Programmed cell death in *C. elegans*

Yi-Chun Wu[1] and Ding Xue[2]

[1]Assistant professor, Ph. D.
Department of Zoology
National Taiwan University
No1 Sec4 Roosevelt Rd
Taipei 10617
Taiwan
Tel: (886) 2-2368-7980
Fax: (886) 2-2363-6837
e-mail: yichun@ccms.ntu.edu.tw

[2]Assistant professor, Ph. D.
Department of MCD Biology
University of Colorado
Boulder, CO 80309
U.S.A.
Tel: (303) 492-0271
Fax: (303) 492-7744
e-mail: Ding.Xue@Colorado.EDU

February 8, 2002

Studies in the nematode *Caenorhabditis elegans* established that programmed cell death is a normal, genetically-determined part of development and is controlled by a number of specific genes. Genetic analyses have ordered these genes into a pathway. This cell death pathway is evolutionarily conserved and provides a basis for understanding programmed cell death in more complex organisms, including humans.

1. *C. elegans* as model organism for studies of programmed cell death

C. elegans is a small (adult animals are approximately 1 mm in length), free living worm with a short generation time (3 days at 20^{o}C) (Figure 1). It can feed on bacteria *Escherichia coli* and is cultivated on Petri dishes in the laboratory (1). Because *C. elegans* is small and transparent, its internal structures can be visualized with the light microscope. Furthermore, using high magnification Nomarski differential interference contrast optics cell divisions and cell deaths can be observed and followed in living animals (2-4). For these reasons, *C. elegans* has been an excellent model organism for experimental analyses and has proven to be exceptionally well suited for the study of programmed cell death.

During the development of the *C. elegans* adult hermaphrodite from the fertilized egg, 1090 somatic nuclei are generated by essentially invariant patterns of cell divisions. Of these 1090 somatic cells, 131 undergo programmed cell death (2-4). When observed with Nomarski optics, the dying cell adopts a refractile and raised, flattened button-like appearance (Figure 2). When viewed using an electron microscope, the cells that undergo programmed cell death in *C. elegans* display characteristic features of apoptosis observed in mammals, including cell shrinkage, chromatin aggregation, and phagocytosis of cell corpse (5). The entire process of cell death from the birth to the disappearance of the cell by phagocytosis occurs within approximately an hour (2,6).

2. The genetic pathway for programmed cell death

Genetic approaches have been taken to identify genes that are involved in controlling different aspects of *C. elegans* programmed cell death. Genetic analyses have placed these genes in five sequential and distinct steps: first, the decision of individual cells whether to live or die; second, the activation of the cell-killing machinery in a cell that is committed to die; third, the execution of the cell-killing process; fourth, the phagocytosis of the dying cell by its neighboring cell; and lastly, the degradation of the dead cell (7) (Figure 3). Genes acting in the first step affect only specific cells. By contrast, genes that function in the later four steps appear to affect all somatic cell deaths and are likely act in a pathway common to all cell deaths.

In the following sections, we will first review our current understanding of genes that function as global cell-death regulators during the cell death activation and then discuss how these regulators may be controlled by cell-fate specification genes to commit the life vs. death fates of specific cells. Last, we will focus on genes that function in the cell death execution and in the engulfment and the degradation of cell corpses.

3. Activation of cell death

3.1 The genetic pathway for cell-killing

Three death-promoting genes *egl-1*, *ced-3* and *ced-4* are required for most, if not all, programmed cell death in *C. elegans*. Strong loss-of-function (lf) mutations in any of these genes lead to the survival of essentially all cells that normally undergo programmed cell death during the development of wild-type animals (8,9). Genetic mosaic analyses were carried out to determine whether these death-promoting genes act in cells that are doomed to die to mediate or promote a cell suicide process or

instead function in adjacent cells to promote the death of dying cells as “murders”. In these experiments, mosaic animals that contain both genotypically wild-type cells and genotypically mutant *ced-3* (or *ced-4*) cells were generated (10). It was found that in these mosaic animals cells that are genotypically wild-type are capable of undergoing programmed cell death and those cells that are genotypically mutant for the *ced-3* (or the *ced-4*) gene fail to die. These findings indicate that both *ced-3* and *ced-4* genes function within dying cells to cause cell death and provide the first genetic evidence that cells undergoing programmed cell death are killed by an intrinsic suicide mechanism.

In contrast to those death-promoting genes, the activity of the *ced-9* gene protects a majority of cells from undergoing programmed cell death during *C. elegans* development (11). Loss-of-function mutations in *ced-9* cause embryonic lethality, as a consequence of massive ectopic deaths of cells that normally live (11).

To understand how *egl-1*, *ced-3*, *ced-4* and *ced-9* coordinate to regulate programmed cell death, two approaches have been taken to order their functions in a pathway leading to cell death. First, genetic epistasis analyses have been performed to define the relationships of the killer genes *egl-1*, *ced-3* and *ced-4* with respect to the protector gene *ced-9*, taking advantage that the phenotype of mutants defective in any of these killer genes is opposite to the Ced-9(lf) phenotype. Specifically, double mutant combinations such as *ced-9*; *ced-3*, *ced-4 ced-9,* and *ced-9*; *egl-1* were generated, and the phenotypes of double mutants were compared with those of respective single mutants. For example, *ced-9*; *ced-3* and *ced-4 ced-9* double mutants are viable and have extra surviving cells as observed in *ced-3* and *ced-4* single mutants (11). This result indicates that the ectopic cell deaths and lethality caused by loss-of-function mutations in *ced-9* are suppressed by loss-of-function mutations in

ced-3 or *ced-4* and are dependent on the activities of *ced-3* and *ced-4*. Therefore, *ced-9* likely acts upstream of *ced-3* and *ced-4* to negatively regulate the activities of these two death-promoting genes. By contrast, *ced-9*; *egl-1* double mutants are lethal and exhibit a large amount of ectopic cell deaths as observed in *ced-9(lf)* single mutants, indicating that loss of *egl-1* function does not suppress ectopic deaths caused by loss of *ced-9* function (9). Therefore, *egl-1* likely acts upstream to negatively regulate *ced-9*.

Transcriptional overexpression experiments have been used to help order the functions of death-promoting genes *egl-1*, *ced-3* and *ced-4*. Overexpression of *egl-1*, *ced-3* or *ced-4* in *C. elegans* can induce deaths of cells in which one of these genes is ectopically expressed (12). The resulting killing effects vary in different genetic backgrounds. For example, the cell killing caused by overexpression of *egl-1*is greatly reduced in *ced-3(lf)* or *ced-4(lf)* mutants (9), consistent with the model that *ced-3* and *ced-4* act genetically downstream of *egl-1*. Similarly, the cell killing caused by overexpression of *ced-4* is greatly reduced in *ced-3(lf)* mutants (12). By contrast, cell killing mediated by overexpression of *ced-3* does not seem to be affected by the absence of the *ced-4* activity (12). These observations indicate that *ced-3* likely acts genetically downstream of *ced-4*.

3.2 Molecular identities of *egl-1*, *ced-3*, *ced-4* and *ced-9*

CED-9 is similar to the product of human proto-oncogene *bcl-2* (13), which plays a similar role in preventing apoptosis in mammals (14-18). *ced-9* and *bcl-2* are two members of an expanding gene family that play important roles in regulating apoptosis in diverse organisms (for review, see references19 and 20). All members of this gene family contain at least one Bcl-2 homology region (BH domain), and some members, such as CED-9 and Bcl-2, consist of up to four BH domains (19). The *C.*

elegans killer gene *egl-1* encodes a relatively small protein of ninety-one amino acids with a potential BH3 motif that has been found in all death-promoting Bcl-2/CED-9 family members (9).

CED-3 belongs to a family of cysteine proteases called caspases (cysteine aspartate-specific protease), which cleave their substrates exclusively after an aspartate amino acid (10,21). CED-3, like many other caspases, is first synthesized as a 56 kDa proenzyme and can be proteolytically activated to generate an active cysteine protease that contains two protease subunits derived from the cleavage products (21,22, Figure 4D). Protease activity assay of several mutant CED-3 proteins showed that the extents of reduction of CED-3 *in vitro* protease activities correlate directly with the extents of reduction of *ced-3 in vivo* killing activities (22). These findings indicate that the CED-3 protease activity is important for *ced-3* to cause programmed cell death in *C. elegans*. The *ced-4* gene encodes a protein similar to mammalian Apaf-1, which is an activator of caspases during apoptosis in mammals (23-26).

3.3 Molecular model for the killing process

Molecular studies of *C. elegans* killer and protector genes not only reveal molecular identities of these genes but also facilitate further biochemical and immunocytochemical analyses of these genes. These studies have provided important insights into how EGL-1, CED-3, CED-4 and CED-9 proteins function in a protein interaction cascade leading to the activation of programmed cell death.

CED-4 has been shown to physically interact with CED-9 *in vitro* (27). Endogenous CED-4 and CED-9 proteins are co-localized at mitochondria in *C. elegans* embryos as detected using antibodies against CED-4 and CED-9 proteins (28). In embryos in which cells had been induced to die by overexpression of *egl-1*,

CED-4 assumed a perinuclear instead of mitochondrial localization (28). This translocalization of CED-4 is mediated through interaction between EGL-1 and CED-9, as biochemical assays show that EGL-1 can bind CED-9 (9, 29) and this binding induces release of CED-4 from CED-9/CED-4 complex (30) (Figures 4A and 4B). The *ced-9* gain-of-function mutation *n1950*, which substitutes glycine 169 with glutamate, impairs the binding of EGL-1 to CED-9 but does not affect association of CED-9 with CED-4. As a result, this mutation inhibits the EGL-1-induced translocation of CED-4 and results in inhibition of programmed cell death (30).

In addition to interacting with CED-9, CED-4 has been shown to interact with CED-3 (31, 32). The binding of CED-3 and CED-9 to CED-4 is not mutually exclusive (31, 32). This observation leads to the hypothesis that in living cells CED-3, CED-4 and CED-9 may co-exist as a ternary protein complex in which CED-3 remains an inactive proenzyme. However, the CED-3 subcellular localization has not yet been determined. It is also possible that CED-3 does not associate with CED-4/CED-9 complex and exists as inactive monomer elsewhere in the cell. In either case, EGL-1-induced dissociation of CED-4 from CED-9 may allow the formation of CED-3/CED-4 complex (Figure 4). In addition to translocating to the perinuclear region, CED-4 may also undergo self-oligomerization, which appears to be important for CED-3 activation, as mutant CED-4 proteins that can not self-oligomerize fail to activate CED-3 killing activity in mammalian cells (31). These findings lead to the hypothesis that CED-4 oligomerization may bring CED-3 zymogens to close proximity and thus facilitate intermolecular proteolytic cleavage between CED-3 zymogens to activate the CED-3 protease (Figures 4C and 4D). Activated CED-3 proteases may cause cell death by cleaving key substrates and hence lead to systematic cell disassembly and the eventual recognition and phagocytosis of the cell

corpse by its neighboring cell.

In addition to CED-4, CED-3 can interact with CED-9. Moreover, CED-9 is an excellent substrate of the CED-3 protease *in vitro*. Mutations that destroy both CED-3 cleavage sites in CED-9 markedly reduce the death protective activity of CED-9 *in vivo*, suggesting that CED-3 cleavage sites are important for CED-9 death protective function (33). Cleavage of CED-9 by CED-3 generates a carboxyl-terminal product that resembles Bcl-2 in sequence and is sufficient to mediate interaction with CED-4 (33). These results suggest that CED-9 may inhibit cell death in *C. elegans* by two distinct mechanisms. First, CED-9 may directly inhibit the CED-3 protease activity through its CED-3 cleavage sites, probably acting as a competitive inhibitor. Second, CED-9 may indirectly inhibit the activation of CED-3 by forming a complex with CED-4 through its carboxyl-terminal Bcl-2 homology regions.

4. Specification of the life vs. death fates

Genetic studies in *C. elegans* suggest that the life vs. death decisions of individual cells are controlled by cell-type specific regulatory genes (34). These regulatory genes appear to control cell deaths by regulating the expression or activities of key components in the central cell-killing pathway. The control of the life vs. death fates of two specific cell types, HSNs and NSM sister cells, will be discussed below.

4.1 HSN neurons

The hermaphrodite-specific neurons (HSN) of *C. elegans* which control the egg laying behavior in hermaphrodites are generated embryonically in both hermaphrodites and males but undergo programmed cell death specifically in males, since they are not needed in males (4). In these sexually dimorphic HSNs, the cell-killer gene *egl-1* is under the direct transcriptional control of the *C. elegans* sex

determination pathway. TRA-1A, a zinc finger protein, is the terminal global regulator of somatic sexual fate and binds to the *egl-1* gene *in vitro* (35). Specific mutations in a *cis*-regulatory element of the *egl-1* gene that disrupt the binding of the TRA-1A to the *egl-1* gene result in transcriptional activation of *egl-1* in the HSNs and subsequent deaths of HSNs not only in males but also in hermaphrodites (35). Therefore, *tra-1* helps to prevent the cell-death fate of the HSNs in hermaphrodites by transcriptionally repressing the expression of the *egl-1* gene (Figure 3).

4.2 Sister cells of NSM neurons

Two genes, *ces-1* and *ces-2* (cell death specification), are important in determining the life vs. death fates of the sister cells of the two NSM (neurosecretory motor) neurons in *C. elegans* pharynx (34). Either the loss-of-function mutation in *ces-2* or gain-of-function mutations in *ces-1* can prevent these two cells from adapting their normal apoptotic cell fates. Interestingly, these cells undergo programmed cell death normally in *ces-1(lf)* mutants and *ces-1(lf)*; *ces-2(lf)* double mutants. These observations suggest that the activity of *ces-1* normally is suppressed to allow NSM sister cells to die and that *ces-2* likely acts upstream of *ces-1* to suppress the activity of *ces-1*.

The *ces-1* gene encodes a Snail family zinc finger protein (36). CES-2, a putative bZIP (basic leucine-zipper) transcription factor (37), can bind to the *ces-1* gene *in vitro* and may thus directly repress *ces-1* transcription (36). These findings suggest that a transcriptional regulatory cascade may control the deaths of NSM sister cells in *C. elegans*.

The relationship of the *ces* genes with the death-promoting genes has mainly been inferred from the phenotype of *ces-1*; *egl-1* double mutants (9). In *ces-1(lf); egl-1(lf)* mutants, NSM sister cells survive as they do in the *egl-1(lf)* mutants. This observation

indicates that *egl-1* likely acts downstream of *ces-1* to cause programmed death and *ces-1* may negatively regulate the activity of *egl-1*in these cells. Therefore, *ces-2, ces-1* and *egl-1* function in a negative regulatory chain to regulate the death fate of NSM sister cells (Figure 3).

5. Engulfment of cell corpses

Once a cell undergoes programmed death, the cell corpse is rapidly engulfed by one of its neighboring cells (2,6). Genetic analyses have identified at least seven genes, *ced-1*, *ced-2*, *ced-5*, *ced-6*, *ced-7*, *ced-10* and *ced-12,* which function in the cell-corpse engulfment process in *C. elegans* (6,38-41). Mutations in any of these genes block the engulfment of many dying cells and lead to persistence of cell corpses. Genetic analysis of double mutant combinations suggests that these seven genes fall into two classes: *ced-1*, *ced-6*, *ced-7* in one and *ced-2*, *ced-5*, *ced-10*, *ced-12* in the other. Single mutants or double mutants within the same class show relatively weak engulfment defects, while double mutants between two classes show much stronger engulfment defects. This finding suggests that dying cells likely present at least two different engulfment-inducing signals which can be recognized by distinct molecules from engulfing cells and both signaling events are required for efficient and complete phagocytosis.

The engulfment process consists of three sequential steps: the recognition of a cell corpse by an engulfing cell, transduction of the engulfing signal to the cellular machinery in the engulfing cell, and the phagocytosis of the cell corpse by the engulfing cell. The genes *ced-1*, *ced-6* and *ced-7*, which define one engulfment pathway, appear to encode components of a signaling pathway involved in cell-corpse recognition. CED-1 is similar to the human scavenger receptor SREC and may function as a corpse-recognizing phagocytic receptor since CED-1 protein was found

to cluster around dying cells (42). CED-7 is similar to ABC (ATP-binding cassette) transporters (42,43) and may play a role in promoting or mediating cell-corpse recognition by CED-1 as CED-1 receptors fail to cluster around dying cells in mutants defective in the *ced-7* gene (42,43). The CED-6 protein contains a PTB (phosphotyrosine-binding) domain (44) and may act as a signaling adaptor downstream of CED-1 and CED-7 (Figure 5).

The *ced-2*, *ced-5*, *ced-10* and *ced-12* genes, which define the other engulfment pathway, also control the migration of specific somatic cells, the gonadal distal tip cells (DTC) of *C. elegans*. These four genes encode conserved components of the Rac GTPase signaling pathway involved in regulating actin cytoskeleton rearrangement essential for cell-corpse phagocytosis and cell migration. CED-2 is a CrkII-like adaptor, consisting of one SH2 and two SH3 domains (45). CED-5 is similar to human DOCK180 that physically interacts with human CrkII (46). CED-10 is a *C. elegans* homologue of mammalian Rac GTPase (45), which controls cytoskeletal dynamics and cell shape change (for review, see reference 47). CED-12 contains a potential PH (pleckstrin-homology) domain and an SH3-binding motif (39-41). Transcriptional overexpression studies suggest that *ced-2, ced-5* and *ced-12* function at the same step upstream of *ced-10* during the engulfment process. Biochemical analysis indicates that CED-2, CED-5 and CED-12 form a ternary complex *in vitro* and so do their human homologues (39-41). Based on these findings, it has been postulated that the engulfing signal induces the formation and translocation of CED-2, CED-5 and CED-12 ternary complex to the plasma membrane of engulfing cells and the subsequent activation of CED-10 GTPase, leading to extension of membrane processes around cell corpses (Figure 5).

6. Degradation of chromosomal DNA in dying cells

Degradation of chromosomal DNA has been thought to be a crucial step and a hallmark of apoptosis. Apoptotic DNA degradation in *C. elegans* has been studied with the aid of DNA-staining techniques. For example, DAPI or Feulgen dye has been used to visualize DNA for *in situ* staining, and the TUNEL technique, which was initially developed to specifically label dying cells (48), has been applied to detect the DNA intermediates with 3'-hydroxyl ends during the degradation process.

When stained with TUNEL, only a small subset of cells that undergo programmed cell death in wild-type *C. elegans* embryos is TUNEL-positive (49), suggesting that DNA degradation is a rapid process and TUNEL only labels apoptotic cells during a transient intermediate stage. Interestingly, mutant embryos defective in *nuc-1,* which encodes a mammalian DNAse II homologue, have many more TUNEL-reactive nuclei than do wild-type embryos (49). This finding indicates that mutations in *nuc-1* allow the generation of TUNEL-reactive DNA breaks but block the subsequent conversion of these TUNEL-reactive DNA ends to TUNEL-unreactive ones. Like *nuc-1* mutations, the mutation in the *cps-6* gene (CED-3 protease suppressor), which encodes a homologue of mammalian mitochondrial endonuclease G, also increases the number of TUNEL-reactive nuclei (50). Interestingly, *cps-6*; *nuc-1* double mutants have more TUNEL-reactive nuclei than *cps-6* or *nuc-1* single mutants, suggesting that *cps-6* and *nuc-1* likely function in a partially redundant fashion to destroy TUNEL-reactive DNA ends. However, *cps-6* and *nuc-1* appear to play different roles during apoptosis. A loss-of-function mutation in the *cps-6* gene not only delays the appearance of embryonic cell corpses during development but also can block the deaths of some cells if the activity of other cell death components is compromised (50), suggesting that *cps-6* is important for the normal progression and execution of apoptosis. In contrast, mutations in *nuc-1* do not appear to affect either the execution

of cell death or the engulfment of cell corpses. Furthermore, *nuc-1* mutants are also defective in the degradation of DNA from ingested bacteria in the intestinal lumens. These observations indicate that *cps-6* is a more specific cell death nuclease and may function at an earlier stage in the apoptotic DNA degradation process than *nuc-1* does. CPS-6 is the first mitochondrial protein that is shown to be important for apoptosis in invertebrates, underscoring the conserved role of mitochondria in regulating apoptosis.

7. Summary

Genetic studies in *C. elegans* have identified more than a dozen genes that function in different aspects of programmed cell death. These genes have defined a programmed cell death pathway that is evolutionarily conserved. In *C. elegans* the life vs. death fates of cells appear to be controlled at the level of transcription. Once the decision to die has been made, a protein interaction cascade involving EGL-1, CED-3, CED-4 and CED-9 is responsible for the activation of the cell death machinery which initiates various cell disassembly processes such as the apoptotic DNA degradation process involving CPS-6 and NUC-1. The dying cell presents at least two distinct engulfing signals to their neighboring cells for phagocytosis. These two signals are mediated by two partially redundant pathways: CED-1, CED-6 and CED-7 in one and CED-2, CED-5 CED-10, and CED-12 in the other. Finally, the dying cell is completely degraded in the engulfing cell.

Figure legends

Figure 1 A *C. elegans* adult hermaphrodite and an embryo viewed using bright-field microscopy.

The embryo is indicated by an arrow. Dorsal is up and anterior is to the left for the adult. The bar represents 0.1 mm.

Figure 2 A Nomarski photomicrograph of an embryo with apoptotic cells.

Three cells indicated by arrows underwent programmed cell death in a bean/comma stage embryo and exhibited a refractile raised-button-like appearance. The bar represents 5 μm.

Figure 3 Genetic pathway of programmed cell death in *C. elegans.*

Five sequential steps of programmed cell death are indicated. In the cell-death specification step, genes involved in regulating the death fates of two specific cell types (HSN neurons and sister cells of NSM neurons) are shown. There are two partially redundant pathways (*ced-1, 6, 7* and *ced-2, 5, 10, 12,* respectively) that mediate the engulfment of cell corpses.

Figure 4 The molecular model for the activation of programmed cell death.

(A) In living cells CED-4 and CED-9 form a complex, which is tethered to mitochondria through CED-9. CED-3 may associate with CED-4 at the mitochondria or exist elsewhere without binding to CED-4. However, in either case, CED-3 remains an inactive proenzyme in living cells. (B) In the cells that are doomed to die, the death initiator protein EGL-1 binds to CED-9 and triggers the release of CED-4 (or the CED-4/CED-3 complex) from CED-9. (C) Released CED-4 proteins undergo oligomerization, which brings two CED-3 proenzymes to close proximity and (D)

leads to CED-3 autoproteolytic activation.

Figure 5 The molecular model for the cell corpse engulfment process.

The engulfment process is mediated by two partially redundant pathways. Molecules in the CED-1, CED-6, and CED-7 pathway are labeled in black. CED-1 and CED-7 act on the surface of engulfing cells to mediate cell-corpse recognition and to transduce the engulfing signal through CED-6 to the cellular machinery of the engulfing cells for engulfment. CED-7 also acts in dying cells. Molecules in the CED-2, CED-5, CED-10 and CED-12 pathway are labeled in gray. The CED-2/CED-5/CED-12 ternary complex mediates the signaling event from unidentified engulfing signal(s) and receptor(s) to activate CED-10 during phagocytosis.

References

1. Brenner, S. (1974) The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.
2. Sulston, J. E. & Horvitz, H. R. (1977) Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Dev. Biol.* **56**, 110-156.
3. Kimble, J. & Hirsh, D. (1979) The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. *Dev. Biol.* **70**, 396-417.
4. Sulston, J. E., Schierenberg, E., White, J. G. & Thomson, J. N. (1983) The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**, 64-119.
5. Robertson, A. G. & Thomson, J. N. (1982) Morphology of programmed cell death in the ventral nerve cord of *Caenorhabiditis elegans* larvae. *J. Embryol.exp. Morph.* **67**, 89-100.
6. Hedgecock, E., Sulston, J. & Thomson, J. N. (1983) Mutations affecting programmed cell deaths in the nematode *Caenorhabditis elegans*. *Science* **220**, 1277-1279.
7. Ellis, R. E., Yuan, J. & Horvitz, H. R. (1991) Mechanisms and functions of cell death. *Annu. Rev. Cell Biol.* **7**, 663-698.

8. Ellis, H. M. & Horvitz, H. R. (1986) Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* **44**, 817-829.
9. Conradt, B. & Horvitz, H. R. (1998) The *C. elegans* protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. *Cell* **93**, 519-529.
10. Yuan, J. Y. & Horvitz, H. R. (1990) The *Caenorhabditis elegans* genes *ced-3* and *ced-4* act cell autonomously to cause programmed cell death. *Dev. Biol.* **138**, 33-41.
11. Hengartner, M. O., Ellis, R. E. & Horvitz, H. R. (1992) *Caenorhabditis elegans* gene *ced-9* protects cells from programmed cell death. *Nature* **356**, 494-499.
12. Shaham, S. & Horvitz, H. R. (1996) Developing *Caenorhabditis elegans* neurons may contain both cell-death protective and killer activities. *Genes Dev.* **10**, 578-591.
13. Hengartner, M. O. & Horvitz, H. R. (1994) *C. elegans* cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene bcl-2. *Cell* **76**, 665-676.
14. Tsujimoto, Y. & Croce, C. M. (1986) Analysis of the structure, transcripts, and protein products of bcl-2, the gene involved in human follicular lymphoma. *Proc Natl Acad Sci U S A* **83**, 5214-5218.
15. Seto, M. *et al.* (1988) Alternative promoters and exons, somatic mutation and deregulation of the Bcl-2-Ig fusion gene in lymphoma. *Embo J.* **7**, 123-131.
16. Cleary, M. L., Smith, S. D. & Sklar, J. (1986) Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t(14;18) translocation. *Cell* **47**, 19-28.
17. Vaux, D. L., Cory, S. & Adams, J. M. (1988) Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* **335**, 440-442.
18. Nunez, G. *et al.* (1990) Deregulated Bcl-2 gene expression selectively prolongs survival of growth factor-deprived hemopoietic cell lines. *J. Immunol* **144**, 3602-3610.
19. Reed, J. C. (1998) Bcl-2 family proteins. *Oncogene* **17**, 3225-3236.
20. Adams, J. M. & Cory, S. (2001) Life-or-death decisions by the Bcl-2 protein family. *Trends Biochem. Sci.* **26**, 61-66.
21. Alnemri, E. S. *et al.* (1996) Human ICE/CED-3 protease nomenclature. *Cell* **87**,

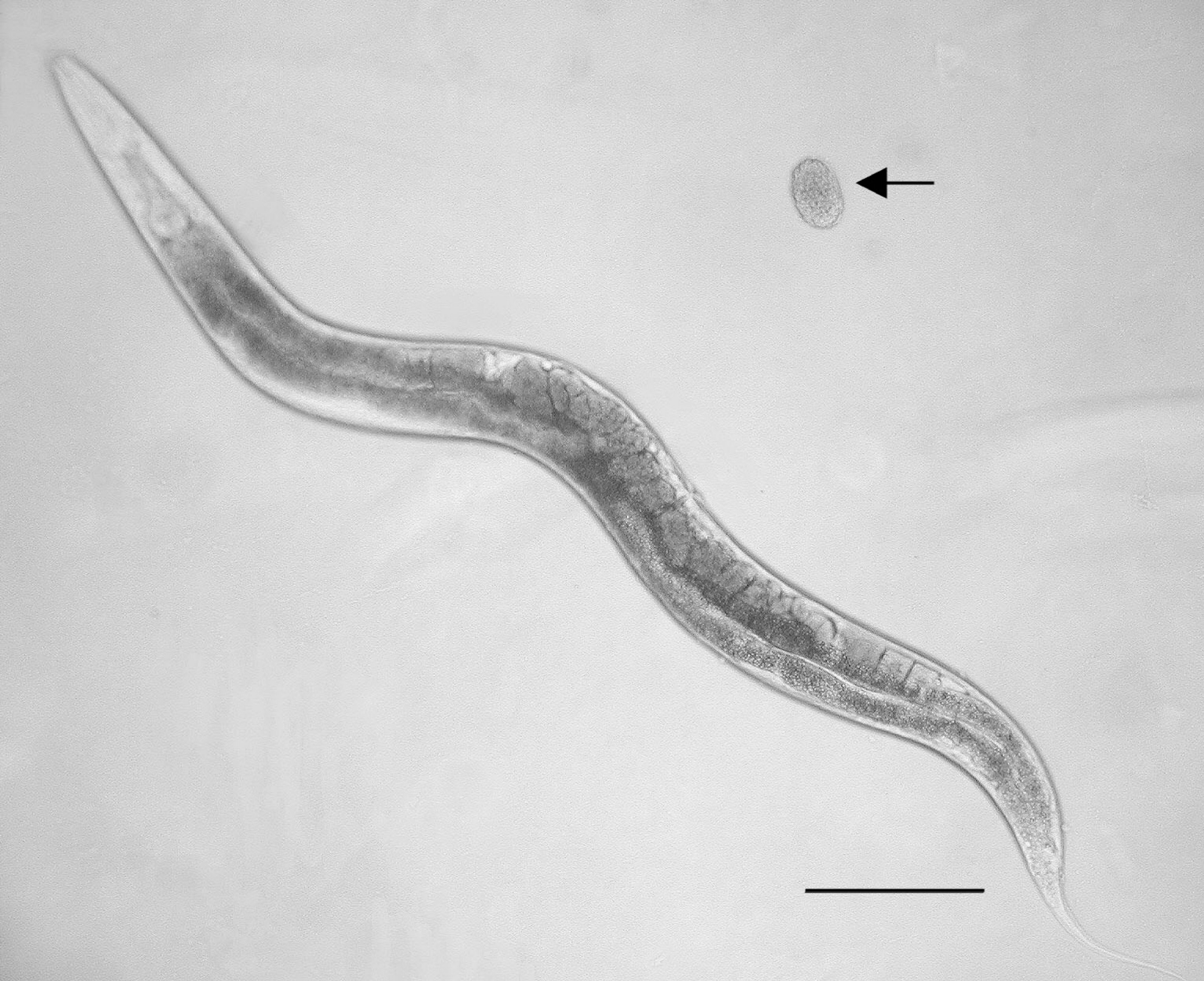
171.

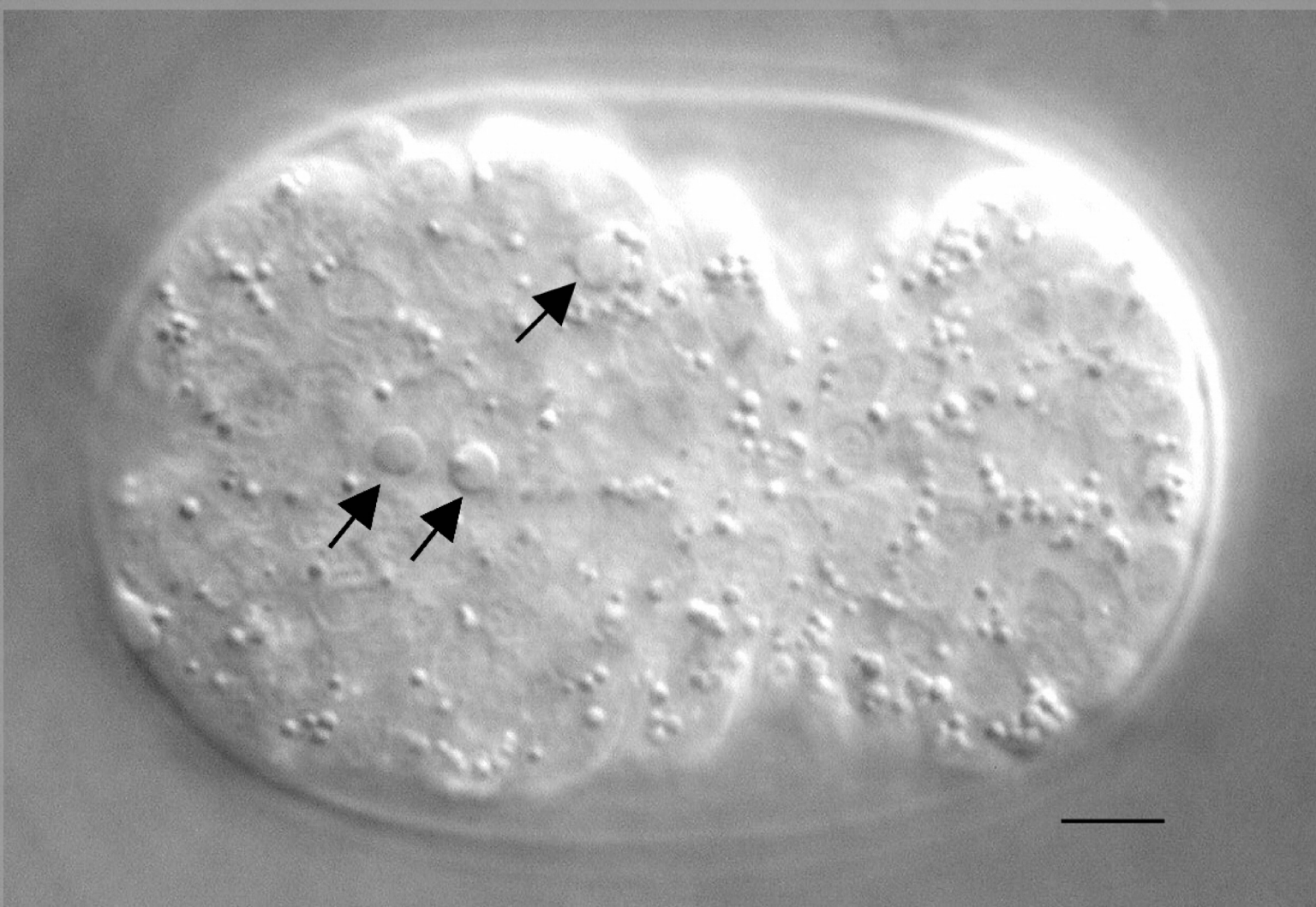
22. Xue, D., Shaham, S. & Horvitz, H. R. (1996) The *Caenorhabditis elegans* cell-death protein CED-3 is a cysteine protease with substrate specificities similar to those of the human CPP32 protease. *Genes Dev.* **10**, 1073-1083.
23. Yuan, J. & Horvitz, H. R. (1992) The *Caenorhabditis elegans* cell death gene *ced-4* encodes a novel protein and is expressed during the period of extensive programmed cell death. *Development* **116**, 309-320.
24. Zou, H., Henzel, W. J., Liu, X., Lutschg, A. & Wang, X. (1997) Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell* **90**, 405-413.
25. Cecconi, F., Alvarez-Bolado, G., Meyer, B. I., Roth, K. A. & Gruss, P. (1998) Apaf1 (CED-4 homolog) regulates programmed cell death in mammalian development. *Cell* **94**, 727-737.
26. Yoshida, H. *et al.* (1998) Apaf1 is required for mitochondrial pathways of apoptosis and brain development. *Cell* **94**, 739-750.
27. Spector, M. S., Desnoyers, S., Hoeppner, D. J. & Hengartner, M. O. (1997) Interaction between the *C. elegans* cell-death regulators CED-9 and CED-4. *Nature* **385**, 653-656.
28. Chen, F. *et al.* (2000) Translocation of *C. elegans* CED-4 to nuclear membranes during programmed cell death. *Science* **287**, 1485-1489.
29. del Peso, L., Gonzalez, V. M. & Nunez, G. (1998) *Caenorhabditis elegans* EGL-1 disrupts the interaction of CED-9 with CED-4 and promotes CED-3 activation. *J. Biol. Chem.* **273**, 33495-33500.
30. Parrish, J., Metters, H., Chen, L. & Xue, D. (2000) Demonstration of the in vivo interaction of key cell death regulators by structure-based design of second-site suppressors. *Proc. Natl. Acad. Sci. U S A* **97**, 11916-11921.
31. Yang, X., Chang, H. Y. & Baltimore, D. (1998) Essential role of CED-4 oligomerization in CED-3 activation and apoptosis. *Science* **281**, 1355-1357.
32. Chinnaiyan, A. M., O'Rourke, K., Lane, B. R. & Dixit, V. M. (1997) Interaction of CED-4 with CED-3 and CED-9: a molecular framework for cell death. *Science* **275**, 1122-1126.
33. Xue, D. & Horvitz, H. R. (1997) *Caenorhabditis elegans* CED-9 protein is a bifunctional cell-death inhibitor. *Nature* **390**, 305-308.
34. Ellis, R. E. & Horvitz, H. R. (1991) Two *C. elegans* genes control the

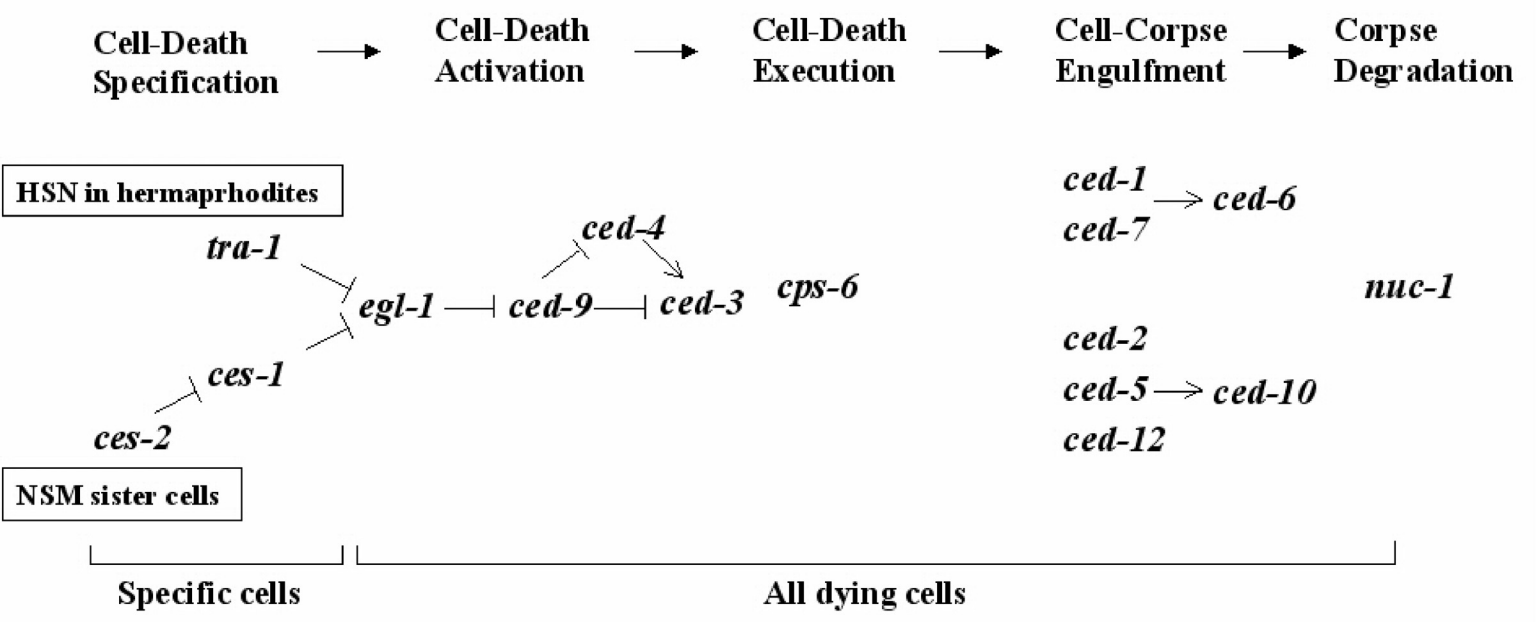
programmed deaths of specific cells in the pharynx. *Development* **112**, 591-603.

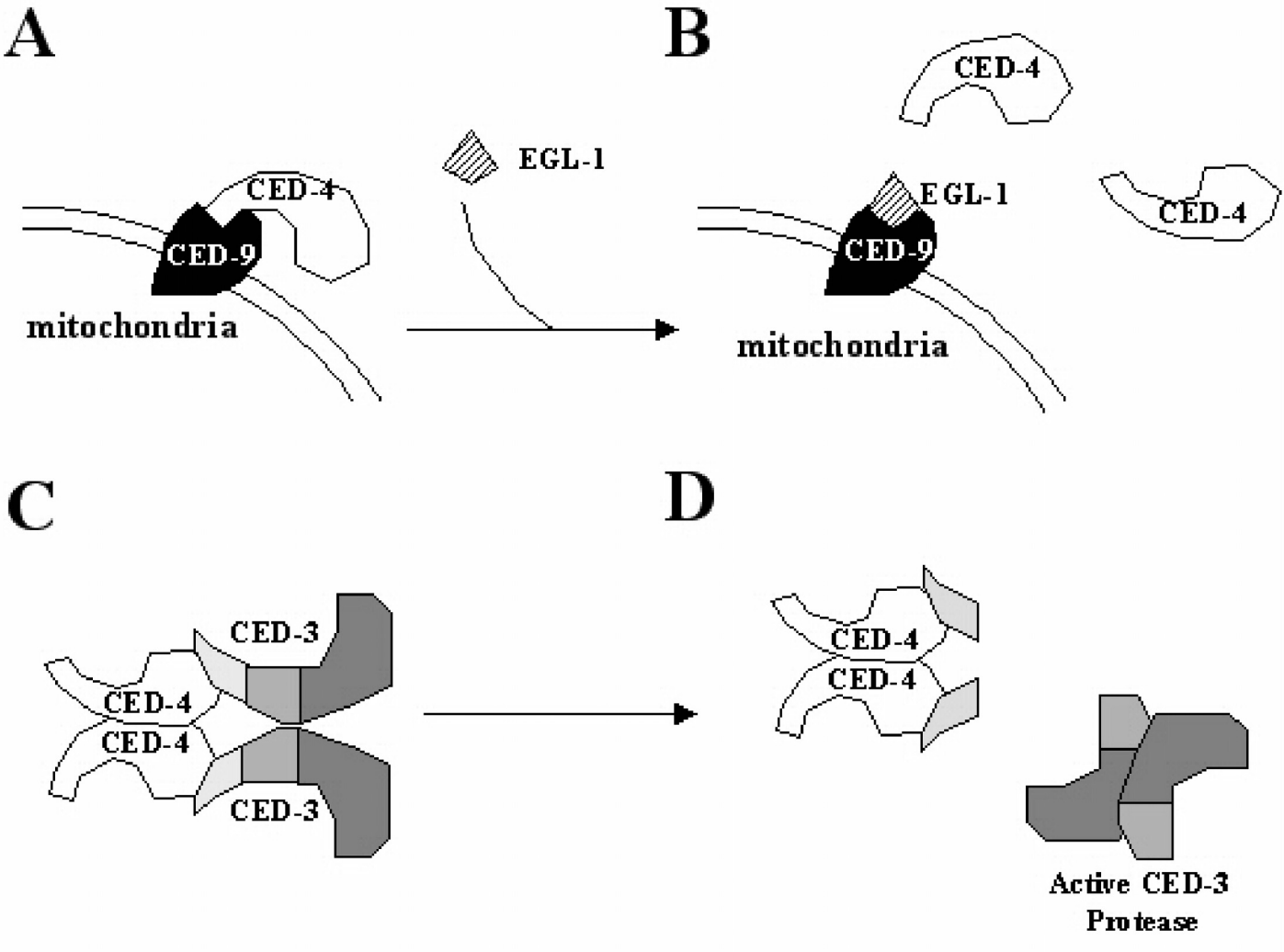
35. Conradt, B. & Horvitz, H. R. (1999) The TRA-1A sex determination protein of *C. elegans* regulates sexually dimorphic cell deaths by repressing the *egl-1* cell death activator gene. *Cell* **98**, 317-327.
36. Metzstein, M. M. & Horvitz, H. R. (1999) The *C. elegans* cell death specification gene *ces-1* encodes a snail family zinc finger protein. *Mol. Cell* **4**, 309-319.
37. Metzstein, M. M., Hengartner, M. O., Tsung, N., Ellis, R. E. & Horvitz, H. R. (1996) Transcriptional regulator of programmed cell death encoded by *Caenorhabditis elegans* gene *ces-2*. *Nature* **382**, 545-547.
38. Ellis, R., Jacobson, D. M. & Horvitz, H. R. (1991) Genes required for the engulfment of cell corpses during programmed cell death in *C. elegans*. *Genetics* **129**, 79-94.
39. Wu, Y. C., Tsai, M. C., Cheng, L. C., Chou, C. J. & Weng, N. Y. (2001) *C. elegans* CED-12 acts in the conserved crkII/dock180/rac pathway to control cell migration and cell corpse engulfment. *Dev. Cell* **1**, 491-502.
40. Zheng, Z., Caron, E., Harwieg, E., Hall, A. & Horvitz, H. R. (2001) The *C. elegans* PH domain protein CED-12 regulates cytoskeletal reorganization via a rho/rac GTPase signaling pathway. *Dev. Cell* **1**, 477-489.
41. Gumienny, T. L. *et al.* (2001) Ced-12/elmo, a novel member of the crkii/dock180/rac pathway, is required for phagocytosis and cell migration. *Cell* **107**, 27-41.
42. Zhou, Z., Hartwieg, E. & Horvitz, H. R. (2001) CED-1 is a transmembrane receptor that mediates cell corpse engulfment in *C. elegans*. *Cell* **104**, 43-56.
43. Wu, Y. C. & Horvitz, H. R. (1998) The *C. elegans* cell corpse engulfment gene *ced-7* encodes a protein similar to ABC transporters. *Cell* **93**, 951-960.
44. Liu, Q. A. & Hengartner, M. O. (1998) Candidate adaptor protein CED-6 promotes the engulfment of apoptotic cells in *C. elegans*. *Cell* **93**, 961-972.
45. Reddien, P. W. & Horvitz, H. R. (2000) CED-2/CrkII and CED-10/Rac control phagocytosis and cell migration in *Caenorhabditis elegans*. *Nat. Cell Biol.* **2**, 131-136.
46. Wu, Y. C. & Horvitz, H. R. (1998) *C. elegans* phagocytosis and cell-migration protein CED-5 is similar to human DOCK180. *Nature* **392**, 501-504.
47. Hall, A. (1992) Ras-related GTPases and the cytoskeleton. *Mol. Biol. Cell* **3**, 475-479.

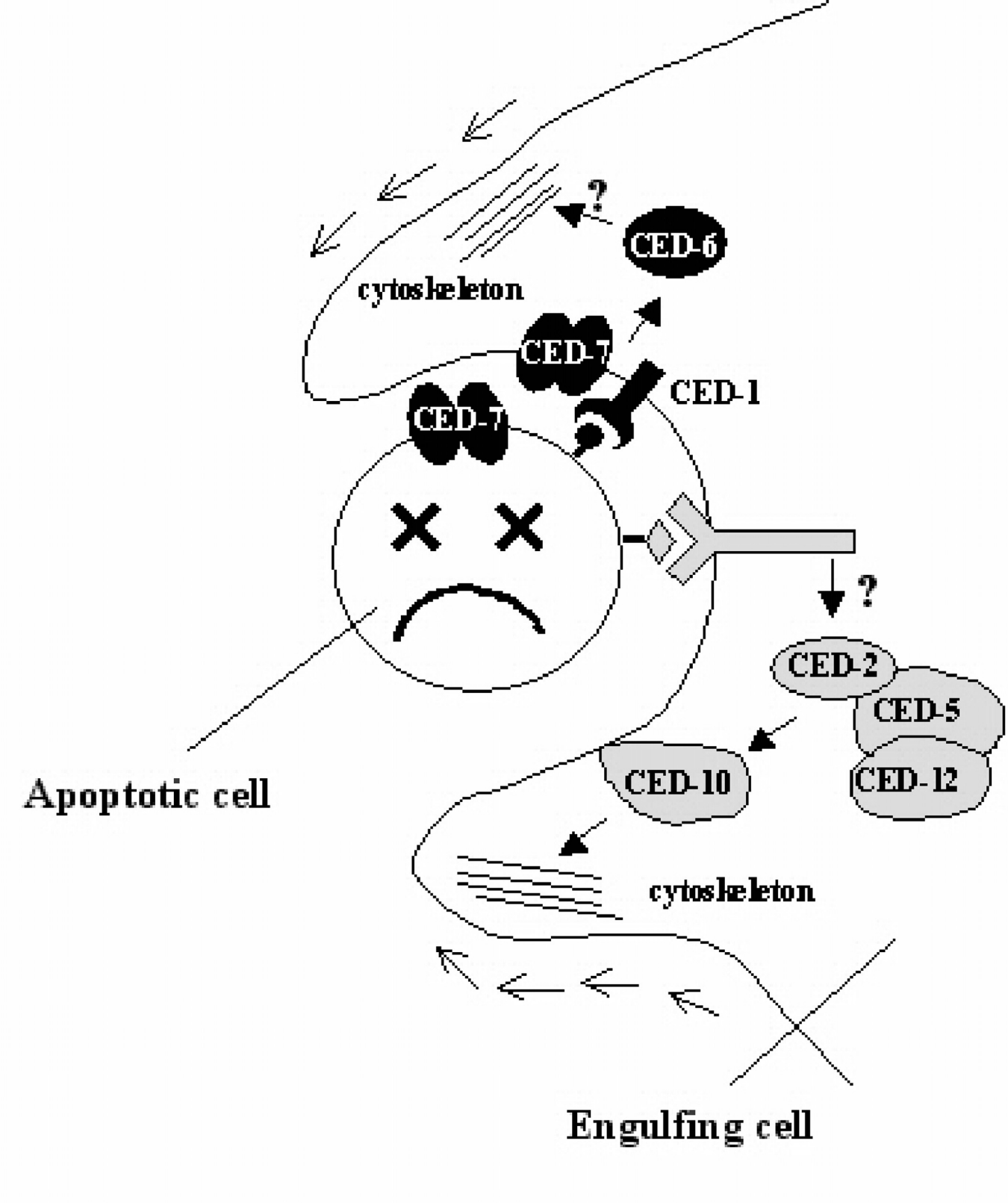
48. Gavrieli, Y., Sherman, Y. & Ben-Sasson, S. A. (1992) Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. *J. Cell Biol.* **119**, 493-501.
49. Wu, Y. C., Stanfield, G. M. & Horvitz, H. R. (2000) NUC-1, a *Caenorhabditis elegans* DNase II homolog, functions in an intermediate step of DNA degradation during apoptosis. *Genes Dev.* **14**, 536-548.
50. Parrish, J. *et al.* (2001) Mitochondrial endonuclease G is important for apoptosis in *C. elegans*. *Nature* **412**, 90-94.






Cell-Death Specification
Cell-Death Activation
Cell-Death Execution
Cell-Corpse Engulfment
Corpse Degradation
HSN in hermaphrodites
tra-1
ces-1
ces-2
NSM sister cells
egl-1
ced-9
ced-4
ced-3
cps-6
ced-1
ced-7
ced-6
ced-2
ced-5
ced-10
ced-12
nuc-1
Specific cells
All dying cells


A
CED-4
CED-9
mitochondria
EGL-1
B
CED-4
EGL-1
CED-9
CED-4
mitochondria
C
CED-3
CED-4
CED-4
CED-3
D
CED-4
CED-4
Active CED-3
Protease


?
CED-6
cytoskeleton
CED-7
CED-1
CED-7
?
CED-2
CED-5
CED-10
CED-12
Apoptotic cell
cytoskeleton
Engulfing cell