Journal of Cellular Biochemistry 60:4–11 (1996)

PROSPECTS

Evolutionary Conservation of a Genetic Pathway of Programmed Cell Death

Junying Yuan

Cardiovascular Research Center, Massachusetts General Hospital, Charlestown, Massachusetts 02129; Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115

Abstract Genetic analysis of programmed cell death in *Caenorhabditis elegans* has led to the identification of 13 genes that constitute a developmental pathway of programmed cell death. Two of the three key genes in this pathway, *ced-9*, a cell death suppressor, and *ced-3*, a cell death inducer, were found to encode proteins that share structural and functional similarities with the mammalian proto-oncogene product Bcl-2 and interleukin-1β converting enzyme, respectively. These results suggest that the genetic pathway of programmed cell death may be evolutionarily conserved from worms to mammals. © 1996 Wiley-Liss, Inc.

The phenomenon of cell death is common during the development of both vertebrate and invertebrate animals [Ellis et al., 1991b]. The regulation of this process is best understood in the nematode *Caenothabditis elegans*. By isolating mutants that are defective in various aspects of programmed cell death process in *C. elegans*, a genetic pathway of programmed cell death has been established [Ellis and Horvitz, 1986; Hengartner et al., 1992; Ellis et al., 1991a; Hedgecock et al., 1983; Sulston, 1976]. Recent results from a number of laboratories [Vaux et al., 1992; Yuan et al., 1993; Miura et al., 1993; Gagliardini et al., 1994; Hengartner and Horvitz, 1994] have revealed that key genes in controlling programmed cell death in *C. elegans* are evolutionarily conserved in mammals and may play a similar role in controling programmed cell death during mammalian development. These discoveries are very exciting because what we know about programmed cell death in *C. elegans* may help us understand the mechanism of programmed cell death in vertebrate animals directly.

Among the 1,090 cells generated during *C. elegans* hermaphrodite development, there are 131 cells undergoing programmed cell death [Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston et al., 1983]. Programmed cell death is a true cell differentiating fate in *C. elegans:* the timing and identities of the cells that undergo programmed cell death are invariant among individual worms. Since the body of *C. elegans* is transparent during embryonic and larval development, it is possible to directly observe cells in the process of undergoing programmed cell death [Sulston and Horvitz, 1977]. Using Nomarski optics, the nuclei of dying cells can be seen as highly refractile round discs. Dying cells disintegrate within an hour or so and are quickly engulfed by neighboring cells. Electron microscopic studies of dying cells in *C. elegans* showed that *C. elegans* cell death exhibits many of the typical features of vertebrate apoptotic cell death [Robertson and Thomson, 1982]. When a *C. elegans* cell dies, its cytoplasm and nucleus condense, and the dying cell fragment into several pieces. Also similar to vertebrate cell death, dead cells are engulfed quickly by neighboring cells in *C. elegans*. Because of the easy accessibility of *C. elegans* to genetic analysis, it was possible to identify a wide variety of mutants that affect different aspects of this process [Ellis et al., 1991b]. Genetic analyses of these mutants have defined a developmental pathway of programmed cell death.

DETERMINATION OF CELL DEATH

A dominant gain-of-function mutation in the gene *ced-9* suppresses all normal programmed

Received October 2, 1995; accepted October 4, 1995.

Address reprint requests to Junying Yuan, Cardiovascular Research Center, Massachusetts General Hospital, 149 13th Street, 4th floor, Charlestown, MA 02129.

© 1996 Wiley-Liss, Inc.

cell death, while recessive loss-of-function mutations in *ced-9* cause many normally living cells to undergo programmed cell death and are lethal to *C. elegans* [Hengartner et al., 1992]. The hypothesis is that in living cells the protein product of *ced-9* acts as a gatekeeper to prevent cells from activating the cell death program. Since *ced-9* function is both sufficient (as shown by the dominant gain-of-function mutation) and necessary (as shown by the recessive loss-of-function mutations) to prevent programmed cell death, *ced-9* may act as a binary switch to control programmed cell death in *C. elegans*.

If the above hypothesis is correct, *ced-9* inactivation will be necessary for cells to undergo programmed cell death. One possibility is that the activity of *ced-9* may be inactivated in cells undergoing programmed cell death by genes that affect the decisions of a small set of cells to live or to die. In wild-type animals, the sister cells of the pharyngeal NSM motor neurons undergo programmed cell death. Two genes, *ces-1* and *ces-2* (*ce*ll death *s*pecification), specifically affect this decision [Ellis and Horvitz, 1991]. Dominant gain-of-function mutations in *ces-1* or recessive loss-of-function mutations in *ces-2* lead to the survival of the NSM sisters. Genetic analysis of *ces-1* and *ces-2* suggests that *ces-2* is a negative regulator of *ces-1,* itself a positive regulator of *ced-9.* Thus, it is possible that in determining cell death of the NSM sisters, *ces-2* inactivates *ced-9* through inhibiting *ces-1. ces-1* and *ces-2* are two of a few genes isolated so far that act in a small subset of cells controlling the determination step of cell death. One may expect that many more genes of this kind may exist that control the determination of cell death in other cells during *C. elegans* development.

Cloning and molecular characterization of the *ced-9* gene showed that *ced-9* encodes a 280-amino acid protein containing sequence and structural similarities to the mammalian proto-oncogene *bcl-2* [Hengartner and Horvitz, 1994]. Overexpression of *bcl-2* in *C. elegans* under the control of a heat-shock promoter can prevent normal programmed cell death, as well as the ectopic cell deaths that occur in *ced-9* loss-of-function mutants [Vaux et al., 1992; Hengartner and Horvitz, 1994]. The amino acid sequence of Ced-9 is 23% identical to that of Bcl-2 throughout their sequences [Hengartner et al., 1994]. The *ced-9(n1950)* gain-of-function mutation results in a glycine-to-glutamate substitution in a region highly conserved in other members of the *ced-9/bcl-2* family [Hengartner and Horvitz, 1994]. Thus, this substitution resulted in activation of *ced-9* function in *C. elegans.* However, the same point mutation in Bcl-2 protein eliminated the ability of Bcl-2 to prevent cell death in *C. elegans,* as well as in 2B4 cells, in which this mutation may even have some dominant negative effect [Yin et al., 1994]. Thus, this substitution in Bcl-2 is a loss-of-function mutation rather than gain-of-function mutation, suggesting that CED-9 and Bcl-2 are not completely functionally equivalent.

The *bcl-2* gene was cloned from the most common chromosomal translocation (t(14,18)(q321q21) in human lymphoid malignancies of follicular B-cell lymphoma [Tsujimoto et al., 1985; Bakhshi et al., 1985; Cleary et al., 1986]. The (t(14,18)(q321q21)) translocation fused the 5′ half of the *bcl-2* transcript, which contains all of the *bcl-2* coding region, to a immunoglobulin heavy chain transcript. This translocation results in an abnormal activation of *bcl-2* expression, which promotes oncogenesis [Seto et al., 1988]. Overexpression of *bcl-2* prevented certain cytokine-dependent cell lines from undergoing cell death induced by the withdrawal of interleukin-3 (IL-3), granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-4 [Vaux et al., 1988; Nunez et al., 1990; Hockenbery et al., 1990]. However, cell survival enhancing activity of *bcl-2* is not universal, as neither IL-2-dependent nor IL-6-dependent cell death can be prevented by *bcl-2.* In normal thymus, *bcl-2* is expressed in mature T cells of medulla [Hockenbery et al., 1991]. In transgenic animals in which *bcl-2* is expressed ectopically in immature cortical thymocytes, *bcl-2* protected these immature thymocytes from glucocorticoid, radiation, and anti-CD3-induced apoptosis [Sentman et al., 1991; Strasser et al., 1991]. However, clonal deletion of T cells that recognize endogenous superantigens still occurs, suggesting that negative selection is not *bcl-2* dependent. Subsequent studies have shown that overexpression of *bcl-2* can protect certain vertebrate cells from an amazing list of insults: sodium azide, glucocorticoids, phorbol ester, methotrexate, heat shock, ethanol, radiation, antimetabolite 1-β-D-arabinofuranosylcytosine, calcium ionophore, vincristine, glucose withdrawl, membrane peroxidation, free radical-induced damage, and tumor necrosis factor-mediated cytotoxicity [Zhong et al., 1993; Hennet et al., 1993; Henderson et al., 1991; Miyashita

and Reed, 1992; Alnemri et al., 1992; Tsujimoto, 1989].

The function of Bcl-2 in mammals is not completely equivalent to that of Ced-9 in *C. elegans*. Homozygous *ced-9* loss-of-function (lf) mutants derived from ced-9(lf)/+ heterozygous mothers showed ectopic cell death, nevertheless grew to normal size, but generated very few eggs, all of which eventually die during embryonic development [Hengartner et al., 1992]. At least part of the reason that such homozygous *ced-9(lf)* mutants can develop is due to the maternal contribution of wild-type Ced-9. The *bcl-2* loss-of-function mutant mice complete normal embryonic development, but exhibit growth retardation and early mortality [Veis et al., 1993]. This is most likely due to redundancy in the mammalian system: additional members of the *bcl-2* family can substitute some functions of *bcl-2*. *ced-9* and *bcl-2* belong to the same family of genes that can act to prevent cell death. However, so far there is no report to indicate that *ced-9* can substitute *bcl-2* and function in vertebrates. These results suggest again that *ced-9* and *bcl-2* are members of the same gene family but are not equivalent.

EXECUTION OF CELL DEATH

The activities of two genes, *ced-3* and *ced-4*, are essential for cells to undergo programmed cell death in *C. elegans* [Ellis and Horvitz, 1986]. Recessive loss-of-function mutations in *ced-3* or *ced-4* prevent all 131 cells from undergoing programmed cell death during *C. elegans* hermaphrodite development. Such mutations can also block the excess cell death and lethality in *ced-9* loss-of-function mutant animals, indicating that *ced-3* and *ced-4* are genetically downstream from *ced-9* [Hengartner et al., 1992]. Genetic mosaic analysis revealed that both genes are most likely to act cell-autonomously within cells to cause cell death [Yuan and Horvitz, 1990]. Thus, the *ced-3* and *ced-4* proteins may be part of the machinery that carry out the execution of the cell death program within dying cells. These results suggest that cells possess a mechanism whose sole function may be to commit suicide if such circumstance is called for.

The functions of both *ced-3* and *ced-4* are essential for cells to undergo programmed cell death in *C. elegans:* mutations in either gene alone are sufficient to eliminate all programmed cell deaths. Thus, the functions of these two genes are not redundant. The genetics of *ced-3* and *ced-4* are slightly different: loss-of-function mutations in *ced-4* are always completely recessive, whereas some mutations in *ced-3* show semidominant phenotype [Ellis and Horvitz, 1986]. Molecular analyses of *ced-3* and *ced-4* (see below) revealed the probable reason behind this difference: all *ced-4* mutations analysed result in complete loss of *ced-4* mRNA transcript [Yuan and Horvitz, 1992], whereas many *ced-3* mutations are of the missense type [Yuan et al., 1993]. Recent genetic evidence suggests that *ced-4* acts upstream of *ced-3* and may be a positive regulator of *ced-3* (S. Shaham and H.R. Horvitz, personal communication).

The *ced-4* gene has been cloned [Yuan and Horvitz, 1992]. The *ced-4* transcript is expressed primarily during embryogenesis, when most cell deaths occur, consistent with the proposed role of *ced-4* in promoting cell death. The 549-amino acid sequence of Ced-4 protein, deduced from cDNA and genomic clones, contain two regions that are similar to the calcium-binding domain known as the EF-hand [Kretsinger, 1987], suggesting that *ced-4* activity and hence, programmed cell death in *C. elegans* may be regulated by calcium. However, in vitro studies have failed to show that Ced-4 protein can bind calcium [Yuan, unpublished data]; thus, even if Ced-4 protein binds calcium in vivo, its affinity is probably low.

The *ced-3* gene has also been cloned [Yuan et al., 1993]. Like *ced-4, ced-3* is also expressed primarily during embryonic development. The Ced-3 protein is 503 amino acids in length and contains a serine-rich middle region of about 100 amino acids. Comparison of the sequence of the Ced-3 protein with the inferred Ced-3 protein sequences from the related nematode species, *C. briggsae* and *C. vulgaris,* revealed that the non-serine-rich region is highly conserved and that the serine-rich region is more variable. The non-serine-rich portions of the Ced-3 protein are similar to human interleukin-1β (IL-1β) converting enzyme (ICE), a cysteine protease that can cleave the inactive 31-kD precursor of IL-1β to generate the active cytokine [Cerretti et al., 1992; Thornberry et al., 1992]. Thus, Ced-3 may act as a cysteine protease in controlling the onset of programmed cell death in *C. elegans.*

Interleukin 1β (IL-1β) is a 17.5-kD polypeptide secreted by monocytes in response to a variety of inflammatory stimuli [Dinarello, 1991; diGiovine and Duff, 1990]. The 17.5-kD mature

Il-1β is cleaved from a 31-kD inactive precursor form. Interleukin-1β converting enzyme (ICE), a cysteine protease, was identified and isolated from a human monocytic cell line that cleaves pIL-1β at Asp^{116}-Ala^{117} to generate mature IL-1β [Kostura et al., 1989; Thornberry et al., 1992; Cerretti et al., 1992]. The amino acid sequence of ICE is 29% identical to that of Ced-3 [Yuan et al., 1993]. The carboxyl half of the Ced-3 protein is the region most similar to ICE. A stretch of 115 residues (amino acids 246–360 of Ced-3) are 43% identical between the Ced-3 and human ICE proteins. This region contains a conserved pentapeptide QACRG (positions 361–365 of the Ced-3 protein), which surrounds a cysteine known to be essential for ICE function. Specific modification of this cysteine in human ICE results in complete loss of activity [Thornberry et al., 1992]. The *ced-3* mutation *n2433* alters the conserved glycine in this pentapeptide and eliminates *ced-3* function, suggesting that this glycine is important for *ced-3* activity and might be an integral part of the active site of ICE. The 5 out of 8 *ced-3* mutations identified altered single amino acids conserved between ICE and Ced-3. Three other mutant alleles of *ced-3* changed amino acids at the very C-terminal of Ced-3 that are not conserved in ICE. The ICE precursor (p45) can be proteolytically cleaved at four sites (Asp^{103}, Asp^{119}, Asp^{297}, and Asp^{316}) to generate two peptides (p20 and p10) necessary for in vitro activity [Thornberry et al., 1992] and three other peptides whose functions remain to be determined. At least two of these cleavage sites are conserved in Ced-3, indicating that the Ced-3 protein might be processed as well.

The similarity between Ced-3 and ICE suggests not only that Ced-3 might function as a cysteine protease but also that ICE might function in programmed cell death in vertebrates. Consistent with this hypothesis is the finding that when murine peritoneal macrophages, after being stimulated with lipopolysaccharide (LPS), were induced to undergo programmed cell death by exposure to extracellular ATP, mature active IL-1β was released into the culture supernatant. However, when cells were injured by scraping, IL-1β was released exclusively as the inactive preform [Hogoquist et al., 1991]. These results suggest that ICE is activated upon induction of programmed cell death.

To be qualified as a bona fide cell death gene, a gene must satisfy three criteria. The first criterion of a cell death gene is that its expression must be able to induce programmed cell death. Since expression of a cell death gene will cause cells to die, no stable cell lines can be established that overexpress the gene. Thus, one must either use an inducible system or a transient assay. Since it can be rather time consuming to establish cell lines using an inducible promoter even when it is working, it is advisable to establish a transient system whenever possible. Miura et al. [1993] used *Escherichia coli lacZ* gene as an indicator of gene expression and constructed fusion of the *mICE* cDNA with the *E. coli lacZ* gene and placed the fused gene under the control of the chicken β-actin promoter. Such constructs were transiently transfected into Rat-1 fibroblast cells and stained for X-gal 24 h later. Overexpression of the murine *Ice* (*mICE*) gene caused Rat-1 cells to undergo programmed cell death efficiently. Point mutations in a region homologous between mICE and CED-3 or truncated *mICE* constructs eliminate the ability of mICE to cause cell death. Cell death caused by overexpression of ICE showed fragmented nuclei, a typical feature of apoptosis. The cell death caused by *mICE* can be suppressed by overexpression of the *crmA* gene, a cowpox virus gene that is a specific inhibitor of ICE [Ray et al., 1992], as well as by *bcl-2*. These experiments showed that ICE can satisfy the first criterion for a cell death gene. These experiments did not show, however, that ICE is indeed responsible for cell death in vivo. Miura et al. [1993] also showed that a similar construct of *ced-3* with *lacZ* fusion also induced Rat-1 cells to die but it is not as efficient as ICE. *bcl-2* and *crmA* can only weakly suppress the cell death induced by *ced-3*. These results suggest that the abilities of *bcl-2* and *crmA* to interfere with *ced-3*-induced cell death are rather limited.

If ICE or its close relative is indeed involved in programmed cell death in vivo, it should be able to satisfy the second criterion of a cell death gene: specific inhibitors of the gene should be able to inhibit cell death in vivo. Among all the available ICE inhibitors, the *crmA* gene of cowpox virus is the best choice [Ray et al., 1992]. Throughout evolution, viruses have developed various mechanisms to prolong their survival. Two of the important mechanisms appear to be anti-inflammatory and anti-apoptotic: both defenses are targeted to eliminate viral infected cells. The *crmA* gene of cowpox virus appears to be able to achieve both goals with one gene. During the development of dorsal root ganglia,

about one-half of the initially generated neurons die. Survival of sensory neurons during the development of vertebrate nervous system is dependent on neurotrophic factors produced by their peripheral and central target fields [Davies, 1987]. Nerve growth factor (NGF) is required for the survival of embryonic chicken dorsal root ganglion neurons both in vitro and in vivo. The removal of NGF in vitro mimics the physiological situation in which neurons die during development or the experimental conditions in which neurons are prevented from innervating their normal targets. An expressing vector of *crmA* was microinjected into DRG neurons cultured in the absence of trophic factors [Gagliardini et al., 1994]. In control conditions (neurons injected with dye alone or vector alone or with the antisense *crmA* cDNA expression vector), more than 80% of neurons die 3 days after trophic factor deprivation, and by 6 days almost all neurons have disappeared. In the presence of trophic factors (NGF and serum), 85% of DRG neurons survived through day 6. The introduction of the *crmA* gene into DRG neurons prevented neuronal death induced by trophic factor deprivation: more than 60% of the *crmA*-injected neurons survived through day 6 in the absence of NGF. This remarkable result suggests that ICE or its close relative is involved in controling neuronal cell death. However, the data do not pinpoint which *Ice* molecule is actually involved, since it is possible that another closely related *Ice* or *Ice*(*s*) are inhibited by CrmA. Indeed, an additional member of *Ice* family has been identified [Kumar et al., 1992, 1994; Wang et al., 1994]. *Nedd-2* (*N*PC *e*xpressed, developmentally down-regulated gene) was cloned by subtraction screening of mouse neural precursor cell (NPC) cDNA library. *Nedd-2* gene is highly expressed in developing central nervous system and other tissues, including kidney. In adult mice, expression levels of *Nedd-2* is lower than that in the embryo but is detectable in all tissues. *Ich-1* (*I*ce and *c*ed-3 *h*omolog) was isolated from a human fetal cDNA library by screening with the C-terminal part of *Nedd-2* coding region as a probe. Structural and functional analysis revealed that *Nedd-2* and *Ich-1* are the same gene. Ich-1/Nedd-2 protein is similar to both ICE and Ced-3 (approximately 28% identity). *Ich-1* mRNA is alternatively spliced into two different forms. One mRNA species encodes a protein product of 435 amino acids, named ICH-1_L, which is homologous to both the P20 and P10 subunits of ICE, as well as the entire Ced-3 protein. The other mRNA encodes a 312-amino acid truncated version of the Ich-1_L protein, named Ich-1_S, that terminates 21-amino acid residues after the pentapeptide QACRG of Ich-1_L. *Ich-1_L* and *Ich-1_S* have opposite functions: overexpression of *Ich-1_L* induces apoptosis, while overexpression of the *Ich-1_S* suppresses Rat-1 cell death induced by serum deprivation. These results suggest that *Ich-1* may play important roles in both positive and negative regulation of programmed cell death. Overexpression of *Ich-1_L* induces programmed cell death in HeLa, Rat-1, and NG108-15, but not in COS cells. *Ich-1_L*-induced cell death can be suppressed effectively by *bcl-2* but only weakly by *crmA*. This result did not rule out, however, that *Ich-1_L* may play a role in controlling DRG neuronal cell death in the experiment mentioned above since the ratio of *crmA* and *Ice*(s) may be important for an effective *crmA* inhibition: in the microinjection experiment, the ratio of *crmA*/*Ice*(s) is likely to be high, whereas in transient assays, the ratio of *crmA*/*Ich-1_L* is low.

The third criterion for a cell death gene is that it should be activated naturally when cells are undergoing programmed cell death. Indication for ICE activation was discovered before *Ice* was identified as a possible cell death gene. As mentioned above, when murine peritoneal macrophages, after being stimulated with lipopolysaccharide (LPS), were induced to undergo programmed cell death by exposure to extracellular ATP, mature active IL-1β was released into the culture supernatant. However, when cells were injured by scraping, IL-1β was released exclusively as the inactive preform [Hogoquist et al., 1991]. This is, however, indirect evidence for ICE activation: since ICE is the enzyme identified responsible for most of IL-1β processing, ICE is likely to be activated in these macrophages.

Having said that ICE satisfies the three essential conditions for a cell death gene, can we state that ICE must be involved in controlling programmed cell death in vertebrates? The answer is far from clear. All three conditions are necessary but not sufficient. If homozygous Ice knock-out mice show defects in cell death, it will be direct evidence that *Ice* itself is indeed the key player of this process. However, if homozygous *Ice* knock-out mice show no defects in cell death, it does not rule out that *Ice* itself is one of the key players of this process: it is still possible that

multiple members of *Ice* family all contribute to the regulation.

Overexpression of *bcl-2* can also prevent DRG neuronal cell death. Of the DRG neurons initially injected with *bcl-2,* 57% survived the NGF-deprived condition through day 6 [Gagliardini et al., 1994]. Thus, the ability of *crmA* to prevent the death of DRG neurons is about the same as that of *bcl-2.* Both *bcl-2* and *crmA* can prevent cell death. If they act through separate pathways, their ability to prevent the death of DRG neurons in the absence of NGF should be additive. Injection of both *bcl-2* and *crmA* expressing vectors into DRG neurons did not increase the survival rate more than either *crmA* or *bcl-2* injection alone (66.6%). Thus, *crmA* and *bcl-2* are likely to act through the same pathway.

A model of genetic cascade for neuronal cell death can be proposed from these data: during neural development, the presence of trophic factors may promote the activity of BCL-2 (and/or its relative) and thereby prevent cell death, while the absence of trophic factors may lead to the inactivation of BCL-2 (and/or its relative) and consequently activation of genes in the ICE/CED-3 family, which in turn cause programmed cell death. Consistent with this hypothesis, expression of Bcl-2 has been detected in postmitotic neurons, whose survival is dependent on the presence of neural trophic factors [Merry et al., 1994], although the relationship between *bcl-2* and neural trophic factors remains to be examined.

ENGULFMENT OF DEAD CELLS

Dead cells are quickly engulfed by neighboring cells in *C. elegans* [Sulston and Horvitz, 1977]. Seven genes (*ced-1, ced-2, ced-5, ced-6, ced-7,* and *ced-10*) have been identified as important for the efficient removal of dead cells by engulfment cells (usually neighboring hypodermal cells) in *C. elegans* [Ellis et al., 1991a; Hedgecock et al., 1983]. Mutations in these genes do not prevent normal programmed cell death, but block the efficient removal of dead cells by engulfment cells. Mutations in any one of these genes prevent some of the dead cells from being engulfed, but many other dead cells are still engulfed properly, suggesting redundancy in engulfment genes. Genetic analysis of double mutant combinations led to the conclusion that these genes can be divided into two groups, which may be involved in two distinct but partially redundant processes that control engulfment. Presumably, one of these groups could encode the signals on dead cells to allow for the recognition, or the receptors on engulfment cells to recognize such signals or the actual engulfment steps in the engulfment cells. These possibilities can be distinguished by genetic mosaic analysis: in the first possibility, the genes will be required in the dying cells for engulfment to occur; in the latter two possibilities, the genes will be required in the engulfment cells.

During embryonic development of vertebrates, dead cells are also quickly engulfed [Dawd and Hinchliffe, 1971]. Antibodies have been isolated that specifically recognize the engulfment cells in chick embryos [Rotello et al., 1994; Fernandez et al., 1994]. The staining pattern of these engulfment cells resembled the staining pattern of an antibody that recognizes chick common leukocyte antigen, suggesting that the engulfment cells may be of common leukocyte origin [Rotello et al., 1994]. Almost all apoptotic cells were found within engulfment cells, indicating that like in *C. elegans,* the engulfment process is very efficient.

DEGRADATION OF DEAD CELLS

The *nuc-1* gene (*nuc*lease deficient) encodes a major DNAase that is used to digest the DNA of dead cells in the engulfment cells as well as the DNA of the bacteria on which *C. elegans* feeds [Sulston, 1976]. Mutations in *nuc-1* do not affect the cell death nor the engulfment process but result in the persistence of DNA of dead cells in engulfment cells. The *nuc-1* nuclease does not require divalent cations for activity. It has not been tested if the DNA of dead cells in *C. elegans* is also cleaved into 180-bp DNA oligomers as it is in vertebrates.

The cleavage of DNA into 180-bp DNA ladders was first discovered in thymocytes induced to die by glucocorticoid [Wyllie, 1980]. Now formation of a DNA ladder has become a standard criterion for apoptosis. However, DNA ladder formation is more likely to be an accessary step of cleaning up the debri of dead cells than an essential step of apoptosis.

FUTURE PROSPECTS

The structural and functional similarities of Ced-9 and Bcl-2 and of Ced-3 and ICE suggest that there may be a common molecular pathway

of programmed cell death in all metazoans. However, the picture in mammals is much more complex: instead of one *ced-3* and one *ced-9*, there are already many *bcl-2*-related genes and likely to be many *Ice*-related genes. Further experiments are needed to determine whether they all control programmed cell death or may have other functions as well. One may expect that additional mammalian programmed cell death genes will be identified that share similarities with other *C. elegans* cell death genes. Isolation of these mammalian genes may allow a better biochemical analysis of the programmed cell death pathway because it is often easier to do biochemistry in mammalian systems than that in *C. elegans*. Such studies in turn may increase our understanding of programmed cell death in *C. elegans*.

ACKNOWLEDGMENTS

I thank the members of my laboratory for stimulating discussions and Robert Friedlander for comments on the manuscript. This work is supported in part by grants to J.Y. from Bristol Myers-Squibb and from the National Institute of Aging.

REFERENCES

Alnemri ES, Fernandes TF, Haldar S, Croce CM, Litwack G (1992): Involvement of BCL-2 in glucocorticoid-induced apoptosis of human pre-B-leukemias. Cancer Res 52:491–495.

Bakhshi A, Jensen JP, Goldman P, et al (1985): Cloning the chromosomal breakpoint of t(14;18) human lymphomas: Clustering around J_H on chromosome 14 and near a transcriptional unit of 18. Cell 41:899–906.

Cerretti DP, Kozlosky CJ, Mosley B, et al (1992): Molecular cloning of the interleukin-1 beta converting enzyme. Science 256:97–100.

Cleary ML, Smith SD, Sklar J (1986): Cloning and structural analysis of cDNAs for bcl-2 and hybrid bcl-2/immunoglobulin transcript resulting from the t(14;18) translocation. Cell 47:19–28.

Davies AM (1987): Molecular and cellular aspects of patterning sensory. Development 100:185–208.

Dawd DS, Hinchliffe JR (1971): Cell death in the "opaque patch" in the central mesenchyme of the developing chick limb: A cytological, cytochemical and electron microscope analysis. J Embryol Exp Morphol 26:401–424.

diGiovine FS, Duff GW (1990): Interleukin 1: The first interleukin. Immunol Today 11:13.

Dinarello CA (1991): Interleukin-1 and interleukin-1 antagonism. Blood 77:1627–1652.

Ellis HM, and Horvitz HR (1986): Genetic control of programmed cell death in the nematode *Caenorhabditis elegans*. Cell 44:817–829.

Ellis, RE, and Horvitz, HR (1991): Two *C. elegans* genes control the programmed deaths of specific cells in the pharynx. Development 112:591–603.

Ellis RE, Jacobson DM, Horvitz HR (1991a): Genes required for the engulfment of cell corpses during programmed cell death in *Caenorhabditis elegans*. Genetics 129:79–94.

Ellis RE, Yuan J, and Horvitz HR (1991b): Mechanisms and functions of cell death. Annu. Rev. Cell Biol. 7:663–698.

Gagliardini V, Fernandez P-A, Lee RKK, Drexler HCA, Rotello RJ, Fishman M, Yuan J (1994): Prevention of vertebrate neuronal death by the *crmA* gene. Science 263:826–828.

Hedgecock EM, Sulston JE, Thomson JN (1983): Mutations affecting programmed cell death in the nematode *Caenorhabditis elegans*. Science 220:1277–1279.

Henderson S, Rowe M, Gregory C, et al (1991): Induction of bcl-2 expression by epstein-Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. Cell 65:1107–1115.

Hengartner M, Horvitz HR (1994): *C. elegans* cell survival gene ced-9 encodes a functional homolog of the mammalian proto-oncogene bcl-2. Cell 76:665–676.

Hengartner M, Ellis RE, Horvitz HR (1992): *Caenorhabditis elegans* gene ced-9 protects cells from programmed cell death. Nature 356:494–499.

Hennet T, Bertoni G, Richter C, Peterhans E (1993): Expression of BCL-2 protein enhances the survival of mouse fibrosaroid cells in tumor necrosis factor-mediated cytotoxicity. Cancer Res 53:1456–1460.

Hockenbery D, Nunez G, Milliman C, Schreiber RD, Korsmeyer SJ (1990): Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 348:334–336.

Hockenbery DM, Zutter M, Hickey W, Nahm M, Korsmeyer SJ (1991): BCL2 protein is topographically restricted in tissues characterized by apoptotic death. Proc Natl Acad Sci USA 88:6961–6965.

Hogoquist KA, Nett MA, Unanue ER, Chaplin DD (1991): Interleukin 1 is processed and released during apoptosis. Proc Natl Acad Sci USA 88:8485–8489.

Kimble J, Hirsh D (1981): The post-embryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev Biol 81:208–219.

Kostura MJ, Tocci MJ, Limjuco G, Chin J, Cameron P, Hillman AG, Chartrain NA, Schmidt JA (1989): Identification of a monocyte specific pre-interleukin-1β convertase activity. Proc Natl Acad Sci USA 86:5227–5231.

Kumar S, Tomooka Y, Noda M (1992): Identification of a set of genes with developmentally down-regulated expression in the mouse brain. Biochem Biophys Res Commun 185: 1155–1161.

Kumar S, Kinoshita M, Noda M, Copeland NG, Jenkins NA (1994): Induction of apoptosis by the mouse *Nedd2* gene, which encodes a protein similar to the product of the *Caenorhabditis elegans* cell death gene *ced-3* and the mammalian IL-1β-converting enzyme. Genes Dev 8:1613–1626.

Kretsinger RH (1987): Calcium coordination and the calmodulin fold: Divergent versus convergent evolution. Cold Spring Harbor Symp Quant Biol 52:499–510.

Merry DE, Veis DJ, Hickey WF, Korsmeyer SJ (1994): bcl-2 protein expression is widespread in the developing nervous system and retained in the adult PNS. Development 120:301–311.

Miura M, Zhu H, Rotello R, Hartwieg EA, Yuan J (1993): Induction of apoptosis in fibroblasts by IL-1β-converting enzyme, a mammalian homolog of the *C. elegans* cell death gene ced-3. Cell 75:653–660.

Miyashita T, Reed JC (1992): bcl-2 gene transfer increases relative resistance of S49.1 and WEHI7.2 lymphoid cells to cell death and DNA fragmentation induced by glucocorticoid and multiple chemotherapeutic drugs. Cancer Res 52:5407–5411.

Nunez G, London L, Hockenbery D, Alexander M, McKearn JP, Korsmeyer SJ (1990): Deregulated *bcl-2* gene expression selectively prolongs survival of growth factor-deprived hemopoietic cell lines. J Immunol 144:3602–3610.

Oppenheim RW (1991): Cell death during development of the nervous system. Annu Rev Neurosci 14:453–501.

Ray CA, Black RA, Kronheim SR, et al (1992): Viral inhibition of inflammation: Cowpox virus encodes an inhibitor of the interleukin-1β converting enzyme. Cell 69:597–604.

Robertson AMG, Thomson JN (1982): Morphology of programmed cell death in the ventral nerve cord of *Caenorhabditis elegans* larvae. J Embryol Exp Morphol 67:89–100.

Rotello R, Fernandez PA, Yuan J (1994): Anti-apogens and anti-engulfens: Monoclonal antibodies reveal specific antigens on cells undergoing programmed cell death in developing chick embryos. Development 120:1421–1431.

Sentman CL, Shutter JR, Hockenbery D, Kanagawa O, Korsmeyer SJ (1991): bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. Cell 67:879–888.

Seto M, Jaeger U, Hockett RD, Graninger W, Bennett S, Goldman P, Korsmeyer SJ (1988): Alternative promoters and exons, somatic mutation and deregulation of the Bcl-2-Ig fusion gene in lymphoma. EMBO J 7:123–131.

Strasser A, Harris AW, Cory S (1991): bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship. Cell 67:889–899.

Sulston JE (1976): Post-embryonic development in the ventral cord of *Caenorhabditis elegans*. Philos Trans R Soc Lond Ser B 275:287–298.

Sulston JE, Horvitz HR (1977): Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. Dev Biol 82:110–156.

Sulston JE, Schierenberg E, White JG, Thomson N (1983): The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev Biol 100:64–119.

Thornberry NA, Bull HG, Galaycay JR, et al (1992): A novel heterodimeric cysteine protease is required for interleukin-1b processing in monocytes. Nature 356:768–774.

Tsujimoto Y (1989): Stree-resistance conferred by high level of bcl-2 alpha protein in human B lymphoblastoid cell. Oncogene 4:1331–1336.

Tsujimoto Y, Cossman J, Jaffe E, Croce CM (1985): The t(14;18) chromosome translocation involved in B-cell neoplasms result from mistakes in VDJ joining. Science 228: 1440–1443.

Vaux DL, Cory S, Adams JM (1988): Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 335:440–442.

Vaux DL, Weissman IL, Kim SK (1992): Prevention of programmed cell death in *Caenorhabditis elegans* by human bcl-2. Science 258:1955–1957.

Veis DJ, Sorenson CM, Shutter JR, Korsmeyer SJ (1993): Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys and hypopigmented hair. Cell 75:229–240.

Wang L, Miura M, Bergeron L, Zhu H, Yuan J (1994): *Ich-1*, an *Ice/Ced-3* related gene, encodes both positive and negative regulators of programmed cell death. Cell 78:739–750.

Wyllie AH (1980): Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 284:555–556.

Yin X-M, Oltvai ZN, Korsmeyer J (1994): BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. Nature 369:321–323.

Yuan J, Horvitz HR (1990): Genetic mosaic analyses of *ced-3* and *ced-4*, two genes that control programmed cell death in the nematode *C. elegans*. Dev Biol 138:33–41.

Yuan J, Horvitz HR (1992): The *Caenorhabditis elegans* cell death *ced-4* encodes a novel protein and is expressed during the period of extensive programmed cell death. Development 116:309–320.

Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR (1993): The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1β converting enzyme. Cell 75:641–752.

Zhong L-T, Sarafian T, Kane DJ, et al (1993): bcl-2 inhibits death of central neural cells induced by multiple agents. Proc Natl Acad Sci USA 90:4533–4537.