# Chapter 4: A Deficiency Screen to Isolate Novel Regulators of DIAP1

## 4.1 Abstract

DIAP1, a ubiquitin E3 ligase, is the key *Drosophila* regulator of the family of cysteine proteases known as caspases. Because DIAP1 is crucial for cell viability, determining how DIAP1 functions in healthy and dying cells is of importance. We have created an eye-specific *diap1 Drosophila* mutant using RNAi, and this mutant, which has a small eye due to increased cell death, provides a sensitized background in which to screen for modifiers of DIAP1. A deficiency screen of the *Drosophila* genome isolated two suppressors and seven enhancers. One enhancer was mapped to a narrow region within the genomic area 30F and tests are being performed to determine the possible role of the ribosomal protein RpL13. A suppressor in the region of 90F has been narrowed down to approximately ten candidate genes. Due to the fact that each deficiency isolated contains no known apoptotic regulators, this screen provides a means to identify novel regulators of cell death.

## 4.2 Introduction

Elimination of cells through programmed cell death is crucial for the development and viability of an organism. Though several mechanisms for cell death exist, apoptosis still remains the best characterized mode of cell death. Caspases, a family of cysteine proteases, comprise a key switch in activation of apoptosis. Activated caspases cleave several protein substrates, leading to gross morphological changes in the cell and eventual death. The first caspase identified was the *ced-3* gene in *C. elegans*, and caspases have since been found throughout metozoa.

In *Drosophila* and mammals, IAPs are the key caspase inhibitors. In part, IAPs are characterized by their BIR domains and some members also have the characteristic E3 ubiquitin ligase RING domain. DIAP1 in flies and XIAP in mammals contain RING domains and act to inhibit caspases through ubiquitin-mediated protein degradation (Suzuki et al., 2001; Wilson et al., 2002).

Several proteins promote cell death by counteracting the function of the anti-apoptotic IAPs. In *Drosophila*, the most famous of these are the small proteins HID, GRIM, REAPER, and SICKLE. Each contains a small hydrophobic motif that binds to a BIR domain (Wu et al., 2001). Once bound to these small proteins, DIAP1 promotes its own degradation and releases the caspase from its grasp (Wang et al., 1999; Yoo et al., 2002). UBCD1 and MORGUE, two components of the ubiquitin protein-degradation

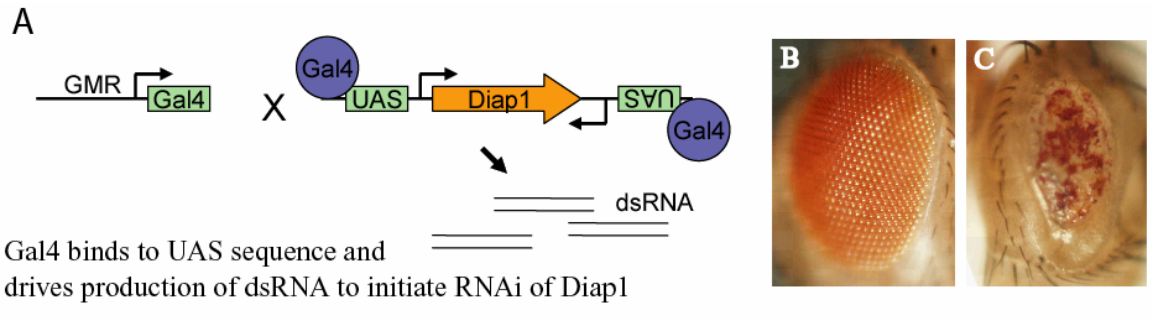


Figure 1. Diap1 RNAi knockdown in the *Drosophila* retina. A) Schematic of the production of dsRNA specifically in the eye by the GMR-gal4 driver. dsRNA serves as a substrate for the RNAi machinery to target and reduce DIAP1 levels. B) Adult wild type eye. C) Small eye phenotype in GMR-diap1(RNAi) flies.

machinery, have also been shown to promote the down regulation of DIAP1 protein levels (Ryoo et al, 2002; Hays et al., 2002; Wing et al., 2002).

While some of the regulators of DIAP1 in dying cells are known, two questions arise: Are there others and what factors aid DIAP1 function in healthy cells? To address these questions, we have screened for modulators of DIAP1 function in a sensitized background. Knockdown of *Diap1* by RNAi in the *Drosophila* eye gives a moderately small eye phenotype (Fig. 1C). By screening though a collection of genetic deficiencies, we were able to isolate several genomic regions that dominantly alter the eye size of the *diap1*(RNAi) mutant. Secondary screens eliminated many of the loci and left nine loci that contained genuine modifiers. Two genomic regions were selected in hopes of isolating one genetic enhancer and one suppressor.

## 4.3 Material and Methods

### Fly Genetics

Drosophila stocks were maintained at 25 $^{o}$C. Deficiencies and mutants were obtained from the Bloomington Stock Center (Bloomington, IN). UAS-Q78, UAS-EGFR(dn), and UAS-Debcl, encoding a pro-apoptotic Bcl family member, were obtained from N. Bonini, K. Moses, and S. Kumar, respectively, and recombined to either the GMR-gal4 or ptc-gal4 chromosome. GMR-ec(RNAi) was reported in a previous chapter. GMR-P35, GMR-Diap1, *thread*[5] have been previously reported. Transgenic flies were created by microinjection into embryos. Constructs were injected by Rainbow Transgenic Flies (Newbury Park, CA). For each injected construct, several lines were balanced and tested for modification of the GMR-diap1(RNAi) small eye phenotype.

### Molecular Biology

Mutant *diap1*(RNAi) flies were made by driving dsRNA production from UAS elements flanking the coding region (Giordano et al., 2002). Two *diap1*(RNAi) lines were generated. The strong line has a small eye phenotype, while the weaker has mild disruption in the eye ommatidia. To generate an eye-specific RNAi mutant of the ribosomal gene *RpL13,* a 22nt sequence from the gene replaced the stem sequence of the microRNA mir-6 (Table 1). The resulting miR-6::RpL13 hybrid was cloned into pGMR for gene expression in the eye. In a similar manner, GMR-miPP2A-B', GMR-miCG14314, GMR-miCG14316, GMR-miCG7940, GMR-miCG7985, GMR-miCG7156, GMR-miCG7183, GMR-miCG7215, GMR-miCG7993 were made (Table 1).

| Gene | Sequence |
|---|---|
| RpL13 | TGATCGTTCTGTCTACAACATC |
| PP2A-B' | GGAGCTGTTCATACAGAAGATC |
| CG14314 | ACACTAGCTGTCAGCCCAAAAC |
| CG14316 | GAACCCTTTTACAAAGTCTAAT |
| CG7940 | TGAAAGCACCGAAAAATACATG |
| CG7985 | GGATAATACGAATGTTAACAGC |
| CG7156 | AGGCACTATTTGCTTTGCTATT |
| CG7183 | AAAGGCATACCCTTCATTTATA |
| CG7215 | AAATTTTACGGCAATTGCTATG |
| CG7993 | TATGTCGCTTTTACGCATCAGG |

Table 1. Genes and sequences used for RNAi as described in Materials and Methods section.

## 4.4 Results

### Screen for diap1(RNAi) modifiers

DIAP1 is the key inhibitor of apoptosis in *Drosophila*. To screen for regulators of DIAP1 function, we created a sensitized background by RNAi knockdown of *Diap1* specifically in the eye. The coding region of *Diap1* was flanked by UAS enhancer elements to drive expression of dsRNA in the presence of a gal4 driver line (Fig. 1). Using the GMR-gal4 line, a mutant *diap1*(RNAi) line was created that resulted in a small eye phenotype and an increase of apoptosis. This line was used to screen a set of autosomal deficiencies covering approximately 80% of the *Drosophila* genome.

Initial results from this screen resulted in 19 genetic deletions that when present as heterozygotes modified the small eye phenotype (Table 2). The effectiveness of our screen was confirmed by the isolation of two enhancers that covered *thread* (*Diap1*). Interestingly, no deficiencies covering the caspase *Dronc* were isolated as modifiers.

To reduce the number of false positives from the list of modifying genomic loci, each deletion was crossed to GMR-gal4>UAS-Egfr(dn), GMR-gal4>UAS-Q78, and GMR-gal4>UAS-ec(RNAi). Though each of these lines has been implicated in regulating cell

| Deficiency | Genomic Location | Enhancer/ Suppressor | GMR>ec(RNAi) | GMR>Q78 | GMR>Egfr(dn) | Gene |
|---|---|---|---|---|---|---|
| Df(2L)net-PMF | 21A1-21B8 | E | - | - | - | |
| Df(2L)BSC37 | 22D2-22F2 | E | + | + | + | |
| Df(2L)sc19-4 | 25A-25E5 | E | + | nd | nd | |
| Df(2L)XE-3801 | 27E2-28D1 | E | - | - | - | |
| Df(2L)BSC17 | 30C3-30F1 | E | - | + | - | |
| Df(2L)TW161 | 38A-40AB1 | E | - | - | - | |
| Df(2R)M41A4 | 41A | E | + | nd | + | |
| Df(2R)cn9 | 42E-44C | E | + | nd | nd | |
| Df(2R)stan1 | 46D7-47F16 | E | + | nd | nd | |
| Df(2R)Jp8 | 52F-53A1 | E | nd | - | + | |
| Df(2R)017/ Df(2R)AA21 | 56F 56F9-57D12 | E | - | - | - | |
| Df(2R)X58-12 | 58D1-2;59A | E | + | + | + | |
| Df(2R)59AD | 59A1-59D4 | E | - | - | + | |
| Df(3L)BSC35 | 66F1-67B3 | S | - | nd | nd | |
| Df(3L)vin7 | 68C8-69B5 | E | - | - | - | |
| Df(3L)brm11/ Df(3L)st-f13 | 71F1-72D10 72C1-73A4 | E | - | - | - | th |
| Df(3L)ri-XT1 | 77E4-78A4 | E | - | - | - | |
| Df(3R)DG2/ Df(3R)Cha7 | 89E-91B2 90F1-91F5 | S | - | + | + | |
| Df(3R)H-B79 | 92B3-92F13 | E | - | - | - | |

Table 2. A list of deficiencies isolated as modifiers of the GMR-diap1(RNAi) small eye phenotype. Deficiencies are listed as enhancers (E) or suppressors (S). GMR-ec(RNAi) is RNAi knockdown of the deubiquitinating enzyme Echinus, using the same RNAi technique as GMR-diap(RNAi). Both GMR-Q78 and GMR-EGFR(dn) use GMR-gal4 to express the UAS construct of each gene. Genetic interactions are listed as none (-), positive (+), or not determined (nd).

death in various ways, they provide a secondary screen to eliminate deletions that alter either GMR function or the gal4/UAS system. This round of crosses eliminated all but nine of the genomic loci – two suppressors and seven enhancers (Table 2).

**Enhancer of *diap1*(RNAi), the Df(2L)BSC17 Deletion**

One of the deletions isolated, Df(2)BSC17, enhanced the *diap1*(RNAi) small eye phenotype. When crossed to the *diap1*(RNAi) mutant, heterozygotes for Df(2)BSC17 resulted in pupal lethality. A weaker *diap1*(RNAi) mutant, which by itself causes a mild phenotype, was crossed to Df(2L)BSC17 and resulted in a smaller, rougher eye (Fig. 3).

Df(2)BSC17 spans the chromosomal 30 region and deletes approximately 100 genes. A series of small overlapping deficiencies narrowed the potential modifier to the right distal

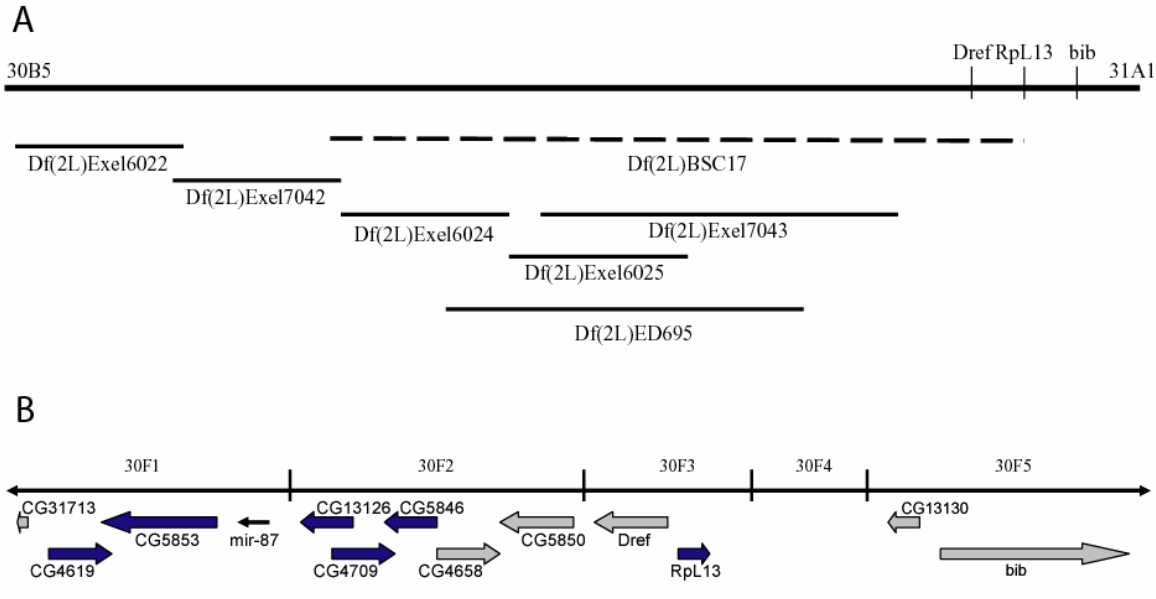


Figure 2. (A) Genomic region of Df(2L)BSC17. Df(2L)BSC17 is shown as a modifier (dashed line) while other non-interacting deficiencies (solid) are shown. Right breakpoint of Df(2L)BSC17 lies between the genes Dref and bib. (B) A view of the region containing the modifier. Genes in grey have been excluded. Genes in blue are candidates.

tip of Df(2)BSC17 (Fig. 2A). The right breakpoint of Df(2)BSC17 was mapped between the genes *Dref* and *bib* by the fact that *Dref* fails to complement and *bib* does complement. The modifier, thus, lies in the 30F region between CG31713, the last gene removed by Df(2L)Exel7043 and *bib* (Fig. 2B). Mutants for the genes CG13130, *Dref*, *bib*, CG5850, and CG4658 failed to phenocopy Df(2)BSC17.

One candidate modifier in the 30F region is the gene encoding the ribosomal protein *RpL13*. An eye-specific RNAi mutant was created by replacing the stem structure of the microRNA mir-6 with a 22 nt sequence specific for *RpL13*, and placing the hybrid sequence under the control of the GMR enhancer elements. Transgenic flies for the *RpL13* knockdown, miRpL13, showed small eye phenotype (Fig. 3). The miRpL13 mutant causes lethality in both the strong and weak *diap1* RNAi lines. These results underscore that loss of *RpL13* enhances loss of *diap1* and remains a potential regulator of DIAP1 function. Transgenic flies with overexpressed *RpL13* are now being generated to eventually test for rescue in a *diap1* mutant fly.

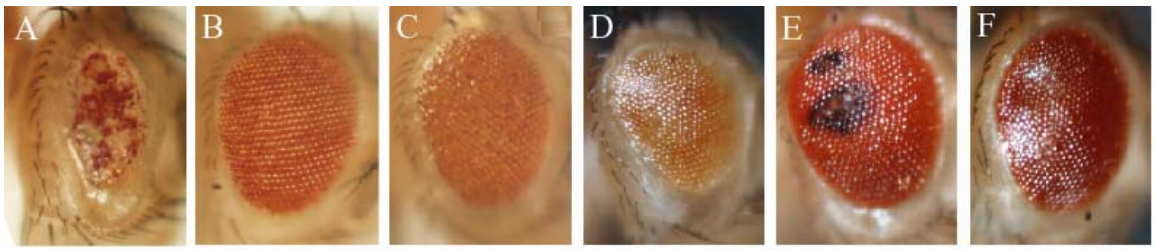


Figure 3. RNAi of the ribosomal gene RpL13 causes a small eye phenotype that can partially suppressed by Diap1 and p35. miRpL13 causes lethality when in trans to GMR-diap1(RNAi). Fly eye pictures of the interaction between Df(2L)BSC17 and RpL13 with GMR-diap1(RNAi). (A) GMR-diap1(RNAi) [strong] (B) GMR-diap1(RNAi) [weak] (C) GMR-diap1(RNAi) [weak]; Df(2L)BSC17/+ (D) GMR-miRpL13 (E) GMR-p35; GMR-miRpL13 (F) GMR-diap1; GMR-miRpL13

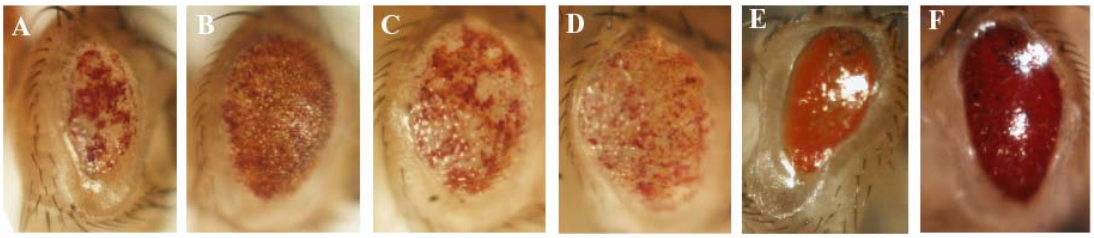


Figure 4. Deficiency Df(3R)DG2 contains two suppressors of the GMR-diap1(RNAi) small eye phenotype. Both *glass* and Df(3R)DG4 overlap Df(3R)DG2, but complement each other. Fly eyes showing the interactions between GMR-diap1(RNAi) and deficiencies of the 90F region. (A) GMR-diap1(RNAi) (B) GMR-diap1(RNAi); Df(3R)DG2/+ (C) GMR-diap1(RNAi); Df(3R)DG4/+ (D) GMR-diap1(RNAi); *gl*[60j]/+ (E) GMR-gal4; UAS-debcl (F) GMR-gal4; UAS-debcl/ Df(3R)DG2

**A potential suppressor in 90F that is not *glass***

In the screen both the Df(3R)DG2 and Df(3R)Cha7 deficiencies were isolated as moderate suppressors of the eye phenotype (Fig. 4B, data not shown for Df(3R)Cha7). Both Df(3R)DG2 and Df(3R)Cha7 include *glass* (*gl*), a transcription factor used to drive gene expression in GMR, as both deficiencies failed to complement the *glass* mutant $gl^{60j}$. As loss of *gl* function would prevent the GMR construct from driving gene expression, we tested whether *glass* mutants could suppress the *diap1*(RNAi) construct. Heterozygotes for $gl^{60j}$ moderately increased the eye size in the *diap1* knockdown flies, proving that disruption of the GMR enhancer element can alter the eye size in GMR-diap1(RNAi) mutants (Fig. 4C).

A second modifier might exist within these modifying chromosomes, despite the fact that both Df(3R)DG2 and Df(3R)Cha7 span the *glass* locus. A series of smaller deficiencies covering the entire 90 chromosomal region, but importantly not covering the *glass* locus, were obtained and tested for suppression of *diap1*(RNAi). Interestingly, Df(3R)DG4 acted similar to Df(3R)Cha7, but complemented *gl*. Based upon crosses with other

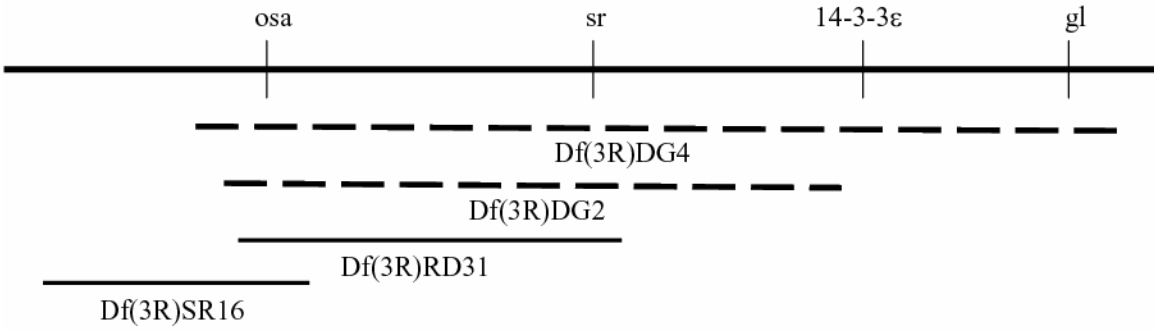


Figure 5 Genomic region of Df(3R)DG2. Df(3R)DG2 and Df(3R)DG4, suppressors of the GMR-diap1(RNAi) eye phenotype, are shown as dashed lines while non-suppressing deficiencies are shown as solid lines.

deficiencies and mutants, the potential suppressor was narrowed down to the genomic area between the genes *stripe* and 14-3-3ε (Fig. 5). Hypomorphic mutants for *stripe*,

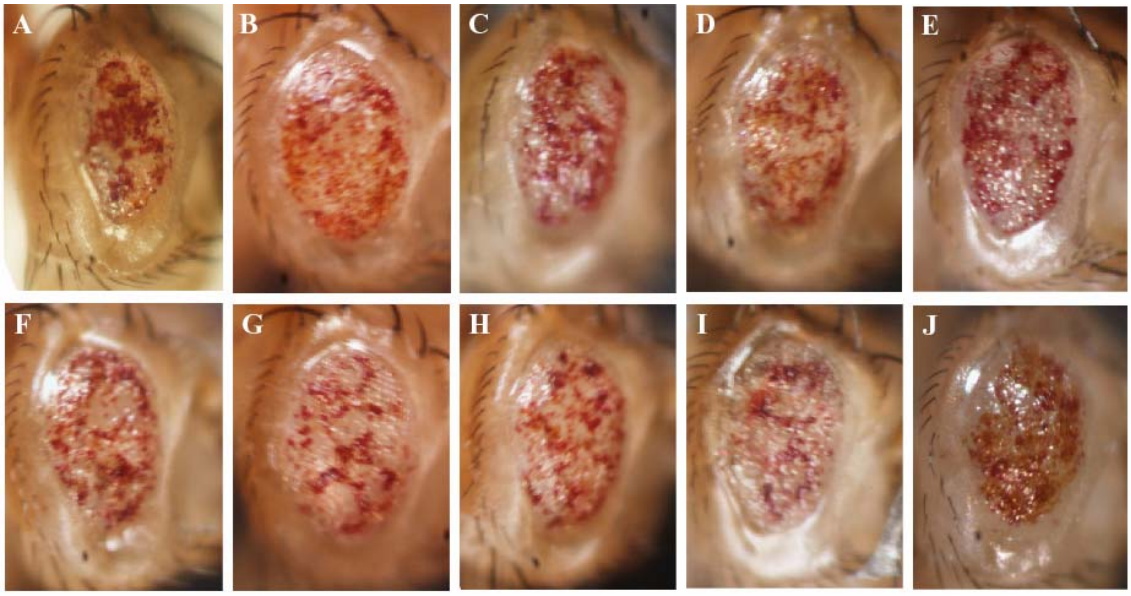


Figure 6 Fly eye pictures of candidate genes in 90F. Each candidate has been knockdown in the eye using the microRNA method described in text. No candidate RNAi lines had dominant eye phenotypes. (A) GMR-diap1(RNAi) (B) GMR-diap1(RNAi); GMR-CG14314 (C) GMR-diap1 (RNAi); GMR-miPP2A-B' (D) GMR-diap1(RNAi); GMR-CG7940 (E) GMR-diap1(RNAi); GMR-CG7215 (F) GMR-diap1(RNAi); GMR-mi7156 (G) GMR-diap1(RNAi); GMR-CG14316 (H) GMR-diap1(RNAi); GMR-CG7183 (I) GMR-diap1(RNAi); GMR-CG7993 (J) GMR-diap1(RNAi); GMR-CG7985

*cadmus*, *Trap80*, *Ssdp*, *repo*, and *14-3-3ε* failed to phenocopy the suppression by Df(3R)DG4. Ectopic expression of CG14315, CG7217, CG7208, CG7998 with P-elements carrying UAS elements also failed to modify the small eye phenotype. RNAi gene knockdown was employed to test several other genes in the region using the microRNA hybrid method mentioned above. Testing nine of them, however, showed no modification of the *diap1*(RNAi) eye phenotype (Fig. 6).

**4.5 Discussion**

In this study a deficiency screen was performed to isolate dominant modifiers of a *diap1*(RNAi) eye phenotype. After a secondary screen to remove regulators of the Gal4/UAS system and RNAi machinery, two suppressors and seven enhancers were isolated. Two overlapping deletions covering *thread*, which encodes the *Diap1* gene, were pulled out of the screen, thus confirming the ability of the screen to identify modifiers.

Sensitized screens are commonly employed as a means of uncovering novel regulators of gene function. In this situation lowered levels of *Diap1* by RNAi caused an increase in apoptosis and reduced the size of the eye, allowing for the recovery of dominant modifiers that regulate DIAP1 function. Deficiency screens provide a quick and easy means of screening the genome, but it is tedious to pinpoint the actual modifier. A P-element screen would be a better means for screening due to the relative ease in

identifying the transposon genomic location and thus a smaller number of candidate genes. P-element screens, though, do not necessarily span the entire genome and are more labor intensive. A small P-element screen has recently been performed in the lab with the results pending.

**RpL13, the potential enhancer in Df(2L)BSC17**

In our screen, we isolated seven different enhancers, all of which are lethal when combined with GMR-diap1(RNAi). The deficiency Df(2L)BSC17 was a strong enhancer, and the potential modifying gene was narrowed down to a group of seven candidates in the 30F genomic region. RNAi of the ribosomal gene *RpL13*, one of the seven candidates, showed a small eye phenotype, making *RpL13* a good candidate modifier. Loss of *RpL13* specifically in the eye enhanced both the strong and weak loss of *diap1* lines, which further proves that *RpL13* is the responsible modifier in Df(2L)BSC17. Finally, overexpression of *Diap1* or the viral caspase inhibitor P35 slightly rescued the mutant *RpL13* eye phenotype. Tests are currently underway to determine whether overexpression of *RpL13* can rescue loss of *diap1*. Nevertheless, based on the observation that loss of *RpL13* phenocopies heterozygosity of Df(2L)BSC17, we strongly suspect that *RpL13* is the responsible gene for modulating DIAP1 levels.

It is interesting to hypothesize about the connection between apoptosis and ribosomal proteins. A recent study in zebra fish showed that eleven ribosomal proteins were haploinsufficient tumor suppressors, including the ortholog for RpL13 (Amsterdam et al., 2004). In *Drosophila* cell culture, depletion of ribosomal proteins by RNAi did not kill

the cells, and even prevented the entry of internal ribosome entry site (IRES) viruses (Cherry et al., 2005). In both cases novel roles for the ribosome were discovered. In the zebra fish study, loss of ribosome led to tumor formation, though the role of the ribosome in apoptosis or even growth control remains unclear. An interesting hypothesis would be that loss of ribosome serves as a protection against apoptosis. Thus, loss of the ribosome allows the cell to bypass growth controls and to initiate tumorigenesis. In support of this model, our lab has seen that the loss of some ribosomal proteins protects S2 cells against *diap1* RNAi induced cell death (I. Muro, unpublished data). This model, however, contradicts the observation that *RpL13* knockdown in the *Drosophila* retina either prohibits cell proliferation or promotes cell death. Does loss of the ribosome promote uncontrolled growth or promote apoptosis, or is there a context in which both these models hold true? For one thing, nothing is known mechanistically of how loss of the ribosome promotes tumor formation in zebra fish or inhibits proper *Drosophila* eye development. Secondly, we do not know if these observations depend on the tissue assayed or the specific ribosomal proteins lost. In both S2 cell lines and in the zebra fish experiments, proliferating cells were depleted of ribosomes, while in the *Drosophila* retina, the cells are essentially post-mitotic. These observations do however point to the fact that there is an uncharacterized connection between the ribosome and control of apoptosis, and the isolation of RpL13 as a modifier in the deficiency screen adds another tie.

**A suppressor in the 90F region**

From the screen, we also isolated a potential pro-apoptotic molecule in the 90F region. The deficiency Df(3R)DG4 suppressed the *diap*(RNAi) eye phenotype, while several overlapping deficiencies showed no suppression. Df(3R)DG4 complemented both 14-3-3ε and *glass*, and only the genes located between *stripe* and 14-3-3ε were left as candidates. This genomic deficiency also suppressed the phenotype of ectopically expressed *Debcl*, a pro-apoptotic Bcl family member whose function is still unknown.

The phosphatase PP2A-B' is a particularly interesting candidate in the region between *stripe* and 14-3-3ε. This enzyme belongs to the class of regulatory subunits for the PP2A phosphatase, and has been shown to inhibit Bcl2 function in mammalian cell culture (Chiang et al., 2001; Deng et al., 1998). The phosphatase, however, is not the suppressor; both RNAi knockdown and a P-element mutant, though located in an intron, show no genetic interaction with loss of *diap1* in the eye (Fig. 6, data not shown). Other candidates were excluded because their knockdown by RNAi failed to alter the small eye phenotype. All ten remaining genes encode novel genes so their likely function and potential connection to regulation of apoptosis remains elusive. Constructs to generate knockdown lines of these remaining candidates are currently being made.

**4.6 Conclusions**

In this study, loss of *diap1* in the eye by RNAi provided a sensitized background for a deficiency screen. With the exception of the deletions spanning the Diap1 locus *thread*,

the isolated deficiencies cover novel regulators of apoptosis. One enhancer shows a potential role for ribosomal proteins in mediating cell death, while another genomic region containing a pro-apoptotic molecule has been narrowed down to ten candidates.

## 4.7 Acknowledgments

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## 4.8 References

**Amsterdam, A., Sadler, K. C., Lai, K., Farrington, S., Bronson, R. T., Lees, J. A., Hopkins, N.** (2004) Many ribosomal protein genes are cancer genes in Zebrafish. *PLoS Biol*. **2**: 690–698.

**Cherry, S., Doukas, T., Armknecht, S., Whelan, S., Wang, H., Sarnow, P., and Perrimon, N.** (2005) Genome-wide RNAi screen reveals a specific sensitivity of IRES-containing RNA viruses to host translation inhibition. *Genes Dev*. **19**: 445–452.

**Chiang, C. W., Harris, G., Ellig, C., Masters, S. C., Subramanian, R., Shenolikar, S., Wadzinski, B. E., and Yang, E.** (2001) Protein phosphatase 2A activates the

proapoptotic function of BAD in interleukin-3-dependent lymphoid cells by a mechanism requiring 14-3-3 dissociation. *Blood* **97**: 1289–1297.

**Deng, X., Ito, T., Carr, B., Mumby, M., and May Jr., W. S.** (1998) Reversible phosphorylation of Bcl2 following interleukin 3 or byrostatin 1 is mediated by direct interaction with protein phosphatase 2A. *J. Biol. Chem*. **273**: 34157–34163.

**Giordano, E., Rendina, R., Peluso, I., and Furia, M.** (2002) RNAi triggered by symmetrically transcribed transgenes in *Drosophila melanogaster*. *Genetics* **160**: 637–648.

**Hays, R, Wickline L, Cagan R.** (2002) Morgue mediates apoptosis in the *Drosophila melanogaster* retina by promoting degradation of DIAP1. *Nat. Cell. Biol*. **4**, 425–31.

**Ryoo, H. D., Bergmann, A., Gonen, H., Ciechanover, A., and Steller, H.** (2002) Regulation of *Drosophila* IAP1 degradation and apoptosis by reaper and ubcD1. *Nat. Cell Biol*. **4**, 432–438.

**Suzuki, Y., Nakabayashi, Y., and Takahashi, R.** (2001) Ubiquitin-protein ligase activity of X-linked inhibitor of apoptosis protein promotes proteasomal degradation of Caspase-3 and enhances its anti-apoptotic effect in Fas-induced cell death. *Proc. Natl. Acad. Sci. USA* **98**, 8662–8667.

**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.

**Wilson, R., Goya, L., Ditzel, M., Zachariou, A., Baker, D. A., Agapite, J., Steller, H., and Meier, P.** (2002) The DIAP1 RING finger mediates ubiquitination of Dronc and is indispensable for regulating apoptosis. *Nat. Cell Biol*. **4**, 445–450.

**Wing, J. P., Schreader, B. A., Yokokura, T., Wang, Y., Andrews, P. S., Huseinovic, N., Dong, C. K., Ogdahl, J. L., Schwartz, L. M., White, K., and Nambu, J. R**. (2002) *Drosophila* Morgue is an F box/ubiquitin conjugase domain protein important for *grim-reaper* mediated apoptosis. *Nat. Cell Biol*. **4**, 451–456.

**Wu, J. W., Cocina, A. E., Chai, J., Hay, B. A., and Shi, Y.** (2001) Structural analysis of a functional DIAP1 fragment bound to Grim and Hid peptides. *Mol. Cell*. **8**: 95-104.

**Yoo, S. J., Huh, J. R., Muro, I., Yu, H., Wang, L., Wang, S. L., Feldmann, R. M. R., Clem, R. J., Muller, H. A. J., and Hay, B. A**. (2002) Hid, Grim, and Rpr negatively regulate DIAP1 levels through distinct mechanisms. *Nat. Cell Biol*. **4**, 416–424.