family. Assuming that this primordial suicide program is conserved throughout evolution, it holds that an analogous paradigm is recapitulated in mammals. The molecular framework that we have proposed gives rise to a number of areas for future study, including the identification of a possible mammalian CED-4 homolog. Though we have established a working framework, the mechanism by which CED-4 activates the caspases and how this is regulated by Bcl-2 family members is unknown. If a mammalian CED-4 homolog does exist, it would be a possible target for therapeutic modulation in diseases of dysregulated apoptosis.

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30. Cell death assays were done as previously described (*26–28*). Transfection of 293T and MCF7 cells was done by calcium phosphate precipitation and lipofectamine, respectively.
31. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
32. Expression constructs were made with pcDNA3 (Invitrogen) or pcDNA3.1(-)/MycHis (Invitrogen) and proteins epitope tagged at the COOH-terminal unless otherwise stated. Coimmunoprecipitation analyses were done essentially as described (*26, 27*). Briefly, a 50% confluent 100-mm plate of 293T cells was transfected by calcium phosphate precipitation with 5 to 10 μg of total DNA and harvested 24 to 48 hours later depending on the experiment. Cells were lysed in 1 ml of lysis buffer (50 mM Hepes, 150 mM NaCl, 1 mM EDTA, 1% NP-40, and a protease inhibitor mixture). Lysate was divided and immunoprecipitated with a control mAb or specified mAbs for 2 to 4 hours at 4°C as previously described (*29*). The beads were washed with lysis buffer (adjusted to 500 mM NaCl) three times, and precipitated immune complexes were resolved on a 12.5% SDS-polyacrylamide gel and transferred to nitrocellulose. Subsequent protein immunoblotting was performed as described (*29*).
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34. Supported by the Medical Scientist Training Program (A.M.C. and B.R.L.) and NIH grant 07863 (A.M.C. and B.R.L.). We thank H. R. Horvitz for the *ced-4, ced-4L*, and *ced-3* clones, M. O. Hengartner for the *ced-9* clone and sharing unpublished data, E. Koonin for suggesting mutations in CED-4, G. Chinnadurai for the HA-BAX, HA-BAK, and HA-BIK expression constructs, and E. H.-Y.Cheng and J. M. Hardwick for the Bcl-x_L constructs. For technical help, advice, and reagents, we also thank the following members of the V.M.D. laboratory: I. Jones (preparation of figures),Y. Kuang, H. Duan, M. Muzio, J. Pan, J. McCarthy, E. Humke, T. Brtva, and C. Vincenz.

26 November 1996; accepted 22 January 1997

Interaction and Regulation of Subcellular Localization of CED-4 by CED-9

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The *Caenorhabditis elegans* survival gene *ced-9* regulates *ced-4* activity and inhibits cell death, but the mechanism by which this occurs is unknown. Through a genetic screen for CED-4–binding proteins, CED-9 was identified as an interacting partner of CED-4. CED-9, but not loss-of-function mutants, associated specifically with CED-4 in yeast or mammalian cells. The CED-9 protein localized primarily to intracellular membranes and the perinuclear region, whereas CED-4 was distributed in the cytosol. Expression of CED-9, but not a mutant lacking the carboxy-terminal hydrophobic domain, targeted CED-4 from the cytosol to intracellular membranes in mammalian cells. Thus, the actions of CED-4 and CED-9 are directly linked, which could provide the basis for the regulation of programmed cell death in *C. elegans*.

Programmed cell death (PCD) plays an essential role in animal development and homeostasis (*1*). Genetic studies in the nematode *Caenorhabditis elegans* have identified several components of the death pathway, some of which are conserved in vertebrates, including humans (*2, 3*). The nematode *ced-9* gene was discovered by analysis of gain-of-function mutants, and it was subsequently shown that *ced-9* protects cells that normally survive from undergoing PCD (*4*). In contrast, loss-of-function mutations in the *ced-3* gene cause all 131 cells that normally die to survive, indicating that *ced-3* is required for PCD (*3*). Both *ced-9* and *ced-3* have mammalian homologs. The *ced-9* gene encodes a protein with significant sequence homology to the vertebrate Bcl-2 and Bcl-x_L survival proteins, whereas the *ced-3* product is homologous to the interleukin-1β–converting enzyme (ICE), which is a member of a growing family of ICE-like proteases, called caspases (*5, 6*). The mammalian *bcl-2* gene can functionally substitute for *ced-9* in *C. elegans* (*5, 7*), suggesting that Bcl-2 is a mammalian homolog of CED-9. Another *C. elegans* gene, *ced-4*, is required for developmental cell death in the worm, and its overexpression can cause cells that normally survive to undergo PCD (*8, 9*). Genetic experiments have indicated that *ced-9* controls cell death by preventing the activation of the death genes *ced-3* and *ced-4* (*4, 9*) and have suggested that *ced-3* acts downstream of *ced-4* and that *ced-9* acts upstream of *ced-4* (*4, 9*). Consistent with this, Bcl-2 and Bcl-x_L can inhibit the activation of ICE-like proteases and therefore appear to function upstream of the death proteases in the mammalian apoptotic pathway (*10*).

To search for proteins that bind to CED-4, we screened a *C. elegans* cDNA library for CED-4–interacting proteins by using GAL4–CED-4S as a "bait" in the yeast two-hybrid assay (*11*). In a screen of 2×10^6 library clones, four cDNAs were found to interact with the GAL4–CED-4 but not with control baits. One of the CED-4–interacting cDNAs encoded the entire CED-9 coding region fused in-frame to the GAL4 transcriptional activation domain. The association of CED-4 with CED-9 was specific in that CED-4 was unable to interact with empty vector or several plasmids encoding irrelevant baits (Fig. 1A) (*12*). To further characterize the CED-4–CED-9 interaction, we determined the ability of CED-4 to associate with three natural CED-9 mutants (Fig. 1A). In the yeast two-hybrid system, CED-4 interacted with the

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wild type and a gain-of-function CED-9 mutant but not with two loss-of-function mutants (Fig. 1B). These results indicate that the protective activity of CED-9 correlates with its ability to interact with CED-4 and strongly suggest that a physical interaction of CED-9 with CED-4 is critical for the regulation of PCD in *C. elegans*.

The interaction of CED-4 with CED-9 could not be assessed in *C. elegans* cells in culture, because no such cells are currently available. To verify that CED-4 associates with CED-9 in vivo, we transiently cotransfected a 293T human kidney cell line with expression plasmids producing a Myc epitope– tagged CED-4 and hemagglutinin (HA)– tagged CED-9 protein (*13*). Immunoprecipitates were prepared with rabbit antibody to Myc (anti-Myc) and subjected to SDS–polyacrylamide gel electrophoresis (PAGE). Immunoblotting with a monoclonal antibody to HA (anti-HA) revealed that CED-9 was coimmunoprecipitated specifically with CED-4 (Fig. 2A). Control immunoblotting with anti-HA and anti-Myc confirmed that CED-9 and CED-4 proteins were expressed in total lysates of cells transfected with the corresponding plasmids but not in cells transfected with control plasmids (Fig. 2A). To further verify the interaction, we performed reciprocal experiments with anti-HA to immunoprecipitate CED-9, followed by immunoblotting with rabbit anti-Myc. In agreement with the reverse experiment, CED-4 coimmunoprecipitated specifically with CED-9 (Fig. 2B). The CED-9 protein contains a conserved COOH-terminal hydrophobic tail that may serve as an anchoring domain to intracellular membranes (*5*). We engineered a mutant form of CED-9 with a deletion of the COOH$^-$ hydrophobic domain (CED-9ΔTM) to determine if the putative membrane anchoring region is required for CED-9 to localize to intracellular membranes in mammalian cells (*13*). Immunoprecipitation analysis revealed that the CED-9ΔTM mutant protein retained its ability to associate with CED-4, indicating that the COOH$^-$ hydrophobic domain of CED-9 is not required for the interaction with CED-4 (Fig. 2B). In addition, CED-4 was specifically coimmunoprecipitated with Bcl-x_L, a mammalian homolog of CED-9 (Fig. 2C) but not with Bcl-x_S, a mutant form of Bcl-x_L lacking an internal region of 62 amino acids that contain the conserved Bcl-2 homology region 1 (BH1) and BH2 domains (Fig. 2D) (*13*).

To assess the subcellular localization of wild-type CED-9, we transfected an HA–*ced-9* expression plasmid into 293T cells and determined the labeling pattern of CED-9 by immunostaining and confocal microscopy (*15*). Analysis of labeled cells revealed that HA–CED-9 displayed a compact, granular, and extranuclear staining pattern consistent with a localization confined to membranes of intracellular organelles and the perinuclear region (Fig. 3A). In contrast, the labeling pattern of the CED-9ΔTM mutant was diffuse and cytoplasmic, which is consistent with a cytosolic localization (Fig. 3B). Analysis of cells transfected with a Myc–CED-4 construct with anti-Myc revealed a cytoplasmic labeling pattern that was distinct from wild-type CED-9 but similar to that of the CED-9ΔTM mutant (Fig. 3C). Transfection with a control GATA-1 expression plasmid revealed a nuclear labeling pattern as expected for a nuclear factor (Fig. 3F). Significantly, coexpression of CED-9 with CED-4 strongly altered the distribution of CED-4 to a compact granular, perinuclear pattern similar to that observed for CED-9 (compare panels C and D in Fig. 3). The change in subcellular localization of CED-4 induced by coexpression with CED-9 was specific in that it was not observed by cotransfection with an expression plasmid that encodes the nuclear GATA-1 (*16*). The labeling pattern of CED-4 was not affected by coexpression of the CED-9ΔTM mutant

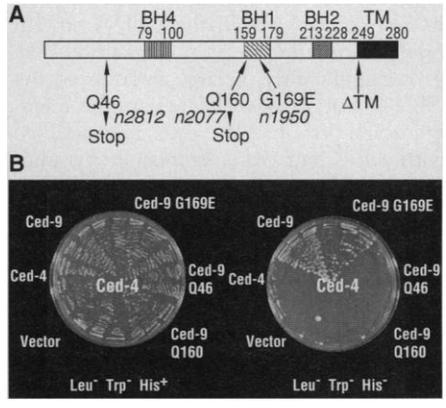

Fig. 1. Interaction of CED-4 with wild-type CED-9 and CED-9 mutants in yeast. (**A**) Schematic representation of wild-type and mutant CED-9 proteins. BH1, BH2, and BH4 boxes depict conserved regions shared with Bcl-2 family members. TM represents the conserved COOH-terminal hydrophobic tail. The position of point mutations in *n2077* and *n2812* (loss-of-function) as well as *n1950* (gain-of-function) mutations of *ced-9* are shown (*5*). Q indicates Gln residues. G169E represents $Gly^{169} \rightarrow Glu^{169}$. ΔTM represents an engineered CED-9 mutation lacking residues 250 through 280. (**B**) Interaction of CED-4 with wild-type and mutant CED-9 proteins. A plasmid expressing CED-4 fused to the GAL4 DNA-binding domain was cotransfected with plasmids encoding wild-type CED-9, CED-9 mutants, wild-type CED-4, or empty vector sequences fused to a GAL4 transcriptional activation domain. Growth of yeast in the absence of leucine, tryptophan, and histidine is indicative of protein-protein interaction. Growth in the absence of leucine and tryptophan is shown as a control. The results are representative of three independent experiments.

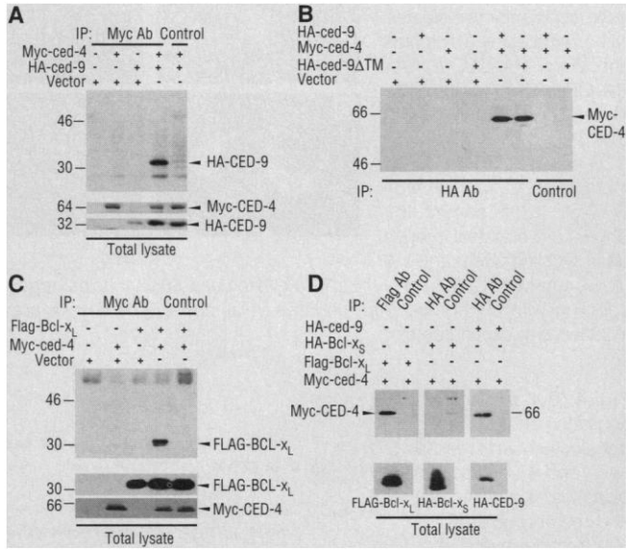

Fig. 2. Interaction of CED-4 with CED-9, CED-9ΔTM mutant, and human Bcl-x proteins in mammalian cells. (**A**) 293T cells (5×10^6 per 100-mm plate) were transiently transfected with 2.5 μg of the indicated Myc- or HA-tagged expression plasmids or empty vector. In the case of transfection with a single plasmid, cells were cotransfected with 2.5 μg of empty vector so that the total amount of transfected plasmid DNA was always 5 μg. Lysates were immunoprecipitated with rabbit anti-Myc or normal rabbit serum. Immunoprecipitates were immunoblotted with mouse anti-HA to detect the HA–CED-9 protein. In the lower two panels, aliquots of total protein lysates were immunoblotted with anti-Myc or anti-HA. Molecular size markers are indicated in kilodaltons. (**B**) Anti-HA or isotype control immunoprecipitates were immunoblotted with anti-Myc to detect Myc-tagged CED-4. The amount of plasmid DNA and number of transfected cells were identical to that described for (A). (**C**) Anti-Myc or control immunoprecipitates were blotted with anti-FLAG. In the lower two panels, aliquots of total protein lysates were immunoblotted with anti-FLAG or anti-Myc(**D**) Anti-FLAG, anti-HA, or control immunoprecipitates were blotted with anti-Myc. In the lower panel, aliquots of total protein lysates were immunoblotted with anti-FLAG (to detect Bcl-x_L) or anti-HA (to detect Bcl-x_S or CED-9). The results are representative of three independent experiments.

(Fig. 3E), indicating that the membrane-anchoring domain is required for CED-9 to alter the subcellular localization of CED-4.

We performed subcellular fractionation of protein lysates in which cytosolic and membrane fractions were prepared and analyzed by immunoblotting for the presence of CED-4 and CED-9 proteins (*17*). Analysis of cellular lysates revealed that the majority of CED-9 was contained within the membrane fraction, whereas the CED-9ΔTM mutant protein was located mainly in the cytosolic fraction (Fig. 4A). Similar subcellular localizations have been reported, respectively, for the CED-9 mammalian homolog Bcl-2 and its ΔTM mutant (*18*). In contrast to CED-9, the CED-4 protein resided mostly in the cytosolic fraction (Fig. 4B). Significantly, a large fraction of the CED-4 protein was directed to the membrane fraction in 293T cells cotransfected with *ced-4* and *ced-9* expression plasmids (Fig. 4B). However, the cytosolic distribution of CED-4 was largely unaffected in cells that expressed the ΔTM mutant form of CED-9 (Fig. 4C), which is consistent with the confocal analysis shown in Fig. 3. These results indicate that CED-9 requires its conserved COOH-terminal hydrophobic region for both attachment to intracellular membranes and targeting of CED-4 to intracellular membranes in mammalian cells.

Genetic studies have shown that *ced-9* controls cell death by preventing the activation of the death genes *ced-3* and *ced-4* (*4*, 9). Two forms of *ced-4*—*ced-4S* and *ced-4L*—that exhibit different functions have been identified (*19*). Here, we studied *ced-4S*, the most abundant form of *ced-4* expressed in C. *elegans* cells (*19*). Our studies showed that CED-4 and CED-9 proteins interact in yeast and mammalian cells and presumably in C. *elegans* cells. Thus, we propose that CED-9 regulates cell death at least in part by binding and inactivating CED-4. This could be accomplished by sequestering CED-4 from its otherwise cytosolic distribution to specific intracellular membrane sites. The removal of cytosolic CED-4 could result in the reduced ability of CED-4 to activate downstream effector molecules such as CED-3, thereby promoting cell survival. This model is most consistent with genetic analysis in C. *elegans*, where it has been shown that *ced-9* inhibits *ced-3* activity at least in part through *ced-4* (9). We have shown that CED-4 interacts with Bcl-x_L, a mammalian CED-9 homolog. These results would predict that the mammalian CED-9 homologs Bcl-2 and Bcl-x_L will regulate cell death at least in part by interacting with a mammalian CED-4 counterpart. The membrane localization of CED-9 may be important for the regulation of CED-4 activity, because Bcl-2 mutants lacking the COOH-terminal membrane-anchoring tail exhibit a greatly reduced anti-apoptotic activity when compared with wild-type Bcl-2 (*20*). Bcl-2 associates with and targets the cytosolic protein kinase Raf-1 to mitochondrial membranes (*21*). Unlike the pro-apoptotic CED-4, Raf-1 functions to improve Bcl-2–mediated resistance to apoptosis, perhaps indicating that the Bcl-2 family may play a more general role in the targeting or sequestration of specific apoptotic regulatory proteins to intracellular membranes.

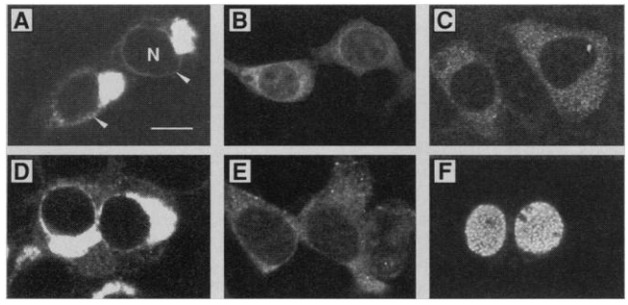

Fig. 3. Subcellular localization of CED-9 and CED-4 proteins in 293T cells. Cells were transiently transfected with expression plasmids encoding HA–CED-9 (**A**), HA–CED-9ΔTM mutant (**B**), Myc–CED-4 (**C**), HA–CED-9 plus Myc-CED-4 (**D**), Myc–CED-4 plus HA–CED-9ΔTM mutant (**E**), or FLAG–GATA-1 as a control (**F**). Shown are confocal images after labeling with anti-HA (A, B), anti-Myc (C, D, E) or anti-FLAG (F). Arrowheads, perinuclear region. N, nucleus. Scale bar, 10 μm. Labeling with control IgG was negative in all cases. The results are representative of at least two independent experiments.

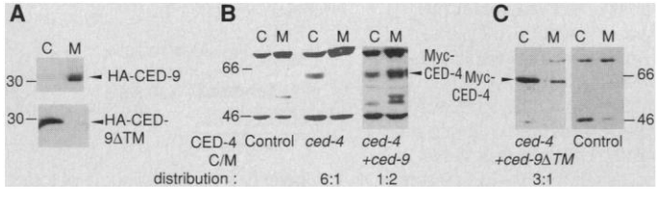

Fig. 4. Subcellular fractionation of 293T cells transfected with *ced-9* and *ced-4* expression plasmids. Cytosolic (C) and membrane (M) protein fractions were analyzed for CED-9 or CED-4 protein expression by immunoblotting using monoclonal anti-HA or polyclonal anti-Myc. Protein lysates from an equal number of cells were loaded in the C and M lanes. (**A**) Cells were transfected with an HA-*ced-9* plasmid (top panel) or an HA–*ced-9* mutant plasmid (ΔTM). Molecular size markers are indicated in kilodaltons. (**B**) Cells were transfected with empty vector (control), Myc–*ced-4*, or Myc–*ced-4* plus HA–*ced-9*. (**C**) Cells were transfected with Myc–ced-4 plus HA–ced-9ΔTM or control plasmids. The relative ratio of CED-4 in the cytosolic or membrane fractions was quantitated by densitometry analysis of the corresponding bands. The results are representative of three independent experiments.

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11. A *C. elegans* cDNA library fused to the GAL4 activating domain of the pACT vector (a gift of Robert Barstead) was screened with the HF7c yeast reporter strain for proteins that interact with CED-4S (*22*). A bait plasmid expressing *ced-4S* in pGBT-8 was constructed by polymerase chain reaction (PCR) amplification of plasmid cDNA to incorporate restriction sites, followed by ligation of the amplified DNA fragment in-frame with the GAL4 DNA-binding domain of pGBT-8. Positive library plasmids were selectively recovered from bacteria colonies by growth in media lacking leucine. cDNA inserts in the plasmid were characterized by restriction enzyme mapping and nucleotide sequence analysis on an automated DNA sequencer (Applied Biosystems model 373A). Plasmids encoding *ced-9* mutants in pGBT-8 were generated by PCR amplification of *ced-9* cDNA template, using 3′ primers that included the natural translation termination sequences as described (*4*, *5*). Authenticity of all constructs was confirmed by dideoxy sequencing.
12. D. Wu, H. D. Wallen, G. Nuñez, data not shown.
13. An HA–ced-9 insert was constructed by introducing an HA epitope tag at the NH2-terminus of CED-9 by PCR. An HA-tagged mutant form of CED-9 (ΔTM) lacking residues 250 through 280 of CED-9 was generated by PCR with wild-type ced-9 as a template. A Myc tag was inserted at the COOH-terminus of CED-4 by PCR amplification with a *ced-4S* cDNA template. Similarly, an HA epitope was tagged to the NH2-terminus of Bcl-x_S by PCR. The HA- and Myc-tagged inserts were ligated to the Eco RI and Kpn I sites of pcDNA3, respectively (Invitrogen). Authenticity of all constructs was confirmed by dideoxy sequencing. The construct to express FLAG–Bcl-x_L has been described (*23*). To assess protein interactions in mammalian cells, we transfected culture dishes containing 3×10^6 293T cells with amounts (indicated in figure legends) of plasmid DNA by the calcium phosphate method. The expression of HA–CED-9, HA–CED-9ΔTM, and Myc–CED-4 was de-

termined in total lysates by immunoblotting (*23*). For immunoprecipitations, cells were lysed in Nonidet P-40 isotonic lysis buffer (*23*) at 24 hours after transfection, and soluble lysates were incubated with either 1 μg/ml anti-HA (clone 12CA5, Boehringer Mannheim); 1 μg/ml rabbit anti-Myc (Santa Cruz), anti-FLAG (clone M2, Scientific Imaging Systems), or isotype-matched mouse IgG (anti-HA control); or normal rabbit serum (anti-Myc control) overnight at 4°C. Protein A–Sepharose 4B (Zymed Laboratories) was added [5% (v/v)] for an additional hour of incubation by rotation. Immune complexes were centrifuged and washed with excess cold Nonidet P-40 isotonic lysis buffer at least four times, separated on a 15% SDS-polyacrylamide gel, and immunoblotted with rabbit anti-Myc, anti-HA or anti-FLAG. The proteins were detected with an ECL system (Amersham).

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15. 293T cells were transfected with pcDNA3-HA–*ced-9*, pcDNA3-HA–*ced-9*ΔTM, pcDNA3-Myc–*ced-4S*, or pcDNA3-Flag–*gata-1* as described (*13*). At 24 hours after transfection, cells were incubated with anti-HA, anti-Myc (clone 9E10, Santa Cruz), anti-FLAG, or control mouse IgG for 1 hour at 23°C, and the labeling was visualized with fluorescein-conjugated goat anti-mouse IgG. After washing, the cells were mounted in Slowfade (Molecular Probes) and examined with a Bio-Rad MRC 600 scanning confocal microscope equipped with an argon-xenon laser (*24*).
16. D. Wu and G. Nuñez, unpublished results.
17. 293T cells (10^7) were transiently transfected with the indicated expression plasmids (10 μg). The cells were washed twice with phosphate-buffered saline and then incubated for 10 min in 1 ml of cold hypotonic buffer containing 1 mM phenylmethylsulfonyl fluoride, 1 μg antipain, and leupeptin (0.3 μg/ml) as described (*25*). The swollen cells were lysed by 15 strokes in a tight-fitting Douncer homogenizer, and the samples were centrifuged at 1000*g* for 6 min to remove nuclei. The resulting supernatant was centrifuged for 1 hour at 100,000*g*. The supernatant portion of the latter centrifugation was the soluble cytosolic (C) fraction; the pellet was the membrane and cytoplasmic organelle (M) fraction. The proteins from the C and M fractions were dissolved in 1 ml of lysis buffer, and samples of equal size were analyzed by immunoblotting.
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26. We thank R. Horvitz for plasmids and R. Barstead for the two-hybrid *C. elegans* cDNA library; C. Edwards and B. Donohoe for expert advice in confocal microscopy; M. Clarke and R. Ellis for stimulating discussions; and M. Benedict, P. Simonian, D. Ekhterae, M. González-García, Y. Hu, and N. Inohara for critical review of the manuscript. Supported in part by NIH grant CA-64556, Immunopathology NIH predoctoral training grant T32A107413-03 (H.D.W.), and an NIH Research Career Development Award (G.N.).

11 December 1996; accepted 22 January 1997

Prevention of Apoptosis by Bcl-2: Release of Cytochrome c from Mitochondria Blocked

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Bcl-2 is an integral membrane protein located mainly on the outer membrane of mitochondria. Overexpression of Bcl-2 prevents cells from undergoing apoptosis in response to a variety of stimuli. Cytosolic cytochrome c is necessary for the initiation of the apoptotic program, suggesting a possible connection between Bcl-2 and cytochrome c, which is normally located in the mitochondrial intermembrane space. Cells undergoing apoptosis were found to have an elevation of cytochrome c in the cytosol and a corresponding decrease in the mitochondria. Overexpression of Bcl-2 prevented the efflux of cytochrome c from the mitochondria and the initiation of apoptosis. Thus, one possible role of Bcl-2 in prevention of apoptosis is to block cytochrome c release from mitochondria.

Genetic studies of programmed cell death in *Caenorhabditis elegans* have identified two genes, *ced-9* and *ced-3*, that play important roles in regulating and executing apoptosis (*1*). *ced-9* and its mammalian counterpart *bcl-2* prevent cells from undergoing apoptosis (*2*, *3*). *ced-3*, which encodes a protease of the interleukin-1β–converting enzyme (ICE), or caspase-1, family, is required for apoptosis (*4*, *5*). The mammalian protein CPP32 (caspase-3) shares sequence similarity and substrate specificity with CED-3 (*6*).

CPP32 is a cytosolic protein that normally exists as a 32-kD inactive precursor. It is cleaved proteolytically into a 20-kD and a 10-kD active heterodimer in cells undergoing apoptosis (*7*). Activated CPP32 cleaves poly(adenosine diphosphate–ribose) polymerase (PARP) (*7*), sterol regulatory element–binding proteins (*8*), and several other cellular proteins (*9*). Bcl-2, which seems to work upstream of CPP32, prevents activation of CPP32 in response to staurosporine and other agents that cause apoptosis (*10*). The mechanism by which Bcl-2 regulates apoptosis is unknown; however, the location of Bcl-2 on the outer membrane of mitochondria raises the possibility that its function may be related to the function of mitochondria, which have been implicated in apoptosis (*11*–*13*).

We previously developed an in vitro assay in which cytosol from normally growing cells induces the activation of CPP32 and fragmentation of DNA after incubation with deoxyadenosine triphosphate (dATP) (*14*). Fractionation of this cytosol preparation revealed that cytochrome c, which was released into the cytosol during homogenization, is required for activation of CPP32 and DNA fragmentation (*14*). We also observed a marked increase of cytochrome c in the cytosol of cells undergoing apoptosis induced by staurosporine, a broad-range protein kinase inhibitor that induces apoptosis in a variety of cell types (*14*).

Cytochrome c is encoded by a nuclear gene and translated by cytosolic ribosomes as apopcytochrome c (*15*). Apocytochrome c is subsequently translocated into the mitochondria where a heme group is attached covalently to form holocytochrome c (*15*). The increase in cytosolic holocytochrome c upon apoptosis suggests that mitochondria may participate in apoptosis by releasing cytochrome c. This observation raises the possibility that Bcl-2, located on the outer membrane of mitochondria, may prevent cell death by blocking the release of cytochrome c. We tested this possibility in the following experiments.

Human acute myeloid leukemia (HL-60) cells were transfected with a retroviral vector containing a neomycin resistance gene (*neo* cells), or the same vector containing a cDNA encoding human Bcl-2 (Bcl-2 cells). The latter cells, which overexpress Bcl-2, resist apoptosis induced by clinically used anticancer drugs such as arabinosylcytosine (Ara-C), etoposide, and mitoxantrone hydrochloride (*16*). We isolated the mitochondria and cytosol from the two cell lines by differential centrifugation (*17*) and confirmed overexpression of Bcl-2 on the mitochondria by protein im-

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