Targeted expression of *ced-3* and *Ice* induces programmed cell death in *Drosophila*

Ayako Shigenaga¹, Yoshimistu Funahashi²,
Ken-ichi Kimura³, Yoshitaka Kobayakawa¹, Shinji Kamada²,
Yoshihide Tsujimoto² and Teiichi Tanimura⁴

¹ Department of Biology, Faculty of Science, Kyushu University, Ropponmatsu, Fukuoka 810, Japan

² Department of Medical Genetics, Biomedical Research Center, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565, Japan

³ Laboratory of Biology, Hokkaido University of Education, Iwamizawa Campus, Iwamizawa 068, Japan

⁴ corresponding author: Teiichi Tanimura, Department of Biology, Faculty of Science, Kyushu University, Ropponmatsu, Fukuoka 810, Japan
tel: 81-92-726-4759; fax: 81-92-726-4644;
e-mail: tanimura@rc.kyushu-u.ac.jp

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Abstract

CED-3 is a cysteine protease required for programmed cell death in the nematode, *Caenorhabditis elegans*, and shares a sequence similarity with mammalian ICE (interleukin-1 β converting enzyme) family proteases. Both CED-3 and ICE family proteases can induce programmed cell death in mammalian cells. Structural and functional similarities between CED-3 and ICE family proteases indicate that the mechanism of cell death is evolutionarily conserved, suggesting the presence of a similar mechanism involving CED-3/ICE-like proteases in *Drosophila*. Here we determined whether CED-3 or ICE functions to induce programmed cell death in *Drosophila*. We have generated transformant lines in which *ced-3* or *Ice* is ectopically expressed using the GAL4-UAS system. Expression of CED-3 and ICE can elicit cell death in *Drosophila* and the cell death was blocked by coexpressing the *p35* gene which encodes a viral inhibitor of CED-3/ICE proteases. Results support the idea that the mechanism of programmed cell death controlled by CED-3/ICE is conserved among widely divergent animal species including *Drosophila*, and the system described provides a tool to dissect cell death mechanism downstream of CED-3/ICE proteases.

Keywords: *ced-3*; *Ice*; programmed cell death; apoptosis; GAL4-UAS system; *Drosophila*

Abbreviations: *rpr*, reaper; *hid*, head involution defective; ICE, interleukin-1 β converting enzyme; *sca*, scabrous; UAS, upstream activating sequence; TUNEL, TdT-mediated dUTP nick end labelling; PNS, peripheral nervous system

Introduction

Programmed cell death, or apoptosis, occurs throughout the course of normal development in both vertebrates and

invertebrates (Steller, 1995; White, 1996; Chinnaiyan and Dixit, 1996). It is an essential process in most organisms, required to remove cells that are either superfluous or harmful during development. This process often requires RNA and protein synthesis, so that active gene-directed processes are thought to be involved in mechanisms of cell death (Martin *et al*, 1988; Oppenheim *et al*, 1990; Raff, 1992). A number of genes participating in different steps of cell death have been isolated in *Caenorhabditis elegans* (Steller, 1995). Several genes involved in cell death have also been isolated in mammals; some genes act as inhibitors, whereas others act as accelerators or executors of the cell death (Steller, 1995; White, 1996; Chinnaiyan and Dixit, 1996). It is still unclear, however, how these cell death genes modulate programmed cell death, interacting with each other to induce cell death in a particular region at particular time. In order to solve these problems, it is necessary to uncover and isolate more cell death genes and investigate the role of each gene in the cascade of gene interactions. *Drosophila melanogaster* provides an ideal system to elucidate the molecular mechanism of cell death, because genes acting upstream or downstream of a given signal pathway can be identified by systematically isolating mutants. We can then examine the *in vivo* function of a given gene by employing sophisticated genetic strategies available in this organism.

In *Drosophila*, two genes, *reaper* (*rpr*) and *head involution defective* (*hid*), that induce programmed cell death in the embryo have been identified from genetic screens for abnormal patterns of cell death in homozygotes for chromosomal deletions (White *et al*, 1994; Grether *et al*, 1995). Recently, a third cell death gene, *grim*, was found in the genomic region between *rpr* and *hid* (Chen *et al*, 1996). These genes are expressed in regions of embryo where cell death normally occurs, and ectopic expression of these genes can induce cell death in the cells that normally survive during development (White *et al*, 1996; Grether *et al*, 1995; Chen *et al*, 1996). Among them, the *rpr* gene has been well investigated and appears to play a key role in regulating cell death during *Drosophila* development (Steller and Grether, 1994; White *et al*, 1994; Nordstrom *et al*, 1996). Although expression of *rpr* is sufficient to induce cell death, the gene cascade functioning downstream of *rpr* is not known. A recent study using a protease inhibitor indicates that *rpr*-induced cell death requires a functional ICE-like protease *in vitro* (Pronk *et al*, 1996).

ICE was initially found as a cysteine protease which cleaves the pro-interleukin-1 β into the biologically active interleukin-1 β (Cerretti *et al*, 1992; Thornberry *et al*, 1992). It shares a sequence similarity with CED-3 (Yuan *et al*, 1993). CED-3 is a protein required for the programmed cell death in *C. elegans* and the loss of the *ced-3* gene function results in a failure of cell to die (Ellis and Horvitz, 1986). The sequence similarity between CED-3 and ICE suggested that ICE might function as a cell death gene in vertebrates. In fact, overexpression of ICE or CED-3

causes rat fibroblast cells to undergo programmed cell death (Miura *et al*, 1993). In mammals, several other genes with structural and functional similarities to *ced-3/Ice* have been identified (Kumar, 1995; Chinnaiyan and Dixit, 1996). The *ced-3/Ice* family can be classified into three main subfamilies (CED-3, ICE, NEDD2) based on their sequence similarities (Chinnaiyan and Dixit, 1996). Some genes belonging to the *ced-3/Ice* family have also been shown to be involved in executing cell death (Kumar *et al*, 1994; Wang *et al*, 1994; Fernandes-Alnemri *et al*, 1994). In *Drosophila*, however, the existence of a *ced-3/Ice* homologue has not been described.

Here, we have investigated whether nematode CED-3 and human ICE can induce cell death in *Drosophila*. Using the GAL4-UAS system, we induced ectopic *ced-3/Ice* expression in specific cells and examined whether cell death occurs in the corresponding region. We have confirmed that *ced-3/Ice* elicits cell death in *Drosophila* which was rescued by coexpressing the viral antiapoptotic p35 protein. Our findings indicate that *ced-3/Ice* can function in *Drosophila* and provide a way to isolate cell death genes through isolating mutants acting downstream of CED-3/ICE proteases.

Results and Discussion

Targeted expression of *ced-3/Ice* by the GAL4-UAS system

In order to examine whether CED-3 and ICE elicit cell death in *Drosophila*, we used the GAL4-UAS system to induce *ced-3* or *Ice* expression in specific cell types or tissues at particular developmental stages. GAL4 is a yeast transcriptional regulator that can activate transcription of any gene if the gene is preceded by a GAL4 upstream activating sequence (UAS) containing GAL4 binding sites (Brand and Perrimon, 1993). The GAL4 and its target gene which is preceded by UAS, are initially separated in two different transgenic lines. In one line, the activator protein, GAL4, is expressed in a strain-specific manner but has no target gene to be activated. In the second line, the target gene is silent in the absence of GAL4. When the two lines are crossed, the ectopic expression of the target gene is induced only in the progeny of the cross.

We have established a number of independent transformant lines carrying either a UAS-*ced-3* or a UAS-*Ice* construct. When we crossed UAS-*ced-3* or UAS-*Ice* transgenic lines to several available GAL4 lines, we observed various phenotypic abnormalities depending on the particular UAS-*ced-3/Ice* and GAL4 lines used. These results suggested that the observed abnormality was due to

induced cell death. Among the GAL4 lines, we used the *sca*-GAL4 line for further studies, because much is known about the expression pattern of the *sca* gene and we could readily observe an adult phenotype caused by ectopic expression of *ced-3* and *Ice* driven by *sca*-GAL4. In the *sca*-GAL4 line, the *scabrous* (*sca*) enhancer activates GAL4 expression in the anlage of the peripheral nervous system during embryonic and larval development (Mlodzik *et al*, 1990). This line was derived from an enhancer trap screen and it has subsequently been confirmed that the GAL4 vector is inserted in the *sca* locus (Kramer *et al*, 1995). As a result, homozygous flies lack a few bristles on the notum. In our study, we always used heterozygous flies for *sca*-GAL4 so the bristle phenotype was not observed.

The progeny from crosses between *sca*-GAL4 and various UAS-*ced-3/Ice* lines were mostly pupal lethal (Table 1). A few combinations produced embryonic lethality. The lethal phase was not always at one restricted stage. In one of the larval lethal lines and several of the pupal lethal lines, some individuals also showed pupal or larval lethality respectively. For the UAS-*ced-3* lines, we chose to analyze five UAS-*ced-3* lines (7-3, 15-11, 6-6, 12-1 and 17-3) that showed lethality at different stages. One line (UAS-*ced-3* 7-3) was embryonic lethal, three lines (UAS-*ced-3* 15-11, 6-6, 12-1) were pupal lethal, and the fifth line (UAS-*ced-3* 17-3) was viable. The hatching ratio of embryos decreased to 8.5% (79/927) in the embryonic lethal line (*sca*-GAL4/UAS-*ced-3* 7-3), while the value was 91.9% (626/681) in the control *w;sca*-GAL4/+ flies. This indicates that the embryonic lethality is a result of ectopic expression of *ced-3* but does not result from GAL4 expression alone. Concerning the UAS-*ced-3* 17-3 line, we needed to examine whether *ced-3* is really expressed because the progeny from the cross between this line and *sca*-GAL4 line were viable, and failed to show morphological abnormality. We crossed this line to other GAL4 lines and confirmed that the abnormal phenotype was induced. This result shows that the transgene of the UAS-*ced-3* 17-3 line is not silent and that *ced-3* is expressed as well as in other UAS-*ced-3* lines used.

Differences in the stage of lethality among UAS-*ced-3* lines depend on the amount of *ced-3* expression

In order to confirm directly that *ced-3* is expressed in the progeny between UAS-*ced-3* lines and *sca*-GAL4, we performed RT-PCR using *ced-3* primers (Figure1). We also examined at the same time whether there was a difference in the mRNA expression levels among UAS-*ced-3* transformant

Table 1 Lethal stages among progenies crossed between *sca*-GAL4 and various UAS-*ced-3/Ice* lines

Transgene	Lethal stage				Total number of strains examined
	embryo	larva	pupa	viable	
UAS- <i>ced-3</i>	3	2	15	2	22
UAS- <i>Ice</i>	5	0	16	1	2

Note. Concerning the pupal lethal lines, we confirmed that bristles on the notum were missing except for one line that showed early pupal lethality. The transgene in the viable progeny is not silent, because we observed lethality when these lines were crossed to other GAL4 lines

Figure 1 Comparison of the levels of *ced-3* mRNA expression between *sca-GAL4/UAS-ced-3 7-3* (which shows embryonic lethality) and *sca-GAL4/UAS-ced-3 12-1* lines (which shows pupal lethality). As a molecular weight marker, lane 1 shows the bands corresponding to the *Hind*III digest of λ phage and the *Hae*III digest of $\phi \times 174$. The expression of *ced-3* in *sca-GAL4/UAS-ced-3 7-3* embryos (lane 4) is more than twice as great as that in *sca-GAL4/UAS-ced-3 12-1* embryos (lane 3). In *w* embryos as a negative control, *ced-3* was not amplified (lane 2). We used three different sets of primers to amplify *ced-3* and all three resulted in a greater amplification of the amount of *ced-3* in *sca-GAL4/UAS-ced-3 7-3* embryos than that in *sca-GAL4/UAS-ced-3 12-1* embryos (data not shown).

lines when the lines were crossed to the *sca-GAL4* line. The differences in the stage of lethality among various transformant lines was expected to be caused by the difference in the amount of *ced-3* expression, depending upon where the gene was inserted into the chromosome. To compare the amount of *sca-GAL4*-driven *ced-3* mRNA between the pupal lethal and the embryonic lethal lines, we selected two *UAS-ced-3* lines, *UAS-ced-3 12-1* line (showing pupal lethality) and *UAS-ced-3 7-3* line (showing embryonic lethality). We used *w* as a negative control. The results showed that *ced-3* was induced in both *sca-GAL4/UAS-ced-3 12-1* and *sca-GAL4/UAS-ced-3 7-3* embryos (Figure 1 lanes 3,4) but not in *w* embryos (Figure 1 lane 2). The *ced-3* mRNA is actually induced in the progeny from crosses between these *UAS-ced-3* lines and *sca-GAL4*. The amount of *ced-3* expression in *sca-GAL4/UAS-ced-3 7-3* embryos (Figure 1 lane 4) was more than twice that found in *sca-GAL4/UAS-ced-3 12-1* embryos (Figure 1 lane 3), which indicates that the difference in the stage of lethality among the *UAS-ced-3/lce* lines depends on the amount of *CED-3/ICE* expression.

The pattern of cell death coincides with that of *GAL4* expression

An important question is whether lethality is really caused by cell death as the result of the ectopic expression of *ced-3*. We performed TUNEL to determine whether ectopic expression of *ced-3* could induce cell death. In embryos, *GAL4* driven by the *sca* enhancer is normally expressed in an ectodermal region that will give rise to the peripheral nervous system, PNS, so that *ced-3* was expected to be overexpressed in the PNS of *sca-GAL4/UAS-ced-3 7-3* embryos. Compared with control *w*; *sca-GAL4/+* embryos (Figure 2b), *sca-GAL4/UAS-ced-3 7-3* embryos showed an increased amount of ectopic cell death (Figure 2a).

Evidence of ectopic cell death could also be seen morphologically; the pattern of the PNS was irregular (Figure 2c, compare with Figure 2d), which is consistent with the loss of some PNS cells during development. These results allow us to conclude that *ced-3* elicits cell death in the embryonic PNS, and that this should lead to embryonic lethality.

We next investigated cell death in anlagen of the sensory cells within wing imaginal discs because *sca* is also expressed in these cells (Mlodzik *et al*, 1990). We used *sca-GAL4/UAS-ced-3 12-1* larvae that survive until the late pupal stage. To confirm the pattern of *GAL4* expression, the *sca-GAL4* line was crossed to a *UAS-lacZ* line and *lacZ* expression was monitored by staining with a monoclonal antibody (mAb) against β -galactosidase. The pattern of *GAL4* expression in the notum anlage of the wing disc certainly corresponds with the region of anlagen of sensory cells (Figure 3a). The cell death pattern in the corresponding region of the wing disc in the control line is shown in Figure 3c. Only a few dying cells were observed in this region in the control, compared with which, many more dying cells were observed in *sca-GAL4/UAS-ced-3 12-1* larvae (Figure 3b, compare with Figure 3c), in a pattern of cell death that was similar to that of *GAL4* expression (Figure 3a,b). The cell death induced in sensory cell anlagen is predicted to result in a severe defect in the development of sensory bristles, and we did indeed observe that bristles on the notum were missing at the late pupal stage (Figure 3d, compare with Figure 3e). We further noticed that almost all bristles were missing, including those on the head, abdomen (Figure 3d, compare with Figure 3e) and compound eyes (Figure 3f, compare with Figure 3g). Similar results were also obtained in those *sca-GAL4/UAS-ced-3 15-11* and *sca-GAL4/UAS-ced-3 6-6* pupae that survived until the late pupal stage (data not shown).

During the larval stage, *GAL4* driven by the *sca* enhancer is also expressed in the central nervous system. Pupal lethality may therefore be caused by a direct influence of the ectopic expression of *ced-3* in the nervous system, which might affect eclosion behavior.

GAL4 is also expressed in the precursor cells of the R8 photoreceptors in the eye imaginal disc of the *sca-GAL4* line (Kramer *et al*, 1995), but ectopic cell death was not observed by TUNEL in the eye disc of *sca-GAL4/UAS-ced-3 12-1* larvae. The external morphology of the pupal eye

Figure 2 Pattern of cell death in an embryonic lethal line. (a) Pattern of cell death in the embryo in which ectopic expression of *ced-3* was induced (stage 12). The *sca-GAL4* line was crossed to *UAS-ced-3 7-3* and their progeny were examined for cell death by TUNEL. The *UAS-ced-3 7-3* line showed the strongest cell death phenotype among *UAS-ced-3* lines. (b) The wild-type pattern of cell death in *w; sca-GAL4/+* embryo at the stage 12. (c) The disrupted pattern of cell death in the PNS caused by the ectopic expression of *ced-3* (late stage embryo). As in (a), the embryo was obtained by crossing the *sca-GAL4* line and *UAS-ced-3 7-3* line. Compared with the wild-type embryo (d), the PNS pattern is severely disrupted and the embryo is shorter. (d) The wild-type pattern of PNS in a *w; sca-GAL4/+* embryo at stage 17. Stages of embryogenesis are according to Campos-Ortega and Hartenstein (1985). Embryos are oriented with anterior to the left and dorsal up.

was normal and the number of ommatidia was not affected. In addition, similar results were obtained when *UAS-ced-3* lines were crossed to other *GAL4* lines in which *GAL4* is expressed in the precursors of photoreceptor cells (AS *et al.* unpublished data). Perhaps the developing photoreceptor cells are resistant to cell death mediated by *ced-3*. These results suggest that there are differences in sensitivity between tissues in the cell death mediated by *ced-3* when this is induced in various tissues. These differences may be due to post translational modulation or the stability of CED-3.

Ice* can induce cell death in *Drosophila

We investigated *UAS-Ice* lines in the same way as for *UAS-ced-3* lines, and obtained similar results with respect to lethality (Table 1). The progeny from crosses between *sca-GAL4* and various *UAS-Ice* lines were mostly pupal lethal. We performed TUNEL to examine the pattern of ectopic cell death in these progeny and confirmed that the pattern of *Ice*-induced cell death was similar to the pattern of *ced-3*-induced cell death (data not shown). These results lead us to conclude that both CED-3 and human ICE are able to induce cell death in *Drosophila*. In many crosses, however, the number of *Ice*-induced cell death was fewer than that of *ced-3*-induced cell death and not all bristles on the notum were missing at the late pupal stage (data not shown). This difference suggests that in *Drosophila* *ced-3* can induce cell death more effectively than *Ice*. A putative *Drosophila* *ced-3/Ice* homologue might therefore be more similar to the members of the *ced-3* subfamily than to those of the *Ice* subfamily. It is difficult to clarify our presumption further, however, because we have to

take into account the different amounts of CED-3/ICE expression that occur, depending on the *UAS-ced-3/Ice* insertion sites.

p35 blocks cell death induced by *ced-3/Ice*

Analysis with TUNEL revealed that cell death occurred exactly where CED-3 or ICE was ectopically expressed. To determine whether the cell death is inhibited by p35, we coexpressed the p35 protein in the same place as *ced-3* and *Ice*. p35 is a baculovirus-encoded protein which prevents cell death by directly inhibiting CED-3/ICE family proteases including ICE and CED-3 (Bump *et al.*, 1995; Xue and Horvitz, 1995). We established flies having both *UAS-p35* and *UAS-ced-3/Ice* transgenes together with *sca-GAL4*, using *UAS-ced-3 12-1* and *UAS-Ice 2-2*, both of which produced pharate adults lacking sensory bristles when crossed to the *sca-GAL4* line (Figure 3d and 4a). As shown in Figure 4b, the coexpressed p35 protein rescued the phenotype induced by both *ced-3* and *Ice*: viable flies resulted and sensory bristles on the notum were normal. We also found that cell death induced by *ced-3/Ice* was suppressed by p35 in combinations of other *GAL4* lines with *ced-3/Ice* lines (data not shown). These results support the view that both CED-3 and ICE might function as cysteine proteases to induce cell death in our experiments.

Previous studies have shown that *ced-3/Ice* can function in mammalian cell lines. Our study is the direct demonstration that the *ced-3/Ice* gene induces cell death in an organism. The ability of CED-3 or ICE to induce cell death in *Drosophila* suggests that the mechanism of cell death is shared in very diverse organisms and implies that there is a

Drosophila homologue of the *ced-3/lce* gene. It would be interesting to know whether the *Drosophila ced-3/lce* homologue forms a family as in mammals, how the *rpr*-mediated signal activates the *ced-3/lce* pathway, and how

ced-3/lce transmits the death signal downstream. Genetic systems in which induction of cell death is targeted have been proven to be useful in identifying genes involved in cell death (Hay *et al*, 1995). We now expect to be able to

Figure 3 GAL4-dependent cell death in third-instar wing discs resulting from *ced-3* expression. (a) GAL4 expression pattern in the anlage of the wing disc notum. *sca*-GAL4 line was crossed to a UAS-*lacZ* line and their progeny were stained for β -galactosidase with an anti β -galactosidase antibody. This pattern corresponds to the *scabrous* expression previously described (Mlodzik *et al*, 1990), where GAL4 is expressed in patches of cells that give rise to the bristles in the notum. (b) Pattern of cell death in the wing disc with the ectopic expression of *ced-3*. *sca*-GAL4 line was crossed to one of the weaker UAS-*ced-3* lines, UAS-*ced-3* 12-1, and their progeny were examined for cell death by TUNEL. The pattern of cell death coincides with the GAL4 expression pattern. (c) Pattern of cell death of a wild-type wing disc. Only a few apoptotic cells can be seen. (d) The notum of the pupa in which the ectopic expression of *ced-3* was induced. As in (b) pupae were obtained by crossing the *sca*-GAL4 line and UAS-*ced-3* 12-1 line. Note absence of bristles. (e) The wild-type pattern of the bristles on the notum of the *w* pupa. (f) The bristles of the pupal compound eyes with the ectopic expression of *ced-3*. As in (b) and (d), the pupa was obtained by crossing the *sca*-GAL4 line and UAS-*ced-3* 12-1 line. Most ommatidial bristles are missing, but the external morphology of the eyes looks normal. (g) The wild-type pattern of bristles in *w* pupal compound eyes. One mechanosensory bristle occurs per ommatidial unit and the ommatidia are aligned in a crystalline array. (a–c) Anterior to the left.

Figure 4 Rescue of the *ced-3/lce*-induced cell death by coexpression of p35. Scanning electron micrographs of dorsal views of notum are shown. (a) The notum of the pharate adult with ectopic expression of *ced-3* (*sca-GAL4/UAS-ced-3 12-1*). Note absence of all the bristles. (b) The notum of the fly with coexpression of p35. Loss of sensory bristles is rescued by p35. The UAS-*p35* transgene was introduced into flies with UAS-*ced-3 12-1* and *sca-GAL4*. The number and location of most bristles are normal.

identify the genes involved in *ced-3/lce*-mediated cell death, and these studies should provide further insight into the molecular mechanisms of cell death not only in *Drosophila* but also in other organisms.

Materials and Methods

Drosophila strains

Flies were raised on a cornmeal-yeast medium at 25°C. Because *GAL4* expression is sensitive to temperature, flies were maintained under strict conditions of temperature. *w*¹¹¹⁸ was used as a control strain and the UAS-*lacZ* line was used to confirm the *GAL4* ectopic expression pattern. As a *GAL4* activator line, we used the *scabrous* (*sca*)-*GAL4* line (i.e. *sca-GAL4*) provided by Y Hiromi. This line was originated from an enhancer trap screen and it was confirmed that *GAL4* inserted in the *sca* locus on the second chromosome. To examine the anti-apoptotic effect of the *p35* gene on CED-3 and ICE-induced cell death, we first established lines having a genotype of *w*; UAS-*p35*/*SM5*; UAS-*ced-3 12-1* or UAS-*lce 2-2/TM3*, *Sb*. This line was crossed to *w*; *sca-GAL4/sca-GAL4* and progeny having neither *SM5* nor *TM3* balancer chromosomes were examined for suppression of cell death. The UAS-*p35* (2Y) line was kindly provided by BA Hay.

P-element transformation and ectopic expression

The UAS-*ced-3* and UAS-*lce* vectors were constructed as follows. A *C. elegans ced-3* cDNA of 2.5 kb containing the entire protein-coding region was obtained from a cDNA library constructed from wild-type

worms (strain N2). A human *lce* cDNA of 1.3 kb containing the entire coding region was isolated from a B cell cDNA library. Nucleotide sequences for coding regions of the *ced-3* and *lce* cDNAs were confirmed by DNA sequencing. The cDNA fragments were cloned in the *EcoRI* site of a pUAST vector and DNA constructs with the right orientation were used to generate transgenic flies. Transgenic flies were generated by standard P-element-mediated germ-line transformation techniques (Rubin and Spradling, 1982) using *w*; *TMS*, *Sb* *P[ry⁺, Δ2-3]* 99B/*Dr* as the recipient strain. Transgenic stocks were kept as homozygotes. In this paper, we used strains that were homozygous for a single UAS-*ced-3* or UAS-*lce* insertion.

Measurement of relative *ced-3* RNA concentrations by RT-PCR

Total RNAs were isolated from *w*, *sca-GAL4/UAS-ced-3 7-3* and *sca-GAL4/UAS-ced-3 12-1* embryos (Stage, 13–15) and further enriched for poly(A)⁺ RNA using a QuickPrep Micro mRNA purification kit (Pharmacia Biotech). First-strand cDNAs were synthesized from 2 μg mRNA (T-primed First-Strand Kit; Pharmacia Biotech). PCR reactions were performed with one-tenth of the total cDNAs produced and 10 μM of each oligonucleotide primer mixture. The 5' primers consisted of 20 mers (5'-TGCTCTTCTCTCGGATACAG-3') and the complementary strand 3' primer consisted of 20 mers (5'-AACACTTCACAGACGGCTTG-3') for amplifying *ced-3* cDNAs. As an internal control, the 5' primer consisted of 21 mers (5'-ACGAAGCAGTGCAACATCGAC-3') and the complementary strand 3' primer consisted of 21 mers (5'-ATCCTGCTCGATGAAGGGACG-3') for amplifying *Dras* DNAs. The PCR reaction was carried out under the following conditions, 1 min at 94°C followed by 30 cycles of 1 min at 94°C,

0.5 min at 55°C and 1.5 min at 72°C. We confirmed that amplified *ced-3* DNAs did not reach saturation before 30 cycles. Images of an agarose gel after ethidium bromide staining were captured by a CCD camera and the amount of DNAs was quantified by a computerized system (Densitograph, ATTO).

Detection of cell death

The TUNEL to identify dying or dead cells based on the presence of damaged DNA was carried out essentially as described in White *et al* (1994) with the following modification: embryos were incubated with proteinase K (2 µg/ml in 10 mM Tris/HCl, pH 8.0) for 5 min at 25°C before the incubation with terminal transferase.

Immunocytochemistry

Immunohistochemistry was done according to Usui and Kimura (1993) using a mAb against β -galactosidase (Promega) and neuronal antigen (22C10).

Scanning electron microscopy

Adult flies were dehydrated in a graded acetone series and dried. Mounted flies were sputter coated with platinum and observed with a Hitachi S-4100 scanning electron microscopy.

Note added in proof

Drosophila caspase-1 (DCP-1) has now been cloned (Song Z, McCall K and Steller H (1997) DCP-1, a *Drosophila* cell death protease essential for development. *Science* **275**: 536–540).

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