The EMBO Journal vol.13 no.9 pp.2023-2028, 1994

Baculovirus *p35* prevents developmentally programmed cell death and rescues a *ced-9* mutant in the nematode *Caenorhabditis elegans*

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Communicated by J.Hodgkin

Programmed cell death, or apoptosis, occurs throughout the course of normal development in most animals and can also be elicited by a number of stimuli such as growth factor deprivation and viral infection. Certain morphological and biochemical characteristics of programmed cell death are similar among different tissues and species. During development of the nematode *Caenorhabditis elegans*, a single genetic pathway promotes the death of selected cells in a lineally fixed pattern. This pathway appears to be conserved among animal species. The baculovirus p35-encoding gene (*p35*) is an inhibitor of virus-induced apoptosis in insect cells. Here we demonstrate that expression of *p35* in *C.elegans* prevents death of cells normally programmed to die. This suppression of developmentally programmed cell death results in appearance of extra surviving cells. Expression of *p35* can rescue the embryonic lethality of a mutation in *ced-9*, an endogenous gene homologous to the mammalian apoptotic suppressor *bcl-2*, whose absence leads to ectopic cell deaths. These results support the hypothesis that viral infection can activate the same cell death pathway as is used during normal development and suggest that baculovirus *p35* may act downstream or independently of *ced-9* in this pathway.

Key words: apoptosis/baculovirus/*bcl-2*/*Caenorhabditis elegans*/programmed cell death

Introduction

Programmed cell death (apoptosis) is used in virtually all metazoans as a mechanism to dispose of unneeded or potentially deleterious cells (reviewed by Ellis *et al.*, 1991b; Raff, 1992). In many animals cell death is essential for normal development, homeostasis and defense against pathogens. For example, cell death occurs widely during development of nervous systems to eliminate neurons that fail to make appropriate connections (Oppenheim, 1991). Most thymocytes produced in vertebrate immune systems undergo programmed death as a way of eliminating cells with inappropriate immune specificity (Golstein *et al.*, 1991; Cohen *et al.*, 1992; Rothenberg, 1992). Ionizing radiation and other DNA-damaging agents are also known to promote apoptosis (e.g. Clarke *et al.*, 1993; Lowe *et al.*, 1993), presumably to prevent propagation of mutant cells. Moreover, infection by some viruses induces apoptotic death of the host cells (e.g. Clem *et al.*, 1991; Hershberger *et al.*, 1992; Rao *et al.*, 1992). This defensive response of the infected cells limits the spread of viral infection in the host organism. Several of the morphological and biochemical characteristics of programmed cell death appear similar irrespective of whether it occurs in a developmental or pathological context.

Some viruses oppose the host apoptotic defense mechanism by producing suppressors of cell death. This counter-measure presumably increases the efficiency of virus replication. The baculovirus gene *p35* is an inhibitor of virus-induced apoptosis in insect cells (Clem *et al.*, 1991; Hershberger *et al.*, 1992; Kamita *et al.*, 1993). The product of this gene is encoded by *Autographa californica* multiply embedded nuclear polyhedrosis virus (Ac*M*NPV) (Friesen and Miller, 1987; Clem *et al.*, 1991) and by *Bombyx mori* nuclear polyhedrosis virus (Kamita *et al.*, 1993), both members of the virus family Baculoviridae. Replication of these viruses in the nucleus of host insect cells culminates with the production of occluded virus particles and cell lysis. Viral mutants that lack *p35* promote premature death of insect cells by virus-induced apoptosis (Clem *et al.*, 1991; Hershberger *et al.*, 1992; Kamita *et al.*, 1993), indicating that *p35* normally represses apoptosis. In the case of Ac*M*NPV, inhibition of apoptosis by *p35* correlates with increased viral replication and higher yields of virus progeny (Hershberger *et al.*, 1992).

To determine whether *p35* can prevent not only virally induced apoptosis, but also developmentally programmed cell death, we tested the effect of *p35* expression in *C.elegans*. This animal is an amenable system in which to assay presumptive inhibitors of developmentally programmed cell death. The somatic cell lineage of *C.elegans* has been completely characterized and found to be invariant: of the 1090 somatic nuclei generated in hermaphrodites, 131 undergo programmed cell death in a predictable pattern (Sulston and Horvitz, 1977; Sulston *et al.*, 1983). The resultant cell corpses are easily recognized in developing animals using Nomarski microscopy (Sulston and Horvitz, 1977).

A single genetic pathway is responsible for all of the developmentally programmed cell deaths in *C.elegans* (Ellis and Horvitz, 1986; Ellis *et al.*, 1991b). Molecular components of this pathway appear to be conserved among animal species. Programmed cell death can be blocked in nematodes by expression of the human proto-oncogene *bcl-2* (Vaux *et al.*, 1992), which inhibits apoptosis in mammals (Vaux *et al.*, 1988; Sentman *et al.*, 1991; Bissonnette *et al.*, 1992; Garcia *et al.*, 1992). Moreover, *ced-9*, a general cell death suppressor in *C.elegans* is structurally similar to *bcl-2* (Hengartner and Horvitz, 1994). Finally, the *ced-3* gene product, which is essential for cell death in *C.elegans*, has sequence similarity to mammalian interleukin-1β-converting enzyme (ICE) (Yuan *et al.*, 1993) and both *ced-3* and mouse

ICE can induce programmed cell death of rat fibroblast cells (Miura *et al.*, 1993).

Here we report that Ac*M*NPV *p35* suppresses developmentally programmed cell death in *C.elegans* and can rescue a *ced-9* loss-of-function mutant. These results suggest that *p35* has the capacity to suppress cell death independently of *ced-9/bcl-2* in an evolutionarily conserved cell death pathway.

Results

Expression of p35 in C.elegans decreases cell corpse number

To express Ac*M*NPV *p35* in *C.elegans*, we placed the *p35* open reading frame under control of the heat-shock-responsive promoter from the *hsp16-1/48* gene of *C.elegans* (Russnak and Candido, 1985). An *hsp16-1/48–p35* plasmid (*hs-p35*) or a control *hsp16-1/48–lacZ* plasmid (*hs-lacZ*) (Stringham *et al.*, 1992), was microinjected into adult germlines along with a plasmid carrying the dominant genetic marker, *rol-6(su1006)*, which causes mature animals to roll when moving (Kramer *et al.*, 1990). The constructs were transformed into a *ced-1* mutant, in which an engulfment defect causes cell corpses to persist, thereby facilitating scoring of cells that have undergone programmed cell death (Hedgecock *et al.*, 1983). Transformants were recognized by their rolling (Rol) phenotype.

The progeny of transgenic and control adults were examined for cell corpses with and without heat-shock. Heat-shock was given during early gastrulation (100–200 min after first cleavage), well before the onset of programmed cell death (i.e. 230 min). The number of cell corpses was scored at 'comma' stage (~400 min), by which time more than half of the cell deaths have occurred in wild-type embryos (Sulston *et al.*, 1983). Histograms of the number of embryos containing a given number of cell corpses in control and *p35*-expressing animals are compared in Figure 1.

The distributions of cell corpse number in embryos produced by *ced-1* and *ced-1;hs-lacZ* controls were similar and neither was substantially altered by heat-shock treatment (Figure 1A and B). Embryos from the *ced-1;hs-p35* transgenic strain, which were not heat-shocked, also showed a similar distribution (Figure 1C, hatched bars). In contrast, heat-shock treatment of the progeny of *ced-1;hs-p35* animals resulted in a marked decrease in the number of cell corpses. This was reflected in a broadening of the distribution and a shift toward lower numbers of corpses (Figures 1C and 2). In fact, no cell corpses were observed in four of 87 heat-shocked embryos obtained from *ced-1;hs-p35* mothers (see Figure 2), whereas among hundreds of control embryos (from *ced-1* or *ced-1;hs-lacZ* parents) none completely lacked cell corpses (Figure 1; A.Sugimoto, R.Hozak and J.Rothman, unpublished data; see also Vaux *et al.*, 1992).

The average numbers of cell corpses in *p35*-expressing and control animals are presented in Figure 3. The effect of *p35* was more striking when the results from embryos that became Rol adults were averaged (Figure 3). Thus, heat-shocked progeny of *ced-1;hs-p35* that showed a Rol phenotype had only 7.1 ± 5.5 corpses (mean ± standard deviation), whereas non-Rol animals had a significantly higher number of corpses (12.1 ± 4.1; Figure 3). However,

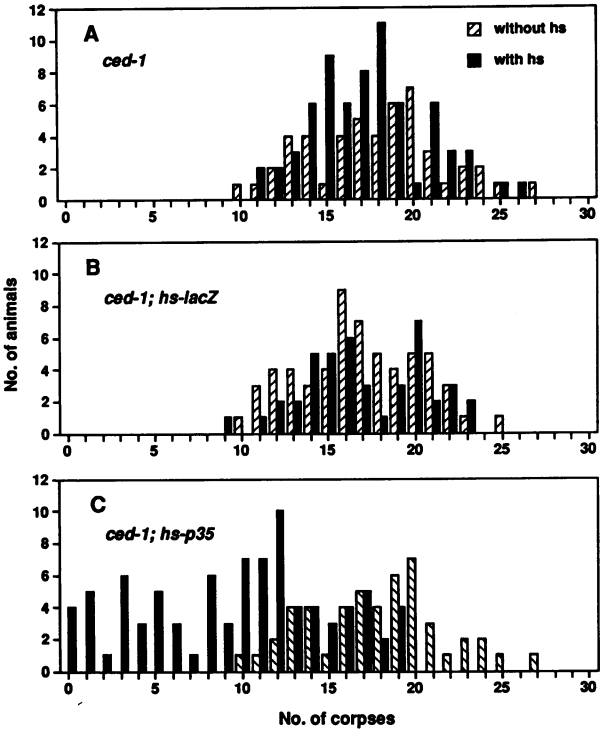

Fig. 1. Effect of *p35* on number of cell corpses in *C.elegans* embryos. For each histogram, the *x*-axis shows the number of cell corpses observed per embryo either without (hatched) or with (filled) prior heat-shock treatment, and the *y*-axis shows the number of embryos with a given number of cell corpses. Cell corpses were counted at approximately the comma stage [~400 min after the first cleavage (Sulston *et al.*, 1983)]. (**A**) *ced-1* embryos, (**B**) embryos from the *ced-1;hs-lacZ* transgenic line and (**C**) embryos from the *ced-1;hs-p35* transgenic line.

even these non-Rol animals showed fewer cell corpses than the non-heat-shocked control (17.8 ± 3.8), or either of the control strains (18.6 ± 3.3 and 16.8 ± 3.5 without heat-shock, 17.4 ± 3.3 and 17.0 ± 3.2 with heat-shock, for all progeny of *ced-1* and *ced-1;hs-lacZ*, respectively). Rol and non-Rol progeny of the *ced-1;hs-lacZ* control gave similar results (Figure 3). Many non-Rol animals from *ced-1;hs-p35* gave rise to Rol progeny (not shown), indicating that the array was transmitted to the germ line of some animals despite the non-Rol phenotype. This effect presumably reflects a combination of genetic mosaicism of the extrachromosomal arrays and incomplete penetrance of the Rol phenotype. The heat-shock-dependent decrease in cell corpse number was seen in two independent *hs-p35* transformants but not in two independent *hs-lacZ* transformants (not shown). We conclude that expression of *p35* reproducibly reduces the number of cell corpses accumulating in *ced-1* embryos.

p35 expression leads to extra surviving cells

The *p35*-dependent decrease in cell corpse number could have arisen either from suppression of the engulfment defect of the *ced-1* mutant or instead from inhibition of programmed cell death. To distinguish between these possibilities, we asked whether heat-shocked *ced-1;hs-p35* transgenic worms contained extra cells, which would correspond to survivors that had escaped cell death. In this experiment, we examined nuclei in the anterior pharynx, which are readily scored. In

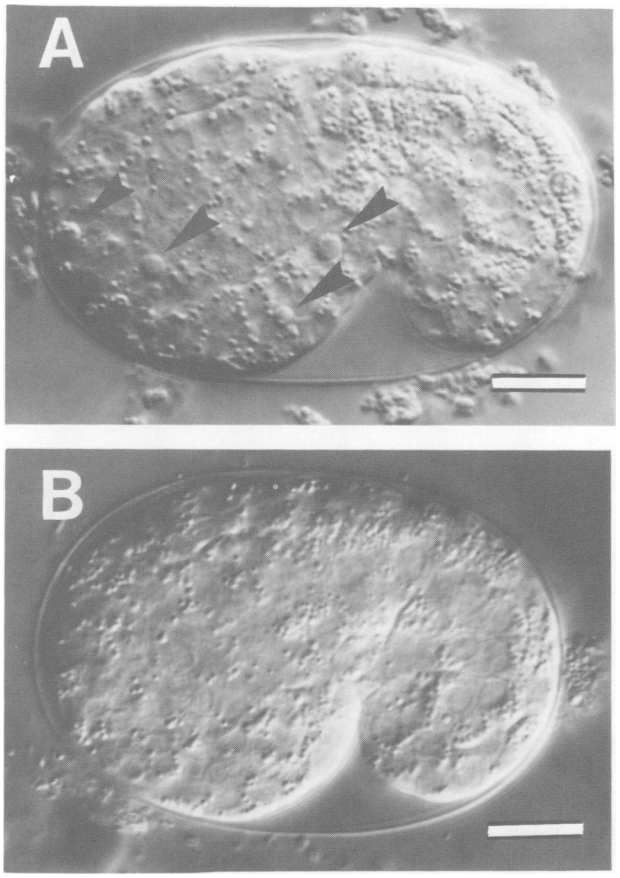

Fig. 2. Absence of cell corpses in *p35*-expressing embryos. Shown are Nomarski micrographs of developing comma-stage embryos. Anterior is to the left, dorsal at the top. Scale bar, 10 μm. (**A**) Embryo without heat-shock treatment. Cell corpses are indicated by arrowheads. A total of 15 corpses were seen in all focal planes of this animal. (**B**) Embryo with heat-shock treatment. Although no corpses were visible in this focal plane, a total of two corpses were seen in this embryo.

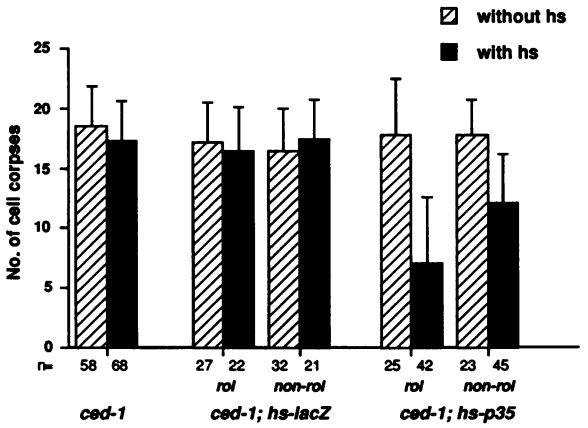

Fig. 3. Mean number of cell corpses in embryos that became Rol or non-Rol adults. The numbers of cell corpses (mean ± SD) without (hatched) and with (filled) heat-shock. The numbers were obtained from the embryos scored in Figure 1. The number of animals scored is shown at the bottom of each bar. The embryos of the transgenic lines were classified into two groups (Rol and non-Rol) based on the phenotype observed at late larval and adult stages. However, many of the non-Rol animals often produced Rol progeny (27/68 of *ced-1;hs-p35* and 34/53 of *ced-1;hs-lacZ*), showing that the array was present in the germ line in many of these animals. The mean numbers of corpses in Rol and non-Rol heat-shocked progeny of *ced-1;hs-p35* were significantly different (*t*-test, $P<0.0001$).

ced-3, *ced-4* or *ced-9(gf)* mutants, in which virtually no cell deaths occur, ~14 extra surviving nuclei are observed in the anterior pharynx in addition to the normal 49 present in wild-type (Hengartner *et al.*, 1992). The embryos from the *ced-1;hs-p35* line were given a heat-shock, and nuclei in the anterior pharynx were counted at late larval stages (Table I). Extra nuclei were observed in 15 out of 18 animals (Table I and Figure 4b and c). As many as 11 extra nuclei (average of 4.1 ± 3.0) were counted in these animals. The extra nuclei were in a similar position to those found in *ced-3*, *ced-4* or *ced-9(gf)* mutants (Hengartner *et al.*, 1992), suggesting that, as in these mutants, the extra cells in *p35*-expressing animals are those that escaped cell death. These extra survivors were observed at least 30 h beyond the time that cells in the pharynx normally die in wild-type embryos. In contrast, generally no extra cells were found in control animals (Table I and Figure 4a). Thus, *p35* reduces cell corpse number by preventing programmed cell death and not by enhancing engulfment of cell corpses *per se*.

Suppression of a ced-9(lf) mutation by p35

To examine the stage in the cell death pathway at which *p35* acts, we tested whether *p35* expression could rescue a *ced-9* loss-of-function mutation. At 25°C, all embryos from *ced-9(n1950n2161)* homozygous mothers die before hatching as the result of excess cell deaths (Hengartner *et al.*, 1992). Since mosaic expression of *p35* might diminish suppression of this maternal-effect lethality, we isolated a line in which the *hs-p35* construct was integrated into a chromosome (see Materials and methods). The resulting integrant was then crossed into *ced-9(n1950n2161)/+* heterozygotes. The percentage of hatching embryos from *ced-9* homozygous mothers was scored without and with heat-shock treatment at early gastrulation stage. The data in Table II demonstrate that expression of *p35* by heat-shock rescued the maternal-effect lethality of *ced-9*. The transgenic line showed weak rescue even without heat-shock, probably as a result of a low basal level of *p35* expression. Notably, two out of 12 heat-shocked *ced-9;hs-p35* embryos that hatched became viable adults. This suggests either that *p35* protein is stable or that the function of *p35* is required only during early development to rescue the *ced-9* defect. In control *ced-9* animals, no embryos hatched out of 316 examined (Table II). These findings suggest that *p35* acts downstream or independently of *ced-9*, rather than upstream in the *C.elegans* cell death pathway.

Discussion

During the development of *C.elegans*, a single genetic pathway promotes the death of a determinate set of cells (Sulston and Horvitz, 1977; Sulston *et al.*, 1983; Ellis *et al.*, 1991b). In *ced-3* or *ced-4* loss-of-function mutants and a *ced-9* gain-of-function mutant, virtually no deaths occur (Ellis and Horvitz, 1986; Hengartner *et al.*, 1992). In contrast, *ced-9* loss-of-function mutations cause ectopic cell deaths, which are suppressed by *ced-3* or *ced-4* mutations (Hengartner *et al.*, 1992). Thus, wild-type *ced-9* functions as a cell death suppressor whereas *ced-3* and *ced-4* act as

Table I. Extra nuclei in the anterior pharynx

Strain	Heat-shock	Animals with extra nuclei	Mean No. of extra nuclei[a]
ced-1	–	0/19	0
	+	0/19	0
ced-1;hs-lacZ	–	1/14[b]	0.07 ± 0.3
	+	1/20[b]	0.05 ± 0.2
ced-1;hs-p35	–	0/17[b]	0
	+	15/18[c]	4.1 ± 3.0

[a]Mean ± SD.
[b]Rol animals at L3 or L4 stage were selected and scored.
[c]Animals showing fewer cell corpses at comma stage were selected and scored at L3 or L4 stage.

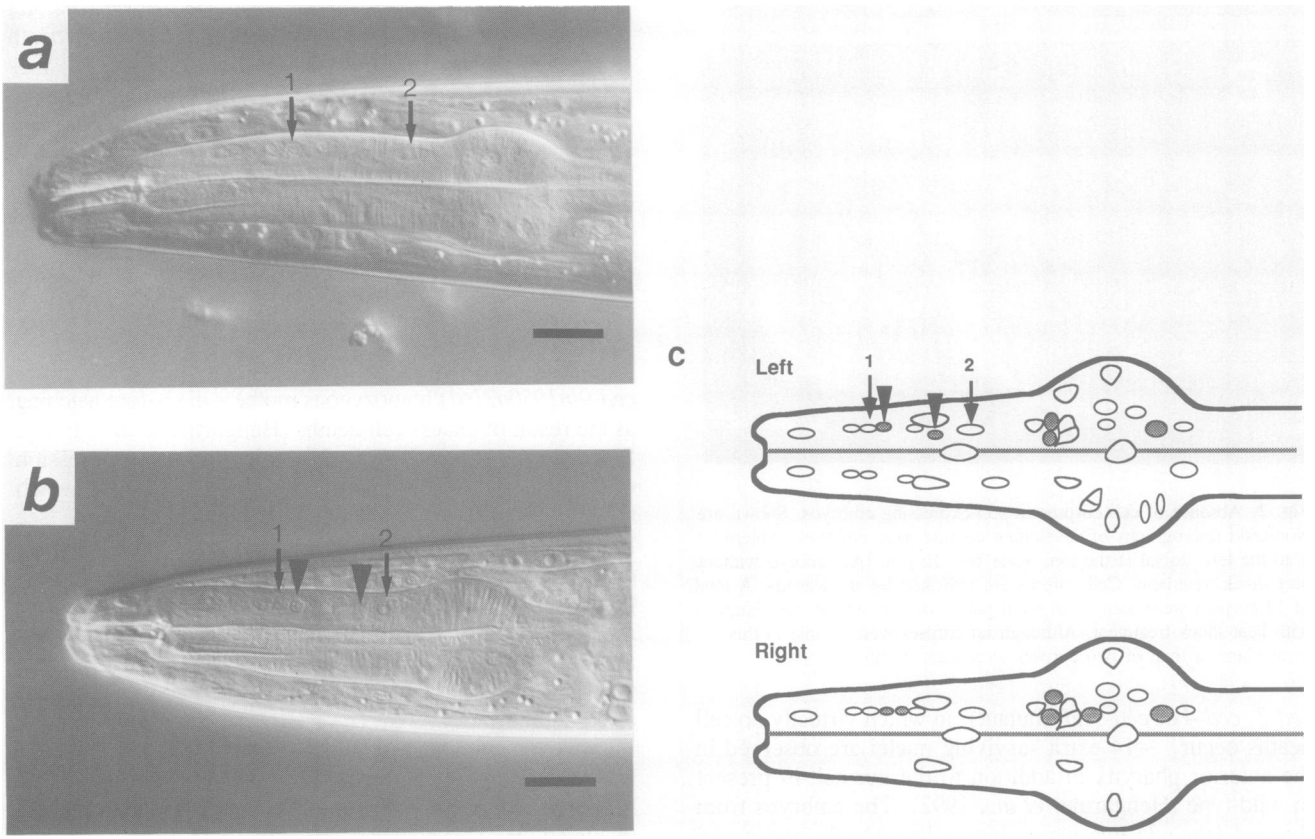

Fig. 4. Extra cells in the anterior pharynx of heat-shocked *ced-1;hs-p35* animals. Ventral sides are at the top. (**a** and **b**) Nomarski micrographs of the anterior pharynx at early L4 stage. Scale bar, 10 μm. Two nuclei are indicated for comparison: arrow 1, nucleus of m2 (pharyngeal muscle cell); arrow 2, nucleus of e3 (pharyngeal epithelial cell) (Albertson and Thomson, 1976). (a) Animal from the *ced-1;hs-lacZ* line that had not been heat-shocked. (b) Animal from the *ced-1;hs-p35* line that had been heat-shocked during early gastrulation. Two of 11 extra nuclei found in this animal are shown (arrowheads). (**c**) The approximate positions of the extra nuclei (shaded) of the pharynx shown in (b). The nuclei present in the wild-type animal are also shown (open). Arrows and arrowheads indicate the extra nuclei shown in the micrographs in (b).

killing genes. It was shown that human *bcl-2* could inhibit cell death in *C.elegans* (Vaux *et al.*, 1992) and that *ced-9* shares sequence similarities with *bcl-2* (Hengartner and Horvitz, 1994). Thus, this developmentally programmed cell death pathway appears to be conserved throughout evolution.

The work reported here extends this conclusion by showing that virally induced and developmentally programmed cell death are subject to molecularly conserved controls: Ac*M*NPV *p35* prevents embryonic cell death in a non-host organism, *C.elegans*. The capacity of *p35* to inhibit programmed cell death in *C.elegans* shows that it can suppress virus-induced apoptosis directly and does not require participation of other baculovirus-encoded genes or factors specific to the host cell. Our results suggest that a single cell death pathway operates both during the course of normal animal development and in response to viral infection. Such a conclusion is supported by the finding that the human proto-oncogene *bcl-2* inhibits developmentally programmed cell death (Vaux *et al.*, 1992) as well as apoptosis induced by infection with Sindbis virus (Levine *et al.*, 1993).

We have also demonstrated that *p35* rescues the maternal-effect lethality of a loss-of-function mutation in *ced-9*, a cell death-suppressing gene in *C.elegans*. This suggests that *p35* does not act through *ced-9* to suppress cell death, but rather by a mechanism that is distinct from that of *ced-9*. Although

Table II. Rescue of maternal-effect lethality of a *ced-9* mutation by *p35*

Strain	Heat-shock	Total No. of hatched embryos	% Hatched embryos
N2 (wild type)	−	181/184	98
	+	114/115	99
ced-9(n1950n2161)	−	0/172	0
	+	0/144	0
ced-9(n1950n2161);hs-p35	−	4/257	1.6
	+	12/196	6.1

the ability of *p35* to rescue the *ced-9* mutant was apparent, we did not observe complete suppression of the *ced-9* phenotype. The heat-shock promoter we used is active in most tissues; however, it shows variability in expression levels (Stringham *et al.*, 1992). It is possible that in some tissues the amount of *p35* was insufficient to overcome the lack of *ced-9* product. In addition, transient expression of *p35* from the heat-shock promoter might not be enough to suppress *ced-9* completely owing to instability of the p35 protein.

What is the mechanism of cell death suppression by *p35*? Because *p35* can rescue a *ced-9(lf)* mutation, it seems likely that it functions at the same point or downstream of *ced-9*. [However, since *ced-9(n1950n2161)* is not a null allele (Hengartner *et al.*, 1992), we cannot exclude the possibility that *p35* enhances the ineffectual activity of the *ced-9* mutant protein.] *ced-9* has sequence similarity to the mammalian cell death suppressor *bcl-2*, which was recently implicated in control of superoxide radicals (Hockenbery *et al.*, 1993; Kane *et al.*, 1993). *p35* may act in the same regulatory pathway. However, the products of *p35* and *bcl-2* reside in different intracellular compartments: Bcl-2 protein is associated with various intracellular membranes (Chen-Levy *et al.*, 1989; Hockenbery *et al.*, 1990; Jacobson *et al.*, 1993), whereas p35 is cytosolic in infected cells (Hershberger *et al.*, 1994). This may indicate that they have distinct mechanisms of action. Alternatively, *p35* may directly or indirectly inhibit *ced-3* or *ced-4* activity. Recently *ced-3* was shown to encode a protein similar to IL-1β-converting enzyme (ICE), a cysteine protease (Yuan *et al.*, 1993). A viral gene, *crmA* gene of cowpox virus, encodes an inhibitor of ICE. Like *p35*, *crmA* can act as a cell death suppressor: *crmA* prevents cell death that results from overexpression of ICE or *ced-3* in rat fibroblasts (Miura *et al.*, 1993).

Many viruses encode suppressors of programmed cell death. Presumably, this is strategically beneficial for viruses to facilitate their effective replication. Although the general tactics of viruses are similar in this regard, the functions of virally encoded cell death suppressors are diverse. For example, Epstein−Barr virus LMP1 protein inhibits apoptosis of infected cells by inducing *bcl-2* expression (Henderson *et al.*, 1991). Some viruses encode Bcl-2-like proteins—BHRF1 in the case of Epstein−Barr virus (Cleary *et al.*, 1986; Henderson *et al.*, 1993) and LMW5-HL in African Swine Fever virus (Neilan *et al.*, 1993). As mentioned above, cowpox virus-encoded *crmA* might be an inhibitor of the *ced-3* homologue of host cells (Miura *et al.*, 1993). Another baculovirus, *Cydia pomonella* granulosis virus has a gene called *iap* that can inhibit apoptosis induced by *p35*-defective Ac*M*NPV (Crook *et al.*, 1993). *iap* does not show significant homology to *p35*, but instead contains a zinc finger-like motif, suggesting that it may have DNA binding activity. Since *p35* appears to be unrelated to any known cell death suppressor, this gene provides a new target of intervention in the *C.elegans* programmed cell death pathway, and may assist in dissecting the molecular steps of this pathway.

Materials and methods

General methods and strain maintenance

Worms were grown on agar plates seeded with *E.coli* strain OP50 as described (Brenner, 1974). Unless otherwise indicated, strains were grown at 20°C.

Strains used

The following mutations and chromosomal aberration were used: LGI: *ced-1(e1735)* (Hedgecock *et al.*, 1983); LGIII: *ced-9(n1950n2161)* (Hengartner *et al.*, 1992); *unc-69(e587)/qC1*.

Plasmid construction

pASP35-3 includes the promoter region [nucleotides 3054−3362, (Russnak and Candido, 1985)] of the *hsp16-1/48* gene of *C.elegans*, and the complete open reading frame and polyadenylation signal of *p35* [nucleotides −15 to 1005 relative to the ATG start codon, +1 (Friesen and Miller, 1987)]. The *hsp16-1/48* promoter region was amplified from plasmid pHS16.48-1/B (provided from P.Candido) by PCR using oligonucleotides AS12 (5′-TAG AGA ATG ATC AGT AAG CAC TTG-3′) and AS13 (5′-TAG TTT GAA GAT CTC ACA ATT AGA G-3′) as primers. Both primers have one base change (bold) from the original genomic sequence in order to incorporate *Sau*3AI sites for cloning.

Strain constructions

ced-1 transgenic strain. To construct transgenic lines, a marker plasmid, pRF4 (Mello *et al.*, 1992) containing the dominant *rol-6(su1006)* mutation and either plasmid pPC16.48-1 (Stringham *et al.*, 1992) *(hs-lacZ)* or pASP35-3 *(hs-p35)* were co-injected into adult *ced-1(e1735)* (Hedgecock *et al.*, 1983) hermaphrodite gonads. pRF4 was injected at a concentration of 20 μg/ml and the heat-shock constructs at 180 μg/ml. Co-injected plasmids are believed to assemble into a single extrachromosomal array that segregates in a non-Mendelian pattern (Mello *et al.*, 1992). Two independent extrachromosomal arrays of *hs-p35* (*wEx14* and *wEx15*), and two of *hs-lacZ* (*wEx9* and *wEx10*) were generated. The arrays were followed by observing the phenotype of late larvae and adults: transgenic animals generally exhibit a Rol phenotype as a result of expression of the *rol-6(su1006)* marker DNA. The expression of pPC16.48-1 in transgenic animals was confirmed by immunofluorescence of heat-shocked embryos using an anti-β-galactosidase antibody (not shown). The presence of pASP35-3 was confirmed by PCR amplification of *p35* sequences (not shown). In both *ced-1;hs-lacZ* (*wEx9*) and *ced-1;hs-p35* (*wEx15*) transgenic lines, which were used to generate the data in Figures 1−3, ~50% of the progeny from a Rol mother showed the Rol phenotype.

ced-9(lf) transgenic strain. To construct the *ced-9;hs-p35* transgenic line, the *ced-1;wEx15* strain was irradiated with γ-rays (3500 rads), and integrants in which the transgene was stably transmitted were isolated. One integrant, designated *wIs3*, was used in this study. The integrated construct was backcrossed five times to the parental *ced-1* strain and then crossed into a *ced-9(n1950n2161) unc-69(e587)/qC1* strain to generate the *ced-9(n1950n2161) unc-69(e587)/qC1;wIs3* strain, which is homozygous

for the integrated *hs-p35*. Homozygous *ced-9 unc-69* worms were recognized by their Unc phenotype.

Cell corpse assays

For heat-shock experiments, gravid Rol hermaphrodite adults were cut open and embryos were collected and given a heat-shock at 32°C for 15 min. After 3.5–4.5 h, embryos at around the comma stage were collected and the number of cell corpses scored by Nomarski microscopy (Sulston and Horvitz, 1977). The comma stage was chosen for scoring because it allows precise staging of embryos and allows ready scoring of cell corpses owing to the absence of movement which occurs shortly after this stage. Because the engulfment defect of the *ced-1* mutant is incomplete, most corpses are eventually engulfed (Hedgecock *et al.*, 1983; Ellis *et al.*, 1991a). Therefore, the number of corpses observed is less than the number of cell deaths that occurred. After scoring of cell corpses, embryos were recovered from the slides, grown at 20°C and the Rol phenotype of the recovered worms and their progeny were scored.

Scoring pharynx nuclei

Embryos were heat-shocked and cell corpses scored as described above. For *ced-1;hs-p35* embryos, those with fewer corpses than wild-type were recovered from the slide. After 2 days, L3 or L4 larvae were anesthetized by mounting them on an agar pad containing 30 mM NaN_3 (Avery and Horvitz, 1987); the numbers of extra nuclei in the anterior pharynx (procorpus and metacorpus, Ellis *et al.*, 1991a) were then counted using Nomarski microscopy (Sulston and Horvitz, 1977). In control experiments (*ced-1;hs-lacZ* and *ced-1;hs-p35* without heat-shock), Rol animals were used to score extra nuclei.

Suppression test of ced-9(lf) by p35

ced-9 homozygotes produced by *ced-9 unc-69/qC1* mothers were recognized by the Unc phenotype. The *ced-9* homozygous adults were cut open with a scalpel and eggs were collected. For heat-shock treatment, embryos at early gastrulation stage were selected under a dissecting microscope and heat-shock was given at 32°C for 15 min on 35 mm agar plates. After 24 h, percentage hatching was scored. The plates were observed every 24 h in order to determine the terminal stage of the hatched animals.

Acknowledgements

We thank Peter Candido for providing us with the heat-shock plasmids and Ron Ellis for assistance with scoring pharynx nuclei and helpful discussions. We thank Judith Kimble, Dave Pilgrim and members of the Rothman lab for critically reading the manuscript. A.S. was supported by a long-term fellowship from the Human Frontier Science Program Organization. This work was supported by grant # 1RO1-GM48137 from the National Institutes of Health (to J.H.R.), and by a Steenbock Career Development Award and an award from the Chicago Community Trust/Searle Scholars Program (to J.H.R.).

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Received on December 22, 1993; revised on February 2, 1994