

C. elegans EIF-3.K Promotes Programmed Cell Death through CED-3 Caspase

Chun-Yi Huang1, Jia-Yun Chen1,2, Shu-Chun Wu1, Chieh-Hsiang Tan1, Ruei-Ying Tzeng1, Pei-Ju Lu2, Yu-Feng Wu1, Ruey-Hwa Chen2,3,4, Yi-Chun Wu1,4,5,7*, Chun-Yi Huang1, Jia-Yun Chen1,2, Shu-Chun Wu1, Chieh-Hsiang Tan1, Ruei-Ying Tzeng1, Pei-Ju Lu2, Yu-Feng Wu1, Ru...
or are CED-3 substrates, such as DCR-1 [21], are important for the timing or progression of programmed cell death. The eukaryotic translation initiation factor 3 (eIF3) plays essential roles in the initiation of translation [22]. The mammalian eIF3 complex contains 10–13 subunits, including five highly conserved core subunits and five to eight less conserved non-core subunits [23,24]. The 20 kDa human eIF3k protein was originally identified as a non-core subunit of the eIF3 complex [25]. An in vitro reconstitution experiment showed that eIF3k is not required for the formation of the active eIF3 complex [26]. Interestingly, eIF3k is conserved among metazoans, including C. elegans, D. melanogaster, M. musculus, and H. sapiens, but is absent in S. cerevisiae, suggesting a specialized role for eIF3.K in multicellular organisms [25,27]. In addition, human eIF3 is associated with dynactin [27], cyclin D3 [28], the 5-HT7 receptor [29], and keratin K18 [30], suggesting the involvement of eIF3k in processes that are unrelated to translation. Recently, we reported an apoptosis-promoting function for eIF3k in simple epithelial cells [30]. Upon apoptotic stimuli, keratin K18 is cleaved by caspase 3, resulting in the collapse of K8/K18 intermediate filaments into apoptotic bodies and the sequestration of caspase 3 in keratin-containing inclusions [31]. eIF3k binds to keratin inclusions, which in turn leads to the release of keratin-associated caspase into the cytosol to facilitate the execution of apoptosis [30]. Keratin K8/K18 is the major intermediate filament in epithelial cells [31]. It is not clear whether eIF3k may potentiate apoptosis in other cell types, such as neurons or muscle cells, where intermediate filaments other than keratin are present. In addition, it is unclear whether the apoptosis-promoting function of eIF3k has been conserved throughout evolution.

In this work, we characterized the function of eIF3.K in C. elegans and showed that its apoptosis-promoting function has indeed been conserved throughout evolution. Furthermore, we identified a new function for the WH domain of EIF-3.K in the promotion of programmed cell death.

Materials and Methods

Strains

All strains were maintained at 20°C on NGM (nematode growth medium) agar seeded with Escherichia coli OP50 bacteria as previously described [32]. The wild-type strain was the Bristol strain N2. The following mutations were used: linkage group (LG) I, cer-1(e1735) [33], csp-3(tm2486) [14]; LGII, cer-1(tm2873) [17]; mll1(dpy-10(e128)mIs14); LGIII, cer-7(n1996) [33], cer-4(n1162, n2273) [4,34], cer-6(n2995) [33]; LGIV, cer-5(n1812), cer-2(n1994) [33], cer-3(n177, n2427) [4,35], cer-2(tm3077) [13]; LGV, eif-3.K(gk126) (C. elegans knockout consortium); unc-76(e911) [30]; unc-1(n1392) [37]; LGX, nIs106 [38]. The following integrated lines were used: nl50[Pmyo-2::GFP] [33], bzIs8[Pmec-3::GFP] [39] and smIs1[Pmec-7::eif-3.K] (30) and subsequently Phsp[eif-3.K] (34) were cloned into pPD52.102 (Andy Fire) to generate Plet-858-eif-3.K or Pmec-7-eif-3.K.

Cell Death Assays

Cell corpse numbers in embryos or germline of indicated mutants were scored as previously described [41]. Extra surviving cells in the anterior pharynx were scored at the late L3 or early L4 larval stage, as previously described [41]. To assay extra surviving cells in the ventral cord, the integrated nl106 (Pmec-7::GFP) transgene was utilized [38]. The nl106 transgene was crossed to cer-2 or cer-7 single mutants or cer-2; eif-3.K or cer-7; eif-3.K double mutants. The extra Pn.aap cells in the P2, P9, P12-derived regions of the transgenic mutants were scored at the L4 stage by the fluorescence microscopy as previously described [38]. The TUNEL assay was carried out using an in situ cell-death detection kit (Roche) as previously described [42]. To assay the UV-C radiation-induced cell death in the germline, adult worms (24 h post the L4 stage) were exposed to 254 nm UV-C light at150 J/m² using a Stratalinker UV crosslinker (Stratagene, model 2400) as previously described [43], and the cell corpses in the gonadal arms were scored 24 hours after the treatment.

Molecular Biology

To determine the 5′ end of eif-3.K mRNA, we performed an RT-PCR experiment using nested primers 5′-GATGAGCAACTTGGGGAGAG-3′ and 5′-CTTGTTCCTTGAGCACTGACC-3′ in combination with either the SL1 primer or SL2 primer and sequenced the resulting product. The sequence confirmed the 5′ end of the eif-3.K coding sequence shown on the Wormbase and revealed that eif-3.K mRNA was trans-spliced to either SL1 or SL2. To generate the eif-3.K cDNA construct, the full-length eif-3.K coding region was amplified by RT-PCR using primers 5′-ATGTGTGGTGGAAACTCTGG-3′ and 5′-GTAAGTTGGGACAATGAGAAAT-3′ and subsequently inserted into the pSTBlue vector (Novagen) at the EcoRV site. To generate Pmec-7:eif-3.K, the eif-3.K cDNA was inserted into heat shock vectors pPD49.78 and pPD49.83 (different tissue specificity). To generate Pmec-7-eif-3.K or Pmec-3:eif-3.K, eif-3.K was inserted to the pPD118.25 plasmid containing Pmec-7 [44] or the pPD95.77 plasmid containing Pmec-3 [45], respectively. We generated mutant eif-3.K cDNA encoding truncated EIF-3.K protein without the HAM domain (amino acids 23–120) or WH domain (amino acids 148–208) by inverse PCR and inserted the mutant cDNA into the vector containing Pmec-7::GFP to generate Pmec-7::eif-3.K::HAM or Pmec-7::eif-3.K::WH. eif-3.K::HAM cDNA was also inserted to vectors pPD49.78 and pPD49.83 to yield Pmec-7::eif-3.K::HAM. To generate Pmec-7::WH or Pmec-3::WH, the cDNA corresponding to the WH domain (amino acids 148–208) was inserted into the vector containing Pmec-7::GFP or Pmec-3::GFP, respectively. The egl-1 [5] or ced-4 [34] cDNA was cloned into pPD52.102 (Andy Fire) to generate Pmec-7::egl-1 or Pmec-3::ced-4, respectively.

Transgenic Animals

Germine transformation experiments were performed as previously described [46]. For the rescue experiment or structure function analysis of EIF-3.K, the indicated constructs (50 µg/ml) were injected into eif-3.K(gk126) animals with the coinjection marker pTG96 plasmid. The pTG96 plasmid contains sur-5::GFP that is expressed in almost all somatic cells [47]. To overexpress egl-1, ced-4, or eif-3.K in the touch neurons, Pmec-7::egl-1, Pmec-7::ced-4, or Pmec-7::eif-3.K (50 µg/ml) was injected into unc-76(e911); bzIs8 animals with the coinjection marker p76-16B (100 µg/ml), which rescues the unc-76 phenotype [36]. To overexpress ced-3 in touch neurons in the bzIs8 transgenic worms, nl50 carrying the integrated transgene Pmec-7::ced-3 (ced-3.A line) [35] was crossed to the bzIs8 strain to generate nl530; bzIs8 double transgenic worms. To express accED-3 in touch neurons, the integrated transgene smIs1 [40] carrying both Pmec-7::ced-3 and Pmec-7::GFP was used [40]. To coexpress eif-3.K and ced-3 in touch neurons Pmec-7::eif-3.K (50 µg/ml) was injected into bzIs8; nl530 animals with the coinjection marker Pmec-7::GFP (2 µg/ml), which expresses GFP in the pharynx [48].

Heat Shock Experiments

To overexpress the wild-type or mutant eif-3.K cDNA or human eIF3K cDNA, young adults carrying the respective transgene were allowed to lay eggs overnight, and the laid embryos were cultured at 20°C (non-heat shock) or at 33°C (heat shock) for 1 hr, which was followed by a 20°C recovery for at least 1.5 hrs. The embryos
were scored for cell corpses at the comma and 1.5-fold stages under DIC optics.

**Antibodies, Immunostaining and Immunoblotting**

To generate anti-EIF-3.K antibodies, the *eif-3.K* cDNA corresponding to 45–240 amino acids was cloned into the pGEX-4T expression vector. GST- EIF-3.K(45–240) fusion protein was expressed in *E. coli* and further purified using 10% SDS-PAGE. GST-EIF-3.K(45–240) protein was excised from the gel and used to immunize rabbits. Immune serum was further purified by EIF-3.K-conjugated Affi-Gel as described by the manufacturer’s manual (Bio-Rad).

For immunostaining, embryos and worms were collected off plates and treated with hypochlorite (10 NaOH and NaOCl) to enrich embryos. Embryos were then washed with ddH2O for three times and fixed in fixation buffer (2% paraformaldehyde, 90% methanol, 10% EGTA, 1 M spermine, 100 mM spermidine, and 0.5 M PIPEs) overnight at -80°C as described by Guenther and Garriga [49]. After fixation, embryos were thawed, washed with Tris-Trition buffer (100 mM Tris-HCl pH7.4, 1% Triton X-100, and 1 mM EDTA) and blocked with 5% bovine serum albumin in PBS. Treated embryos were incubated with purified antibodies against EIF-3.K overnight at 4°C. After washing with wash buffer (1X PBS, 1% BSA, 0.5% Triton X-100, 0.05% NaN3, and 1 mM EDTA), embryos were then incubated with rhodamine-conjugated donkey secondary antibodies against rabbit (Jackson Immune Research Laboratories). After incubation for 2 hr at room temperature, antibodies were washed off using wash buffer three times for 5 min each, with DAPI included in the first wash. For MitoTracker staining, embryos were collected from worms grown in the dark on NGM agar plates containing MitoTracker® Red 580 (1 μg/mL, Molecular Probes). Stained embryos were mounted with VECTASHIELD® mounting medium H-1000 (Vector Laboratories) and observed using confocal laser scanning microscopy (Leica TCS SP2 Confocal Spectral Microscope).

For western blot analysis, total protein extracts of indicated genotypes were resolved by 12% SDS-PAGE and transferred to nitrocellulose membranes. The blot was incubated with affinity-purified EIF-3.K antibodies (1:2500) and monoclonal anti-α tubulin antibodies (Abcam). ECL detection system (pierce) was used for detection.

**Bacteria-mediated RNAi**

Induction RNA interference (RNAi) experiments were carried out using a bacterial feeding protocol [50]. L4 larvae were transferred to the control (pPD129.36) or indicated RNAi plates and cultured at 20°C. F1 embryos laid approximately 48 hours later were picked for phenotypic analysis. The *eif-3.K* RNAi clone was obtained from the Ahringer RNAi library.

**Results**

**The Loss of *eif-3.K* Causes Reduced Cell Death in Both Somatic and Germline Cells**

We obtained the cDNA for *eif-3.K* through reverse transcription polymerase chain reaction (RT-PCR) using total RNA from mixed-stage worms. The predicted full length amino acid sequence of EIF-3.K is 35% identical and 57% similar to that of human eIF3k (Figure 1A). We next characterized the *eif-3.K* mutant allele *gk126*, which was isolated by the *C. elegans* Gene Knockout Consortium. This allele contains a 538 base pair (bp) long deletion from 119 bp upstream of the start ATG codon to the second exon in the *eif-3.K* locus (Figure 1B). No EIF-3.K protein was detected in the *eif-3.K(gk126)* mutant by western blotting or immunostaining analyses using purified anti-EIF-3.K antibodies (Figure 2), suggesting that the *gk126* allele is null. Because *eif-3.K(gk126)* and *eif-3.K(RNAi)* mutant worms were viable and had normal development and growth rates (Table S1), we concluded that *eif-3.K* is not an essential component of the general translation machinery in *C. elegans*.

We next examined whether *eif-3.K(RNAi)* or *eif-3.K(gk126)* embryos have defective programmed cell death. A time course analysis of embryonic cell corpses using differential interference contrast (DIC) microscopy showed that *eif-3.K(RNAi)* or *eif-3.K(gk126)* embryos had fewer cell corpses than wild-type embryos throughout embryogenesis (Figure 3A). To determine whether this decrease in cell corpse number corresponded with a reduction in cell death or was simply due to abnormal corpse morphology, we further analyzed the embryos using the TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) assay. The degradation of DNA in dying cells is a hallmark of apoptosis and can be detected in situ using TUNEL staining [42,51]. As shown previously [42], wild-type embryos had very few TUNEL-positive corpses (Figure 3B); however, embryos lacking the *nuc-1* gene, which codes for a protein similar to DNAse II that is involved in DNA degradation [42], had many more TUNEL-positive corpses (Figure 3B). The *eif-3.K* embryos, like the wild-type embryos, had few TUNEL-positive corpses; however, *eif-3.K; nuc-1* double mutant embryos had fewer TUNEL-positive corpses than *nuc-1* single mutant embryos, indicating that apoptosis.

DNA degradation is compromised in the *eif-3.K* mutants. This result, together with the observed decrease in cell corpse number (Figure 3A), indicates that apoptosis is compromised in *eif-3.K* mutants during embryogenesis.

Like somatic cells, germline cells also undergo apoptosis in *C. elegans* [52]. Because few germ cell corpses can be observed in the wild-type adult gonad at any given time due to the prompt removal of cell corpses by the gonadal sheath cells [52], we utilized *cod-1(e1735)* mutant worms, in which cell corpses are not efficiently removed and therefore accumulate, to increase our chances of detecting cell corpses. We found that *cod-1(e1735); eif-3.K(gk126)* double mutants had significantly fewer germ cell corpses than *cod-1(e1735)* single mutants at all adult stages (Figure 3C). Therefore, *eif-3.K* is also important for programmed cell death in germline cells.

**The Loss of *eif-3.K* Enhances Cell Survival in Sensitized Mutants**

We next examined whether a loss of *eif-3.K* function could prevent cell death and result in an accumulation of surviving cells. Two assays were used to score the surviving cells in various regions of the animal [53]. First, superfusional surviving cells that were present in the anterior pharynx were scored using DIC optics. As was previously shown [54], in the presence of a strong loss-of-function mutation in the pro-apoptotic gene *ced-3(n717)*, which blocks nearly all cell deaths, resulted in approximately 10 additional surviving cells in the anterior pharynx (Figure 3D). Animals harboring the weak *cod-3(n2427)* mutation had only 1.2 additional surviving cells (Figure 3D). We found that the *eif-3.K(RNAi)* or *eif-3.K(gk126)* single mutant animals had 0.2 or 0.5 extra surviving cells, similar to the wild-type animals (Figure 3D), indicating that the loss of *eif-3.K* could not detectably block apoptosis in these cells; however, the *eif-3.K(RNAi)* or *eif-3.K(gk126)* mutation did enhance cell survival in the weak *cod-3(n2427)* mutant animals. The *cod-3(n2427); eif-3.K(RNAi)* or *cod-3(n2427); eif-3.K(gk126)* double mutants had 2.7 or 2.1 additional surviving cells in the anterior pharynx (Figure 3D). This is significantly more than *cod-3(n2427)*, *eif-3.K(RNAi)* or *eif-3.K(gk126)* single mutants.
Moreover, the RNAi-mediated inactivation of eif-3.K also significantly enhanced cell survival in the worms lacking the ced-8 gene (Figure 3D), which controls the timing of programmed cell death [19]. These results show that the loss of eif-3.K enhances cell survival in sensitized mutants.

We further analyzed the identities of the surviving cells in these mutants. The extraneous surviving cells observed in ced-3(n2427) single mutants and ced-3(n2427); eif-3.K(gk126) double mutants appeared similar and included sisters of muscle cells m1 and m2 and neurons I1, I2, and MC (Figure S1). It is possible that these cells are more likely to survive than others when the apoptotic machinery is compromised. Consistent with this hypothesis, m1 and m2 sister cells were occasionally observed to survive in the wild-type or eif-3.K(gk126) animals (Figure S1).

We also compared the identities of surviving cells in the ced-3(n2427) animals that were treated with either the eif-3.K or control RNAi. Compared to the control RNAi, eif-3.K RNAi enhanced the survival of the niece of the epithelial cell e1, the sister of the neuron I1 and those cells that were also enhanced by the eif-3.K(gk126) mutation in the ced-3(n2427) single mutants, including sisters of m1, m2, I1, I2, and MC cells (Figure S1). Because the eif-3.K null allele did not enhance the total number of extra surviving cells in the strong ced-3(n717) mutants (Figure 3D), eif-3.K likely functions with ced-3 in the same genetic pathway to promote most, if not all, programmed cell death. Additionally, because the identities of apoptotic cells can be inferred from the cell fates of their differentiated sister cells [55], our observations suggest that eif-3.K exerts a cell death-promoting function in multiple cell types, including neuron, muscle and epithelial cells during development.

Secondly, we scored superfluous surviving cells in the ventral cord in larvae. In contrast to the extra surviving cells we observed in the anterior pharynx, which are generated during embryogenesis [56], extra surviving cells in the ventral cord are generated during larval development [57]. In strong ced-3(n717) mutants, five cells P2.aap and P9–P12.aap in the ventral cord survive [38]. These Pn.aap cells are known to differentiate into VC motor neuron-like cells and express the Plin-11gfp reporter (Table 1) [38].

We scored extra surviving Pn.aap cells using the Plin-11gfp transgene as a marker and found that only 2% of eif-3.K(gk126) worms exhibited extra Pn.aap cells (Table 1). However, the eif-3.K(gk126) mutation increased the average number of extra surviving Pn.aap cells in ced-3(n2427) mutants from 2.6 to 3.6 (Table 1). A previous study showed that strong mutations in genes essential for the removal of apoptotic cells, such as ced-2 or ced-7, block cell death, albeit at low efficiency, as apoptotic cell removal is involved in the death of cells [38,58]. The frequency of extra Pn.aap cell survival in these mutants can be enhanced by a weak mutation in the core programmed cell death genes ced-3, ced-4, or egl-1 [38]. Therefore, we tested whether the loss of eif-3.K enhanced the frequency of superfluous Pn.aap cell survival in strong ced-2 or ced-7 mutants. We found that the eif-3.K(gk126) mutation increased the frequency of Pn.aap cell survival in ced-2
The Loss of eif-3.K Partially Suppresses the Ectopic Cell Deaths Induced by the Overexpression of egl-1 or ced-4

We next tested whether eif-3.K genetically interacts with the core programmed cell death genes ced-3, ced-4, and egl-1. Previous studies have shown that cell-specific expression of these three genes under the control of the Pmec-7 promoter, which is expressed in six touch neurons (AVM, ALMR/L, PVM, and PLMR/L), promotes these neurons to undergo programmed cell death. The observed decreased cell death in eif-3.K mutants (Figures 3A–3C) as well as the enhanced cell survival observed in the anterior pharynx (Figure 3D) and ventral cord (Table 1) of sensitized mutants shows that eif-3.K is a positive mediator of programmed cell death.

Therefore, the efficient apoptosis of touch neurons induced by the overexpression of egl-1 or ced-4 requires eif-3.K. This result showed that eif-3.K functions downstream of or in parallel to egl-1 or ced-4 to promote cell death.

In contrast, eif-3.K(gk126) failed to suppress the apoptosis of touch neurons induced by ced-3 overexpression. The overexpression of ced-3 resulted in the death of approximately 34% of the PVM neurons in the wild-type animals, similar to the percentage (40%) of cell death observed in the eif-3.K(gk126) mutants (Figure 4C). When activated CED-3 (acCED-3) was expressed in touch neurons via the Pmec-7acCED-3 transgene [40], 40% and 43% of the PVM neurons were killed in the wild-type and eif-3.K(gk126) worms, respectively (Figure 4D). This result showed that eif-3.K also fails to inhibit apoptosis caused by the overexpression of activated CED-3. Similar results were observed in other touch neurons expressing either the Pmec-7ced-3 or the Pmec-7acCED-3 transgene (Figures 4C and 4D).

We next examined if overexpression of ced-3 using the heat shock promoter Phsp was able to rescue the cell death defects caused by the eif-3.K(gk126) mutation. Heat shock-induced ced-3 overexpression rescued the defect at the comma and 1.5-fold stages, but it also slightly elicited ectopic cell killing at the comma stage (Table 2). The eif-3.K(gk126) embryos carrying the Phspced-3 transgene had approximately 7.0 cell corpses at the comma stage under non-heat shock condition. The heat shock-induced overexpression of ced-3 in the transgenic embryos at the same developmental stage increased the cell corpse number to 10.7, which was significantly more than the 8.4 cell corpses that were observed in the wild-type embryos carrying the control Phspgfp transgene under the same conditions (Table 2). This result supports the model that ced-3 acts downstream of eif-3.K to execute programmed cell death.

![Figure 2. EIF-3.K protein expression.](image-url)

(A) Western blot analysis of EIF-3.K protein expression. Affinity-purified anti-EIF-3.K antibodies were used to probe a blot of embryonic extracts from wild-type and eif-3.K(gk126) worms (above). Equal loading of the two extracts was confirmed by anti-α tubulin antibodies (below). The sizes of molecular weight markers and the positions of EIF-3.K and α tubulin are indicated. (B–D) Images of an eif-3.K(gk126) mutant early embryo (B), a wild-type early embryo (C) and a wild-type newly hatched larva (D) that were co-stained with anti-EIF-3.K antibodies and DAPI. Merged images are also shown. Scale bar = 10 μm.
To determine whether *eif-3.K* promotes programmed cell death, we tested if the overexpression of *eif-3.K* caused cells that would normally live to undergo programmed cell death by overexpressing *eif-3.K* under the control of the heat shock promoter *Phsp* in the wild-type animals. The overexpression of *eif-3.K*, but not of the control *gfp*, slightly but significantly increased the cell corpse number at the 1.5-fold stage, despite a lack of significant ectopic killing at the comma stage (Table 2). Nonetheless, this ectopic killing was significantly suppressed by the strong *ced-3(n717)* or *ced-4(n1162)* mutations (Table 2). This result, in combination with the reciprocal experiment in which the loss of *eif-3.K* suppressed the efficient apoptosis of touch neurons in the presence of *ced-4*...
overexpression (Figure 4 B), suggests a mutual requirement for elf-3.K and ced-4. Additionally, because the loss of elf-3.K failed to suppress the efficient apoptosis of touch neurons in the presence of ced-3 overexpression, our result suggests a unidirectional requirement of elf-3.K for ced-3 to achieve effective ectopic cell death under overexpression conditions.

**elf-3.K Promotes Cell Death in a Cell-Autonomous Fashion**

To determine whether elf-3.K promotes programmed cell death in a cell-autonomous fashion, we tested if the *Pmec-7::elf-3.K* transgene, in which elf-3.K is expressed under the *Pmec-7* promoter in touch neurons, could trigger touch neuron apoptosis in the wild-type animals. Although the overexpression of elf-3.K transgene resulted in a low frequency of individual touch neuron apoptosis, approximately 19.2% of transgenic worms had at least one missing touch neuron (Figure 4E). This result not only reinforced the cell death-promoting function for elf-3.K but also showed that elf-3.K executes this function in a cell-autonomous fashion.

### The Loss of elf-3.K Significantly Reduces Ectopic Cell Deaths in icd-1 Mutants

The inactivation of *icd-1* (inhibitor of cell death-1) by RNAi results in ectopic cell death that can be blocked by the loss of *ced-4* but not *ced-3*, revealing that the cell death in *icd-1*(*RNAi*) embryos is *ced-4*-dependent but *ced-3*-independent [17]. The observation that elf-3.K is required for cell death induced by the overexpression of *ced-4* but not *ced-3* (Figures 4B–4D) prompted us to test whether elf-3.K could suppress ectopic cell death resulting from the loss of *icd-1*. Like the *icd-1*(*RNAi*) embryos described previously [17], *icd-1(tm2873)* embryos had additional cell corpses compared to the wild-type embryos at the comma and 1.5-fold stages (Figure 5A). Although the elf-3.K(*gk126*) mutation significantly reduced the cell corpse number in *icd-1(tm2873)* embryos at both stages, the cell corpse number was not reduced to the extent observed in the elf-3.K(*gk126*) mutants alone (Figure 5B). Instead, the cell corpse number for the double mutant was between that observed in the elf-3.K(*gk126*) and *icd-1(tm2873)* single mutants. This result is consistent with the model that elf-3.K acts in parallel with *icd-1* to promote cell death; however, we cannot rule out the possibility that *icd-1* may in part prevent programmed cell death in an elf-3.K-dependent manner.

**EIF-3.K is Widely Expressed throughout Embryogenesis and Localized to the Cytoplasm**

To determine the localization pattern of EIF-3.K, we raised antibodies against a recombinant EIF-3.K protein (see Experimental Procedures). Using affinity-purified EIF-3.K antibodies and western blot analysis, we detected a band of apparent molecular mass 27 kDa from wild-type worm extracts by western blot analysis (Figure 2A). This protein was absent in extracts from the elf-3.K(*gk126*) mutants (Figure 2A), confirming that the 27 kDa protein is the product of the elf-3.K gene. We used the purified EIF-3.K antibodies to stain embryos and larvae. EIF-3.K was widely expressed in embryos and larvae and was localized to the cytoplasm (Figures 2B–2D). EIF-3.K did not appear to be associated with mitochondria, where several cell death regulators such as CED-9, WAH-1, and WAN-1 are located [7,18,20], because EIF-3.K did not co-localize with MitoTracker Red, a marker of mitochondria (Figure S2).

### The WH Domain of EIF-3.K is Necessary and Sufficient for its Cell Death-Promoting Activity

EIF-3.K contains two distinct domains, the HAM (HEAT Analogous Repeats) and WH (Winged Helix) domains, which have been implicated in protein-protein and protein-RNA interactions, respectively [59]. To test the importance of these domains for EIF-3.K function, we deleted the region corresponding to the HAM or WH domains, respectively [59]. To our surprise, the *Plet-858eif-3.K* deletion region corresponding to the HAM or WH domains, respectively [59], failed to rescue the cell death defects in elf-3.K(*gk126*) embryos by expressing the mutant construct under the control of the ubiquitous let-655 promoter *Plet-858* [44]. To our surprise, the HAM domain, comprising more than one-third of the ELF-3.K protein, was dispensable for elf-3.K activity, as *Plet-858elf-3.K*HAMP completely rescued the cell death defects in elf-3.K(*gk126*) embryos (Table 3). In contrast, *Plet-858elf-3.K*WH failed to rescue the defect (Table 3), suggesting an essential role for the WH domain in the cell death-promoting function of elf-3.K. Because the expression level of the *Plet-858elf-3.K* transgene was lower than that of the endogenous elf-3.K, as detected by western blotting or immunostaining analysis (data not shown), the stronger heat shock

### Table 1. The loss of elf-3.K enhances cell survival in the ventral cord of sensitized mutants.

| Transgene | Genotype | Average number of extra Pn.aap cells | Animals with surviving Pn.aap cells |
|-----------|----------|-------------------------------------|-----------------------------------|
| *Pmec-7::gfp* | wild-type | 0.0 | 0 |
| *Pmec-7::gfp* | ced-3(n717) | 5.0 | 100 |
| *Pmec-7::gfp* | ced-3(n2427) | 2.6 | 99 |
| *Pmec-7::gfp* | elf-3.K(*gk126*) | 0.0 | 2 |
| *Pmec-7::gfp* | ced-3(n2427); elf-3.K(*gk126*) | 3.6 | 100 |
| *Pmec-7::gfp* | ced-2(n1994) | 0.3 | 27 |
| *Pmec-7::gfp* | ced-2(n1994); elf-3.K(*gk126*) | 2.4 | 100 |
| *Pmec-7::gfp* | ced-7(n1996) | 1.6 | 83 |
| *Pmec-7::gfp* | ced-7(n1996); elf-3.K(*gk126*) | 2.1 | 93 |

*Average numbers of fluorescent cells caused by expression from *Pmec-7::gfp* in P2, 9, 10, 11, and 12-derived regions were determined using DIC microscopy equipped with an ultraviolet light source. Greater than 20 larvae of each genotype were analyzed.*

*The percentages of animals that had at least one fluorescent cell in the P2, 9, 10, 11, and 12-derived regions were determined.*

[doi:10.1371/journal.pone.0036584.t001](https://doi.org/10.1371/journal.pone.0036584.t001)
promoter $P_{kph}$ was subsequently used to increase the expression of the mutant $eif-3.K$ construct in an effort to confirm our results. The heat shock-induced expression of the wild-type and mutant $eif-3.K$ genes resulted in slightly higher protein expression levels (Figure S3). The overexpressed proteins exhibited a similar localization as the endogenous $EIF-3.K$ protein, suggesting that these proteins localize normally (Figure S3). Similar to the results obtained using the $P_{let-858}$ promoter, expression under the heat shock promoters revealed that $eif-3.K^{HAM}$, but not $eif-3.K^{WH}$, rescued the $eif-3.K$ mutant phenotype at the comma and 1.5-fold stages (Table 3).

Figure 4. The loss of $eif-3.K$ partially suppresses cell death induced by the overexpression of $egl-1$ and $ced-4$, but not $ced-3$. (A–D) The percentage of animals missing specific touch neurons are shown for the wild-type (white columns) or $eif-3.K^{gk126}$ (black columns) embryos carrying the $P_{mec-7}egl-1$ (A), $P_{mec-7}ced-4$ (B), $P_{mec-7}ced-3$ (C), or $P_{mec-7}acCED-3$ (D) transgenes. The $eif-3.K^{gk126}$ transgenic worms were compared to the analogous wild-type transgenic worms. Comparisons were performed using the unpaired t test ($t^*<0.05$, $t^{**}<0.001$). Data are presented as the mean ± standard deviation. Error bars represent S.D. n.s. indicates no significant difference. More than 100 animals were scored for each strain. doi:10.1371/journal.pone.0036584.g004
These data show that the WH domain, but not the HAM domain, is necessary for the cell death-promoting function of EIF-3.K.

We next tested if the WH domain of EIF-3.K is sufficient to rescue the cell-death defect caused by the eif-3.K mutation. We expressed the WH domain alone using either the Pgo.eis or Pnap promoters in the transgenics Pgo.eisWH or PnapWH, respectively. We found that either promoter rescued the cell death defect in the eif-3.K mutants (Table 3). Moreover, when the PnapWH transgene was expressed in the wild-type animals, it induced ectopic cell deaths. Furthermore, superficial cell corpses were observed at the comma and 1.5-fold stages (Table 3). Therefore, the WH domain is both necessary and sufficient for the cell death-promoting activity of eif-3.K.

Human eIF3k can Partially Substitute for C. elegans EIF-3.K

The human eIF3k mediates apoptosis in simple epithelial cells, likely by binding to keratin K10 via its HAM domain [30]; however, the HAM domain of C. elegans EIF-3.K appears dispensable for its function in cell death. We tested whether the expression of human eIF3k by Pnap was able to rescue the cell death defect caused by the eif-3.K mutation. We found that human eIF3k partially rescued the defective apoptosis in the eif-3.K(gk126) mutants (Table 3). This result indicates that the pro-apoptotic function of EIF-3.K has been conserved through evolution from C. elegans to humans and that the mechanisms by which human eIF3k and C. elegans EIF-3.K promote apoptosis may, in part, be similar.

Discussion

eIF3 is the largest and most complex translation initiation factor, consisting of thirteen subunits in both C. elegans and humans [22]. The RNAi-based or genetic inactivation of ten eIF3 subunits, including eif-3.A, eif-3.B, eif-3.C, eif-3.D, eif-3.E, eif-3.F, eif-3.G, eif-3.H, eif-3.I, or eif-1(eif-3.M), in C. elegans can cause sterility, embryonic lethality or gross developmental defects [60–63]. In contrast, the eif-3.K null mutant is viable and healthy (Table S1), suggesting that eif-3.K is not essential for general translation initiation. Similarly, human eIF3k is dispensable for the formation of an active eIF3 complex in vitro [26]. We have previously shown that human eIF3k promotes apoptosis in cultured simple epithelial cells [30]. In this work, we provide evidence that eif-3.K has a cell death-promoting function at an organizational level and that this function has been conserved through evolution.

In C. elegans, the loss of eif-3.K caused reduced programmed cell death (Figures 3A–3C) and enhanced cell survival in sensitized mutants (Figure 3D and Table 1). In contrast, the overexpression of eif-3.K by the heat shock promoter or a touch neuron-specific promoter resulted in ectopic cell death (Table 2 and Figure 4E). These results demonstrate that eif-3.K promotes programmed cell death. Our results also show that eif-3.K is essential for the efficient cell death that is induced by the overexpression of egl-1 or ced-4, but not ced-3, as the loss of eif-3.K partially suppresses the cell death that is induced by the overexpression of egl-1 or ced-4 only (Figures 3A–3D). In addition, the observation that ced-3 overexpression can rescue the cell death-defective phenotype of eif-3.K mutants and that the ced-3 strong mutation can suppress cell death caused by heat shock-induced eif-3.K overexpression (Table 2) further reinforces the notion that eif-3.K requires ced-3 to promote programmed cell death. Furthermore, the wide range in the identity and type of extraneous surviving cells that are affected by the eif-3.K mutation (Figure S1) suggests that eif-3.K may be involved in the majority of programmed cell death. This is consistent with the ubiquitous expression of EIF-3.K in embryos and larve (Figures 2C and 2D). In addition to physiological cell deaths, DNA damage caused by genotoxic stress such as UV or IR radiation also induces cell deaths in the germline [43,64]. We found that the eif-3.K(gk126) mutation significantly reduced UV-induced cell deaths in the germline (Figure S5), indicating that eif-3.K also mediates DNA damage-induced cell death.

During C. elegans development, the activity of the executioner caspase CED-3 is under both positive and negative regulation. Previous studies have shown that CED-4 facilitates the auto-cleavage of pro-CED-3 to generate the active CED-3 caspase during the promotion of cell death [12,65,66], while the CED-3 paralogs CSP-2 and CSP-3 associate with the CED-3 zymogen and inhibits its auto-activation, thereby protecting cells from inappropriate apoptosis [13,14]. Our observation that neither eIF-3.K nor its WH domain bind to CED-3 or CED-4 in a yeast 2-hybrid system (Figure S4) suggests that EIF-3.K may not promote cell death through a direct association with either protein. In addition, since CED-3 and CED-4 are the only known proteins involved in CED-3 activation from pro-CED-3 [9,12], EIF-3.K likely does not affect this activation process directly. It is possible that EIF-3.K may promote programmed cell death after CED-4-induced CED-3 activation. Human eIF3k has been proposed to

| Table 2. Overexpression of eif-3.K or ced-3 in cell death-defective mutants. |
|-----------------------------------------------|
| Transgene | Genotype | Heat shock | No. of Cell Corpses |
|-----------|----------|------------|-------------------|
| Pgo.eis | wild-type | comma | 8.0±0.9 | 10.8±1.2 |
| Pgo.eis | wild-type | 1.5-fold | 8.4±1.1 | 11.1±1.5 |
| Pgo.eis | ced-3(n717) | comma | 0.0±0.0 | 0.0±0.0 |
| Pgo.eis | ced-3(n717) | 1.5-fold | 0.0±0.0 | 0.0±0.2 |
| Pgo.eis | ced-4(n1162) | comma | 0.0±0.0 | 0.0±0.2 |
| Pgo.eis | ced-4(n1162) | 1.5-fold | 0.1±0.3 | 0.1±0.1 |
| Pgo.eis | eif-3.K(gk126) | comma | 6.7±2.2 | 9.0±2.1 |
| Pgo.eis | eif-3.K(gk126) | 1.5-fold | 6.8±2.0 | 8.9±1.9 |
| Pgo.eis | ced-3 | comma | 7.8±1.1 | 10.8±1.6 |
| Pgo.eis | ced-3 | 1.5-fold | 11.2±2.1** | 12.8±2.2** |
| Pgo.eis | eif-3.K(gk126) | comma | 7.0±1.8 | 8.9±2.0 |
| Pgo.eis | eif-3.K(gk126) | 1.5-fold | 10.7±0.6** | 11.6±1.7** |
| Pgo.eis | eif-3.K | comma | 8.5±2.1 | 11.2±2.4 |
| Pgo.eis | eif-3.K | 1.5-fold | 9.4±1.7 | 13.4±2.9* |
| Pgo.eis | ced-3(n717) | comma | 0.0±0.0 | 0.0±0.2 |
| Pgo.eis | ced-3(n717) | 1.5-fold | 0.2±0.4 | 0.4±0.5* |
| Pgo.eis | ced-4(n1162) | comma | 0.1±0.3 | 0.0±0.2 |
| Pgo.eis | ced-4(n1162) | 1.5-fold | 0.2±0.4 | 0.1±0.2 |

*Transgenic animals were subjected to heat-shock (+) or left at 20°C (–).
†Transgenic embryos were scored for the number of cell corpses 1.5 hrs after heat shock (see Materials and methods). Data are presented as the mean ± standard deviation from two independent stably transmitting lines.
‡Greater than 20 embryos were analyzed from each line except for 1.5-fold embryos carrying Pgo.eis-3(ced-3(n717)) after heat shock due to high lethality.

The transgenic embryos after heat shock were compared to the corresponding transgenic embryos without heat shock. All comparisons were performed using the unpaired t test (*P<0.05, **P<0.001).

doi:10.1371/journal.pone.0036584.t002
promote apoptosis by facilitating the release of active caspases from an inhibitory compartment of intermediate filament-containing inclusions into the cytosol, thereby allowing the released caspase better access to its cytosolic substrates [30]. Although the mechanism by which eIF3k may affect the release of caspases from intermediate filament-containing inclusions is not clear, the binding of eIF3k to intermediate filaments is known to be important for the release process [30]. Similarly, C. elegans eIF-3.K may promote programmed cell death by affecting the distribution of active CED-3, thus facilitating the substrate cleavage and the subsequent execution of cell death. Alternatively, eIF-3.K might promote programmed cell death in parallel with CED-4 by antagonizing CSP-2 or CSP-3, thus facilitating CED-3 auto-activation from the zymogen in germline or somatic cells, respectively [13,14]. To test the latter possibility, we used bzIs8 (Pmec-4gfp), which labels six touch neurons, as marker to monitor the survival of touch neurons and tested the effect of the eif-3.K mutation on the missing cell phenotype of the csp-3(lf) animals. As previously shown [14], in csp-3(lf) animals six touch neurons were lost randomly at a frequency from 2% to 10% (Table S2) and 24% of animals lost at least one touch neuron (Table S2). The eif-3.K(gk126) mutation strongly suppressed this missing cell defect in csp-3 mutants (Table S2). In addition, loss of csp-2 resulted in increased germline cell deaths [13], and this phenotype can also be suppressed by the eif-3.K mutation (Figure S6). These results suggest that EIF-3.K may promote cell death downstream of or in parallel to csp-2 or csp-3 in the germline and somatic cell deaths, respectively.

Human eIF3k co-localizes with keratin and requires keratin for its apoptosis-promoting function in simple epithelial cells [30]. Upon apoptotic stimuli, keratin K18 is cleaved by caspase 3 at VEVD238 of the L1-2 linker region or DALD397 of the C terminal (tail) domain, resulting in a collapse of keratin filaments [67]. C. elegans contains eleven genes that encode cytoplasmic intermediate filaments, including ifa-1, mua-6, ifa-3, ifa-4, ifb-1, ifb-2, ifc-1, ifc-2, ifd-1, and ifp-1 [68]. No detectable change in either the localization or the level of the EIF-3.K protein was observed by immunostaining analysis in mua-6(rh85) or ifb-1(ju71) mutant embryos or in embryos treated with ifa-1, mua-6, ifa-4, ifb-1, ifc-1, or ifd-1 interfering RNAs (data not shown).

Figure 5. eif-3.K partially suppresses the ectopic cell deaths caused by the loss of icd-1. (A) A quantification of the cell corpses present in the wild-type and icd-1(tm2873) embryos at the comma (left) and 1.5-fold (right) stages. (B) A quantification of the cell corpses present in the eif-3.K(gk126) and icd-1(tm2873) single mutant and icd-1(tm2873); eif-3.K(gk126) double mutant embryos at the comma (left) and 1.5-fold (right) stages. The y axis shows the percentage of embryos and the x axis shows the cell corpse number. More than 20 embryos for each genotype at each stage were scored.
doi:10.1371/journal.pone.0036584.g005
Table 3. Structure and function analysis of eif-3. K.

| Transgene     | Genotype    | Heat shock | No. of Cell Corpses ( ± standard deviation) |
|---------------|-------------|------------|--------------------------------------------|
| none          | wild-type   | –          | 8.4 ± 0.9 11.3 ± 1.0                       |
| none          | eif-3.K(kig126) | –       | 6.9 ± 2.1 9.0 ± 2.3                       |
| Phsp eif-3.K(KHAM) | eif-3.K(kig126) | –       | 7.8 ± 1.1 11.0 ± 1.0 **                    |
| Phsp eif-3.K(KWH) | eif-3.K(kig126) | +       | 6.5 ± 1.2 9.1 ± 1.3                       |
| Phsp WH       | eif-3.K(gk126) | –          | 8.1 ± 0.7 11.0 ± 1.2 **                    |
| Phsp ifd-1    | eif-3.K(kig126) | –          | 6.7 ± 2.2 9.0 ± 2.1                       |
| Phsp eif-3.K   | eif-3.K(kig126) | +       | 6.8 ± 2.0 8.9 ± 1.9                       |
| Phsp eif-3.K   | eif-3.K(gk126) | –          | 6.9 ± 2.0 8.8 ± 1.9                       |
| Phsp eif-3.K   | eif-3.K(gk126) | +       | 8.8 ± 1.2 ** 11.3 ± 1.1 **                |
| Phspeif-3.K    | wild-type   | –          | 8.5 ± 2.1 11.2 ± 2.4                      |
| Phspeif-3.K    | eif-3.K(gk126) | +       | 9.4 ± 1.7 13.4 ± 2.9 **                   |
| Phspeif-3.K(KWH) | eif-3.K(kig126) | –       | 6.4 ± 2.1 8.7 ± 1.8                       |
| Phspeif-3.K(KWH) | eif-3.K(gk126) | +       | 6.2 ± 1.1 8.6 ± 0.8                       |
| Phspeif-3.K(KHAM) | eif-3.K(gk126) | –       | 6.5 ± 2.0 8.8 ± 2.0                       |
| Phspeif-3.K(KHAM) | eif-3.K(gk126) | +       | 8.3 ± 1.1 ** 11.2 ± 1.9 **                |
| Phsp WH       | eif-3.K(gk126) | –          | 6.5 ± 2.1 9.0 ± 1.8                       |
| Phsp WH       | eif-3.K(gk126) | +       | 8.3 ± 0.9 ** 10.9 ± 1.0 **                |
| Phsp WH       | wild-type   | –          | 8.4 ± 2.0 11.0 ± 2.4                      |
| Phsp WH       | wild-type   | +       | 10.1 ± 2.2 * 13.1 ± 2.1                   |
| Phspeif3(k(human)) | eif-3.K(kig126) | –       | 6.5 ± 1.9 8.6 ± 1.7                       |
| Phspeif3(k(human)) | eif-3.K(gk126) | +       | 8.7 ± 0.8 ** 10.4 ± 0.6 **                |

*Transgenic animals were subjected to heat-shock (+) or left at 20 °C (–). 
**Transgenic embryos were scored for the number of cell corpses 1.5 hrs after heat shock.

Data are presented as the mean ± standard deviation from two independent stably transmitting lines. Greater than 20 embryos were analyzed from each line.

For the Phass expressing transgene, eif-3.K mutant embryos carrying the transgene were compared to eif-3.K mutant without the transgene.

For the Phass expressing transgene, the transgenic embryos after heat shock were compared to the corresponding transgenic embryos without heat shock. All comparisons were performed using the unpaired t test (**P<0.05, **P<0.01).

doi:10.1371/journal.pone.0036584.t003

Potential CED-3 cleavage sites (DXXD) are found in IFA-1 (DAED), IFC-2 (DNRD), IFD-1 (DNRD and DVDD), and IFP-1 (DSVD). The RNAi-mediated inactivation of ifa-1, but not ifc-2, ifj-1, or ifj-1, reduced the number of cell corpses at the comma stage. For example, the ifa-1(RNAi) worms had on average 6.9 ± 1.8 cell corpses, which is similar to the number of cell corpses observed in eif-3.K mutants; however, whether IFA-1, IFC-2, IFD-1, or IFP-1 are direct targets of the CED-3 caspase or are involved in programmed cell death needs to be evaluated. It is not yet clear whether E2F-3.K localizes to intermediate filaments or mediates programmed cell death through intermediate filaments in C. elegans, similar to human eIF3k. Previously, the pro-apoptotic function of human eIF3k was identified and assayed in simple epithelial cells [30] in which keratin K8/K18 is the major intermediate filament. It will be interesting to determine whether human eIF3k, like C. elegans eIF3k, can promote apoptosis in muscle or neuron cells, as human eIF3k is widely expressed in many tissues, including the brain and muscle [27], where no very little keratin is expressed [69]. In addition, because eIF3k is also present in D. melanogaster, an organism that lacks intermediate filaments, it will be interesting to see if eIF3k plays a role in apoptosis in D. melanogaster as well.

C. elegans E3F-3.K and human eIF3k both contain two conserved domains, the WH and HAM domains. The HAM domain, but not the WH domain, of human eIF3k interacts with keratin 18 in a yeast two-hybrid system and thus may be important for eIF3k localization to keratin [30]; however, in C. elegans, the HAM domain is dispensable for the cell death-promoting function of E3F-3.K and the WH domain alone is sufficient to promote cell death (Table 3). This result suggests that the WH domain may promote programmed cell death by an IF-independent mechanism. The WH domain has been implicated in DNA or RNA binding [59], but how it may promote programmed cell death needs further study. The result that human eIF3k can partially rescue the cell death defect in the eif-3.K mutants suggests that the eIF3k family may promote apoptosis through a conserved mechanism, which may be dependent upon the WH domain.

Supporting Information

Figure S1 The identification of extraneous surviving cells in the mutants. The y axis represents the percentage of animals with specific superfluous surviving cells (x axis). The extra surviving cells are named after their sister or niece cells, such as “c1 sister cell” and “i2 niece cell.” M4, MC, NSM, I1 and I2 are neurons. e1 is an epithelial cell, and m1 and m2 are muscle cells. L: left, R: right. The identities of extraneous surviving cells were determined as previously described [41]. More than 20 worms for each genotype were scored.

(TIF)

Figure S2 E3F-3.K is not associated with mitochondria. A wild-type embryo was co-stained with anti-EIF-3. K antibodies (A) and MitoTracker (B). The merged image is shown in C. Scale bar = 10 μm.

(TIF)

Figure S3 Deletion of the WH domain does not affect the expression pattern or stability of E3F-3.K. The wild-type embryo (A) and eif-3.K mutant embryo (B) with no transgene, and the eif-3.K mutant embryos carrying the transgene Phspeif-3.K(C), Phspeif-3. KHAM (D), or Phspeif-3. KWH (E) were heat shocked and co-stained with anti-EIF-3. K antibodies (red) and DAPI (blue). Representative images of anti-EIF-3. K antibody staining (upper panel) and merged images of anti-EIF-3. K antibody and DAPI staining (lower panel) are shown. Scale bar = 10 μm.

(TIF)

Figure S4 Neither EIF-3.K nor the WH domain alone interacts with CED-3 or CED-4 in a yeast 2-hybrid assay. Pairs of constructs expressing the indicated fusion proteins were transformed into the yeast strain MaV203. The resulting transformants were streaked on SC-Trap-Leu-His or SC-Trap-Leu plates containing 30 mM 3-AT. Growth on the SC-Trap-Leu-His plates indicates an interaction between the fusion proteins. The E2F1 AND RB pair was used as positive control [70], „-“ in the lower panel indicates no insert was present in the AD fusion construct.

(TIF)

Figure S5 Loss of eif-3. K reduced DNA damage-induced apoptosis. Apoptotic germ cell corpses were scored in the wild-type (black columns) and eif-3. K(kig126) (white columns) young adult worms 24 hr following exposure to 150 J/m² UV-C radiation. The eif-3. K(kig126) mutants were compared to the
wild-type using the unpaired t test (***P<0.001). More than 20 gonadal arms were scored for each genotype. (TIF)

Figure S6 Loss of eif-3.K suppressed the increased cell death phenotype of csp-2 mutants in the germline. Germ cell corpses were scored in ced-6(n2095) (white columns), ced-6(n2095); csp-2(tm3077) (gray columns), ced-6(n2095); eif-3.K(kl26) (black columns), ced-6(n2095); csp-2(tm3077); eif-3.K(kl26) (slashed columns) worms 48 hours after entering adulthood. The x axis represents the average number of cell corpses scored in each gonadal arm. The data were compared using the unpaired t test (**P<0.05, ***P<0.001). More than 20 gonadal arms of each genotype were scored. (TIF)

Table S1 eif-3.K is not essential for embryonic or larval development. (DOC)

References
1. Raff MC, Barres BA, Barres BA, Burne JF, Coles HS, Ishizaki Y, et al. (1993) Programmed cell death and the control of cell survival: lessons from the nervous system. Science 262: 693–700.
2. Jacobson MD, Weil M, Raff MC (1997) Programmed cell death in animal development. Cell 88: 347–354.
3. Fuchs Y, Steller H (2011) Programmed cell death and apoptosis in animal development and disease. Cell 147: 742–758.
4. Ellis HM, Horvitz HR (1986) Genetic control of programmed cell death in the nematode C. elegans. Cell 44: 517–529.
5. Wu D, Chen PJ, Chen S, Hu Y, Nunez G, et al. (1999) C. elegans MAC-1, an immunoglobulin superfamily receptor that inhibits development of apoptosis. Genes Dev 13: 503–512.
6. Hengartner MO, Ellis RE, Horvitz HR (1992) Caenorhabditis elegans gene ced-3: a homolog of the mammalian Fas ligand that promotes cell death. Nature 356: 494–499.
7. Hengartner MO, Horvitz HR (1994) C. elegans cell survival gene ced-9 encodes a functional homolog of the mammalian proto-oncogene bcl-2. Cell 76: 665–676.
8. Chen F, Hersh EM, Conradt B, Zhou Z, Riemer D, et al. (2000) Translocation of C. elegans CED-4 to nuclear membranes during programmed cell death. Science 287: 1485–1489.
9. Yang X, Chang HY, Baltimore D (1998) Essential role of CED-4 oligomerization in CED-3 activation and apoptosis. Science 281: 1355–1357.
10. Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR (1995) The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell 75: 641–652.
11. Poukarin E, Gerasimov G, Cormican A (2000) Evidence that CED-9/Becl2 and CED-4/Apaf-1 localization is not consistent with the current model for C. elegans apoptosis induction. Cell Death Differ 7: 406–15.
12. Qi S, Pang Y, Hu Q, Liu Q, Li H, et al. (2010) Crystal structure of the Caenorhabditis elegans apoptosis protein reveals an octameric assembly of CED-4. Cell 141: 446–457.
13. Geng X, Zhou QH, Kage-Nakadai E, Shi Y, Yan N, et al. (2009) Caenorhabditis elegans caspase homolog CSP-2 inhibits CED-3 autoactivation and apoptosis in germ cells. Cell Death Differ 16: 1385–1394.
14. Shen Q, Qin F, Gao Z, Cui J, Xiao H, et al. (2009) Adenine nucleotide excision repair pathway is required for UV-C-induced apoptosis in C. elegans. Mol Cell Biol 13: 1094–1101.
15. Wu D, Chen PJ, Chen S, Hu Y, Nunez G, et al. (1999) C. elegans MAC-1, an essential member of the AAA family of ATPases, can bind CED-4 and prevent cell death. Development 126: 2021–2031.
16. Chen L, McCloskey T, Joshi PM, Rothman JH (2000) CED-4 and proto-oncogene fgl-1 antagonistically regulates cell size and apoptosis in C. elegans. Curr Biol 8: 1025–1033.
17. Blos TA, Witze ES, Rothman JH (2003) Suppression of CED-3-independent apoptosis by mitochondrial betaelNAC in Caenorhabditis elegans. Nature 424: 1066–1071.
18. Chen S, Shi Y, Nakagawa A, Yoshina S, Mitsu M, et al. (2008) Inhibition of CED-3 zymogen activation and apoptosis in Caenorhabditis elegans by caspase homolog CSP-3. Nat Struct Mol Biol 15: 1094–1101.
19. Shi L, Chen PJ, Chen S, Hu Y, Nunez G, et al. (1999) C. elegans MAC-1, an essential member of the AAA family of ATPases, can bind CED-4 and prevent cell death. Development 126: 2021–2031.
20. Chen L, McCloskey T, Joshi PM, Rothman JH (2000) CED-4 and proto-oncogene fgl-1 antagonistically regulate cell size and apoptosis in C. elegans. Curr Biol 8: 1025–1033.
21. Blos TA, Witze ES, Rothman JH (2003) Suppression of CED-3-independent apoptosis by mitochondrial betaelNAC in Caenorhabditis elegans. Nature 424: 1066–1071.
22. Shen Q, Qin F, Gao Z, Cui J, Xiao H, et al. (2009) Adenine nucleotide excision repair pathway is required for UV-C-induced apoptosis in C. elegans. Mol Cell Biol 13: 1094–1101.
23. Shi L, Chen PJ, Chen S, Hu Y, Nunez G, et al. (1999) C. elegans MAC-1, an essential member of the AAA family of ATPases, can bind CED-4 and prevent cell death. Development 126: 2021–2031.
24. Chen L, McCloskey T, Joshi PM, Rothman JH (2000) CED-4 and proto-oncogene fgl-1 antagonistically regulate cell size and apoptosis in C. elegans. Curr Biol 8: 1025–1033.
25. Bloch TA, Witze ES, Rothman JH (2003) Suppression of CED-3-independent apoptosis by mitochondrial betaelNAC in Caenorhabditis elegans. Nature 424: 1066–1071.
26. Shi L, Chen PJ, Chen S, Hu Y, Nunez G, et al. (1999) C. elegans MAC-1, an essential member of the AAA family of ATPases, can bind CED-4 and prevent cell death. Development 126: 2021–2031.
44. Kelly WG, Xu S, Montgomery MK, Fire A (1997) Distinct requirements for somatic and germline expression of a generally expressed Caenorhabditis elegans gene. Genetics 146: 227–238.
45. Lai CC, Hong K, Kinney M, Challie M, Driscoll M (1996) Sequence and transmembrane topology of MEC-4, an ion channel subunit required for mechanotransduction in Caenorhabditis elegans. J Cell Biol 133: 1071–1081.
46. Mello C, Fire A (1995) DNA transformation. Methods Cell Biol 48: 451–482.
47. Gu T, Oriti S, Han M (1998) Caenorhabditis elegans SUR-5, a novel but conserved protein, negatively regulates LET-60 Ras activity during vulval induction. Mol Cell Biol 18: 4556–4564.
48. Okkema PG, Fire A (1994) The Caenorhabditis elegans NK-2 class homeoprotein CEH-22 is involved in combinatorial activation of gene expression in pharyngeal muscle. Development 120: 2173–2186.
49. Garencher C, Garriga G (1996) Asymmetric distribution of the C. elegans HAM-1 protein in neuroblasts enables daughter cells to adopt distinct fates. Development 122: 3509–3518.
50. Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG, Ahringer J (2001) Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in Caenorhabditis elegans. Genome Biol 2: RESEARCH0002.1–10.
51. Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 119: 493–501.
52. Seshagiri S, Miller LK (1997) Caenorhabditis elegans CED-4 stimulates CED-3 processing and CED-3-induced apoptosis. Curr Biol 7: 455–460.
53. Lazarides E (1980) Intermediate filaments as mechanical integrators of cellular space. Nature 283: 249–256.
54. Ahringer J (1998) Asymmetric distribution of the C. elegans HAM-1 protein in neuroblasts enables daughter cells to adopt distinct fates. Development 122: 3509–3518.
55. Suomalainen A, Ahonen S, Ahonen P, Aho K, Ahonen R (1999) Mutational analysis of the Caenorhabditis elegans cell-death gene ced-3. Genetics 153: 1655–1671.
56. Suomalainen A, Ahonen S, Ahonen P, Aho K, Ahonen R (1999) Mutational analysis of the Caenorhabditis elegans cell-death gene ced-3. Genetics 153: 1655–1671.