RNAi-Mediated Knockdown Showing Impaired Cell Survival in Drosophila Wing Imaginal Disc

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Abstract: The genetically amenable organism Drosophila melanogaster has been estimated to have 14,076 protein coding genes in the genome, according to the flybase release note R5.13 (http://flybase.bio.indiana.edu/static_pages/docs/release_notes.html). Recent application of RNA interference (RNAi) to the study of developmental biology in Drosophila has enabled us to carry out a systematic investigation of genes affecting various specific phenotypes. In order to search for genes supporting cell survival, we conducted an immunohistochemical examination in which the RNAi of 2,497 genes was independently induced within the dorsal compartment of the wing imaginal disc. Under these conditions, the activities of a stress-activated protein kinase JNK (c-Jun N-terminal kinase) and apoptosis-executing factor Caspase-3 were monitored. Approximately half of the genes displayed a strong JNK or Caspase-3 activation when their RNAi was induced. Most of the JNK activation accompanied Caspase-3 activation, while the opposite did not hold true. Interestingly, the area activating Caspase-3 was more broadly seen than that activating JNK, suggesting that JNK is crucial for induction of non-autonomous apoptosis in many cases. Furthermore, the RNAi of essential factors commonly regulating transcription and translation showed a severe and cell-autonomous apoptosis but also elicited another apoptosis at an adjacent area in a non-autonomous way. We also found that the frequency of apoptosis varies depending on the tissues.

Keywords: Drosophila RNAi apoptosis JNK caspase-3

Introduction

In recent years, mechanisms controlling apoptosis have been extensively studied, and various factors are known to be involved in the intrinsic and extrinsic apoptotic pathways.1–4 Although these pathways play a pivotal role in the execution of most cases of apoptosis, the apoptosis shown in developing animal tissues is also affected by various growth and differentiation signals to promote or repair organ development.5 In general, inhibition of apoptosis accompanies growth induction, whereas reduction of growth conversely leads to apoptosis. However, we can often find exceptions showing an opposite relationship, such as overgrowth-induced apoptosis6–8 and apoptosis-induced overgrowth,5–11 indicating that we do not fully understand these cell survival controls between apoptosis and growth. In order to systematically investigate the apoptosis phenotype caused by reducing each gene function in the developing animal tissues, we employed a genetically amenable fruit fly Drosophila melanogaster, in which each gene can be knocked down by RNAi,12–14 to observe the effect on apoptosis induction. RNAi provides an easy and powerful technique for reducing the quantity of mRNA derived from endogenous specific genes, and it has recently been applied in many studies to investigate various gene functions.15

In this study, we screened 2,497 protein-coding genes of Drosophila to determine whether they were required for prevention of apoptosis in the wing imaginal disc and found that 47% of them showed an apoptosis induction when their functions were knocked down by RNAi in the developing wing disc. Most of the cases (82%) with detectable Caspase activation were associated with JNK activation, which was
unexpectedly high because JNK has not been observed as essential for all apoptosis. Alternatively, JNK is known to be involved in inducing non-autonomous apoptosis, which occurs in cells distant from the cells associated with the primary cause of apoptosis. Interestingly, a major part of the JNK and Caspase-3 activation found in this study occurred in a non-autonomous manner, suggesting that the non-autonomous pathway is a common way to induce apoptosis. Loss of membrane proteins frequently caused JNK activation, which had also been expected because cell-cell communication is presumed to be important for many developmental processes, including apoptosis in multicellular organisms. These results, as well as the database showing the immunofluorescent data, provide an archival source for survey of genes and for fine analysis of each gene in apoptosis regulation using the *Drosophila* imaginal discs.

## Results

### Rationale for RNAi-mediated screening for genes regulating apoptosis

We induced RNAi in the dorsal compartment of the wing disc and monitored the activities of Caspase-3 and JNK. Caspase-3 plays a central role in most apoptosis, while JNK leads to a subgroup of stress-induced apoptosis. In the *Drosophila* wing disc, JNK activation is usually linked to the activation of Caspase-3. Puc is a protein phosphatase specifically inactivating JNK, and its transcription occurs in response to the JNK signal, thereby making a negative-regulatory circuit. Thus, the expression of *puc* reflects the JNK activity and can be used for monitoring it.

Before expanding the RNAi analyses to the entire genome, we checked whether the mutant phenotypes caused by previously known apoptosis-regulating genes, such as *diap1* and *dark*, were reproduced by their RNAi. When *diap1* (*Drosophila Inhibitor of Apoptosis Protein 1*) was knocked down within the dorsal compartment of the imaginal disc, a local but prominent activation of Caspase-3 was detected (Fig. 1B). The position-specificity may be dependent on the difference in sensitivity in the induction of apoptosis, as described later. In contrast, when *dark* (*Drosophila* Apaf-1-Related Killer) was knocked down, no apoptosis induction was observed (Fig. 1C). Furthermore, the use of this collection of RNAi strains has already been validated, since they were screened for apoptosis phenotype in the compound eye.

For the non-autonomously induced apoptosis during restoration of morphogenesis, for example, we tested whether the apoptosis shown in several RNAi samples really reflected the non-autonomous apoptosis by conventional gene manipulation in previously studies. We manipulated signaling factors for a diffusible extracellular ligand Dpp, a homolog of mammalian BMP (Bone Morphogenetic Protein)–2/4. Mad (Mothers against Dpp), a *Drosophila* homolog of mammalian r-Smad, transmits the intracellular signal caused by Dpp. As shown in Figure 1E, the RNAi of *mad* within the dorsal compartment activated JNK and Caspase-3 in both dorsal and ventral compartments of the central wing disc region, which is a typical example of non-cell-autonomous induction of apoptosis. These features are also quite similar to those seen in the case of overexpression of Dad (Daughters against Dpp), a homolog of anti-Smad, (Fig. 1F) that can repress Dpp signaling. Therefore, RNAi can mimic the conventional gene manipulation in induction of apoptosis, at least in some representative examples.

We describe how to choose the genes for RNAi in the section on Experimental Procedures. During observation of our RNAi results, we focused on six areas of the wing disc (Notum [N], Dorsal wing Blade [DB], DorsoCentral spot [DC], Dorsal wing Margin [DM], Ventral wing Margin [VM], and Ventral wing Blade [VB]), and classified the strength of JNK and Caspase-3 activities in each area into three grades (+, −, +, and ++). The database was constructed by using FileMaker Pro 7 (FileMaker, Inc.) and contains each immunofluorescence image with the above classification of JNK/Caspase-3 activities in each gene page.

First, we noticed that a narrow area in the dorso-central (DC) wing region showed JNK and Caspase-3 activation too sensitively (e.g. Fig. 2), which was not always correlated with RNAi. Consequently, the results that simply reflected this feature were excluded from all of the analyses.

When a survey of all of the RNAi experiments was carried out, both weak and strong activation of JNK and Caspase-3 (shown by + and ++ signs in the database) was observed in 41% and 87% of the cases, respectively (Fig. 3A). These proportions seemed much higher than expected because the imaginal disc cells may not actually express such a large number of genes, and they are thought to express several thousand genes. This large
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**Figure 1.** Similarity of RNAI and conventional gene manipulation in their apoptotic phenotypes. (A–A”) RNAi of *diap1* by expressing its inverted repeat (IR) sequence in the dorsal compartment displayed a reduction of DIAP1 protein levels specifically in the dorsal compartment. Expression of a dorsal compartment marker *apterous* (green), DIAP1 (red), and JNK indicator *puc-lacZ* (blue) are shown. A” shows a pseudocolor image representing DIAP1 protein levels. Arrowhead in A indicates a cluster of high level staining of DIAP1 and *puc-lacZ*, which is caused by the presence of massive apoptotic bodies. (B–F) Expression of *apterous* (green), *puc-lacZ* (magenta), and activated Caspase-3 (blue) are shown. (B–B”) RNAi of *diap1* in the dorsal compartment led to an activation of Caspase-3 at the specific position in the dorsal compartment. When the contrast of B” image is elevated, *puc-lacZ* expression can be observed at around the apoptotic bodies as in A” (not shown). (C–C”) RNAi of *dark* in the dorsal compartment as a negative control experiment. No activation of JNK and faint activation of Caspase-3 were observed. (D–D”) Wild type. JNK and Caspase-3 are not activated in wild type. (E–E”) RNAi of *mad* in the dorsal compartment led to a non-autonomous activation of JNK and Caspase-3 in the medial region of the DV boundary. (F–F”) Overexpression (OE) of *dad* in the dorsal compartment, which results in a reduction of Dpp signaling, induces a shrinkage of the dorsal compartment as well as a non-autonomous activation of JNK and Caspase-3 in the medial region of the DV boundary.
number of strains with weak JNK and/or Caspase-3 activation may reflect false-positive results due to an off-target effect (OTE) or other non-specific effects of dsRNA expression. A part of these IR lines was known to have possible OTs and the frequency of such suspected OTE lines was calculated to be 47% as a maximal estimation. Accordingly, we did not give further consideration to this class of strains and hereafter focused on the results showing high levels of JNK and Caspase-3 activation (shown only by the ++ sign in the database), which amounted to 10% and 47% of cases, respectively (Fig. 3A). The immuno-fluorescence data can be accessed on the website: http://www.shigen.nig.ac.jp/fly/nigfly/index.jsp (see Experimental Procedures).

Screening summary
Among these strong cases of JNK and/or Caspase-3 activation, more than 80% showed a coupling of JNK and Caspase-3 activation to various extents (Fig. 3C). Conversely, there were only two cases in which JNK activation did not accompany Caspase-3 activation (calculated as 0.17%). Therefore, these findings are consistent with the previous observation that the JNK activation precedes Caspase-3 activation and is strongly linked to apoptosis in the Drosophila wing.17 Furthermore, we carefully assessed the non-cell autonomous effect of RNAi by examining the phenotypes in the vicinity of the DV boundary. Most of the RNAi-induced JNK/ Caspase-3 activation showed a striking non-cell autonomy (Fig. 3D), which is similar to the previously known feature in non-cell-autonomous activation of JNK by altered Dpp signaling.17 This suggests that the non-cell-autonomous induction of apoptosis is one of the common patterns in cell death induction.

It has previously been shown that LRR (Leucine Rich Repeat) family cell adhesion proteins contribute
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Figure 3. Categorization of RNAi-induced JNK and Caspase-3 activation. A) Frequency of genes in which RNAi induces JNK activation. Cases with activation only at the dorsocentral spot (e.g. aop shown in Fig. 2) are excluded. B) Frequency of genes in which RNAi induces Caspase-3 activation. Again, cases with activation only at the dorsocentral spot are excluded. C) Relationship between JNK and Caspase-3. Cases showing strong activation of at least either JNK or Caspase-3 are analyzed. Left: Overlap of JNK and Caspase-3 activation. In most cases, both are simultaneously activated. Caspase-3 are sometimes activated solely (e.g. Fig. 1B), whereas JNK activation without Caspase-3 activation is rare. Right: Pattern of relative position of JNK-activating area (red) and Caspase-3-activating area (blue). Overlapping areas are shown in magenta. D) Relation of cell autonomy in JNK activation and that in Caspase-3 activation around the DV boundary in the wing blade. Examples showing rare patterns in the table are RpL17 (α), shotgun (β), CG14122 (γ), CG14072 (δ), and E(spl)m5 (ε). E) Frequencies of each JNK activation pattern with regard to wing disc subdomains. F) Frequencies of each Caspase-3 activation pattern with regard to wing disc subdomains.
to unique cell affinity. Furthermore, alteration of LRR protein functions causes JNK activation followed by Caspase-3 activation.\textsuperscript{30,31} Therefore, transmembrane and secreted proteins are good candidates for mediators of non-cell-autonomous apoptosis. We thus tested the apoptosis-inducing activity by RNAi of putative secreted proteins selected based on the presence of N-terminal signal sequences. Consistently, the RNAi of such genes caused a higher frequency of strong JNK activation (74 out of 296 cases, 25\%) when compared with the frequency of strong JNK activation in all of the RNAi cases (248 out of 2,497 cases, 10\%). Moreover, the RNAi of various transcription factors frequently showed non-autonomous apoptosis, suggesting that they are highly involved in regulation of morphogenesis and that their aberration likely induces non-autonomous apoptosis. These results suggest that a relatively large number of secreted proteins and transcription factors are involved in the prevention of non-cell-autonomous apoptosis. However, functional redundancy may have prevented identification of such molecules.

We also noted that there was a significant tendency for various examples of RNAi-mediated apoptosis to be preferentially found in the wing blade region but not outside of this region. For example, the RNAi of \textit{taf6} (TBP-Associated Factor 6) leads to an autonomous activation of Caspase-3 at high levels in the wing blade region but at lower levels in the wing hinge region (Fig. 4). Similar traits have also been reported in the case of the apoptosis induced by a reduced-Dpp signal.\textsuperscript{17} Furthermore, when we surveyed all of the results, the activation of JNK/Caspase-3 in the wing blade was found to be much more frequent than those in the notum (Figs. 3E and F). Consequently, localization of apoptosis in the wing blade region may not be a feature specific to the alteration of a particular cell signal (such as Dpp) but instead a general

![Figure 4](image-url)
Non-autonomously induced apoptosis

As we reported previously, aberrations of some morphogenetic signaling induce JNK activation followed by Caspase-3 activation at the boundary between cell populations with different levels of signaling intensities. This non-autonomous apoptosis is thought to be important for restoration of abnormally developing tissues. Various examples of apoptosis are probably induced in a similar non-autonomous way. To determine to what extent this applies to non-autonomous apoptosis, we surveyed the relationship in cell autonomy between JNK and Caspase-3 activation by focusing on the DV boundary at which the two cell populations come in contact (Fig. 3D). Around this position, there is an apparent tendency for JNK and Caspase-3 activation to occur simultaneously, which was observed in 471 cases, as shown in the 9 upper-left boxes in Fig. 3D’s grid. As stated above, the most frequent pattern is that both JNK and Caspase-3 are both autonomously and non-autonomously activated. However, 112 out of 196 cases with autonomous JNK activation (57%) displayed a non-autonomous Caspase-3 activation (upper-most row in Fig. 3D grid). In contrast, except for 6 cases, autonomous Caspase-3 activation (331 cases) did not show non-autonomous JNK activation (left-most column in Fig. 3D grid). Accordingly, these data strongly suggest that JNK activation is also crucial for priming non-autonomous apoptosis, whereas Caspase-3 is not.

Furthermore, when observed throughout the wing disc, the activation patterns of JNK and Caspase-3 are different (Figs. 3E and F). JNK activation seems to be found unevenly in the dorsal region \(2 + 42 + 13 = 57\%\), whereas the Caspase-3 activation only within the dorsal region is less \(2 + 5 + 35 = 42\%\). On the other hand, JNK with non-autonomous activation is minor \(0 + 0 + 16 + 27 = 43\%\), whereas non-autonomous Caspase-3 is major \(0 + 0 + 4 + 54 = 58\%\).

We were interested in the fact that some RNAi examples resulted in a non-autonomous apoptosis similar to that seen previously. The RNAi of basal transcription factor Ta\(f\) showed an autonomous activation of Caspase-3 in the blade region and a non-autonomous activation of JNK in the hinge region (Figs. 4A, B), the latter of which was unexpected because Ta\(f\) is known to be necessary for the function of TBP (TATA-Binding Protein), suggesting its ubiquitous requirement for most of the transcription by RNA polymerase II. Accordingly, the RNAi of \(ta\f\) is expected to induce a severe autonomous apoptosis in all of the tissues, as is the case for the RNAi of ribosomal protein genes (e.g. RpS14, Fig. 4C). Thus, the non-autonomy in JNK activation in the hinge region in \(ta\f\) RNAi suggests a morphogenetic function rather than its common transcriptional function and/or a difference in the sensitivity of decreased transcription leading to apoptosis between the tissues. The difference in responses between the blade and hinge regions was previously described in the apoptosis associated with homeotic transformation by overexpression of spineless. 30

Through these RNAi experiments, we discovered numerous cases of non-autonomous apoptosis cases. Among these, there are particular cases in which an autonomous apoptosis must be induced as a primary response (e.g. Rp\(L\)17, shotgun) while an additional non-autonomous apoptosis may be further induced as a secondary response, which is probably caused by a juxtaposition of a normal area and a wide apoptotic area, as proposed previously. 32 Therefore, we tested a model case in which the proapoptotic gene \(rpr\) is temporarily induced within the dorsal compartment by combination with a temperature sensitive-GAL80 33 (Fig. 5). As a result, at around 24 hours after \(rpr\) expression, non-autonomous apoptosis could be observed in the ventral compartment (Fig. 5B), although no similar non-autonomy was observed earlier or later (Figs. 5A and C). Thus, a wide area of autonomous apoptosis induction can cause a secondary non-autonomous apoptosis. This phenomenon seems to be a repair mechanism for fitting the adjacent tissue size. 32

Discussion

We created a database to show the immunofluorescent images for JNK and Caspase-3 activities in each RNAi experiment. In addition to our purpose of surveying genes affecting cell survival, the database may also be useful for searching for genes...
regulating tissue growth and patterning. For example, the RNAi of mad displays an apparent shrinkage of the compartment size without showing severe apoptosis except at the DV boundary (Fig. 1E). This phenotype strongly suggests the involvement of this gene in tissue growth and/or patterning. In contrast, the wing disc in which the dorsal compartment cells overexpress rpr showed a wide and severe apoptosis so that most of the dorsal cells disappeared (Fig. 5D). This case represents a typical phenotype, showing that the gene plays a role exclusively for apoptosis. These findings provide insight into the roles of genes for regulating various developmental processes.

As is the case for the above-mentioned possibility of weak activation of JNK and/or Caspase-3, the OTE should also be considered for all phenotypes. As an effective initial examination, the RNAi phenotype must be ameliorated by addition of the wild type transgene that is targeted by RNAi. Furthermore, two ways to distinguish the real RNAi effect from OTE have been proposed. One is a test of a dsRNA corresponding to the other part of the same mRNA for displaying the
same phenotype. The other is a test of an artificially altered transgene that is not targeted by the dsRNA but that encodes the same amino acid sequence for complete rescue of the phenotype. Of course, classical analysis using the loss-of-function mutant may be another reliable way to judge the involvement of the gene in each phenotype. In either case, further analyses are required to demonstrate each phenotype as a real loss-of-function phenotype of the gene under focus. The database will be updated when we check the phenotype by the above examinations or accumulate the data from other RNAi constructs.

**Experimental Procedures**

**Materials**

Various fly strains harboring a transcribable inverted repeat sequence (IR) driven by UAS (Upstream Activation Sequence) were prepared in the National Institute of Genetics (NIG) in Japan, as previously described. Briefly, a cDNA fragment with nucleotide position 1–500 of the coding sequence was obtained by PCR and was inserted as an IR in a head-to-head manner into a modified Bluescript vector, pSC1. Then IR-fragments were excised by NotI and were subcloned into pUAST, a germline transformation vector containing UAS.

In the earlier stages of this research, we did not select the IR strains but randomly employed them according to the order in which NIG collected them. In the later stages, we preferentially focused on 302 genes that were predicted to encode secretory proteins. Each UAS-IR fly strain was crossed with another strain carrying ap-GAL4, UAS-GFP and puc-lacZ. The offspring larvae possessing these four transgenes were reared at 25 °C on a standard diet and then dissected at the late third instar larval stage for immunological staining.

**Prediction of secreted proteins in the Drosophila genome**

Putative secreted proteins were searched based on the presence of hydrophobic residues in the N-terminal amino acids. When the average hydrophobicity index in the 25 amino acids between positions 6 and 30 exceeded 0.953, the protein was assumed to be secreted. The predicted protein data set from BDGP release 4.2 was searched using the program “Ahiru”, which was written based on the algorithm described in (http://bioinformatics.oxfordjournals.org/cgi/reprint/18/2/298); this yielded a list of 2,184 candidate genes encoding secreted proteins. Among them, 296 genes available in the RNAi strains in NIG were used for the screen.

**How to access the immunofluorescence images**

To access the fluorescent images, go to the middle of the right column of the web page (http://www.shigen.nig.ac.jp/fly/nigfly/index.jsp), and click the line of “Browse All RNAi Stocks”. In the newly appeared page, you can see all of the IR strains. When you click each Stock ID name which has the “wing disc” icon on the right column, you can see a set of immunofluorescence images at the bottom of the further next page.

**Acknowledgments**

We are grateful to Yoshihiro H. Inoue, Guillermo Marques, Enrique Martin-Blanco, Alfonso Martinez-Arias, Tetsuya Tabata, Daisuke Yamamoto, and the Bloomington Stock Center (University of Indiana)

(Cell Signaling Technology, #9661, 1/200 dilution), which is known to bind to the cleaved (activated) forms of mammalian Caspase-3 and its Drosophila homolog Drice. DIAP1 was detected by the rabbit anti-DIAP1 antibody (1/500 dilution). Specific binding of these primary antibodies was visualized by fluorescent secondary antibodies, such as the anti-mouse Ig-Cy3 (#715–165–151, Jackson ImmunoResearch) and anti-rabbit Ig-Cy5 (#711–175–152, Jackson ImmunoResearch) antibodies. Incubation with these primary or secondary antibodies was performed at 37 °C for 1 hour or at 4 °C overnight. Microscopic observation was performed without antifade reagents by the Leica deconvolution system Q550FW.
Disclosure
The authors report no conflicts of interest.

References
1. Domingos PM, Steller H. Pathways regulating apoptosis during patterning and development. Curr Opin Genet Dev. 2007;17:294–9.
2. Hay BA, Huh JR, Guo M. The genetics of cell death: approaches, insights and opportunities in Drosophila. Nat Rev Genet. 2004;5:911–22.
3. Putcha GV, Johnson EM Jr. Men are but worms: neuronal cell death in C elegans and vertebrates. Cell Death Differ. 2004;11:38–48.
4. Steller H. Regulation of apoptosis in Drosophila. Cell. Dev. Disc. 2008;15:1132–8.
5. Jacobson MD, Weil M, Raff MC. Programmed cell death in animal development. Cell. 1997;88:347–54.
6. Gastman BR, et al. Tumor-induced apoptosis of T cells: amplification by a mitochondrial cascade. Cancer Res. 2000;60:6811–7.
7. Poggi A, et al. Tumor-induced apoptosis of human IL-2-activated NK cells: role of natural cytotoxicity receptors. J Immunol. 2005;174:2653–60.
8. Zilian O, et al. double-time is identical to discs overgrown, which is required for cell survival, proliferation and growth arrest in Drosophila imaginal discs. Development. 1999;126:5409–20.
9. Huh JR, Guo M, Hay BA. Compensatory proliferation induced by cell death in the Drosophila wing disc requires activity of the apical cell death caspase Dronc in a nonapoptotic role. Curr. Biol. 2004;14:1262–6.
10. Perez-Garrio A, Martin FA, Morata G. Caspase inhibition during apoptosis causes abnormal signalling and developmental aberrations in Drosophila Development. 2004;131:5591–8.
11. Ryoo HD, Gorenc T, Steller H. Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. Dev Cell. 2004;7:491–501.
12. Fire A, et al. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998;391:806–11.
13. Kennerdell JR, Carthew RW. Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. Dev Cell. 1998;95:1017–26.
14. Kennerdell JR, Carthew RW. Heritable gene silence in Drosophila using double-stranded RNA. Nat Biotechnol. 2000;18:896–9.
15. Dietzl G, et al. A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature. 2007;448:151–6.
16. Adachi-Yamada T, O’Connor MB. Morphogenetic apoptosis: a mechanism for correcting discontinuities in morphogen gradients. Dev Biol. 2002;251:74–90.
17. Adachi-Yamada T, O’Connor MB. Mechanisms for removal of developmentally abnormal cells: cell competition and morphogenetic apoptosis. J Biochem (Tokyo). 2004;136:13–7.
18. Kanda H, Miura M. Regulatory roles of JNK in programmed cell death. J Biochem (Tokyo). 2004;136:1–6.
19. Martín-Blanco E, et al. puckered encodes a phosphatase that mediates a feedback loop regulating JNK activity during dorsal closure in Drosophila. Genes Dev. 1998;12:557–70.
20. Hay BA, Wassarman DA, Rubin GM. Drosophila homologs of baculovirus inhibitor of apoptosis proteins function to block cell death. Cell. 1995;83:1253–62.
21. Kanuka H, et al. Control of the cell death pathway by Dapaf-1, a Drosophila Apaf-1/CED-4-related caspase activator. Mol Cell. 1999;4:757–69.
22. Rodriguez A, et al. Dark is a Drosophila homologue of Apaf-1/CEP-4 and functions in an evolutionarily conserved death pathway. Nat Cell Biol. 1999;1:272–9.
23. Zhou L, et al. HAC-1, a Drosophila homolog of APAF-1 and CED-4 functions in developmental and radiation-induced apoptosis. Mol Cell. 1999;4:745–55.
24. Leulier F, et al. Systematic in vivo RNAi analysis of putative components of the Drosophila cell death machinery. Cell Death Differ. 2006;13:1633–74.
25. Sekelsky JJ, et al. Genetic characterization and cloning of mothers against dpp, a gene required for decapentaplegic function in Drosophila melanogaster. Genetics. 1995;139:1347–58.
26. Tsuneizumi K, et al. Daughters against dpp modulates dpp organizing activity in Drosophila wing development. Nature. 1997;389:627–31.
27. Butler MJ, et al. Discovery of genes with highly restricted expression patterns in the Drosophila wing disc using DNA oligonucleotide microarrays. Development. 2003;130:659–70.
28. Halder G, Callaerts P, Gehring WJ. Induction of ectopic eyes by targeted expression of the eyeless gene in Drosophila. Science. 1995;267:1788–92.
29. Klebes A, et al. Expression profiling of Drosophila imaginal discs. Genome Biology. 2002;3:RESEARCH0038.
30. Adachi-Yamada T, et al. Wing-to-Leg homeosis by spineless causes apoptosis regulated by Fish-lips, a novel leucine-rich repeat transmembrane protein. Mol Cell Biol. 2005;25:3140–50.
31. Milan M, Perez L, Cohen SM. Short-range cell interactions and cell survival in the Drosophila wing. Dev Cell. 2002;2:797–805.
32. Milan M, Campuzano S, Garcia-Bellido A. Cell cycling and patterned cell proliferation in the wing primordium of Drosophila. Proc Natl Acad Sci U S A. 1996;93:640–5.
33. McGuire SE, et al. Spatiotemporal rescue of memory dysfunction in Drosophila Science. 2003;302:1765–8.
34. Ma Y, et al. Prevalence of off-target effects in Drosophila RNA interference screens. Nature. 2006;443:539–63.
35. Echeverri CJ, et al. Minimizing the risk of reporting false positives in large-scale RNAi screens. Nat Methods. 2006;3:777–9.
36. Leulier F, et al. Inducible expression of double-stranded RNA reveals a role for dFADD in the regulation of the antibacterial response in Drosophila adults. Curr Biol. 2002;12:996–1000.
37. Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development. 1993;118:401–15.
38. Sakurai KT, et al. Differential control of cell affinity required for progression and refinement of cell boundary during Drosophila leg segmentation. Dev Biol. 2007;309:126–36.
39. Kuranaga E, et al. Drosophila IKK-related kinase regulates nonapoptotic function of caspases via degradation of IAPs. Cell. 2006;126:583–96.
40. Bannai H, et al. Extensive feature detection of N-terminal protein sorting signals. Bioinformatics. 2002;18:298–305.