The Genetics of Programmed Cell Death in the Nematode *Caenorhabditis elegans*

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Cancerous growth often results from an increased rate of cell proliferation caused by the abnormal activation of a signal transduction pathway that normally stimulates cell division only in response to growth factor signals; many of the proto-oncogenes that have been characterized function in or respond to such intercellular signaling pathways (for review, see Cooper 1990). Recent findings indicate that cancerous growth also can result from a decreased rate of cell loss. The most striking example is provided by human B-cell follicular lymphomas. These cancers are often associated with t(14;18) chromosomal translocations that cause the proto-oncogene *bcl-2* (*bcl*, *B* cell *l*ymphoma), normally located on chromosome 18, to be adjacent to and regulated by an enhancer of the immunoglobulin heavy-chain locus, normally located on chromosome 14 (Bakhshi et al. 1985; Cleary and Sklar 1985; Tsujimoto et al. 1985). The resulting overexpression of a normal Bcl-2 protein in the B-cell lineage leads to the cancerous growth of B cells (McDonnell and Korsmeyer 1991). Studies of *bcl-2* have indicated that this gene acts to protect cells from undergoing programmed cell death and that the oncogenic activity of *bcl-2* is a consequence of its allowing B cells that would normally die instead to survive and subsequently proliferate (for review, see Korsmeyer 1992).

Other findings have also associated cancerous growth with the process of programmed cell death. For example, overexpression of the tumor suppressor gene *p53* can trigger programmed cell death (Yonish-Rouach et al. 1991), and, on the basis of studies of *p53*-deficient mice, *p53* is required for at least radiation-induced programmed cell death (Lowe et al. 1993; Clarke et al. 1993). These findings suggest that a loss of *p53* function could contribute to cancerous growth by causing cells that normally die instead to live. The proto-oncogene c-*myc* also can trigger programmed cell death (Evan et al. 1992; Shi et al. 1992). This observation has led to the hypothesis that *myc* functions by preventing cellular differentiation and can cause either proliferation or death, depending on whether appropriate survival factors are or are not present (G. Evan, pers. comm.). These relationships between cancer genes and programmed cell death suggest that an understanding of the mechanisms responsible for programmed cell death could be of central importance to an understanding of human cancer.

To identify and characterize the genes and proteins involved in programmed cell death, we have been analyzing this process in the nematode *Caenorhabditis elegans*. During *C. elegans* development, the generation of the total of 959 non-germ-line nuclei present in the adult hermaphrodite is accompanied by the generation and subsequent death of 131 additional cells (Sulston and Horvitz 1977; Sulston et al. 1983). These cell deaths display certain morphological features characteristic of the apoptotic programmed cell deaths seen in mammals, including cell shrinkage, nuclear condensation, and the phagocytosis of cell corpses while cellular organelles remain intact (Wyllie et al. 1980; Robertson and Thomson 1982). Furthermore, as we discuss below, at least some of the genes that function in *C. elegans* programmed cell deaths have counterparts that function in mammalian cell deaths. These findings suggest that the pathway of programmed cell death has been conserved from nematode to human and that studies of this pathway in *C. elegans* may well facilitate an understanding of the mechanisms that can lead to cancerous growth in humans.

GENETIC PATHWAY FOR PROGRAMMED CELL DEATH

Mutations that affect programmed cell deaths in *C. elegans* have defined a four-step genetic pathway (Fig. 1). Three genes—*ces-1* (*ces*, *c*ell death *s*pecification), *ces-2*, and *egl-1* (*egl*, *e*gg-*l*aying abnormal)—define the first of these steps (Trent et al. 1983; Ellis and Horvitz 1986; Ellis and Horvitz 1991). These genes can mutate to perturb the life-versus-death decision of only a few cells, either causing specific cells that normally die instead to live or causing specific cells that normally live instead to undergo programmed cell death. These three genes appear to regulate in a cell-specific fashion the process of programmed cell death, which involves 10 known genes and is responsible for killing cells, for removing cell corpses, and for degrading the cellular debris of these corpses.

Three genes—*ced-3* (*ced*, *c*ell *d*eath abnormal), *ced-4*, and *ced-9*—can mutate to cause the survival of all 131 cells that normally undergo programmed cell death (Ellis and Horvitz 1986; Hengartner et al. 1992). The surviving or "undead" cells generated when cell death

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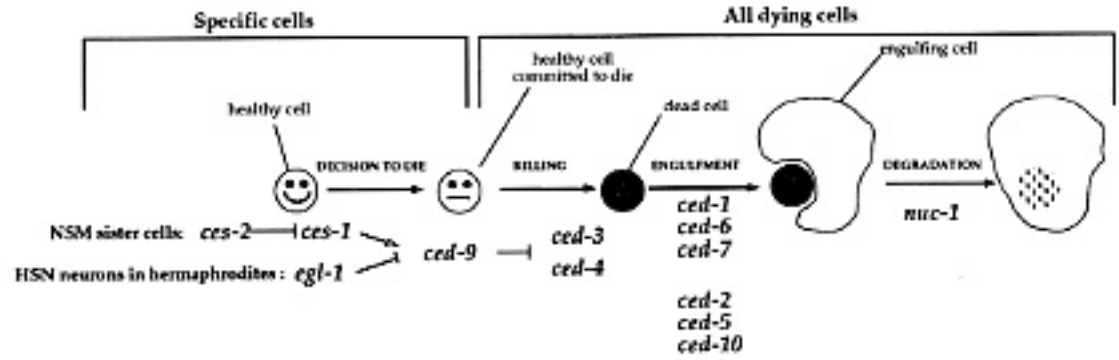

Figure 1. The genetic pathway for programmed cell death in *C. elegans*. See text for details. (Adapted from Ellis et al. 1991b.)

is blocked generally differentiate into recognizable cell types that can be, in at least some cases, capable of functioning (Ellis and Horvitz 1986; Avery and Horvitz 1987; White et al. 1991). These three genes define a killing or execution step of programmed cell death.

Six genes—*ced-1*, *ced-2*, *ced-5*, *ced-6*, *ced-7*, and *ced-10*—can mutate to disrupt the process of phagocytosis that normally acts to remove cell corpses from the body of the developing animal (Hedgecock et al. 1983; Ellis et al. 1991a). These genes define an engulfment step of programmed cell death. One gene—*nuc-1* (*nuc*, *nu*clease abnormal)—can mutate to prevent the degradation of DNA in the cell corpses formed by programmed cell death (Sulston 1976; Hedgecock et al. 1983). *nuc-1* defines a terminal step in programmed cell death in which the cellular debris of cell corpses is degraded. We discuss each step in the pathway of programmed cell death in more detail below.

SPECIFIC GENES REGULATE THE LIFE-VERSUS-DEATH DECISIONS OF SPECIFIC CELLS

The genes *ces-1* and *ces-2* control the decisions of the sister cells of the two serotonergic NSM neurons of the pharynx to undergo programmed cell death (Ellis and Horvitz 1991). Whereas these cells normally die, mutations that result in increased or altered *ces-1* function or reduced *ces-2* function cause the NSM sister cells to survive and differentiate into serotonergic cells with the morphology of the NSM neurons. Genetic analyses have suggested that *ces-1* can act to prevent NSM sister cell deaths, that *ces-2* acts as a negative regulator of *ces-1*, and that both *ces-1* and *ces-2* act genetically upstream of the cell survival gene *ced-9* and the cell killer genes *ced-3* and *ced-4* (see below). Mutations in *ces-1* that prevent the deaths of the NSM sister cells also prevent the programmed deaths of the sisters of the two pharyngeal I2 neurons. Most, and possibly all, other cell deaths appear to occur normally in *ces-1* and *ces-2* mutants, suggesting that these genes control the life-versus-death decision of only one or two cell types. However, the phenotypes that result from a complete loss of *ces-1* or *ces-2* function are unknown, so it is possible that one or both of these genes act more broadly.

Mutations in the gene *egl-1* cause the deaths in hermaphrodites of the serotonergic HSN motor neurons, which innervate the vulval muscles and stimulate egg laying (Trent et al. 1983; Ellis and Horvitz 1986). For this reason, *egl-1* mutants are defective in egg laying. Six *egl-1* alleles have been isolated, and all six have a dominant effect, causing the deaths of the HSN neurons in *egl-1*/+ heterozygotes as well as in *egl-1*/*egl-1* homozygotes. Since in *egl-1*/+ heterozygotes the HSN neurons die, but in *nDf41*/+ heterozygotes, which carry a deficiency that deletes the *egl-1* gene, these cells survive, the existing *egl-1* alleles probably do not simply reduce or eliminate *egl-1* function (M. Hengartner and H.R. Horvitz, unpubl.). How these *egl-1* mutations affect *egl-1* activity and the life-versus-death decision of the HSN neurons remains to be determined.

The functions and cellular specificities of action of *ces-1*, *ces-2*, and *egl-1* are not yet known. Nonetheless, the studies of these genes to date suggest that specific genes regulate the life-versus-death decisions of specific cells and that many more genes of this class may exist, with each such gene controlling the life-versus-death decisions of only a small number of cells.

THE *ced-9* GENE PROTECTS CELLS AGAINST PROGRAMMED CELL DEATH

The gene *ced-9* was initially defined by a gain-of-function mutation, *n1950*, that causes all 131 cells that normally undergo programmed cell death instead to live (Hengartner et al. 1992). Mutations that reduce or eliminate *ced-9* function have the opposite effect, causing cells that normally live instead to undergo programmed cell death. For example, animals homozygous for a *ced-9* loss-of-function (*lf*, *l*oss of *f*unction) allele generated as the progeny of *ced-9(lf)*/+ heterozygous mothers hatch and grow to normal size but lack a variety of cells normally present in wild-type animals; these cells are absent because in *ced-9(lf)*/*ced-9(lf)* homozygous animals they (or their progenitor cells) undergo programmed cell death (Fig. 2a). These *ced-9(lf)*/*ced-9(lf)* homozygous animals generate *ced-9(lf)*/*ced-9(lf)* embryos that arrest development during

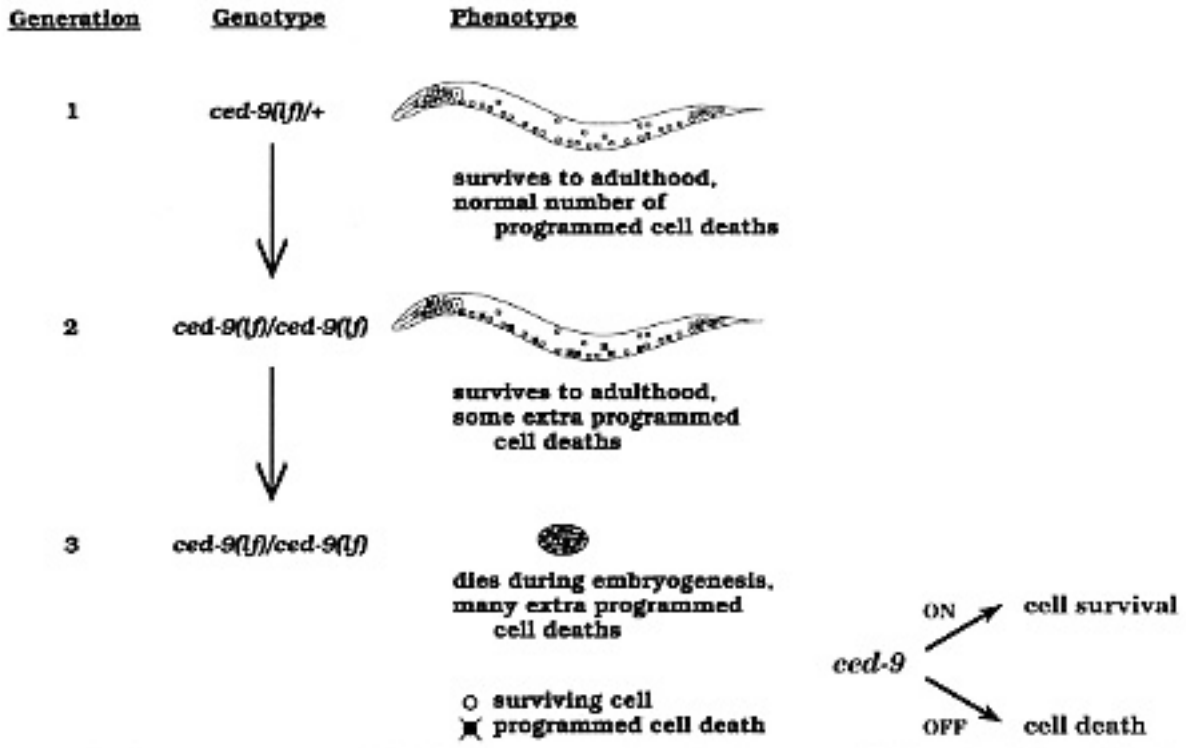

Figure 2. (*Left*) The phenotype of *ced-9(lf)/ced-9(lf)* homozygotes depends on the genotype of their mothers. *lf*, loss-of-function. (*Right*) *ced-9* acts as a binary switch to regulate programmed cell death in *C. elegans* (see text for details). (Reprinted, with permission, from Hengartner et al. 1992.)

embryogenesis; this developmental arrest occurs because large numbers of cells undergo programmed cell death. Thus, in animals homozygous for *ced-9* loss-of-function alleles, cells that normally survive instead undergo programmed cell death, and the extent to which these deaths occur depends on whether the animal is derived from a mother that was heterozygous or homozygous for the *ced-9(lf)* mutation. This maternal effect presumably reflects a contribution by a *ced-9(lf)/+* mother of wild-type *ced-9* activity (in the form of RNA or protein) to a genotypically *ced-9(lf)* oocyte, allowing a *ced-9(lf)/ced-9(lf)* embryo to survive to adulthood; in contrast, a *ced-9(lf)/ced-9(lf)* embryo derived from a *ced-9(lf)/ced-9(lf)* mother would not contain wild-type *ced-9* activity and would die.

The observation that a reduction in *ced-9* function causes cells that should live to undergo programmed cell death indicates that *ced-9* acts to protect cells from programmed cell death. Conversely, overexpression of a wild-type *ced-9* transgene under the control of a *C. elegans* heat-shock promoter can prevent programmed cell deaths (Hengartner and Horvitz 1994a). Together these findings demonstrate that *ced-9* acts as a binary switch gene to regulate programmed cell death in *C. elegans*, causing cells in the developing animal to live when active and to die when inactive (Fig. 2b). Remarkably, it seems that many, and possibly all, of the cells that survive during *C. elegans* development do so because *ced-9* protects them from undergoing programmed cell death.

ced-9 ANTAGONIZES THE ACTIONS OF *ced-3* AND *ced-4*

ced-9, which protects cells from programmed cell death, acts oppositely to *ced-3* and *ced-4*, which cause cells to undergo programmed cell death (see below). *ced-9* might function by preventing the action of either or both of the killer genes *ced-3* and *ced-4*. Conversely, *ced-3* and *ced-4* might cause programmed cell death by preventing the action of *ced-9*. To distinguish between these alternatives (which are not mutually exclusive), Hengartner et al. (1992) constructed double mutants in which both *ced-9* and *ced-3* or *ced-9* and *ced-4* activities were reduced or eliminated. If *ced-9* protected cells by antagonizing *ced-3*, then *ced-9* activity would not be important for cell survival in an animal lacking *ced-3* function, i.e., in a *ced-9; ced-3* double mutant, cells that should die would live, just as they do in the *ced-3* single mutant. If, however, *ced-3* killed by antagonizing *ced-9*, then *ced-3* activity would not affect programmed cell deaths in an animal lacking *ced-9* function; i.e., in a *ced-9; ced-3* double mutant, cells that should live would die, just as they do in the *ced-9* single mutant. These double mutant studies demonstrated that in *ced-9; ced-3* and *ced-4; ced-9* double mutants, all cells—i.e., both those that usually die and those that die when *ced-9* activity is reduced—live, strongly supporting the hypothesis that *ced-9* acts by antagonizing the actions of *ced-3* and *ced-4*.

These gene interaction studies indicate that for *ced-9*

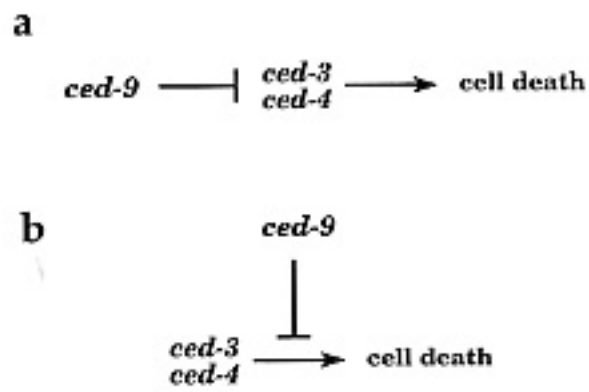

Figure 3. Two models of ways in which *ced-9* might act as a negative regulator of *ced-3* and *ced-4* function. Gene interaction studies indicate that *ced-9* inhibits the function of *ced-3* and/or *ced-4* and could do so either (*a*) by directly preventing the expression or action of *ced-3* and/or *ced-4* or (*b*) by preventing the action of any function that acts in response to *ced-3* and *ced-4* (see text for details). (Based on data from Hengartner et al. 1992.)

activity to have an effect, *ced-3* and *ced-4* must be functional, so that the effect of *ced-9* activity is to decrease the activities of *ced-3* and/or *ced-4*. Thus, *ced-9* functions in a genetic sense as a negative regulator of *ced-3* and *ced-4*. Biochemically, a CED-9 protein could function either before or after the formation of a CED-3 or CED-4 protein; e.g., a CED-9 protein might repress transcriptionally *ced-3* or *ced-4* expression, might modify and thereby inhibit posttranslationally the activity of a CED-3 or CED-4 protein, or might inhibit the activity of any function that acts in response to CED-3 and CED-4 to effect the killing of cells by programmed cell death. The first two of these alternatives are consistent with the model that these three genes act in a linear pathway for programmed cell death, whereas the third requires a branched pathway in which *ced-9* does not act directly on *ced-3* and *ced-4* but rather prevents their effect (Fig. 3).

ced-9 ENCODES A FUNCTIONAL HOMOLOG OF THE PROTO-ONCOGENE *bcl-2*

The cloning of the *ced-9* gene revealed that the inferred 280-amino-acid protein product of *ced-9* is similar in sequence to the protein product of the mammalian proto-oncogene *bcl-2* (Hengartner and Horvitz 1994a). As discussed above, *bcl-2* had been shown to delay or prevent the programmed cell deaths of a variety of cells exposed to a variety of stimuli and, for this reason, was postulated to cause B-cell follicular lymphoma by allowing the survival and subsequent proliferation of B cells in the immune system (for review, see Korsmeyer 1992). The finding that *ced-9*, the normal in vivo function of which is to protect cells from programmed cell death, and *bcl-2* were members of a gene family strongly supported the hypothesis that the normal function of *bcl-2* is to protect cells against programmed cell death.

However, the degree of overall sequence identity between the CED-9 and Bcl-2 proteins is only 23%. Is this similarity meaningful? A number of additional findings also indicate that *ced-9* and *bcl-2* are homologs (Hengartner and Horvitz 1994a). First, the sequence similarity between the protein products of these two genes is highest in those regions that are most conserved, and hence likely to be of the greatest functional significance, among all members of a growing *ced-9/bcl-2* gene family (for review, see Vaux 1994; see below). Similarly, the regions most conserved among *bcl-2* genes from different vertebrate species are also the regions most conserved between the *ced-9* genes of *C. elegans* and of another *Caenorhabditis* species, *C. briggsae*. Second, based on hydrophobicity plots, the CED-9 protein contains a hydrophobic tail like that thought to be important (Alnemri et al. 1992; Tanaka et al. 1992; Hockenbery et al. 1993) for *bcl-2* localization and/or function. Third, the last *ced-9* intron is in precisely the same position of the open reading frame as that of the last intron in many *ced-9/bcl-2* family members.

To determine if *bcl-2* is sufficiently similar to *ced-9* to function in *C. elegans* to protect worm cells against programmed cell death, we expressed a human *bcl-2* transgene in *C. elegans* under the control of a *C. elegans* heat-shock promoter (Hengartner and Horvitz 1994a). These experiments revealed that *bcl-2* can prevent the deaths of *C. elegans* cells that normally undergo programmed cell death. Similar results were obtained by Vaux et al. (1992). We further showed that expression of human *bcl-2* can prevent ectopic cell deaths that occur in *ced-9(lf)* mutants, consistent with the hypothesis that *bcl-2* is capable of substituting for *ced-9* (Hengartner and Horvitz 1994a). It should be noted that *bcl-2* is not the only gene that can act in this way: The baculovirus gene *p35*, an inhibitor of virus-induced programmed cell death in insect cells, also can act in *C. elegans* to protect against programmed cell death and prevent the ectopic cell deaths that occur in *ced-9(lf)* mutants (Sugimoto et al. 1994). *p35* is not an obvious member of the *ced-9/bcl-2* gene family, underscoring the fact that the conclusion that *ced-9* and *bcl-2* are functional homologs depends on the combined structural and functional evidence.

ced-9 is one of a growing number of *C. elegans* genes known to be transcribed as a polycistronic RNA (Hengartner and Horvitz 1994a). The *ced-9* polycistronic RNA also encodes a protein similar to cytochrome b560 of complex II of the mitochondrial respiratory chain (Yu et al. 1992), indicating that the transcription of *ced-9* and this cytochrome gene can be coordinately regulated. In this regard, it is interesting to note that Bcl-2 protein has been found localized to mitochondria (Hockenbery et al. 1990) and has been suggested to function in an antioxidant pathway to prevent cell death by acting at sites of free radical generation, such as mitochondria (Hockenbery et al. 1993; Kane et al. 1993; Veis et al. 1993).

The *bcl-2* mutations that have been associated with

follicular lymphoma are all translocations that result in the overexpression of a normal Bcl-2 protein in B cells (Bakhshi et al. 1985; Cleary and Sklar 1985; Tsujimoto et al. 1985; Cleary et al. 1986; Tsujimoto and Croce 1986). Like these mutations, the *ced-9(n1950)* mutation dominantly suppresses programmed cell death, which suggests, by analogy, that *ced-9(n1950)* may be a chromosomal rearrangement that leads to the overexpression of a normal CED-9 protein. Indeed, overexpression of a normal CED-9 protein can protect cells from programmed cell death (Hengartner and Horvitz 1994a). Sequence analysis, however, revealed that the *ced-9(n1950)* mutation is a missense mutation that causes a substitution of a glutamate for a glycine that is conserved among all *ced-9/bcl-2* gene family members (Hengartner and Horvitz 1994b). This same substitution inactivates rather than activates human *bcl-2* when assayed in both mammalian cells (Yin et al. 1994) and *C. elegans* (Hengartner and Horvitz 1994b), indicating that *ced-9* and *bcl-2* are not completely equivalent. The site of the *n1950* mutation may identify a protein domain that regulates the activities of proteins of the *ced-9/bcl-2* family, leading to *ced-9* activation and *bcl-2* inactivation when appropriately perturbed. The finding that a missense mutation can activate *ced-9* suggests that similar mutations in other members of the *ced-9/bcl-2* family might lead to oncogenic activation. In this context, it is noteworthy that a chicken *bcl-2* cDNA isolated from a B-cell lymphoma contains a valine instead of the glycine found in the genomic sequence at this site (Cazals-Hatem et al. 1992; Eguchi et al. 1992). Perhaps this chicken lymphoma resulted from an activation of *bcl-2* caused by this missense mutation.

bcl-2 is only one of a number of *ced-9/bcl-2* gene family members found in vertebrates (Boise et al. 1993; Kozopas et al. 1993; Lin et al. 1993; Oltvai et al. 1993). In contrast, *ced-9* is the only such family member as yet described for *C. elegans*. Of the vertebrate gene family members, some (e.g., *bcl-2*) can protect against programmed cell death, some (*bax*) can promote programmed cell death, and some (*bcl-x*) encode both death-inhibiting and death-promoting forms (Boise et al. 1993; Oltvai et al. 1993). Genetic analyses of *ced-9* have suggested that *ced-9* also might encode a death-promoting activity as well as its well-characterized death-inhibiting activity (Hengartner and Horvitz 1994b).

THE *ced-3* AND *ced-4* GENES CAUSE CELLS TO UNDERGO PROGRAMMED CELL DEATH

Mutations that reduce or eliminate the functions of the genes *ced-3* or *ced-4* cause cells that normally undergo programmed cell death instead to survive, showing that the activities of both of these genes are needed for the process of programmed cell death (Ellis and Horvitz 1986). Genetic mosaic experiments suggested that *ced-3* and *ced-4* are expressed within cells that will die and act within those cells to cause them to undergo programmed cell death (Yuan and Horvitz 1990). Thus, programmed cell death can be considered, at least to some extent, to be an active process on the part of dying cells. As discussed above, both *ced-3* and *ced-4* act genetically downstream from or in parallel to *ced-9* in the pathway of programmed cell death.

The cloning of *ced-4* (Yuan and Horvitz 1992) and *ced-3* (Yuan et al. 1993) indicated that each encodes a single mRNA that is expressed predominantly during embryogenesis, when 113 of the 131 programmed deaths occur. *ced-3* gene function is not required for *ced-4* gene expression, nor is *ced-4* gene function required for *ced-3* gene expression. Thus, *ced-3* and *ced-4* do not control the onset of programmed cell death by acting sequentially in a transcriptional regulatory cascade.

The sequence of the inferred 549-amino-acid CED-4 protein shows no significant similarities to the sequences of other known proteins (Yuan and Horvitz 1992). Although we previously noted that two regions of the CED-4 protein show some similarity to the EF-hand calcium-binding motif, comparison with a more recent compilation of a larger number of EF-hand sequences (A. Bairoch, pers. comm.) indicates that this similarity is unlikely to be meaningful. Furthermore, relevant residues within these two regions are not conserved in the related *Caenorhabditis* species *C. briggsae* and *C. vulgaris*, and we have mutated by site-directed mutagenesis residues that might be expected to be functionally important within these regions without disrupting *ced-4* function (S. Shaham and H.R. Horvitz, unpubl.). Thus, we do not have any evidence that *ced-4* encodes a calcium-binding protein.

ced-3 ENCODES A PROTEIN SIMILAR TO THE HUMAN CYSTEINE PROTEASE ICE

The sequence of the inferred 503-amino-acid CED-3 protein is similar to that of the human cysteine protease interleukin-1β-converting enzyme, or ICE (Yuan et al. 1993). The CED-3 and ICE proteins are 29% identical over their entire lengths and 43% identical over a 115-amino-acid region that includes a completely conserved pentapeptide (QACRG), which contains the active-site cysteine of ICE. ICE was identified and purified on the basis of its ability to cleave the inactive 31-kD precursor of interleukin-1β (IL-1β) to generate the active 17.5-kD cytokine (Cerretti et al. 1992; Thornberry et al. 1992). ICE was inferred to be a cysteine protease from inhibitor studies. Active human ICE is composed of two subunits (p20 and p10) that appear to be cleaved autoproteolytically from a single 45-kD proenzyme. Both the precursor of IL-1β and the ICE proenzyme are cleaved after aspartate residues, although the precise amino acid sequence that defines a good cleavage target site is not known.

The similarity between CED-3 and ICE strongly suggests that CED-3 functions as a cysteine protease in controlling programmed cell death in *C. elegans*. CED-3 might act either by activating another protein or set of

proteins that cause cells to die or by inactivating a protein or set of proteins that protect cells from death. In the latter case, such a protective protein could not be CED-9 or a protein that acts in response to CED-9, since, as discussed above, genetic studies indicate that *ced-9* acts upstream of or in parallel with rather than downstream from *ced-3*. CED-9 could act by negatively regulating CED-3 activity, for example, either by preventing the proteolytic activation of a pro-form of CED-3 or by inhibiting the activity of a CED-3 protease once it has been generated. CED-4 could be a target of CED-3 or, alternatively, could function to activate CED-3. Targets of CED-3 might include not only proteins that function in causing or preventing programmed cell death, but also proteins that function in later aspects of the process of programmed cell death, such as the phagocytosis of cell corpses (which involves the presumptive proteins CED-1, CED-2, CED-5, CED-6, CED-7, CED-10) or in the degradation of these corpses (which involves the protein NUC-1).

The similarity between CED-3 and ICE also suggests that ICE or a cysteine protease in the CED-3/ICE family might function in controlling programmed cell death in vertebrates. This hypothesis is supported by two additional findings. First, expression of either *ced-3* or ICE in rat fibroblasts can cause these cells to undergo apoptotic cell death (Miura et al. 1993). Thus, this class of cysteine proteases can cause mammalian cells to undergo programmed cell death. Second, *crmA* (*crm*, cytokine response *m*odifier), a protein inhibitor of ICE encoded by a cowpox virus, can block the apoptotic death of chick dorsal root ganglion cells that have been deprived of nerve growth factor (Gagliardini et al. 1994). The finding that an inhibitor of CED-3/ICE-like proteases can block an endogenous pathway of vertebrate cell death indicates that a protease of this class functions in this pathway. Although these observations indicate that a CED-3/ICE-like cysteine protease functions in vertebrate programmed cell death, they do not reveal whether IL-1β is the relevant target of the cysteine protease or whether it is ICE or one or more related cysteine proteases that act in this process.

The CED-3 protein contains a region that is very rich in serines: 32 of the 99 amino acids from residue 107 to residue 205 are serines (Yuan et al. 1993). This region is not highly similar to the corresponding region of ICE, which is 68 amino acids in length, contains 10 serines, and is overall 21% identical to that of CED-3. The functional significance of the serine-rich region in the CED-3 protein is unknown.

SIX GENES AFFECT THE PHAGOCYTOSIS OF CELL CORPSES BY NEIGHBORING CELLS

Cells that undergo programmed cell death in *C. elegans* are engulfed by neighboring cells (Robertson and Thomson 1982; Sulston et al. 1983). Six genes have been identified that have functions needed for this process of engulfment or phagocytosis to occur: *ced-1*, *ced-2*, *ced-5*, *ced-6*, *ced-7*, and *ced-10*, which we will refer to collectively as the engulfment *ced* genes (Hedgecock et al. 1983; Ellis et al. 1991a). (A seventh gene previously thought to be needed for engulfment, *ced-8*, now seems likely not to be involved in this process; G. Stanfield et al., unpubl.) Mutations in each of the engulfment *ced* genes can cause dying cells to remain unengulfed. Some or all of these genes might function in the process of engulfment per se, for example, by encoding cytoskeletal proteins that act in the extension of pseudopodia by the engulfing cell. Alternatively, these genes might function in the initiation of the process of engulfment, for example, by encoding proteins that act in an intercellular signaling system that triggers the engulfing cell to initiate phagocytosis in response to a signal from the dying cell.

Genetic studies suggest that the engulfment *ced* genes may control two parallel and partially redundant processes. These genes appear to define two sets, such that animals with mutations in genes of only one set have relatively few unengulfed cell corpses, whereas animals with mutations in genes of both sets have substantially more unengulfed corpses. One set consists of the genes *ced-1*, *ced-6*, and *ced-7*, and the other set consists of the genes *ced-2*, *ced-5*, and *ced-10*. Thus, for example, *ced-1*, *ced-2*, or *ced-6* single mutant animals have few unengulfed corpses, as do *ced-1; ced-6* double mutant animals, but *ced-1; ced-2* and *ced-6; ced-2* double mutant animals have many unengulfed corpses. One interpretation of such genetic interactions is that each of these sets of genes defines a pathway that can function to cause phagocytosis and that these two pathways are partially redundant, so that disrupting one pathway leaves the other, and hence the process of phagocytosis, mostly intact; only if both pathways are disrupted is phagocytosis blocked.

When the process of phagocytosis is blocked by appropriate double mutant combinations, those cells that normally undergo programmed cell death still die and form morphologically distinct cell corpses. This observation indicates that phagocytosis is not what kills cells during programmed cell death. Rather, phagocytosis is a downstream event that removes cell corpses that have been generated by a process that depends on the activities of *ced-3* and *ced-4*.

A NUCLEASE CONTROLLED BY THE GENE *nuc-1* DEGRADES THE DNA IN CELL CORPSES

The final step in programmed cell death is the removal of the cellular debris that constitutes the cell corpse. To date, only one gene has been identified of the presumably many genes that function in this step. The gene *nuc-1*, which controls the activity of an endodeoxyribonuclease, is required for the degradation of the DNA in dying cells (Sulston 1976; Hedgecock et al. 1983; Hevelone and Hartman 1988). In *nuc-1* mutant

animals, cell death proceeds normally, except that the DNA of dying cells remains in pyknotic bodies. This observation suggests that the processes of killing and engulfment precede and are necessary for the process of DNA degradation controlled by *nuc-1*.

The observation that cell corpses form in *nuc-1* animals reveals that the process of DNA degradation controlled by this gene, like the process of phagocytosis, is not what kills cells during programmed cell death in *C. elegans*. Nuclease activity is also involved in the programmed deaths of cells in other organisms, and a basic issue concerning this activity has been whether it is causally responsible for killing cells (see, e.g., Arends et al. 1990; Oberhammer et al. 1993). If so, and if programmed cell death in *C. elegans* involves a similar mechanism, there must be an as yet unidentified nuclease in the worm that functions in the killing step of programmed cell death and acts to cleave the DNA in dying cells to a state that allows that DNA to remain visible within the pyknotic bodies present in *nuc-1* animals.

THE PATHWAY FOR PROGRAMMED CELL DEATH MAY BE CONSERVED FROM NEMATODE TO HUMAN

As discussed above, of the three genes that act in the killing step of programmed cell death in *C. elegans*, at least two—*ced-9* and *ced-3*—have counterparts that act comparably in vertebrates. Specifically, *ced-9* and the human gene *bcl-2* both act to protect cells against programmed cell death and are members of a gene family, and *ced-3* and the mammalian gene that encodes the cysteine protease ICE both can act to cause programmed cell death and are also members of a gene family. Furthermore, the human *bcl-2* gene can act in *C. elegans* to prevent worm cells from undergoing programmed cell death and can even substitute for *ced-9* in mutant worms deficient in *ced-9* function. Similarly, the *C. elegans ced-3* gene can act in mammalian cells to cause them to undergo programmed cell death. That *bcl-2* can act in worms and *ced-3* can act in mammals indicates that the pathways in which these genes function are highly similar in these different organisms. In short, these observations strongly support the hypothesis that the pathway for programmed cell death is conserved from nematode to human.

CELL DEATH GENES MAY DEFINE NEW CLASSES OF PROTO-ONCOGENES AND TUMOR SUPPRESSOR GENES

That *bcl-2* acts as a proto-oncogene by suppressing programmed cell death suggests that other genes that function in the process of programmed cell death could also be responsible for human cancers. For example, mutations in human genes that act like mutations in the *ces* genes and prevent the deaths of specific classes of cells could lead to the uncontrolled proliferation of those cell types. Similarly, mutations that eliminate the activities of cysteine proteases of the CED-3/ICE family or of proteins similar to the *C. elegans* CED-4 protein could prevent the normal process of programmed cell death and thereby promote malignancy, just as does overexpression of *bcl-2*. Thus, the CED-3/ICE family and CED-4 may define new classes of tumor suppressor genes. If indeed cell death genes define new classes of proto-oncogenes and tumor suppressor genes, one could seek as anticancer agents antagonists of genes that function to prevent programmed cell death, such as members of the *ced-9/bcl-2* family, as well as agonists of genes that function to promote programmed cell death, such as members of the *ced-3*/ICE family or *ced-4*. In this way, studies of the genetics of programmed cell death in *C. elegans* could lead to the identification of both drug targets and therapeutic agents that might be important for the development of novel methods for cancer therapy.

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