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The Apoptosome: Molecular Control of Programmed Cell Death in the Nematode *C. elegans*

Abstract

The proapoptotic genes *ced-3* and *ced-4* are essential for programmed cell death in the nematode *C. elegans*. The activity of these killer genes in antagonized by the survival gene *ced-9*. All three genes have mammalian homologs that also function in the apoptotic pathway. Recent work suggests that CED-3, CED-4, and CED-9 form a multiprotein complex — the apoptosome — that regulates apoptosis in *C. elegans*. A similar complex might exist in mammalian cells.

Introduction

Programmed cell death (apoptosis) is an important component of animal development and homeostasis. This process, which removes cells that are not needed or are potentially dangerous, can be observed in a wide variety of tissues in both vertebrates and invertebrates (Ellis et al., 1991; Glücksmann, 1951; Saunders, 1966). Proper control of programmed cell death is very important: breakdown in the regulation of this process appears to be associated with the etiology or pathology of many types of cancer, certain autoimmune diseases, myocardial heart infarct, stroke, and possibly neurodegenerative diseases (Thompson, 1995).

Programmed Cell Death during *C. elegans* Development

The small nematode *Caenorhabditis elegans* has been used with great success as a model organism for the genetic analysis of programmed cell death. Of the 1090 cells generated during *C. elegans* hermaphrodite development, 131 undergo programmed cell death (Kimble and Hirsh, 1979; Sulston and Horvitz, 1977; Sulston et al., 1983). As with most of *C. elegans* development, these deaths are highly reproducible: The identity of the dying cells and the time in development at which these cells die are essentially invariant among individuals. Cells dying by programmed cell death in *C. elegans* undergo a series of morphological changes that, at both the light and electron microscopy levels, show many features that are characteristic of apoptotic deaths

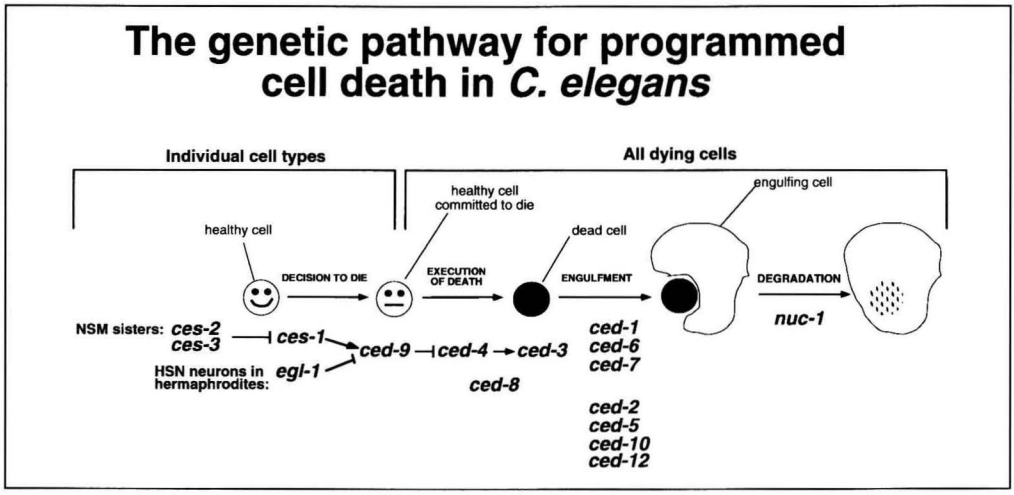

Fig. 1. The genetic pathway for programmed cell death in *C. elegans*. Mutations in 14 genes affect programmed cell deaths. These mutations divide the process of programmed cell death into four steps; genes that act in the last three steps are common to all programmed cell deaths, while mutations in 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., 1991a).

in mammals, suggesting that the subcellular events that are occurring with the dying mammalian and nematode cells are similar (Robertson and Thomson, 1982; Sulston and Horvitz, 1977; Wyllie et al., 1980).

Genetic dissection of programmed cell death in *C. elegans* has led to the identification of a large number of mutations that affect this process (reviewed by Driscoll, 1992; Hengartner and Horvitz, 1994). These mutations identify 14 genes that function in programmed cell death and that can be placed into a genetic pathway (Figure 1). Mutations in 11 genes affect all 131 programmed cell deaths. These genes divide the death process into three distinct steps: execution of the death sentence, engulfment of the dying cells by neighboring cells, and degradation of the engulfed cell. Three additional genes, *ces-1*, *ces-2*, and *egl-1*, have been identified that act upstream of the general cell death pathway: These genes affect the decisions of a very small number of cells whether to live or die. One attractive hypothesis is that these genes are involved in the cell type-specific control of the activation of the death program (reviewed by Driscoll, 1992; Hengartner, 1997; Hengartner and Horvitz, 1994).

Three genes act in the execution step of the cell death pathway (Figure 1). The activities of two of these three genes, *ced-3* and *ced-4* (**ce**ll **d**eath abnormal), are necessary for programmed cell deaths to occur: Mutations that inactivate either *ced-3* or *ced-4* result in the survival of all 131 cells that normally die during hermaphrodite development (Ellis and Horvitz, 1986). Both genes act cell-autonomously, indicating that the dying cell plays a central role in bringing forth its own demise and suggesting that programmed cell deaths in *C. elegans* might not be 'murders' but rather 'suicides' (Yuan and Horvitz, 1990). The third gene acting in this step, *ced-9*, is required

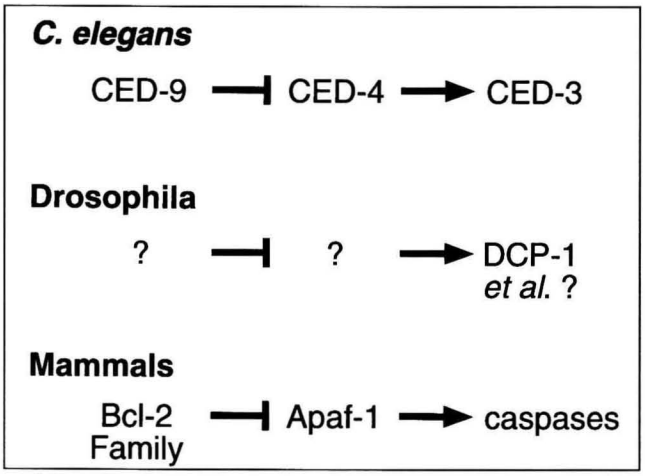

Fig. 2. Conservation of the genetic pathway for apoptosis between *C. elegans*, Drosophila, and mammals. All three of the key cell death regulators identified in *C. elegans* have homologs in mammals that perform similar genetic functions in apoptosis. While CED-9/Bcl-2 and CED-4/Apaf-1 homologs have not yet been identified in Drosophila, the requirement for caspases for programmed cell death in this species suggest that a similar pathway for apoptotic death also exists in insect.

to prevent activation of the cell death program in cells that should live: Either a gain-of-function mutation in the *ced-9* gene or overexpression of wild-type *ced-9* results in the survival of cells that normally die; by contrast, mutations that reduce *ced-9* function cause many cells that normally survive to undergo programmed cell death.

Mutations in seven genes (*ced-1*, *2*, *5*, *6*, *7*, *8*, *10*) affect the engulfment of dying cells in *C. elegans* (Ellis et al., 1991; Hedgecock et al., 1983b). In these mutants, many dying cells fail to be engulfed and persist for many hours or even days. Absence of any one of these genes does not completely block the engulfment process: While some cell corpses accumulate, most dying cells are still engulfed properly, suggesting an element of redundancy among the various genes. Indeed, analysis of all double mutant combinations led to the division of these seven genes into two subgroups, which are proposed to be involved in two distinct but partially redundant processes that act in the engulfment of cell corpses (Ellis et al., 1991b).

The gene *nuc-1* (nuclease abnormal) is involved in the last step of the cell death pathway (Figure 1): *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 (Hedgecock et al., 1983), suggesting this nuclease is not required for killing cells, but rather is involved in the subsequent 'cleaning up'.

Conservation of the Genetic Pathway for Programmed Cell Death between Nematodes and Mammals

The molecular characterization of the *C. elegans* cell death genes revealed that *ced-3*, *ced-4*, and *ced-9*, the three key genes involved in the control and execution of the death sentence, are similar in sequence to mammalian cell death genes, suggesting the cell death program found in *C. elegans* also functions in mammals (Figure 2; reviewed by Hengartner, 1997; Hengartner and Horvitz, 1994).

For example, we have found that the CED-9 protein shows significant similarity to the Bcl-2 family of cell death regulators. Like CED-9 *in C. elegans*, several members of this family, including Bcl-2, Bcl-Xl, and Bcl-W, protect cells from apoptotic death (Reed, 1997). Similarly, CED-3 shows significant sequence similarity to the caspase family of cysteine proteinases (Yuan et al., 1993). Mammalian caspases, like CED-3 in *C. elegans*, are essential positive mediators of apoptosis. Finally, Wang and colleagues recently reported the isolation of a mammalian homolog of CED-4, called Apaf-1 (Zou et al., 1997). Like CED-4 in *C. elegans* (Shaham and Horvitz, 1996; Yuan and Horvitz, 1992), Apaf-1 is a pro-apoptotic protein, and acts upstream of caspases.

The involvement of conserved functional homologs in the process of apoptosis in both *C. elegans* and humans strongly suggest that nematodes and mammals share a common molecular pathway for programmed cell death. If so, then it seems likely that not only CED-3, CED-4, and CED-9, but also the rest of the cell death pathway that has been characterized in *C. elegans* will be conserved through evolution. This common genetic program for cell death presumably predates the evolutionary separation of nematodes and vertebrates and thus seems likely to be of ancient origin. Consistent with this hypothesis, caspases homologs also have been identified in Drosophila (Fraser and Evan, 1997; Song et al., 1997). As is the case in worms and mammals, at least some of these fly caspases are essential for apoptosis, as overexpression of the caspase antagonist p35 prevents cell death during eye development (Hay et al., 1994).

Dissecting the Cell Death Machinery: the Apoptosome

How do CED-3, CED-4, and CED-9 act to regulate cell death in C. elegans? Early genetic studies have suggested that the genetic order of function of the three genes is *ced-9* > *ced-4* > *ced-3*, but gave no hints as to the molecular interpretation of this genetic ordering. However, recent work from a number of groups, including our own, have led to the emergence of a simple model for the control of apoptosis in *C. elegans*.

The first series of experiments, published nearly simultaneously by several groups, revealed that the cell death suppressor CED-9 binds tightly and specifically to the killer protein CED-4 (Chinnaiyan et al., 1997; James et al., 1997; Spector et al., 1997; Wu et al., 1997). This interaction, which can be detected in vitro, in yeast (*both S. cerevisiae* and *S. pombe*), and in mammalian cells, appears to be fairly stable, as co-expression of CED-4 with CED-9 leads to recruitment of the CED-4 protein to the subcellular localization observed for CED-9. CED-9 mutants defective in their ability to prevent apoptosis are also impaired in their ability to interact with CED-4, suggesting that the binding of CED-9 to CED-4 is biologically relevant (James et al., 1997; Spector et al., 1997; Wu et al., 1997).

How does CED-4, once 'freed' from CED-9's clutches, promote apoptosis? Recent work by three groups has demonstrated that CED-4 efficiently promotes the processing of the inactive proCED-3 proenzyme into the active enzyme (Chinnaiyan et al., 1997; Seshagiri and Miller, 1997; Wu et al., 1997). CED-4 exerts this activating effect by directly binding to proCED-3, suggesting that CED-4 might act as a

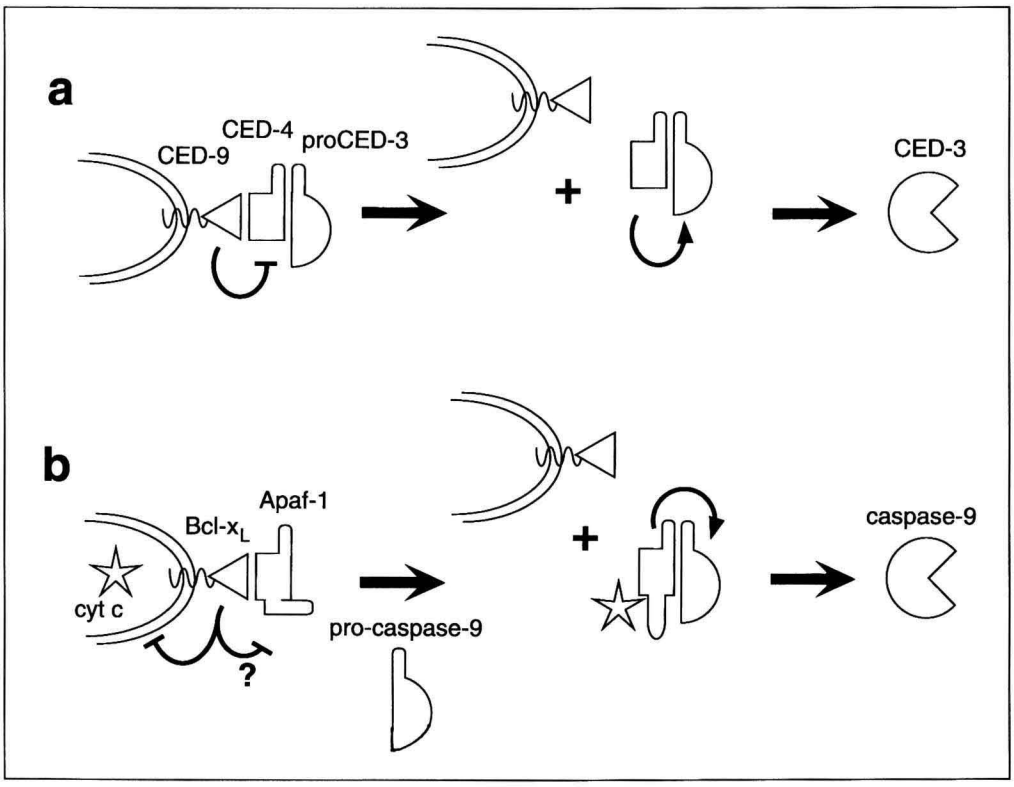

Fig. 3. The *C. elegans* apoptosome: a model for the mechanism of action of the cell death machinery. The cell death regulators CED-3, CED-4, and CED-9 are predicted to be stably associated in a multiprotein complex localized, by analogy with Bcl-2 family members in mammals, to the outer surface of mitochondria. This complex would be expected to be present in all cells, but is inactive. In cells fated to die, a proapoptotic stimulus modifies the complex, possibly resulting in the dissociation of CED-3/4 from CED-9. Once freed, CED-4 allows the CED-3 proenzyme to autoactivate. The active protease then cleaves the relevant apoptotic substrates, bringing on the death of the cell. Adapted from Hengartner (1997).

chaperonin or co-factor in the activation of CED-3. As for the CED-9/CED-4 interaction, the binding of CED-4 to CED-3 is crucial for its ability to promote CED-3 activation: point mutations that inactivate the proapoptotic activity of CED-4 also abolish both interaction with proCED-3 and stimulation of CED-3 activation.

As might be expected from the available genetic data, binding of CED-9 to CED-4 abolishes its ability to promote CED-3 activation (Chinnaiyan et al., 1997; Seshagiri and Miller, 1997; Wu et al., 1997). However, CED-9-bound can still interact with CED-3. Thus, in normal *C. elegans* cells, all three key cell death proteins are likely to be associated together in a multiprotein complex, which has been termed the 'apoptosome', that controls cell death (Figure 3). In cells that are fated to die, a proapoptotic stimulus would be expected to somehow modify one or several components of the apoptosome either through post-translational modification, protein-protein interaction, or modification in protein levels), thereby abolishing CED-9's negative influence on

CED-4. One simple way in which this could occur is by promoting a physical dissociation of CED-4/3 from CED-9 (as shown in Figure 3). However, a simple conformational change might also be sufficient.

Is there a Mammalian Apoptosome?

Since all three components of the *C. elegans* apoptosome have mammalian homologs, it is worth asking whether a similar death complex might exist in mammalian cells. So far, the evidence is rather limited. A direct interaction between Bcl-2 family member Bcl-Xl and CED-4 has been reported, presumably mimicking an interaction with an endogenous CED-4 family member. The nature of this interactor has, however, not yet been determined.

The most obvious similarity is at the level of the CED-4/CED-3 interaction. In a very elegant series of papers, Wang and his colleagues have purified Apaf-1, a mammalian homolog of CED-4, based on its activity to promote the activation of caspase-9 (Apaf-3), a mammalian homolog of CED-3 (Zou et al., 1997). Indeed, Apaf-1 and caspase-9 can interact directly, as do CED-4 and CED-3. However, unlike the situation in *C. elegans*, this interaction requires the presence of another protein, cytochrome c (Apaf-2). While this twist does not invalidate the concept that interaction between CED-4 family members and caspases promotes the autocatalytic activation of the latter, it clearly indicates that we do not yet have the full picture in view, and many more embellishments to the basic model are to be expected.

Conclusion

Genetic studies in *C. elegans* have been very successful in identifying key regulators of the apoptotic machinery. Because this machinery is conserved, we predict that not only *ced-3*, *ced-4*, and *ced-9*, but also all the other cell death genes that have been identified in *C. elegans* will have mammalian homologs that perform similar functions in the control of apoptosis.

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