED-4 and promotes the activation of the apical caspase Caspase-9 (Zou et al., 1997). *ced-9* is a member of the Bcl-2 family of mitochondrial proteins and inhibits ced-4 (Hentgartner and Horvitz, 1994).

While the genes involved in apoptosis are highly conserved, the inhibition of caspases appears to vary in different organisms. In flies and mammals, caspases are inhibited by the IAP group of proteins. Removal of IAPs causes rapid cellular destruction, thus proving that they are crucial for maintaining cell viability (Wang et al., 1999). Importantly, no functional caspase inhibitor has yet been identified in *C. elegans*. The two genes with structural motifs similar to other IAPs in worms, the genes *bir-1* and *bir-2*, are not involved in apoptosis, but instead are required for midzone spindle formation and cytokinesis (Fraser et al, 1999, Speliotes, et al, 2000). To explain how CED-3 is kept from chewing up the cell, two models have been proposed. One model states that an

alternative anti-apoptotic splice form of CED-4 keeps CED-3 down, though this splice form is relatively rare when compared to the pro-apoptotic one (Shaham and Horvitz, 1996). Another model suggests that the Bcl-like protein CED-9 binds CED-3 and CED-4 in a ternary complex and prevents CED-3 activation (Xue and Horvitz, 1997). A necessary tenet of this model is that CED-3 is localized to the mitochondria along with CED-4 and CED-9, yet no one has shown the localization pattern of CED-3. Furthermore, recent work in a heterologous assay has shown that CED-9 cannot inhibit CED-3 mediated cell death (Jabbour et al., 2004).

If these models are flawed, the question remains: What keeps CED-3 from killing the cell? To probe this question, we used a function-based assay in *S. cerevisiae* to screen for inhibitors of CED-3. Two *C. elegans* genes were isolated from this screen, though subsequent studies in *Drosophila* eliminated one candidate from consideration. The remaining candidate, Y39B6A.12, weakly suppressed death induced by the Bcl2 gene *Debcl* in the *Drosophila* eye. Though Y39B6A.12 still needs to be characterized further in *C. elegans*, this heterologous screen provides a novel way to screen for regulators of the caspase CED-3.

3.3 Materials and Methods

Yeast protocols

S. cerevisiae were transformed using a standard LiAc protocol. The W303α strain (*MATα, can1-100, leu2-3, his3-11, -15, ura3-1, ade2-1*) was grown on selective media containing 2% glucose or 2% galactose/1% raffinose for gene induction. *ced-3* and *ced-4* were induced using an 815 bp fragment of the Gal1 promoter region (Hawkins et al., 1997). The caspase-lacZ reporter assay used to test caspase suppression independent of galactose has been previously described (Hawkins et al., 1997).

C. elegans cDNA library construction

Mixed-stage *C. elegans* polyA+ RNA was converted into cDNA using the Superscript cDNA synthesis kit (GIBCO). cDNAs were size-fractioned to select clones greater than 500 bp, and were ligated into the NotI–SalI sites of the gpat-his vector. Transformation of the ligation mix into the bacteria strain DH10B (GIBCO) yielded approximately 5 x 10^5 colonies. The cDNA library was amplified by growing the colonies in LB with carbecillin and isolating their plasmid DNA (Qiagen).

Drosophila genetics

Drosophila strains were grown at 25 ^{0}C. CG7967, T23G11.7b, and Y39B6A.12 were subcloned into the UASt vector and injected using standard embryo microinjection techniques. Recombinants of the UAS transgenics with the GMR-gal4 chromosome were made. UAS-Q78 was kindly provided by N. Bonini. UAS-Wg and UAS-pygo were provided by K. Cadigan. Pictures of Drosophila retina were taken using an Olympus DP10 digital camera.

C. elegans RNAi

cDNAs for T23G11.7b and Y39B6A.12 were cloned into the BamHI–XbaI sites of pPD129.36. RNAi knockdown of these genes was conducted by feeding *C. elegans* on bacteria producing dsRNA from these constructs.

3.4 Results

Isolation of two *ced-3* suppressors in yeast

CED-3 is the only *C. elegans* caspase known to be involved in programmed cell death. To test whether *C. elegans* does encode a novel CED-3 inhibitor we screened an inducible C. elegans cDNA library for genes that suppress *ced-3* in *S. cerevisiae*. As previously reported, overexpression of *ced-3*, under the control of the galactose-inducible promoter, kills *S. cerevisiae* cells (Figure 1, Jabbour et al., 2004). This killing is complete when two Gal-*ced-3* plasmids are present. The *C. elegans* genes T23G11.7b and Y39B6A.12 were both repeatedly isolated as suppressors of CED-3 mediated death (Fig. 1B, C). Reintroduction of the cDNAs encoding Y39B6A.12 and T23G11.7b back into *S. cerevisiae* verified their suppression.

The CARD domain found in the prodomain of CED-3 is necessary for its regulation, though not essential for proteolytic activity. Overexpression of CED-3 lacking its CARD

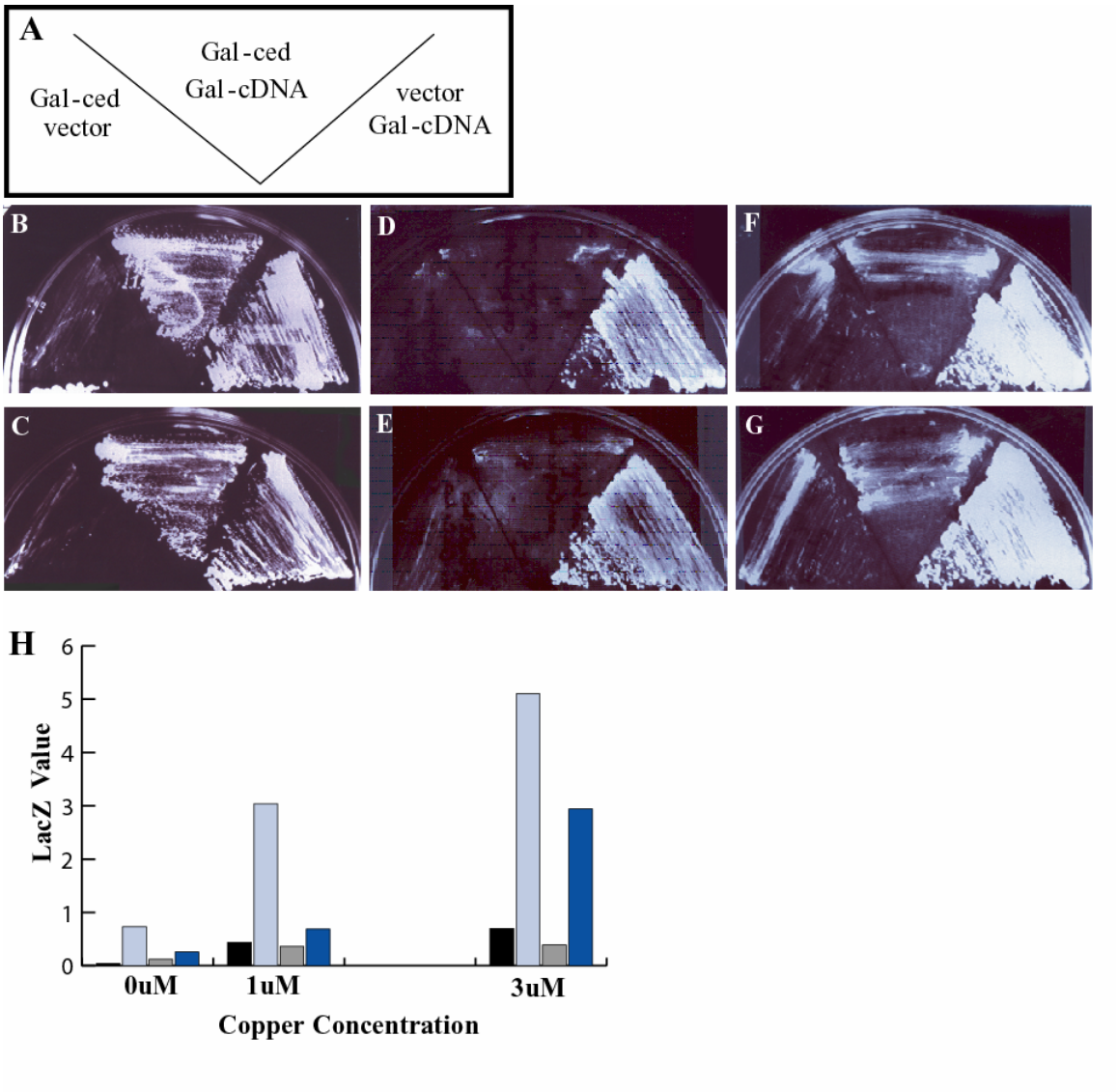

Figure 1. Y39B6A.12 and T23G11.7b were isolated as CED-3 suppressors in yeast. Panel A is the schematic for the plate growths in panels B-G where the 'ced' gene is either ced-3 (B,C), ced-3 lacking its prodomain (D,E), or ced-4 (F,G) and the cDNA are the C. elegans cDNAs Y39B6A.12 (B,D,F) or T23G11.7b (C,E,G). (B,C) Both cDNAs suppress CED-3-dependent death. cDNAs fail to suppress death induced by CED-3 lacking its prodomain (D,E) or death induced by CED-4 (F,G). (H) Caspase CED-3 was induced by the copper-inducible promoter and its activity measured by lacZ (see Materials and Methods for details). Black bars represent P35 suppression of CED-3 activity, light blue bars represent empty vector, light grey represent presence of Y39B6A.12, and dark blue represents T23G11.7b. Note that the isolated cDNAs suppress CED-3 activity independent of the Gal1 promoter, and that Y39B6A.12 suppresses as well as the viral P35.

domain (*ced-3* Δprodomain) can still kill yeast cells. Interestingly, both T23G11.7b and Y39B6A.12 can no longer function as CED-3 inhibitors when the CED-3 CARD domain is missing, suggesting that the CARD domain is important for regulation (Fig 1D, E). CED-4 also encodes a CARD domain, and CED-4 has been shown to activate apoptosis and kill *S. pombe* (James et al., 1997). In *S. cerevisiae*, galactose-induced *ced-4* can also kill, though this death cannot be suppressed by T23G11.7b or Y39B6A.12 (Fig 1F, G).

Because the two cDNAs failed to suppress CED-3 (Δprodomain) and CED-4 dependent death in yeast, it is reasonable to suggest that the cDNAs are CED-3 inhibitors and not suppressors of the galactose-inducible promoter. To test this hypothesis another way, we

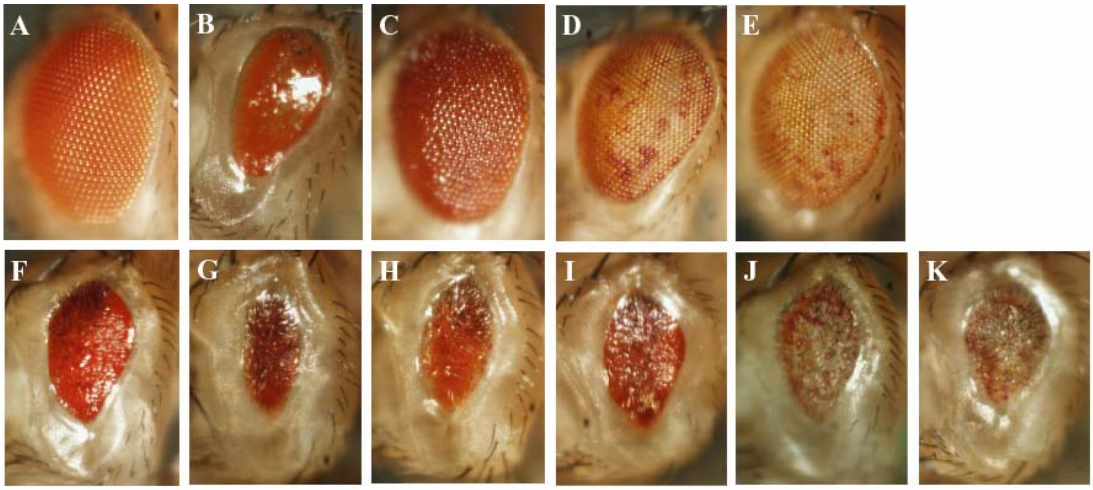

Figure 2. CG7967 is the *Drosophila* ortholog of *C. elegans* T23G11.7b and suppresses ectopic Debcl, but not GMR-dronc, GMR-hid, GMR-grim, GMR-reaper. (A) GMR-gal4, UAS-CG7967 shows no phenotype on its own. (B) GMR-gal4,UAS-Debcl (C) GMR-gal4, UAS-Debcl; UAS-CG7967 (D) GMR-gal4; GMR-dronc (E) GMR-gal4,UAS-CG7967; GMR-dronc (F) GMR-gal4; GMR-hid (G) GMR-gal4, UAS-CG7967; GMR-hid (H) GMR-gal4; GMR-reaper (I) GMR-gal4, UAS-CG7967; GMR-reaper (J) GMR-gal4; GMR-grim (K) GMR-gal4, UAS-CG7967; GMR-grim

tested whether T23G11.7b and Y39B6A.12 could suppress *ced-3* controlled by the copper-inducible promoter. Increasing the concentration of copper led to an increase in CED-3 activity. Y39B6A.12 and T23G11.7b, as well as the pan-caspase suppressor P35, suppressed CED-3 activity independent of the Gal1 promoter (Fig. 1H). Notably, Y39B6A.12 suppressed just as well as P35.

T23G11.7b/ CG7967 suppresses the Gal4/UAS system and does regulate apoptosis

To determine whether the isolated cDNAs are genuine caspase suppressors *in vivo*, we used the *Drosophila* eye for our assays. Using the GMR (Glass multiple repeat)-gal4 line to drive gene expression in the eye, ectopic expression of CG7967, the *Drosophila* ortholog of T23G11.7b, showed no phenotype (Fig. 2A). *hid*, *grim*, and *reaper* are

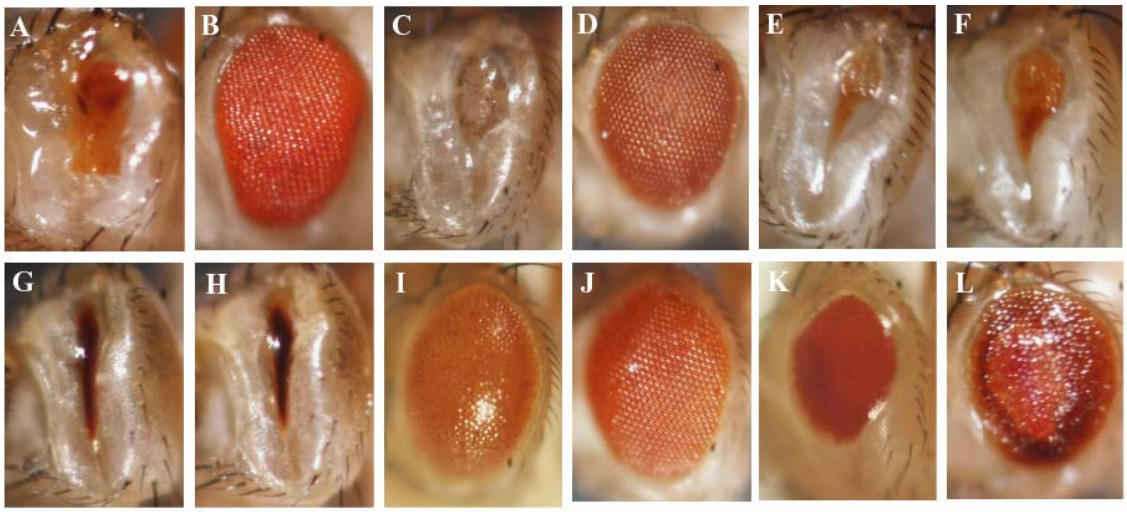

Figure 3. The Drosophila gene CG7967 suppresses members of the JNK pathway, *pygopus (pygo)*, and *fat facets (faf)*. (A) GMR-gal4; UAS-Eiger (B) GMR-gal4; UAS-Eiger/ UAS-CG7967 (C) GMR-gal4; UAS-dTak1 (D) GMR-gal4; UAS-dTak1/ UAS-CG7967 (E) GMR-gal4; UAS-hep (F) GMR-gal4; UAS-hep/ UAS-CG7967 (G) GMR-gal4; UAS-Wingless (Wg) (H) GMR-gal4; UAS-Wg/CG7967 (I) GMR-gal4; UAS-pygo (J) GMR-gal4; UAS-pygo/ UAS-CG7967 (K) GMR-gal4; *faf*(EP381) (L) GMR-gal4; *faf*(EP381)/ UAS-CG7967. CG7967 suppresses the ablated eye phenotype of all lines except Wingless (Wg).

activators of apoptosis and cause a small eye phenotype when overexpressed using the GMR enhancer. Crosses of GMR-gal4, UAS-CG7967 to GMR-hid, GMR-grim, and GMR-reaper showed no changes in eye size (Fig 2). In a similar fashion, expression of GMR-Dronc, which also induces death, could not be suppressed by GMR-gal4, UAS-CG7967 (Fig 2D, E). Though the Bcl proteins are much more extensively characterized in humans and mice, the *Drosophila* members, *Debcl* and *Buffy*, have been shown to regulate apoptosis. Ablation of the eye by overexpressed *Debcl* can be restored by

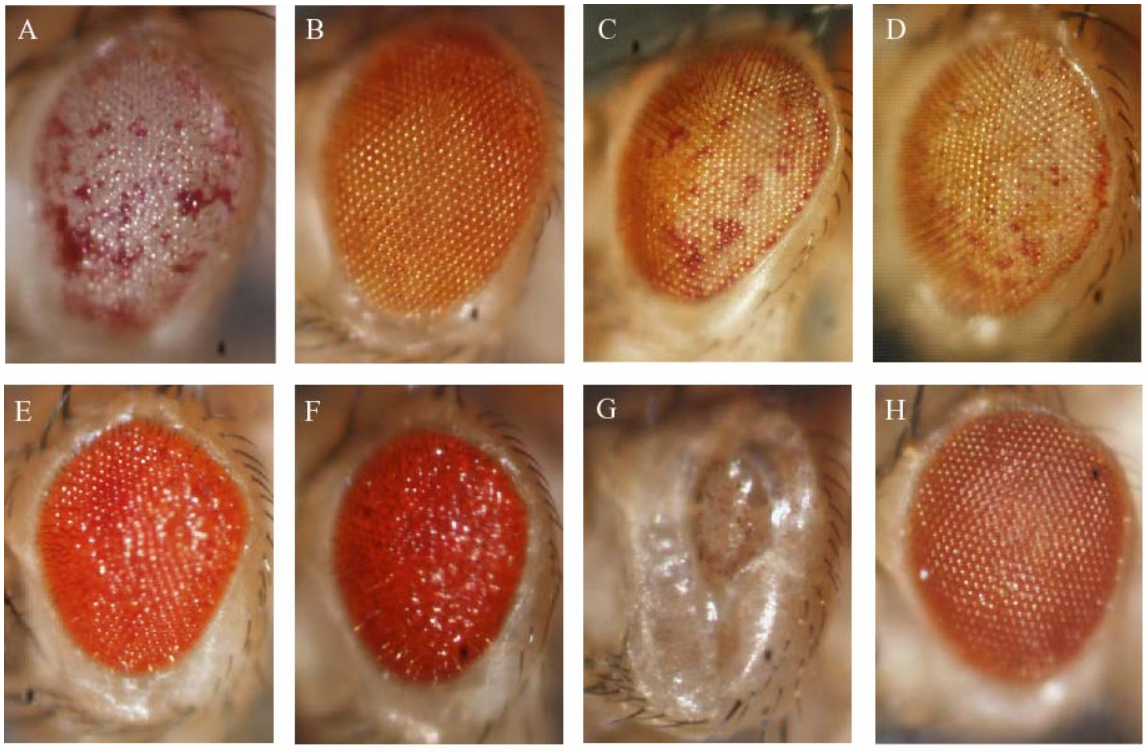

Figure 4. The *Drosophila* gene CG7967 regulates Gal4 dependent expression. CG7967 suppresses death induced by GMR-gal4, UAS-Dronc, but not GMR-Dronc (A-D). CG7967 also fails to suppress ectopically expressed mouse Tak1, but yet suppresses *Drosophila* dTak1 driven by GMR-gal4 (E-H). (A) GMR-gal4, UAS-Dronc (B) GMR-gal4, UAS-Dronc; UAS-CG7967 (C) GMR-gal4; GMR-Dronc (D) GMR-gal4; GMR-Dronc/ UAS-CG7967 (E) GMR-mTak1 (F) GMR-mTak1; GMR-gal4, UAS-CG7967 (G) GMR-gal4; UAS-dTak1 (H) GMR-gal4, UAS-CG7967; UAS-dTak1

overexpression of *Buffy* (Quinn et al., 2003). CG7967 can suppress excess death phenotypes associated with ectopically expressed *Debcl* both in the eye and the wing (Fig 2, data not shown).

During the course of experiments, it became apparent that CG7967 could suppress genes expressed using the Gal4/UAS system, but not genes controlled directly by the GMR enhancer (Fig 2, 3). Once again, to test whether CG7967 and T23G11.7b are genuine inhibitors of apoptosis, we tested their ability to suppress genes expressed using the GMR enhancer and the Gal4/UAS system. The caspase Dronc expressed using the Gal4/UAS system gives a stronger phenotype than GMR-Dronc. CG7967 could not suppress GMR-Dronc, as previously mentioned, though it could suppress GMR-gal4, UAS-Dronc (Fig. 4). Along with other members of the JNK pathway, overexpression of the kinase dTak1 and its mouse counterpart mTak1 activates apoptosis in the *Drosophila* eye. Overexpression of CG7967 failed to suppress GMR-mTak, while it suppressed GMR-gal4, UAS-dTak (Fig. 4). Taken together, these results show that CG7967, and, by implication, T23G11.7b are not genuine regulators of apoptosis.

Y39B6A.12, a possible caspase inhibitor

To determine whether Y39B6A.12 is a pan-caspase inhibitor, we resorted to the *Drosophila* eye once again. In yeast, Y39B6A.12 proved to be a CED-3 inhibitor. *Drosophila* encodes for seven caspases, with the downstream caspases *DrICE*, *decay*, and *dcp-1* showing homology to *ced-3* in their catalytic domains. Since at the time of this writing, recombinants of GMR-gal4; GMR-DrICE, -Decay, and –dcp-1 do not exist, we

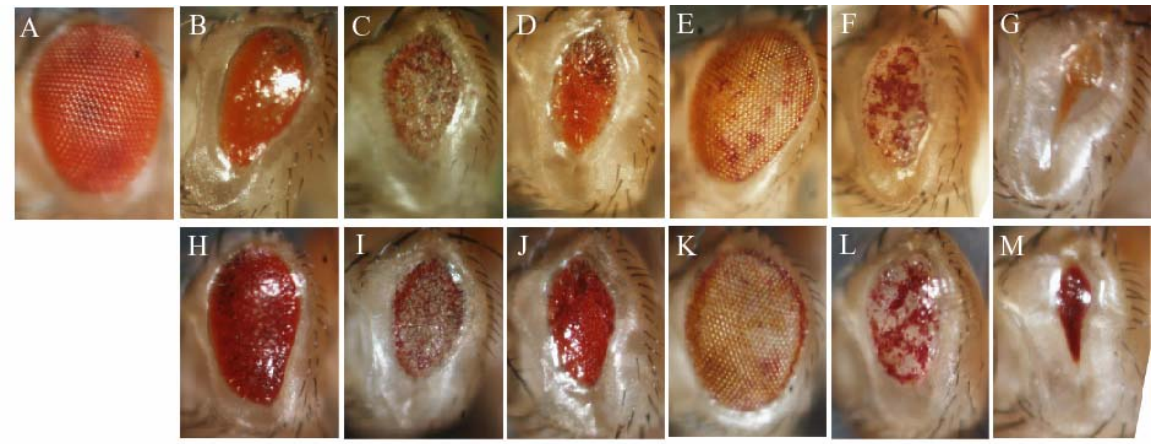

Figure 5. Ectopically expressed C. elegans Y39B6A.12 in the Drosophila eye suppresses Debcl. Y39B6A.12 shows no suppression of overexpressed grim, hid, Dronc, diap1(RNAi), or hep. UAS-Y39B6A.12 in panels H-M. (A) GMR-gal4, UAS-Y39B6A.12 (B) GMR-gal4; UAS-debcl (C) GMR-gal4; GMR-grim (D) GMR-gal4; GMR-hid (E) GMR-gal4; GMR-dronc (F) GMR-gal4, UAS-diap1(RNAi) (G) GMR-gal4; UAS-hep (H) GMR-gal4; UAS-debcl, UAS-Y39B6A.12 (I) GMR-gal4; GMR-grim, UAS-Y39B6A.12 (J) GMR-gal4; GMR-hid/ UAS-Y39B6A.12 (K) GMR-gal4; GMR-dronc, UAS-Y39B6A.12 (L) GMR-gal4, UAS-diap1(RNAi); UAS-Y39B6A.12 (M) GMR-gal4; UAS-hep/ UAS-Y39B6A.12

sought to test whether Y39B6A.12 could suppress activators of these caspases. GMR-gal4, UAS-Y39B6A.12 could not suppress ectopic expression of *grim* nor *hid* (Fig. 5). The upstream caspase Dronc contains an N-terminal CARD motif, though with little similarity to the CARD domain in CED-3, and has been shown to activate DrICE. Like *hid* and g*rim*, overexpression of *Dronc* could not be suppressed by ectopically expressed Y39B6A.12. However, Y39B6A.12 did slightly suppress the ablated eye caused by overexpression of Debcl (Fig. 5). Finally, unlike T23G11.7b, Y39B6A.12 failed to counteract the JNK pathway components, *Eiger*, *hep*, and *dTak1* (Fig. 5, data not shown). Crosses to establish the GMR-gal4, UAS-Y39B6A.12 recombinant are being made, so that we can test Y39B6A.12 suppression against the effector caspases *DrICE* and *dcp-1*.

To test for the function of Y39B6A.12 in *C. elegans*, worms were fed with bacteria expressing dsRNA to Y39B6A.12. RNAi knockdown of Y39B6A.12 in N2 worms caused a sterile phenotype that was not suppressed by mutants for *ced-3* or *ced-4* (B. Derry, personal communication). Loss of the upstream inhibitor *ced-9* shows partial sterility, among other effects (Hentgartner et al., 1992). The results of the Y39B6A.12 RNAi and epistasis analysis suggest that either Y39B6A.12 is a tissue-specific inhibitor of an unidentified caspase or that the sterility is not related to cell death genes at all.

Protein characteristics and homology

Y39B6A.12 encodes a previously uncharacterized gene. With an ortholog found only in *C. briggsae*, Y39B6A.12 is a nematode-specific gene characterized only by its BED Zn-finger domain. BED Zn-finger proteins are a diverse class of nuclear proteins thought to bind to DNA. *C. elegans* encodes several BED proteins, including *dpy-20* and the transposase *tam-3*. The best-characterized BED proteins in *Drosophila* are the transcriptional activator Dref and the chromatin insulator Beaf. How a DNA-binding protein like Y39B6A.12 functions in relation to CED-3 is yet to be determined.

3.5 Discussion

Y39B6A.12 shows anti-apoptotic activity in yeast and Drosophila

CED-3 remains the only characterized caspase in *C. elegans* apoptosis, and to date no proteins have been identified as CED-3 inhibitors. In this report, we describe the two

genes identified in a yeast-based screen performed to isolate CED-3 suppressors. The *C. elegans* gene T23G11.7b and its *Drosophila* ortholog proved to be general suppressors of gene induction, while preliminary work on the *C. elegans*-specific gene Y39B6A.12 shows conserved function, as it partially suppressed death induced by expression of the pro-apoptotic Bcl2 protein Debcl. Because Y39B6A.12 was isolated in *S. cerevisiae*, which is devoid of the core components of the apoptosis machinery, it stands to reason that Y39B6A.12 functions to inhibit caspases. Though Y39B6A.12 could not suppress the overexpressed *Drosophila* caspase Dronc, Y39B6A.12 may still inhibit caspases that have not been tested. *Drosophila* encodes seven caspases, and the downstream caspases DrICE, DCP-1, and Decay share the most sequence similarity to CED-3. Tests to determine if Y39B6A.12 is a pan-caspase inhibitor and can inhibit any *Drosophila* caspase are currently underway.

Another possibility is that Y39B6A.12 could function only with CED-3 and not show any activity in *Drosophila*. To test for *in vivo* function in *C. elegans*, we knocked-down Y39B6A.12 levels by the feeding RNAi method. If Y39B6A.12 inhibits apoptosis, then loss of its function would cause spurious cell death. Reduced Y39B6A.12 function caused sterility, though this phenotype could not be suppressed by mutants for *ced-3* or *ced-4* (B. Derry, personal communication). Whether this sterility is caused by ectopic cell death is still to be determined. The ability of Y39B6A.12 to prevent cell death in C. elegans is currently being examined.

Complete story of developmental apoptosis in *C. elegans* remains to be resolved

C. elegans was used first to understand the molecular components of programmed cell death. Screens pulled out 13 genes involved in killing the cell and in corpse removal. Even though worms have the simplest cell killing machinery it is not completely understood. For example it is not understood how the fragmentation of the mitochondria, important in mammalian apoptosis, is involved in worm cell death (Jagasia et al., 2005). The homologous apoptotic components AIF (apoptosis inducing factor) and endonuclease G are important in *C. elegans* programmed cell death, though the mechanism of their mitochondrial release has not been determined (Wang et al., 2002; Parrish et al., 2001). Anecdotal evidence also suggests that even the list of players mediating *C. elegans* apoptosis is incomplete. A deficiency screen looking at abnormal corpse numbers isolated many genomic regions containing no previously characterized CED genes (Sugimoto et al., 2001). In addition, mutants for *ced-4* but not *ced-3* suppress the embryonic cell death caused by loss of *icd-1*, a mitochondrial localized βNAC ortholog. Finally, *ced-3* is not essential for the death of the male linker-cells, the so-called “murders”; linker-cell death requires the presence of another cell (Sulston and White, 1980, Ellis and Horvitz, 1986).

3.6 Conclusion

We report the isolation of *C. elegans* genes that can suppress the killing ability of the *C. elegans* caspase CED-3. One of these genes, T23G11.7b and its Drosophila ortholog CG7967 prove to be regulating Gal4 dependent gene expression. Y39B6A.12 is a

nematode-specific gene containing a BED Zn-finger domain. In the *Drosophila* retina, Y39B6A.12 shows weak ability to suppress ectopically expressed *Debcl*. Further tests are being conducted to determine if Y39B6A.12 encodes a novel inhibitor of CED-3.

3.7 Acknowledgments

I would like to thank Chris Hawkins for providing most of the yeast constructs and establishing the caspase-lacZ reporter assay, Jackie Barton and Kim Copeland for use of their radioactive hoods, Brent Derry for performing RNAi analysis for Y39B6A.12 and T23G11.7b, and Jun Huh for providing the JNK pathway strains.

3.8 References

Ellis, H. M. and Horvitz, H. R. (1986) Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* **44**, 817–829.

Fraser, A. G., James, C., Evan, G. I., Hentgartner, M. O. (1999) *Caenorhabditis elegans* inhibitor of apoptosis protein (IAP) homologue BIR-1 plays a conserved role in cytokinesis. *Curr. Biol*. **9**, 292–301.

Hawkins, C. J., Wang, S. L., and Hay, B. A. (1999) A cloning method to identify caspases and their regulators in yeast: Indentification of *Drosophila* IAP1 as an inhibitor of the *Drosophila* caspase DCP-1. *Proc. Natl. Acad. Sci. USA* **96**, 2885–2890.

Hentgartner, M. O., Ellis, R. E., and Horvitz, H. R. (1992) *Caenorhabditis elegans* gene *ced-9* protects cells from programmed cell death. *Nature* **356**, 494–499.

Jabbour, A. M., Ho, P. K., Puryer, M. A., Ashley, D. M., Ekert, P. G., and Hawkins, C. J. (2004) The *Caenorhabditis elegans* CED-9 protein does not directly inhibit the caspase CED-3, in vitro nor in yeast. *Cell Death and Diff*. **11**, 1309–1316.

Jagasia, R., Grote, P., Westermann, B., and Conradt, B. (2005) DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in *C. elegans*. *Nature* **433**, 754–760.

James, C., Gschmeissner, S., Fraser, A., and Evans, G. I. (1997) CED-4 induces chromatin condensation *in Schizosaccharomyces pombe* and is inhibited by direct physical associations with CED-9. *Curr. Biol.* **7**, 246–252.

Metzstein, M. M., Stanfield, G. M., and Horvitz, H. R. (1998) Genetics of programmed cell death in *C. elegans*: past, present, and future. *Trends Genet.* **14**, 410–416.

Parrish, J., Li, L., Klotz, K., Ledwich, D., Wang, X., and Xue, D. (2001) Mitochondrial endonuclease G is important of apoptosis in *C. elegans*. *Nature* **412**, 90–94.

Quinn, L., Coombe, M., Mills, K., Daish, T., Colussi, P., Kumar, S., and Richardson, H. (2003) Buffy, a *Drosophila* Bcl-2 protein, has anti-apoptotic and cell cycle inhibitory functions. *EMBO J*., **22**, 3568–3579.

Seshagiri, S., and Miller, L. K. (1997) *Caenorhabditis elegans* CED-4 stimulates CED-3 processing and CED-3-induced apoptosis. *Curr Biol*. **7**, 455–460.

Shaham, S., and Horvitz, H. R. (1996) An alternatively spliced *C. elegans ced-4* RNA encodes a novel cell death inhibitor. *Cell* **86**, 201–208.

Speliotes, E. K., Uren, A., Vaux, D., and Horvitz, H. R. (2000) The Survivin-like *C. elegans* BIR-1 protein acts with the Aurora-like kinase AIR-2 to affect chromosomes and the spindle midzone. *Mol. Cell* **6**, 211–223.

Sugimoto, A., Kusano, A., Hozak, R. R., Derry, W. B., Zhu, J., and Rothman, J. H. (2001) Many genomic regions are required for normal embryonic programmed cell death in *Caenorhabditis elegans*. *Genetics* **158**, 237–252.

Sulston, J. E., and White, J. G. (1980) Regulation and cell autonomy during postembryonic development of *Caenorhabditis elegans*. *Dev. Biol.* **78**, 577–597.

Wang, S. L., Hawkins, C. J., Yoo, S. J., Muller, H. A. J., and Hay, B. A. (1999) The *Drosophila* caspase inhibitor DIAP1 is essential for cell survival and is negatively regulated by HID. *Cell* **98**, 453–463.

Wang, X., Yang, C., Chai, J., Shi, Y., and Xue, D. (2002) Mechanisms of AIF-mediated apoptotic DNA degradation in *Caenorhabditis elegans*. *Science* **298**, 1587–1592.

Xue, D., and Horvitz, H. R. (1997) *Caenorhabditis elegans* CED-9 protein is a bifunctional cell-death inhibitor. *Nature* **390**, 305–308.

Zou, H., Henzel, W. J., Liu, X., Lutschg, A., and Wang, X. (1997) Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of Caspase-3. *Cell* **90**, 405–413.