Genome-Wide Analyses of Steroid- and Radiation-Triggered Programmed Cell Death in *Drosophila*

Cheng-Yu Lee,[1,2,4,5] Emily A. Clough,[1,5] Paula Yellon,[1] Tanya M. Teslovich,[3] Dietrich A. Stephan,[3] and Eric H. Baehrecke[1,*]
[1]Center for Biosystems Research
University of Maryland Biotechnology Institute
College Park, Maryland 20742
[2]Department of Biology
University of Maryland
College Park, Maryland 20742
[3]Research Center for Genetic Medicine
Children's National Medical Center
111 Michigan Avenue, NW
Washington, DC 20010

Summary

Apoptosis and autophagy are two forms of programmed cell death that play important roles in the removal of unneeded and abnormal cells during animal development [1, 2]. While these two forms of programmed cell death are morphologically distinct, recent studies indicate that apoptotic and autophagic cell death utilize some common regulatory mechanisms [3–5]. To identify genes that are associated with apoptotic and autophagic cell death, we monitored changes in gene transcription by using microarrays representing nearly the entire *Drosophila* genome. Analyses of steroid-triggered autophagic cell death identified 932 gene transcripts that changed 5-fold or greater in RNA level. In contrast, radiation-activated apoptosis resulted in 34 gene transcripts that exhibited a similar magnitude of change. Analyses of these data enabled us to identify genes that are common and unique to steroid- and radiation-induced cell death. Mutants that prevent autophagic cell death exhibit altered levels of gene transcription, including genes encoding caspases, non-caspase proteases, and proteins that are similar to yeast autophagy proteins. This study also identifies numerous novel genes as candidate cell death regulators and suggests new links between apoptosis and autophagic cell death.

Results and Discussion

Transcript Changes during Steroid-Triggered Autophagic Cell Death

Numerous stimuli, including steroid hormones and genotoxic stress, activate programmed cell death. During *Drosophila* development, the steroid hormone 20-hydroxyecdysone (ecdysone) titer rises 10–12 hr after puparium formation, triggering a transcription hierarchy that regulates programmed cell death of salivary glands [2]. This cell death is preceded by markers of apoptosis and is prevented by expression of the caspase inhibitor p35 [6]. While these data indicate that salivary glands die by apoptosis, these dying cells possess autophagic vacuoles that contain organelles and do not appear to be associated with phagocytes during degradation, indicating that they die by autophagic cell death [3].

In order to identify genes and study global patterns of gene transcription during ecdysone-induced programmed cell death, total RNA was extracted from salivary glands dissected from animals that were staged 6 and 12 hr following puparium formation. These stages were selected for genome-wide analyses based on previous Northern blot hybridization analyses [4, 5], as well as on studies using spotted DNA microarrays (data not presented), which indicated that 6- and 12-hr salivary glands exhibit the greatest changes in cell death gene transcript levels. Three independent sets of salivary gland RNA samples were collected from each stage and were used to hybridize Affymetrix *Drosophila* oligonucleotide Genechips. These Genechips contain 13,197 unique gene transcripts, and 2,876 gene transcripts were consistently detected in all three samples of either 6- or 12-hr salivary glands. Of these 2,876 gene transcripts, 484 increased 5-fold or more and 448 decreased 5-fold or more in mRNA levels in 12-hr RNA samples when compared to 6-hr RNA samples (see Figure S1 and Tables S1 and S2 in the Supplementary Material available with this article online). The large number of genes that exhibit increased RNA levels just 3 hr prior to complete cell demise suggests the importance of transcription in these dying cells.

Dying salivary glands exhibit dramatic changes in the transcription of several categories of genes, and a subset of these changes are summarized because of their association with particular biological processes or biochemical pathways in these cells (Tables 1 and S2). Several ecdysone-regulated genes, including *Eip93* (*E93*) and the nuclear hormone receptor family members *Eip75B*, *HR39*, *HR78*, *usp*, and *Eip78C*, were induced in 12-hr salivary gland samples. While these genes are known to be regulated by ecdysone, this is the first report that specifically associates the transcription of many of these genes with ecdysone-triggered cell death. The *E74A* and *BR-C* genes were not consistently detected in 12-hr samples, and this lack of detection is likely because these genes reach peak levels 10 hr after puparium formation in salivary glands [7].

Several genes that function in apoptosis were detected in dying salivary glands (Tables 1 and S2). In addition to the known increases in transcription of the apoptosis genes *rpr*, *W* (*hid*), *ark*, *Nc* (*dronc*), and *crq* [5], we also detected the Bcl-2 family member *buffy*, the caspases *Ice* (*drice*) and *dream* (*strica*), and the DNase *rep4*. The increased levels of *buffy* RNA presents the possibility that a proapoptotic Bcl-2 family member is involved in salivary gland autophagy. The increase in the levels of the caspases *Ice* (*drice*) and *dream* (*strica*) indicate that these caspases, in addition to *Nc* (*dronc*), may be involved in salivary gland destruction. Finally,

*Correspondence: baehreck@umbi.umd.edu
[4]Present address: Howard Hughes Medical Institute, University of Oregon, Institute of Neuroscience, Eugene, Oregon 97403-1254.
[5]These authors contributed equally to this work.

Table 1. Genes that Are Transcribed in Dying Salivary Glands

Category	CG #	Name	Notes	Fold Change	p Value	Gene Ontology	Similar Human Protein
Edysone-Regulated Genes	CG8127	*Eip75B*	nuclear receptor	4.7	0.0339134	nucleic acid binding	NP_006229
	CG18389	*Eip93F*		15.1	0.0520563	unknown	XP_050988
	CG8676	*Hr39*	nuclear receptor	5.9	0.0062732	nucleic acid binding	NP_003813
	CG7199	*Hr78*	nuclear receptor	3.8	0.0062952	nucleic acid binding	NP_003288
	CG4380	*usp*	nuclear receptor	4.3	0.0091751	nucleic acid binding	NP_002948
	CG18023	*Eip78C*	nuclear receptor	9.9	0.0124672	nucleic acid binding	NP_068370
Apoptosis	CG4319	*rpr*		33.4	0.091019	unknown	no significant similarities
	CG5123	*W*		8.5	0.1735617	unknown	no significant similarities
	CG8238	*buffy*	Bcl-2 family	25.1	0.0167123	apoptosis regulator	NP_115904
	CG6829	*ark*	Apaf-1	50.8	0.0036266	enzyme activator	NP_037361
	CG8091	*Nc*	caspase	17.2	0.0040372	enzyme	NP_116756
	CG7788	*Ice*	caspase	9.6	0.0034024	enzyme	NP_001218
	CG7863	*dream*	caspase	7.1	0.0045826	enzyme	NP_001218
	CG4280	*crq*	CD36 family	11.0	0.0277993	signal transducer	NP_005496
	CG9414	*rep4*	DNase	4.6	0.0003345	enzyme	XP_113366
Autophagy	CG1241		APG2-like	14.9	0.0306705	unknown	XP_045612
	CG4428		APG2-like	2.5	0.070662	unknown	NP_037457
	CG6194		APG4-like	50.5	0.0387029	unknown	NP_116241
	CG1643		APG5-like	4.4	0.0334395	unknown	NP_004840
	CG5429		APG6-like	1.6	0.1435074	unknown	NP_003757
	CG5489		APG7-like	7.1	0.0000258	unknown	NP_006386
	CG3615		APG9-like	3.6	0.0164025	unknown	NP_076990
Non-Caspase Proteases and Inhibitors	CG5505		cysteine protease	71.9	0.00194	enzyme	XP_027039
	CG3650		serine protease	37.6	0.1255277	enzyme	NP_071329
	CG4859	*mmp1*	metalloprotease	172.8	0.0495749	enzyme	NP_004986
	CG5663	*dip-C*	metalloprotease	7.5	0.0000664	enzyme	XP_009179
	CG3991	*tppII*	serine protease	7.6	0.001382	enzyme	NP_003282
	CG10149	*rpn6*	proteasome protease	6.4	0.0643783	enzyme	NP_002806
	CG6281	*timp*	metalloprotease inhibitor	−26.5	0.0187313	enzyme inhibitor	NP_003246
	CG15369		cysteine protease inhibitor	−25.5	0.0036482	enzyme inhibitor	no significant similarities
	CG3604		serine protease inhibitor	−24.2	0.0014124	enzyme inhibitor	NP_006278
Transcription Regulators	CG4013	*smr*	corepressor	5.2	0.0175817	ligand binding or carrier	XP_037176
	CG4756		corepressor	6.8	0.007654	ligand binding or carrier	NP_078908
	CG5848	*cact*	IkB	10.8	0.0410213	ligand binding or carrier	NP_065390
	CG6794	*Dif*	NFkB	6.1	0.0486931	nucleic acid binding	NP_002899
	CG5461	*bun*		38.9	0.0002165	nucleic acid binding	XP_038383
	CG3090	*sox14*		25.2	0.0000001	nucleic acid binding	NP_003098
	CG5465	*trap95*		24.8	0.0138889	nucleic acid binding	NP_005472
	CG10897	*tou*	chromatin binding	13.0	0.0673001	nucleic acid binding	no significant similarities
	CG5748	*hsf*		7.0	0.0001696	nucleic acid binding	XP_035368
	CG8319		zinc finger	6.6	0.0095535	nucleic acid binding	XP_012174
Cell Remodeling	CG6998	*ctp*	Dynein light chain	−5.8	0.0916813	motor	NP_542408
	CG7595	*ck*	Myosin ATPase	−6.0	0.0275607	motor	NP_000251
	CG12363	*dlc90F*	Dynein light chain	−5.9	0.00609	enzyme	NP_006510
	CG5701	*RhoBTB*	small GTPase	34.8	0.0405482	enzyme	XP_166144

(*continued*)

Table 1. Continued

Category	CG #	Name	Notes	Fold Change	p Value	Gene Ontology	Similar Human Protein
Cell Remodeling							
	CG8556	*Rac2*	small GTPase	33.6	0.0048393	enzyme	NP_008839
	CG9366	*RhoL*	small GTPase	9.1	0.0000255	enzyme	NP_008839
	CG9635	*RhoGEF2*	nucleotide exchange factor	5.0	0.005114	ligand binding or carrier	NP_055599
	CG4921	*Rab4*	small GTPase	6.0	0.0276963	enzyme	NP_004569
	CG3664	*Rab5*	small GTPase	7.1	0.0101971	enzyme	NP_004153

Categories are based on association with biological or biochemical properties. CG numbers and names of genes are based on Flybase annotation (http://flybase.bio.indiana.edu/). Gene Ontology follows the assignments given by this consortium (http://www.geneontology.org/). Most similar human proteins were determined by using protein BLAST searches of the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/). BLAST values greater than 1.0×10^{-7} were considered insignificant.

the increase in *rep4* transcription is interesting because of the similarity between this gene and *cad*, which encodes the DNase that is activated via a caspase-dependent mechanism during mammalian apoptosis [8].

One of the most striking differences between apoptosis and autophagic cell death are the mechanisms of cell degradation: apoptotic cells are engulfed by phagocytes in which lysosomes degrade the dying cell, while autophagic cells appear to be responsible for most if not all of their own degradation [1, 2]. While the mechanisms of protein degradation during autophagic programmed cell death are not well understood, the process of bulk protein degradation under nutrient-limiting conditions in yeast occurs by autophagy and has been well characterized [9]. It is intriguing that several fly homologs of the yeast autophagy genes are transcribed during salivary gland cell death. The most similar fly genes to yeast *apg2* (*CG1241*), *apg4* (*CG6194*), *apg5* (*CG1643*), and *apg7* (*CG5489*) all increased at the onset of salivary gland cell death, while the fly genes that are most similar to the yeast *apg6*, *apg9*, *apg12*, *aut1/apg3*, *aut7/apg8*, and *aut10* genes were all detected in these dying cells but did not change significantly (Tables 1 and S2).

Salivary glands appear to utilize caspases for degradation [6]. However, inhibition of caspases does not prevent cytoplasmic cell death changes in salivary glands [3]. Thus, it is interesting that several non-caspase proteases and their inhibitors exhibit dynamic changes in transcription in dying salivary glands (Tables 1 and S2). Large increases in transcription of cysteine, serine, and metalloproteases accompany salivary gland cell death, and these changes in transcription are complemented by decreased transcription of cysteine, serine, and metallo protease inhibitors. Human homologs of some of these proteases, such as matrix metalloproteases, have been implicated in tumor progression [10], raising the possibility that genes identified in this screen may have human homologs that are of clinical relevance.

Several transcription regulators were also identified that have not been previously shown to be regulated by ecdysone in dying salivary glands (Tables 1 and S2). The genes encoding the corepressors *Smarter* and *CG4756*, and the chromatin-associated gene *tou*, increase in level in 12-hr salivary glands. The NFkB regulator *cactus*, and the NFkB family member *dif*, both increase just before salivary gland cell death [11]. Furthermore, several other genes encoding DNA binding proteins, such as *bun*, *sox14*, and the zinc finger-encoding gene *CG8319*, all increased in transcription in 12-hr salivary gland samples. Finally, several genes, including the fly gene that is most similar to *trap95* and *hsf*, that encode important components of transcription complexes are transcribed in dying salivary gland cells. TRAP95 was isolated because of its association with the thyroid hormone receptor complex in mammals [12], and its association with ecdysone-induced cell death indicates that this molecule may be broadly associated with nuclear receptor-triggered transcription.

Dynamic changes in cell morphology suggest that cell remodeling is important for salivary gland cell death. We observed a decrease in several genes encoding motor proteins, including *ctp* and *ck*, as well as the *dynein light chain* gene *dlc90F* (Table 1). While numerous genes

Table 2. Genes that Are Induced following Both Radiation- and Steroid-Triggered Cell Death

		Radiation-Induced		Steroid-Induced			
CG #	Name	Fold Change	p Value	Fold Change	p Value	Gene Ontology	Similar Human Protein
CG4319	*rpr*	22.1	0.01011	33.4	0.09102	unknown	no significant similarities
CG10965		4.4	0.00047	15.7	0.00619	unknown	no significant similarities
CG17323		7.1	0.00264	12.6	0.06287	enzyme	NP_003351.1
CG7144	BEST:CK02318	11.6	0.02061	24.4	0.00409	enzyme	NP_005754.2
CG2865	EG:25E8.4	5.7	0.40101	30.0	0.01533	unknown	no significant similarities
CG5254	EG:BACR19J1.2	5.0	0.07949	126.2	0.00051	transporter	NP_085134.1

CG numbers and names of genes are based on Flybase annotation (http://flybase.bio.indiana.edu/). Gene Ontology follows the assignments given by this consortium (http://www.geneontology.org/). Most similar human proteins were determined by using protein BLAST searches of the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/). BLAST values greater than 1.0×10^{-7} were considered insignificant.

encoding cytoskeletal proteins were transcribed during this period, their levels did not change significantly (Table S2). However, members of the Rho, Rac, and Rab families of small guanosine triphosphates (GTPases) exhibited increases in transcription just before salivary gland cell death (Tables 1 and S2). These small GTPases play important roles in regulating assembly and organization of the actin cytoskeleton, and they have been implicated in processes associated with changes in cell shape [13]. While cell remodeling is known to play an important role in phagocyte engulfment of apoptotic cells [14], this is the first molecular evidence linking this important cellular process to autophagic programmed cell death.

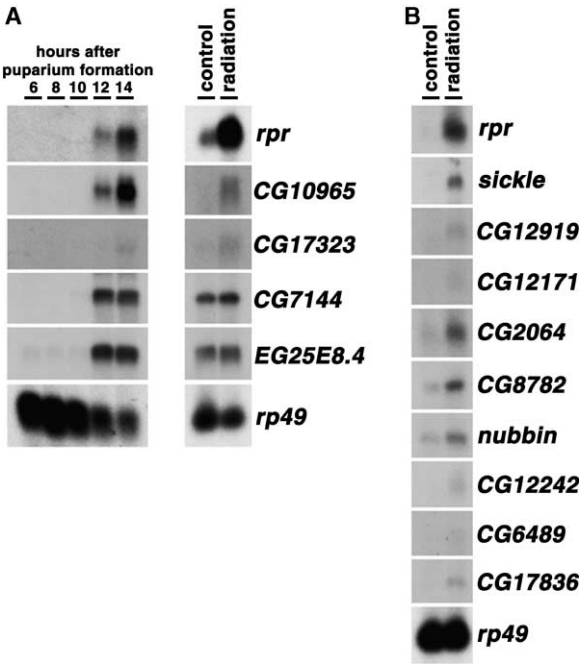

Figure 1. Analyses of Genes that Were Induced following Radiation-Triggered Cell Death

(A) Northern blot analyses of the genes that increased following both steroid- (left) and radiation-triggered (right) programmed cell death. The ecdysone titer rises 10–12 hr after puparium formation in salivary glands.

(B) Northern blot analyses of the genes that only increased following radiation-induced cell death. *rp49* serves as a control for loading and transfer.

Transcript Changes during Radiation-Triggered Apoptosis

Similar to steroid-induced cell death, transcription also plays an important role in radiation-induced cell death in *Drosophila* through the function of the DNA binding protein p53 [15, 16]. In *Drosophila*, p53 activates *rpr* transcription via a radiation-responsive element in the *rpr* promoter [15], and *rpr* function is required for radiation-induced apoptosis [17].

To identify genes that are common and unique to different types of programmed cell death, we compared the profiles of gene expression during steroid-induced autophagy and radiation-induced apoptosis. *Drosophila* larvae were initially selected for these radiation studies because of the similarity to the developmental stage selected for analyses of steroid-triggered cell death. Unfortunately, extreme variation in the amount of cell death per larva and in different tissues (data not presented) led to the use of embryos for studies of radiation-induced apoptosis; radiation induces rapid and widespread apoptosis in *Drosophila* embryos [18].

To examine genomic responses to radiation-induced apoptosis, RNA was extracted from three independent collections of both control unirradiated and irradiated embryos and was used to hybridize Affymetrix *Drosophila* oligonucleotide microarrays. While 5,495 unique gene transcripts were consistently detected in all three samples of either control or irradiated embryos, most of these genes were expressed at near equal levels in both treatments (Table S3), suggesting that most of these genes play an important role in embryogenesis. Surprisingly, there were only 22 genes that increased and 12 genes that decreased 5-fold or greater in the level of RNA in samples extracted from irradiated embryos compared to unirradiated control embryos (Table S3). Consistent with previous reports [19], the *Drosophila* cell death genes *rpr* and *sickle* were induced upon exposure to radiation (Table S3). The difference in the number of genes that were induced by ecdysone and radiation most likely reflects either the presence of maternal RNAs for cell death genes that are deposited in embryos or the presence of cell death proteins in embryos that become activated posttranslation.

The genes that were induced following radiation treatment of embryos were compared to the genes that were induced in salivary glands dying by autophagy. *rpr* was the only known cell death gene that appeared in both data sets (Table 2). However, several genes, including *CG10965*, *CG17323*, *CG7144*, *EG25E8.4*, and *CG5254*,

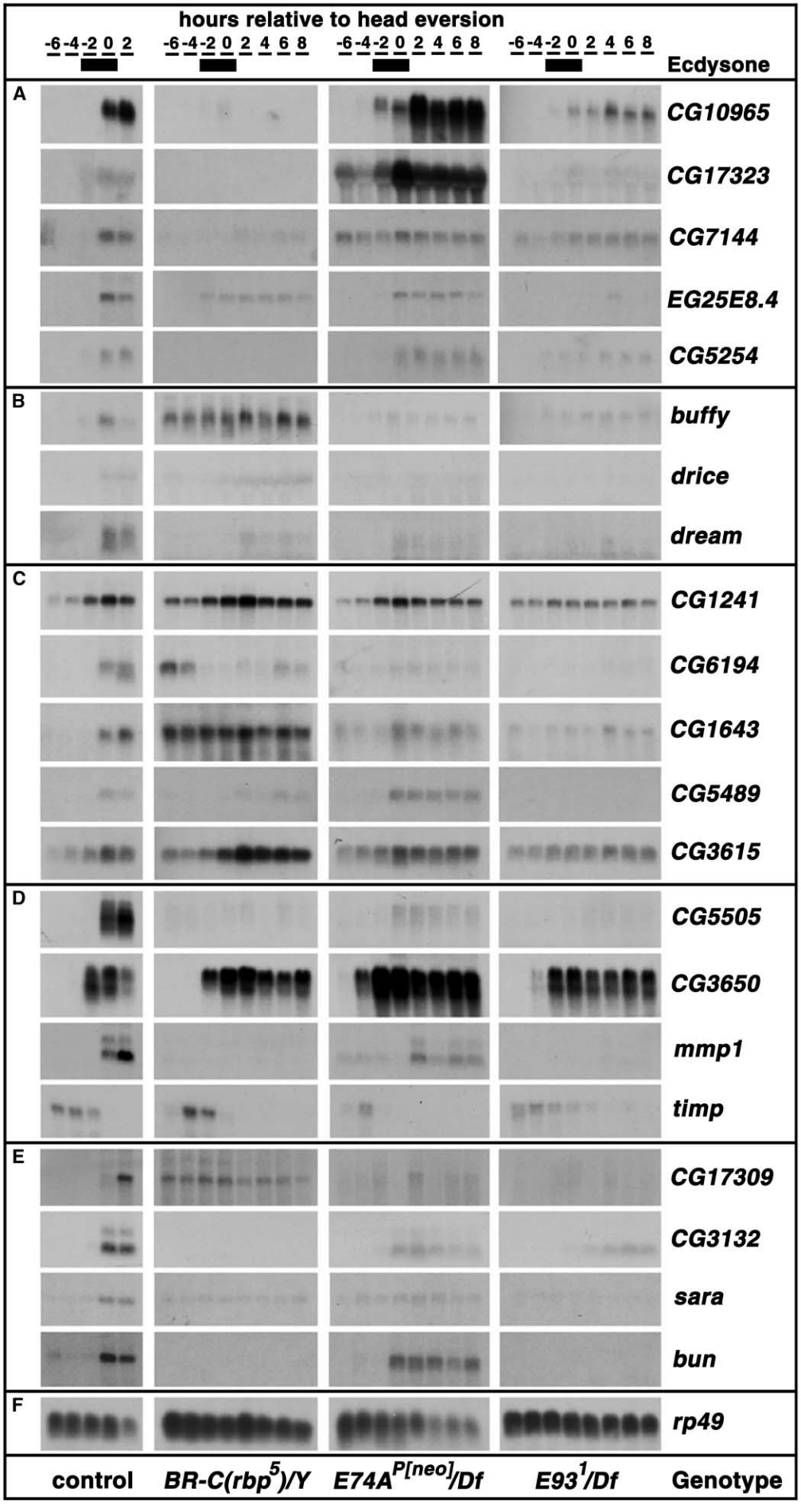
hours relative to head eversion
-6 -4 -2 0 2
-6 -4 -2 0 2 4 6 8
-6 -4 -2 0 2 4 6 8
-6 -4 -2 0 2 4 6 8
Ecdysone
A
CG10965
CG17323
CG7144
EG25E8.4
CG5254
B
buffy
drice
dream
C
CG1241
CG6194
CG1643
CG5489
CG3615
D
CG5505
CG3650
mmp1
timp
E
CG17309
CG3132
sara
bun
F
rp49
control
BR-C(rbp5)/Y
E74AP[neo]/Df
E931/Df
Genotype

exhibit increased levels of transcription in both ecdysone- and radiation-triggered cell death (Table 2). We sought empirical validation of the genes that were induced in both dying salivary glands and following radiation treatment of embryos by hybridization of Northern blots. Consistent with DNA microarray results, *rpr*, *CG10965*, *CG17323*, *CG7144*, and *EG25E8.4* are transcribed following the increase of ecdysone that triggers salivary gland cell death 12 hr after puparium formation (Figure 1A). While there is a low level of *rpr* transcription in unirradiated embryos, *rpr* mRNA is highly induced following radiation treatment. Both *CG10965* and *CG17323* are induced in the irradiated embryos, while there appears to be a very low level of expression in the controls (Figure 1A). *CG7144* and *EG25E8.4* also appear to be induced to higher levels in irradiated embryos compared to the control, although this induction is subtle (Figure 1A). *sickle*, *CG12919*, *CG12171*, *CG2064*, *CG8782*, *nubbin*, *CG12242*, *CG6489*, and *CG17836* are all induced only in irradiated embryos (Figure 1B and Table S3).

Mutations that Prevent Autophagic Cell Death Alter Transcription of Newly Identified Genes

The identification of genes that exhibit significant changes in RNA levels during steroid-triggered autophagic cell death and radiation-induced apoptosis prompted empirical analyses of transcription in mutants that block salivary gland cell death. Mutations in the ecdysone-regulated genes *BR-C*, *E74A*, and *E93* prevent salivary gland programmed cell death and prevent proper transcription of the apoptosis genes *rpr*, *W* (*hid*), *ark*, *Nc* (*dronc*), and *crq* [20]. We examined the transcription of a subset of the newly identified genes in *BR-C*, *E74A*, and *E93* mutants by Northern blot hybridization because of their possible association with apoptosis and autophagy in dying salivary glands. Cohybridization of these Northern blots allows systematic investigation of how *BR-C*, *E74A*, and *E93* might regulate transcription of genes that were identified with Genechips (Figure 2) and provides a possible mechanism to explain steroid regulation of cell death.

The radiation-inducible genes *CG10965*, *CG17323*, *CG7144*, *EG25E8.4*, and *CG5254* are induced in control dying salivary glands at head eversion, and this transcription is altered in mutants that prevent salivary gland cell death (Figure 2A). *CG10965* and *CG17323* are not transcribed in salivary glands of *BR-C* mutants, exhibit elevated levels of transcription in *E74A* mutants, and have reduced RNA levels in *E93* mutants. *CG7144* is transcribed at significantly reduced levels in *BR-C* mutants, is ectopically transcribed before the rise in ecdysone in salivary glands of *E74A* mutants, and may also be ectopically transcribed in *E93* mutants. *EG25E8.4* is not altered in *BR-C* and *E74A* mutants, but this RNA is significantly reduced in salivary glands of *E93* mutants. *CG5254* is not transcribed in *BR-C* mutants, had normal RNA levels in *E74A* mutants, and had reduced RNA levels in *E93* mutants.

Several other categories of genes exhibit interesting patterns of regulation in *BR-C*, *E74A*, and *E93* mutant salivary glands. The Bcl-2 family member *buffy* and the caspases *Ice* (*drice*) and *dream* (*strica*) are induced at head eversion in salivary glands of control animals, and they are altered to different extents in mutants (Figure 2B). Similarly, the *Drosophila* genes that are most similar to the yeast autophagy genes *apg2* (*CG1241*), *apg4* (*CG6194*), *apg5* (*CG1643*), *apg7* (*CG5489*), and *apg9* (*CG3615*) are induced just prior to cell death of wild-type salivary glands, and they are altered to varying extents in *BR-C*, *E74A*, and *E93* mutants (Figure 2C). It is particularly intriguing that *E93* mutants have significantly decreased levels of *CG6194*, *CG1643*, and *CG5489*, as yeast with mutations in *apg4*, *apg5*, and *apg7* are defective in autophagosome formation and size [21], and *E93* mutants exhibit defects in vacuolar changes in dying salivary gland and midgut cells [3, 22]. In addition, the cysteine protease (*CG5505*), serine protease (*CG3650*), and metalloprotease (*mmp1*) all exhibited increases in RNA level immediately following the rise in ecdysone in dying wild-type salivary glands, and this change was accompanied by a decrease in the inhibitor of metalloproteases, *timp* (Figure 2D). It is interesting that *BR-C*, *E74A*, and *E93* mutations affect transcription of the non-caspase protease genes *CG5505*, *CG3650*, and *mmp1* (Figure 2D), since caspase inhibitors do not completely block changes in dying salivary glands, and mutations in these ecdysone-regulated genes prevent degradation of salivary gland cells [3].

Drosophila salivary gland chromosomes were used to predict the first steroid-triggered transcription hierarchy based on chromosome puffing (chromatin decondensation) [23]. In this study, we identified several candidate genes in this signaling pathway based on correlative increases in transcription that are associated with chromosome puffs and with the proximity of binding sites of transcription factors in this pathway. Two putative puff genes, *CG17309* (86E puff) and *CG3132* (87A puff), increased following the rise in ecdysone titer (Figure 2E) and match the puffing patterns of these chromosome loci [23]. *CG17309* RNA is present before the rise in ecdysone in *BR-C* mutants, while it is reduced in salivary glands of *E74A* and *E93* mutants (Figure 2E). *CG3132* appears to encode two transcription units that were either not detected or decreased in salivary glands of *BR-C*, *E74A*, and *E93* mutants (Figure 2E). The Smad anchor for receptor activation *sara* and the transcription regulator *bun* have increased RNA levels in dying salivary glands (Figure 2E) and have *BR-C Z1* [24] and *E74A*

Figure 2. Mutations in Ecdysone-Regulated Genes Impact Transcription of Genes in Salivary Glands

(A–F) Larval salivary glands were dissected from control and *BR-C*, *E74A*, and *E93* homozygous mutant animals that had been staged in 2-hr intervals from −6 to +8 hr relative to the timing of adult head eversion (time 0); the peak of ecdysone occurs between −2 and 0 hr using this staging. Northern blots of each genotype were cohybridized with the same probe to detect (A) radiation-induced genes, (B) apoptosis genes, (C) APG-like genes, (D) non-caspase proteases and protease inhibitors, (E) candidate new puff and puff gene targets, and (F) *rp49* as a control for loading and transfer. Control salivary glands die by 4 hr after head eversion, while mutant salivary glands fail to die and can be analyzed at later stages.

[25] binding sites in the same region of the genome. *sara* was not induced in *BR-C*, *E74A*, and *E93* mutant salivary glands. *bun* RNA was also not detected in *BR-C* and *E93* mutant salivary glands, but it was expressed normally in *E74A* mutant salivary glands. These data provide a direct link between the ecdysone-regulated early genes and target genes.

Conclusions

Developmental cues and genotoxic stress can both trigger programmed cell death. During steroid-triggered autophagic cell death in developing salivary glands, 932 gene transcripts were identified that either decreased or increased 5-fold or greater in RNA level. In contrast, radiation-activated apoptosis in embryos only identified 34 gene transcripts that exhibited a similar magnitude of change. The difference in the number of genes that were induced by these stimuli most likely reflects the presence of maternal RNAs for cell death genes that are deposited in embryos. Alternatively, the apoptotic machinery may exist in cells as proteins waiting to be posttranslationally activated following a death-inducing stimulus. Radiation-induced apoptosis in *Drosophila* embryos can be suppressed by treatment with cyclohexamide [18], suggesting that protein synthesis is necessary for activation of this cell death. In addition, studies of radiation-induced apoptosis have implicated p53, which is known to function as a regulator of transcription in this process [15, 16]. It is also possible that radiation-induced apoptosis is sufficiently asynchronous that it is difficult to detect changes in RNA levels in a very complex cell population. Comparative analyses of cell death microarray data has enabled us to identify a small group of genes that are induced by both ecdysone and radiation. While salivary gland autophagic cell death and radiation-induced apoptosis appear to be quite different, transcription of the common genes *rpr*, *CG10965*, *CG17323*, *CG7144*, *EG25E8.4*, and *CG5254* is altered in mutants that prevent salivary gland cell death (Figure 2), further suggesting that these genes are important for this cell death. In addition, *BR-C*, *E74A*, and *E93* mutants also impacted transcription of numerous genes in salivary glands, including apoptosis regulators, non-caspase proteases and protease inhibitors, cell remodeling factors, and the genes that are similar to the yeast genes that function in protein degradation by autophagy. This study has identified numerous genes that exhibit interesting patterns of transcription during steroid- and radiation-induced programmed cell death, and future genetic studies will determine the importance of these genes in autophagy and apoptosis.

Supplementary Material

Supplementary Material including one figure, three tables, and the Experimental Procedures is available at http://images.cellpress.com/supmat/supmatin.htm.

Acknowledgments

We thank Eric Hoffman for sharing resources and expertise, Paul Spellman for providing his revised Affymetrix gene list, Gerald M. Rubin, Michael Muse, and Damon Harvey for providing the *Drosophila* Gene Collection, and Chris Doe, Steve Mount, and members of the Baehrecke laboratory for helpful comments and discussions. This work was supported by a Howard Hughes Medical Institute Undergraduate Research Fellowship to P.Y. and National Science Foundation grant BES-9908942 and National Institutes of Health grant GM59136 to E.H.B.

Received: November 1, 2002
Revised: December 16, 2002
Accepted: December 18, 2002
Published: February 18, 2003

References

1. Schweichel, J.-U., and Merker, H.-J. (1973). The morphology of various types of cell death in prenatal tissues. Teratology *7*, 253–266.
2. Baehrecke, E.H. (2002). How death shapes life during development. Nat. Rev. Mol. Cell Biol. *3*, 779–787.
3. Lee, C.-Y., and Baehrecke, E.H. (2001). Steroid regulation of autophagic programmed cell death during development. Development *128*, 1443–1455.
4. Jiang, C., Lamblin, A.-F.J., Steller, H., and Thummel, C.S. (2000). A steroid-triggered transcriptional hierarchy controls salivary gland cell death during *Drosophila* metamorphosis. Mol. Cell *5*, 445–455.
5. Lee, C.-Y., Wendel, D.P., Reid, P., Lam, G., Thummel, C.S., and Baehrecke, E.H. (2000). *E93* directs steroid-triggered programmed cell death in *Drosophila*. Mol. Cell *6*, 433–443.
6. Jiang, C., Baehrecke, E.H., and Thummel, C.S. (1997). Steroid regulated programmed cell death during *Drosophila* metamorphosis. Development *124*, 4673–4683.
7. Woodard, C.T., Baehrecke, E.H., and Thummel, C.S. (1994). A molecular mechanism for the stage-specificity of the *Drosophila* prepupal genetic response to ecdysone. Cell *79*, 607–615.
8. Yokoyama, H., Mukae, N., Sakahira, H., Okawa, K., Iwamatsu, A., and Nagata, S. (2000). A novel activation mechanism of caspase-activated DNase from *Drosophila melanogaster*. J. Biol. Chem. *275*, 12978–12986.
9. Klionsky, D.J., and Emr, S.D. (2000). Autophagy as a regulated pathway of cellular degradation. Science *290*, 1717–1721.
10. Coussens, L.M., Fingleton, B., and Matrisian, L.M. (2002). Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science *295*, 2387–2392.
11. Lehmann, M., Jiang, C., Ip, Y.T., and Thummel, C.S. (2002). AP-1, but not NF-kappaB, is required for efficient steroid-triggered cell death in *Drosophila*. Cell Death Differ. *9*, 581–590.
12. Ito, M., Yuan, C.X., Malik, S., Gu, W., Fondell, J.D., Yamamura, S., Fu, Z.Y., Zhang, X., Qin, J., and Roeder, R.G. (1999). Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. Mol. Cell *3*, 361–370.
13. Hall, A. (1998). Rho GTPases and the actin cytoskeleton. Science *279*, 509–514.
14. Hengartner, M.O. (2001). Apoptosis: coralling the corpses. Cell *104*, 325–328.
15. Brodsky, M.H., Nordstrom, W., Tsang, G., Kwan, E., Rubin, G.M., and Abrams, J.M. (2000). *Drosophila* p53 binds a damage response element at the reaper locus. Cell *101*, 103–113.
16. Ollmann, M., Young, L.M., Di Como, C.J., Karim, F., Belvin, M., Robertson, S., Whittaker, K., Demsky, M., Fisher, W.W., Buchman, A., et al. (2000). *Drosophila* p53 is a structural and functional homolog of the tumor suppressor p53. Cell *101*, 91–101.
17. Peterson, C., Carney, G.E., Taylor, B.J., and White, K. (2002). *reaper* is required for neuroblast apoptosis during *Drosophila* development. Development *129*, 1467–1476.
18. Abrams, J.M., White, K., Fessler, L.I., and Steller, H. (1993). Programmed cell death during *Drosophila* embryogenesis. Development *117*, 29–43.
19. Christich, A., Kauppila, S., Chen, P., Sogame, N., Ho, S.I., and Abrams, J.M. (2002). Damage-responsive *Drosophila* gene *sickle* encodes a novel IAP binding protein similar to but distinct from *reaper*, *grim*, and *hid*. Curr. Biol. *12*, 137–140.
20. Lee, C.-Y., Simon, C.R., Woodard, C.T., and Baehrecke, E.H.

(2002). Genetic mechanism for the stage- and tissue-specific regulation of steroid-triggered programmed cell death in *Drosophila*. Dev. Biol. *252*, 138–148.

21. Tsukada, M., and Ohsumi, Y. (1993). Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. FEBS Lett. *1–2*, 169–174.
22. Lee, C.-Y., Cooksey, B.A.K., and Baehrecke, E.H. (2002). Steroid regulation of midgut cell death during *Drosophila* development. Dev. Biol. *250*, 101–111.
23. Ashburner, M. (1967). Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. I. Autosomal puffing patterns in a laboratory stock of *Drosophila melanogaster*. Chromosoma *21*, 398–428.
24. von Kalm, L., Crossgrove, K., Von Seggern, D., Guild, G.M., and Beckendorf, S.K. (1994). The *Broad-Complex* directly controls a tissue-specific response to the steroid hormone ecdysone at the onset of *Drosophila* metamorphosis. EMBO J. *13*, 3505–3516.
25. Urness, L.D., and Thummel, C.S. (1990). Molecular interactions within the ecdysone regulatory hierarchy: DNA binding properties of the *Drosophila* ecdysone-inducible *E74A* protein. Cell *63*, 47–61.