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The ins and outs of programmed cell death during *C. elegans* development

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SUMMARY

During the development of the *C. elegans* hermaphrodite, 131 of the 1090 cells generated undergo programmed cell death. Genetic studies have identified mutations in 14 genes that specifically affect this process. These genes define a genetic pathway for programmed cell death in *C. elegans*. Two genes, *ced-3* and *ced-4*, are required for cells to undergo programmed cell death, while a third gene, *ced-9*, protects cells that should live from undergoing programmed cell death. The proteins encoded by *ced-3* and *ced-9* show significant similarity to proteins that affect programmed cell death in vertebrates, suggesting that the molecular cell death pathway in which *ced-3*, *ced-4*, and *ced-9* act has been conserved between nematodes and vertebrates.

1. THE SETTING

The small nematode *Caenorhabditis elegans* has been widely used over the past two decades to study fundamental problems in developmental biology (Wood 1988). The two characteristics that have contributed most to the widespread use of this organism are its powerful genetics (Brenner 1974) and the simplicity and reproducibility of its development. For example, the pattern of cell divisions generating an adult worm from the one-celled zygote is essentially invariant from one animal to the next (Sulston & Horvitz 1977; Kimble & Hirsh 1979; Sulston *et al.* 1983).

During *C. elegans* development, a number of cells are eliminated, generally shortly after their generation, by undergoing programmed cell death. The number of cell deaths is high: in hermaphrodites, 131 of the 1090 cells generated die, while in males 147 of the 1178 cells generated die. Like the rest of *C. elegans* development, these deaths are highly reproducible: the same number of cells die in all individuals, and the time during development at which a given cell dies is constant. Programmed cell death in *C. elegans* can thus be studied with single-cell resolution.

2. THE CAST

Genetic studies of programmed cell death in *C. elegans* have led to the isolation of many mutations that affect this process. These mutations identify 14 genes that function in programmed cell death and define a genetic pathway for programmed cell death in *C. elegans* (figure 1). This pathway can be divided into four distinct steps: the decision of individual cells whether to undergo programmed cell death or adopt another fate, the actual killing of the cell, the engulfment of the dying cell by a neighboring cell, and the degradation of the dead, engulfed cell. Genes in the last three steps are involved in all programmed cell deaths, whereas genes in the decision step affect only a small number of cells, usually only one or two cell types.

3. THE PROTAGONISTS

(*a*) *The killers*

The activities of two genes, *ced-3* and *ced-4* (cell death abnormal), are necessary for programmed cell deaths to occur: mutations that inactivate either *ced-3* or *ced-4* result in the survival of almost all cells that normally die during development (Ellis & Horvitz 1986). The *ced-4* gene encodes a protein of about 63 kDa and is expressed primarily during embryonic development, when 113 of the 131 programmed cell deaths occur (Yuan & Horvitz 1992).

The ced-3 gene encodes a 503 amino acid protein and also is expressed primarily during embryonic development (Yuan *et al.* 1993). The protein encoded by the *ced-3* gene shows significant similarity to two mammalian proteins: interleukin-1β converting enzyme (ICE) and the product of the *nedd-2* gene (Yuan *et al.* 1993). ICE is a cysteine protease unrelated in sequence to any previously known protease (Cerretti *et al.* 1992; Thornberry *et al.* 1992). ICE recognizes and cleaves the 31 kDa pro-IL-1β into the 17.5 kDa mature IL-1β. The only other known ICE substrate besides pro-IL-1β is ICE itself: the protein is synthesized as an inactive proenzyme,

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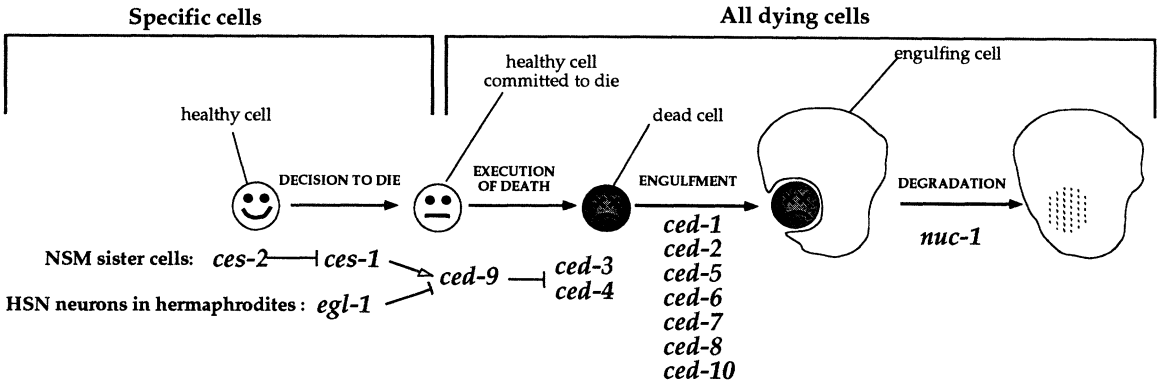

Figure 1. The genetic pathway for programmed cell death in *C. elegans*. Mutations in 14 genes affect programmed cell deaths. These mutations divide the process of programmed cell death into four steps; genes that act in the last three steps are common to all programmed cell deaths, whereas genes that act in the first step affect only a few cells. Regulatory interactions deduced from genetic studies are shown. ⟶, Positive regulation; ⟞, negative regulation. Adapted from Ellis *et al.* (1991).

which can be activated through limited proteolysis by the mature enzyme (Thornberry *et al.* 1992). The mouse *nedd-2* gene, which encodes a protein of unknown function, is expressed during embryonic brain development and is down-regulated in adult brain (Kumar *et al.* 1992). The sequence similarity between CED-3 and ICE suggests that CED-3 might also be a cysteine protease. Interestingly, overexpression of ICE, a vertebrate homologue of CED-3, induced programmed cell death in rat fibroblasts (Miura *et al.* 1993), suggesting that a cysteine protease similar to CED-3/ICE might also be involved in causing programmed cell death in mammals.

(*b*) *The guardian*

The activity of the gene *ced-9* appears to be both sufficient and necessary to protect *C. elegans* cells from undergoing programmed cell death. Either a gain-of-function mutation in the *ced-9* gene (Hengartner *et al.* 1992) or overexpression of wild-type *ced-9* (Hengartner & Horvitz 1994) results in the survival of cells that normally die. By contrast, mutations that inactivate *ced-9* cause many cells that normally survive to undergo programmed cell death (Hengartner *et al.* 1992). The progeny of animals lacking *ced-9* activity

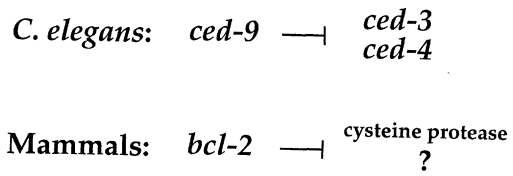

Figure 2. Nematodes and mammals might share a common pathway for programmed cell death. Genetic studies of *C. elegans* have indentified three genes, *ced-3*, *ced-4*, and *ced-9*, involved in the execution of all programmed cell death (top). Homologues of these genes might be involved in a similar pathway in mammals (bottom). Overexpression of CED-9 or Bcl-2 prevents programmed cell death (Vaux *et al.* 1988; Hengartner & Horvitz 1994), while overexpression of CED-3 or ICE induces programmed cell death (Miura *et al.* 1993).

die during embryogenesis, indicating that the protective activity of *ced-9* is essential for *C. elegans* development. The CED-9 protein shows sequence similarity to the product of the mammalian proto-oncogene *bcl-2* (Hengartner & Horvitz 1994). Interestingly, *bcl-2* also shares several functional similarities with *ced-9*: overexpression of *bcl-2* also protects cells from programmed cell death (apoptosis; reviewed by Korsmeyer *et al.* (1993)), and cells that lack *bcl-2* activity are hypersensitive to death-inducing signals (Nakayama *et al.* 1993; Veis *et al.* 1993). Human *bcl-2* can prevent both the normal programmed cell deaths that occur during *C. elegans* development (Vaux *et al.* 1992; Hengartner & Horvitz 1994) and also the ectopic cell deaths observed in mutants lacking *ced-9* function (Hengartner & Horvitz 1994), suggesting that *bcl-2* can substitute for *ced-9* in *C. elegans*. These results have led to the suggestion that *bcl-2* might be a vertebrate homologue of *ced-9*.

4. ACT I

How is the cell death program regulated such that only the correct cells die? The 131 cells that die during *C. elegans* hermaphrodite development have very different developmental origins and differ widely in their cell types, yet they all activate the same genetic pathway that leads to their programmed deaths. It is possible that like mammals (reviewed by Ellis *et al.* 1991), *C. elegans* has more than one way to induce programmed cell death and that distinct, possibly cell-type-specific death-inducing signals converge to activate the same pathway.

Several genes have been identified that affect the deaths of only a small number of cells. For example, the genes *ces-1* and *ces-2* (cell death specification) affect the decision of two cells in the pharynx (the worm's feeding organ) whether to live or die: in the wild-type, these two cells, which are the sister cells of the NSM motor neurons, undergo programmed cell death (Sulston *et al.* 1983). Dominant gain-of-function mutations in the gene *ces-1* and recessive loss-of-function mutations in the gene *ces-2* allow these two cells to survive and adopt

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a fate similar to that of their sisters the NSM neurons (Ellis & Horvitz 1991). A third gene, *egl-1* (egg-laying defective) affects the death of the two sexually dimorphic hermaphrodite-specific HSN neurons. The HSNs are a pair of serotonergic motor neurons that innervate the vulval muscles and drive egg-laying in *C. elegans* hermaphrodites (Desai *et al.* 1988; Desai & Horvitz 1989). In *C. elegans* males, which have no eggs to lay, the HSNs undergo programmed cell death (Sulston *et al.* 1983). Dominant mutations in the *egl-1* gene cause the HSNs to undergo programmed cell death in hermaphrodites, leading to a defect in egg-laying (Trent *et al.* 1983).

What is the function of these cell-death specification genes? One possibility is that they encode genes required for the cells to assume their proper identities: for example, the NSM sisters might survive in the *ces* mutants because they failed to adopt their proper fate and for this reason do not activate the cell death pathway. Alternatively, these genes might encode cell type-specific regulators of general cell death genes such as *ced-3*, *ced-4*, or *ced-9*. In this second model, the NSM sisters for example might be determined to die but fail to properly activate the cell death pathway, possibly as a result of the absence in the cell of one of the components of the general cell death pathway.

5. THE FINAL ACT

What happens once a cell has embarked upon the death process? Although the molecular events downstream of CED-3 and CED-4 are unknown, the structural changes accompanying death have been described (Sulston & Horvitz 1977; Robertson & Thomson 1982; Ellis *et al.* 1991). These changes include nuclear chromatin aggregation (and adoption by the nucleus of a characteristic pycnotic appearance evident at the levels of both the light and electron microscopes), cytoplasmic condensation, membrane whorling, and fragmentation of the cell into membrane-bound fragments. Several of these features are also characteristic of apoptotic deaths in mammals (Wyllie *et al.* 1980), suggesting that some of the molecular events responsible for these changes are common to both processes.

While these events occur within the dying cell, cytoplasmic extensions from a neighboring cell progressively surround the dying cell and engulf it. Mutations in seven genes (*ced-1*, *2*, *5*, *6*, *7*, *8*, *10*) delay or block this enfulfment process to varying degrees: whereas in the wild-type a dying cell is engulfed and degraded within an hour of the appearance of its first morphological changes, dying cells fail to be engulfed for many hours or even days in these mutants, leading to an accumulation of unengulfed, undegraded corpses that are easily observed in the light microscope (Hedgecock *et al.* 1983; Ellis *et al.* 1991).

The gene *nuc-1* (nuclease abnormal) is involved in the last step of the cell death pathway: *nuc-1* mutants lack a nuclease activity that is required to degrade the DNA of the dead cell. In these animals, cells die and are engulfed normally, but the DNA of the dead cells remains undegraded inside the engulfing cell. The enzymic properties of this nuclease (Hevelone & Hartman 1988) suggest that it corresponds to the major lysosomal nuclease, an observation consistent with the fact that this nuclease is required for the degradation of DNA of the bacteria on which the worm feeds (Hedgecock *et al.* 1983).

6. DEATH FROM THE INSIDE

Are programmed cell deaths suicides or murders? The discovery that the genes *ced-3* and *ced-4* are required for killing allowed this question to be addressed experimentally. Genetic experiments by Yuan & Horvitz (1990) suggest that both *ced-3* and *ced-4* genes must be expressed by the cells that die, indicating that the dying cell plays an essential role in bringing forth its own demise. This observation suggests that programmed cell death in *C. elegans* is a suicide process and truly comes 'from the inside'.

7. CHARACTER INTERPLAY

How do *ced-3*, *ced-4* and *ced-9* interact? Genetic studies showed that mutations in *ced-3* and *ced-4* can completely block the ectopic deaths caused by the lack of *ced-9* function (Hengartner *et al.* 1992). If we assume that these genes are involved in a regulatory pathway, then this observation suggests that *ced-9* encodes a negative regulator of *ced-3* and *ced-4* activities. The molecular mechanism of this regulation has yet to be uncovered. One possibility is that CED-9 is a protease inhibitor, binding to CED-3 to block its activity, or binding to the CED-3 pro-enzyme to prevent its proteolytic activation. Mammalian Bcl-2 binds to at least two distinct proteins, Bax (Oltvai *et al.* 1993) and R-ras (Fernandez-Sarabia & Bischoff 1993). Whether *C. elegans* contains homologues of these proteins to which CED-9 could bind remains to be determined.

8. AN OLD PLOT?

Two key players in the execution step, *ced-3* and *ced-9*, are members of gene families that include mammalian genes involved in the regulation of programmed cell death. For example, several members of the *ced-9* gene family, such as Bcl-2 (Vaux *et al.* 1988), Bax (Oltvai *et al.* 1993), and Bcl-x (Boise *et al.* 1993), modulate the susceptibility of mammalian cells to death-inducing signals, much in the same way as CED-9 does in *C. elegans*. Similarly, overexpression of ICE, a vertebrate homologue of CED-3, induced programmed cell death in rat fibroblasts (Miura *et al.* 1993), suggesting that a cysteine protease similar to CED-3 and ICE might cause programmed cell death in mammals.

The involvement of members of the *ced-9*/*bcl-2* and *ced-3*/ICE gene families in programmed cell death in both nematodes and mammals suggests that not only these genes but also the rest of the cell death pathway that has been characterized in *C. elegans* may be conserved through evolution and that such a pathway

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for programmed cell death is of ancient origin and might operate in all metazoans (figure 2).

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