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Fate of the Nuclear Lamina during *Caenorhabditis elegans* Apoptosis

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In vertebrates and in *Drosophila*, lamins and lamin-associated proteins are primary targets for cleavage by caspases. Eliminating mammalian lamins causes apoptosis, whereas expressing mutant lamins that cannot be cleaved by caspase-6 delay apoptosis. *Caenorhabditis elegans* has a single lamin protein, Ce-lamin, and a caspase, CED-3, that is responsible for most if not all somatic apoptosis. In this study we show that in *C. elegans* embryos induced to undergo apoptosis Ce-lamin is degraded surprisingly late. In such embryos CED-4 translocated to the nuclear envelope but the cytological localization of Ce-lamin remained similar to that in wild-type embryos. TUNEL labeling indicated that Ce-lamin was degraded only after DNA is fragmented. Ce-lamin, Ce-emerin, or Ce-MAN1 were not cleaved by recombinant CED-3, showing that these lamina proteins are not substrates for CED-3 cleavage. These results suggest that lamin cleavage probably is not essential for apoptosis in *C. elegans*.

Key Words: **apoptosis; *C. elegans*; emerin; lamin; MAN1; nuclear envelope; programmed cell death.**

INTRODUCTION

Apoptosis (programmed cell death) is a genetically controlled pathway of cellular suicide that is conserved in metazoans. Apoptosis is used during development to regulate cell number and sculpt tissues. Apoptosis is also used as a defense mechanism in response to cell transformation, virus infection, specific drugs, or stress (reviewed in Hetts, 1998; Kidd, 1998; Lincz, 1998; Thompson, 1998). Nuclei undergo specific morphological changes during apoptosis, including proteolytic cleavage of the nuclear lamina, clustering of nuclear pore complexes, collapse and condensation of chromatin, and fragmentation of DNA. The execution phase of apoptosis involves the activation of caspases, cystein proteases that function as effectors of cell death (Kidd, 1998). The proteins targeted by caspase for cleavage and the kinetics of degradation for each targeted nuclear envelope protein reveal the nature and time course of nuclear envelope destruction during apoptosis (reviewed in Cohen *et al.*, 2001).

In *Caenorhabditis elegans*, the genes *egl-1* (*egg l*aying defective), *ced-4*, and *ced-3* (*ce*ll *d*eath abnormality) are required for most if not all somatic apoptotic events, while the *ced-9* gene protects cells from apoptosis. CED-3 is a caspase. CED-3 is activated by CED-4, which is prevented from activating CED-3 by CED-9. Cell death is triggered when EGL-1 inhibits CED-9 (reviewed in Hengartner, 1999; Metzstein *et al.*, 1998). In normal *C. elegans* cells, CED-9 and CED-4 are both localized to the mitochondria. However, when EGL-1 activation triggers apoptosis, CED-4 translocates to the nuclear envelope. This translocation does not depend on CED-3 (Chen *et al.*, 2000). This result suggests that the *C. elegans* nuclear envelope may have a role in apoptosis.

Loss of nuclear structure and function occurs early in apoptosis (Earnshaw, 1995). In vertebrates, both A- and B-type lamins are early targets for cleavage by caspases before either DNA cleavage or chromatin condensation could be detected (reviewed in Cohen *et al.*, 2001). Nuclear integral membrane proteins are also targeted by caspases, including lamin B receptor (LBR) (Buendia *et al.*, 1999; Duband Goulet *et al.*, 1998) and the LEM domain (*L*AP2, *e*merin, *M*an1 shared domain) proteins: lamin-associated polypeptide 2β (LAP2β) (Buendia *et al.*, 1999) and emerin (Columbaro *et al.*, 2001). The nuclear pore proteins NUP153 (Buendia *et al.*, 1999) and POM121 (Kihlmark *et al.*, 2001) are also targeted by caspases. Lamina-associated proteins in the nuclear interior, such as LAP2α, are also early targets for caspase cleavage (Gotzmann *et al.*, 2000). These lamina and lamina-associated proteins are cleaved at specific residues by specific caspases. The

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vertebrate and *Drosophila* lamins are cleaved in the α-helical rod domain, probably by caspase-6/interleukin-1β-converting enzyme (ICE) (Fraser and Evan, 1997; Rao *et al.*, 1996). LAP2β and Nup153 are cleaved by caspase-3 (Buendia *et al.*, 1999). LAP2α is probably cleaved by both caspases-3 and -6 (Gotzmann *et al.*, 2000). Cleaved fragments of lamins and LAP2β remain associated with the nuclear envelope but are not known to play further roles in apoptosis.

An importance of lamin cleavage in the progression of apoptosis was supported by expressing uncleavable mutant lamins A or lamin B in baby rat kidney tissue culture cells (Rao *et al.*, 1996). Cells expressing uncleavable lamins A or B, alone or together, showed a 12-h delay in the morphological onset of apoptosis. Although caspases were activated in these cells, the chromatin failed to condense and oligonucleosomal cleavage was delayed. Caspase activation in the absence of lamin cleavage confirmed that lamin cleavage occurs downstream of caspases. The delay in oligonucleosomal cleavage suggests that lamin proteolysis may facilitate the activation of nucleases responsible for DNA fragmentation (Rao *et al.*, 1996). In addition, preventing the assembly of B-type lamins at the nuclear envelope at the end of mitosis can trigger apoptosis (Steen and Collas, 2001).

Lamin-deficient cells have clustered nuclear pore complexes, detached chromatin and abnormal shapes (Liu *et al.*, 2000; Sullivan *et al.*, 1999). Apoptotic nuclei share some of these morphological features with lamin-deficient cells. The cleavage of lamins and integral proteins of the inner nuclear membrane during apoptosis may allow chromatin to detach from the nuclear lamina and may also allow changes in the shape and rigidity of the nuclear envelope. We examined the fate of the nuclear lamina during *C. elegans* apoptosis to determine how apoptosis proceeds morphologically in this system and to test the importance of lamin degradation.

MATERIALS AND METHODS

Strains, Plasmids, Antibodies, and Indirect Immunofluorescence Staining of C. elegans

Animals were maintained as described (Brenner, 1974).

Strains. *C. elegans* strains used in this study were as follows: Bristol N2, *ced-3(n717)* (Ellis and Horvitz, 1986), *nuc-1(e1392am)* [(*nuc*) abnormal nuclease], (Sulston, 1976), P_{hsp} *egl-1* [*Ex*P_{hsp} *egl-1; ced-1(e1735); egl-1(n1084 n3082) unc-76(e911)*] (Conradt and Horvitz, 1998).

Plasmids. The Ce-lamin plasmid, YK63f8, is described in Liu *et al.* (2000). The complete coding sequences of the *emr-1* or the *lem-2* genes were amplified by PCR and cloned into pCR 2.1-TOPO (Invitrogen, Leek, Netherlands). CED-3-FLAG constructs (wild-type and C358S mutation) are described in Xue *et al.* (1996). The full-length *Drosophila* lamin DM_0 cDNA in the pET20b + vector (Novagen, Madison) is described in Zhao *et al.* (1996).

Antibodies. Rabbit polyclonal antibodies against the rod and tail of Ce-lamin are described in Liu *et al.* (2000). Antiserum against CED-4 is described in Chen *et al.* (2000). Rat antisera against the N-terminal peptide of Ce-lamin (CRKGTRSSRIVTLERSAN; serum 3931) or C-terminal peptide of Ce-lamin (VEFSESESSDPSDPADRC; serum 3933) were produced by Covance, Inc. (Denver, PA). Anti-FLAG M2 monoclonal antibodies were purchased from Sigma (F3165). All fluorescently labeled secondary antibodies were of affinity-purified grade and were purchased from Jackson Laboratories (West Grove, PA).

Antibody staining. Embryos (N2 or P_{hsp} *egl-1*) were obtained and stained as described (Chen *et al.*, 2000). In short, mixed-stage animals were collected from six 90-mm petri dishes, washed with M9, and treated with a hypochlorite solution (1.1% hypochlorite, 0.62 M NaOH) for 10 min at room temperature. Embryos were resuspended in 610 μl ddH_2O, added to 640 μl staining solution (295 μl methanol, 0.4 mM EGTA, 1.6% paraformaldehyde, 35 mM KCl, 8.8 mM NaCl, 4.4 mM Na_2EGTA, 2.2 mM spermidine-HCl, 6.6 mM Pipes, pH 7.4), and incubated for 15 min on ice. The suspension was frozen in liquid nitrogen, thawed under tap water, and incubated for 15 min at room temperature. All further steps were performed at room temperature. The embryos were washed once in TBST (100 mM Tris-HCl, pH 7.5, 1% Triton X-100) and once in PBS containing 0.1% BSA, 0.5% Triton X-100, 1 mM EDTA (PBST-B). Embryos were incubated in PBST-B for 15 min, pelleted, and incubated overnight in primary antibody diluted 1:200 in PBS containing 0.5% Triton X-100, 1 mM EDTA, 1% BSA (PBST-A). Excess antibody was removed by four washes in PBST-B, 25 min each. Embryos were then incubated for 2 h with FITC- or Cy3-conjugated goat secondary antibodies.

Double-label immunostaining for CED-4 and Ce-lamin was performed as follows: Animals were first stained with primary antibodies to CED-4 and Cy3-conjugated secondary antibodies and then washed in PBST-B. Animals were then incubated overnight with antibodies to Ce-lamin, washed as above, and incubated for 2 h with FITC-conjugated secondary antibodies. Double-label for TUNEL (*T*dT-mediated d*U*TP *n*ick *e*nd *l*abeling) and Ce-lamin was performed as follows: animals were first stained for TUNEL (see below), then incubated for 2 h with antibodies to Ce-lamin, washed as above, and incubated for 2 h with Cy3-conjugated secondary antibodies. Excess secondary antibodies were removed by washes in PBST-B, and embryos were mounted in glycerol containing 2% *n*-propylgallate. Embryos were viewed with either an Olympus IX70 microscope equipped with epifluorescence or a Bio-Rad MRC-1024 confocal scanhead coupled to a Zeiss Axiovert 135M inverted microscope with a 63x/NA = 1.3 oil immersion objective. A 100 mW air-cooled argon ion laser run in the multiline mode provided the excitation light. Excitation was at 488 nm. The emission filter in the Cy3 detection channel was a D580/32 interference filter (32 nm bandpass centered on 580 nm). In the FITC channel a D522/35 interference filter (522 nm center wavelength, 35 nm bandwidth) was used. The confocal iris diameter was 2.5–3 mm, with the larger opening used for weaker signals. Vertical resolution was about 1 μm. When necessary, two to four images were averaged to reduce noise.

TUNEL Analysis

TUNEL staining was performed essentially as described in (Wu *et al.*, 2000). Embryos from six 90-mm petri dish plates were prepared from mixed-stage animals as described above and resuspended in 400 μl ddH_2O. Embryos were fixed by adding the embryos to a 1-ml solution containing 80 mM KCl, 20 mM NaCl, 1.3 mM EGTA, 3.2 mM spermine, 7.5 mM sodium Hepes (pH 6.5), 25% methanol, 2% paraformaldehyde, 0.4% glutaraldehyde and immediately frozen in liquid nitrogen. The frozen embryos were thawed under tap water and incubated at room temperature for 25 min. Fixed embryos were washed once in 1 ml of Tris–Triton

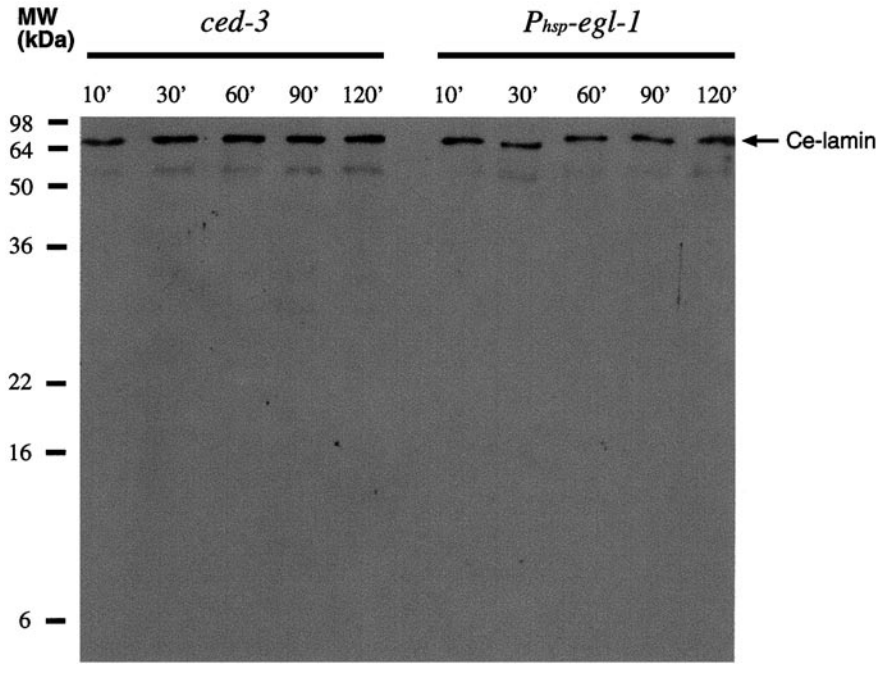


FIG. 1. Ce-lamin is not cleaved early in apoptosis. Either *ced-3* or P_{hsp}*-egl-1* embryos were heat-shocked for 1 h at 33°C, allowed to recover at 20°C for different time periods (10, 30, 60, 90, or 120 min), and then lysed. Ce-lamin was detected with antibodies that recognize the rod and tail domains (Liu *et al.*, 2000). Molecular weight (MW) markers are shown on the left and the position of full-length Ce-lamin is indicated on the right.

buffer (1% Triton X-100, 100 mM Tris-HCl, pH 7.5) and three times, 10 min each, in 1 ml of PBST (PBS containing 0.5% Triton X-100). Approximately 15 μl of packed embryos was used for TUNEL labeling. Embryos were incubated 5 min at room temperature in 25 μl of TdT enzyme buffer (Roche), 0.1% Triton X-100, and 1.5 mM $CoCl_2$. This buffer was then replaced with TdT enzyme buffer containing 0.1% Triton X-100, 1.5 mM $CoCl_2$, 8.2 units of TdT enzyme (Roche), 6.6 nM dUTP, and 3.3 nM fluorescein-12-2′-dUTP (Roche). Embryos were incubated for 2 h at 37°C. After TUNEL staining, embryos were washed in PBST and stained with antibodies as described above.

In Vitro Cleavage of Ce-Lamin, Ce-Emerin, and Ce-MAN1 by CED-3

Bacterial lysates. Preparation of bacterial lysates expressing CED-3 was as described in (Xue *et al.*, 1996). Briefly, overnight cultures of *E. coli* BL21 (*DE3*) carrying either pET-3a-*ced-3* cDNA-FLAG or pET-3a-*ced-3*(C358S) cDNA -FLAG (Xue *et al.*, 1996) were diluted 1:10 in 50 ml LB broth supplemented with 100 μg/ml ampicillin. The bacteria were grown at 37°C to OD_{600} of ~0.6. isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM to induce expression of CED-3 or CED-3(C358S) protein. Induced bacteria were grown 2 h at room temperature, centrifuged at 3000*g* for 5 min, and resuspended in 1/50 vol of CED-3 buffer (50 mM Tris-HCl at pH 8.0, 0.5 mM EDTA, 0.5 mM sucrose, 5% glycerol, 1 mM AEBSF, 5 μg/ml aprotinin, 5 μg/ml pepstatin, and 10 μg/ml leupeptin). Bacteria were lysed by sonication, and the debris was sedimented by centrifugation at 14,000*g* for 10 min. The supernatant was used in the *in vitro* cleavage experiments.

In vitro transcription and translation and CED-3 digestion. Proteins were labeled with [^{35}S]methionine using the TNT-coupled reticulocyte lysate system (Promega, Madison) and full cDNA constructs (see above). One microliter of the labeled protein was incubated at room temperature with 1 μl diluted bacterial lysate and 2 μl CED-3 buffer. The reaction was terminated after 2 h by adding an equal amount of 2X SLB (50 mM Tris-HCl, pH 6.8, 2% SDS, 100 mM dithiothereitol, 10% glycerol). Reaction mixes were separated by SDS–15% PAGE. The gels were dried and exposed to X-ray films.

Time-Course Analysis of Ce-Lamin in Vivo during Apoptosis

Embryos (N2, P_{hsp} *egl-1*, or *ced-3*) were heat-shocked for 1 h at 33°C and allowed to recover at 20°C for varying time intervals. Embryos were suspended in an equal volume of 2X SLB containing the protease inhibitors 0.1 mM AEBSF, 3.4 μg/ml aprotinin, and 50 mM Benzamidine and were frozen in liquid nitrogen. The frozen embryos were crushed by a pestle and the embryo solution was boiled for 10 min at 100°C. Lysates were subjected to SDS–15% PAGE, transferred to nitrocellulose membranes, and probed with Ce-lamin antibodies. Detection was with an ECL Western blot kit (Amersham, England, Cat. No. RPN2106).

RESULTS

Ce-Lamin Is a Late Target for Degradation during Apoptosis

The fate of Ce-lamin during *C. elegans* during apoptosis was analyzed by Western blot analysis following induction of EGL-1 expression (Fig. 1). Following heat-shock-induced expression of EGL-1, embryos were allowed to recover at 20°C for various time periods ranging from 10 min to 2 h and then lysed. The fate of Ce-lamin in these extracts was

determined by using Ce-lamin antibodies directed against the entire tail and rod domain (Liu *et al.*, 2000). We could not detect Ce-lamin cleavage in the P_{hsp} *egl-1* embryos even after 2 h of recovery (Fig. 1), even though ~30% heat-treated P_{hsp} *egl-1* embryos had arrested and contained program cell death corpses, indicating that programmed cell death had been activated. Control wild-type or *ced-3* embryos subjected to the same treatment developed normally and did not have detectable lamin cleavage (Fig. 1 and data not shown). Similar results were obtained with antibodies directed against the N-terminus of lamin (data not shown). These results indicate that in *C. elegans*, lamin is either not cleaved during apoptosis or is a very late target for caspase cleavage.

Ce-Lamin Remains Localized to the Nuclear Envelope during Apoptosis in C. elegans

We used Ce-lamin antibodies to follow the fate of Ce-lamin protein in apoptotic cells *in situ*. We also used CED-4 antibodies to follow CED-4 translocation to the nuclear periphery as a marker for cells induced to undergo apoptosis (Chen *et al.*, 2000).

We induced apoptosis in P_{hsp} *egl-1* embryos and determined the localization of endogenous CED-4 and Ce-lamin. Ce-lamin was localized with antibodies against either the N-terminus (lam-N) or C-terminus (lam-C). Since cleavage of Ce-lamin could result in different behaviors of the N- and C-terminal fragments, we used antibodies in order to follow the fate of both ends of the protein. CED-4 staining colocalized with Ce-lamin at the nuclear periphery following EGL-1 induction (Figs. 2a, and b). The pattern and intensity of staining of Ce-lamin was similar for antibodies directed against the C-terminus (Fig. 2a) or the N-terminus (Fig. 2b) of Ce-lamin. In addition, there was no significant change in the staining pattern for Ce-lamin in the nuclear interior or in the cytoplasm, compared to Ce-lamin staining in wild-type embryos (Liu *et al.*, 2000). Wild-type embryos (Figs. 2c and d), *ced-3* embryos, in which programmed cell death is blocked (data not shown), and P_{hsp} *egl-1* embryos in which apoptosis was not induced by heat shock (data not shown), displayed cytoplasmic staining of CED-4 and nuclear envelope staining of Ce-lamin. These results show that Ce-lamin remains associated with the nuclear envelope during apoptosis.

Ce-Lamin Degradation Occurs Following the Laddering of DNA

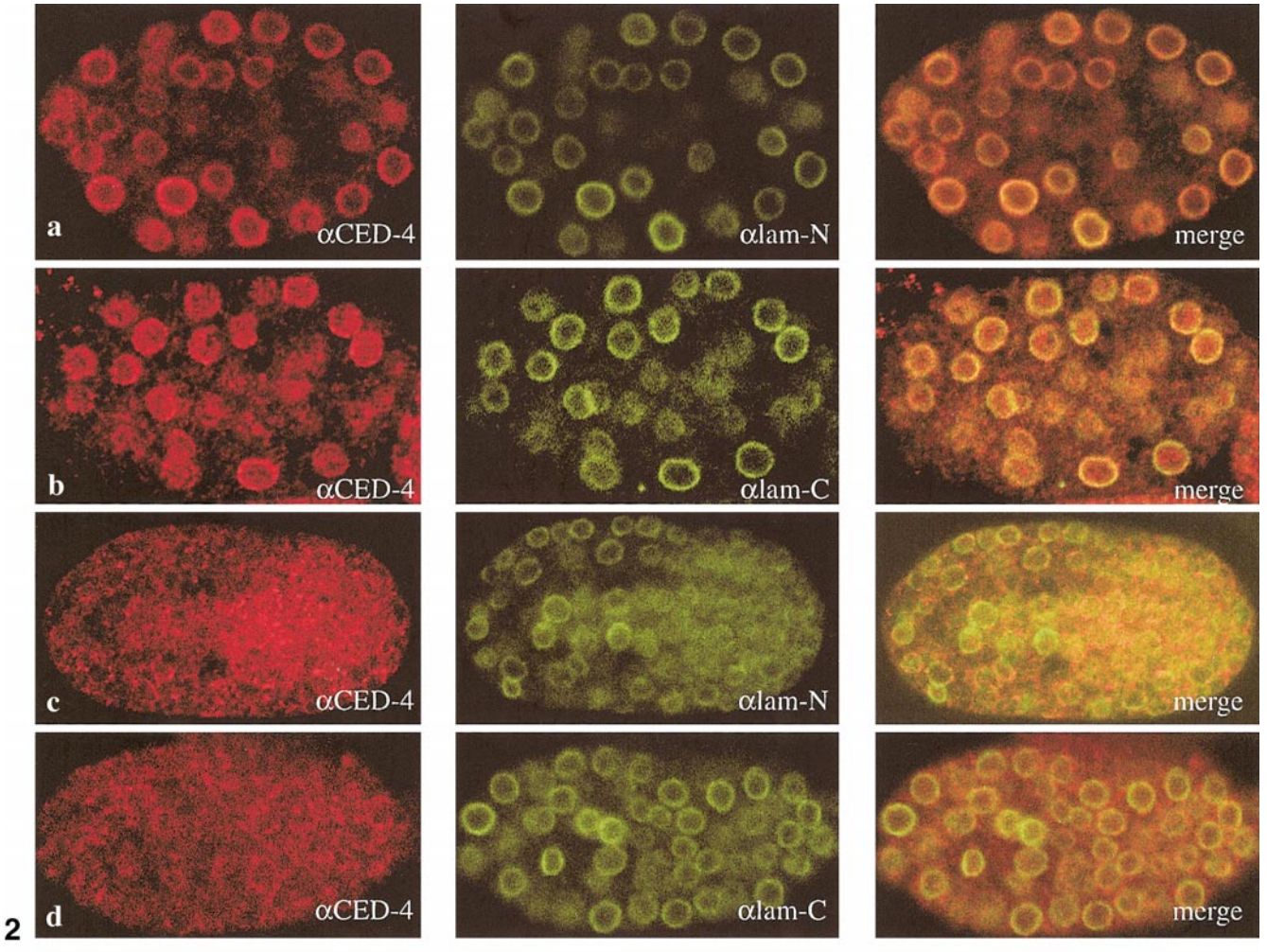
In mammalian cells, expression of mutant lamins that could not be cleaved by caspase-6 inhibited chromatin fragmentation (Rao *et al.*, 1996). We tested whether lamin degradation in *C. elegans* might be detectable only at late stages of apoptosis, when DNA laddering occurs. We stained wild-type and *nuc-1* (G → A transition converting Trp59 to amber stop codon) embryos with Ce-lamin antibodies and assessed DNA laddering by TUNEL labeling (Fig. 3). Mutations in the *nuc-1* gene, a mammalian homolog of the DNase II gene, allow the generation of TUNEL-positive staining but block further DNA degradation, resulting in the accumulation of TUNEL-positive nuclei (Wu *et al.*, 2000). We found many TUNEL-positive nuclei that had either normal (arrow in Fig. 3a) or reduced (arrow in Fig. 3b) levels of Ce-lamin staining. Some TUNEL-positive nuclei completely lacked Ce-lamin staining (arrow in Fig. 3c). Wild-type embryos at the comma or the two-fold stages contained one to three TUNEL-positive nuclei consistent with previous studies (Wu *et al.*, 2000). Most TUNEL-positive nuclei had either residual lamin staining (arrow in Fig. 3d) or no lamin staining (arrows in Figs. 3e and f). In a few TUNEL-positive nuclei the lamin staining at the nuclear envelope resembled lamin staining in normal nuclei (data not shown).

Likewise, embryos ectopically induced to undergo apoptosis contained both TUNEL-positive nuclei with normal, residual, and no Ce-lamin staining at the nuclear envelope (data not shown). These results provided the first evidence that Ce-lamin is actually degraded during late stages of apoptosis in *C. elegans* and suggest that lamin degradation in *C. elegans* occurs after DNA degradation has initiated.

Ce-Lamin Is Not a Substrate for CED-3 Cleavage in Vitro

We next analyzed if the CED-3 caspase can cleave Ce-lamin *in vitro*. Recombinant CED-3-FLAG protein was expressed in bacteria as described (Xue *et al.*, 1996). Control lysates with no CED-3 protease activity were obtained by expressing the CED-3(C358S) protein (CED-3 mutant with a Cys-to-Ser substitution at the active site) in bacteria (Xue *et al.*, 1996). We verified that CED-3 lysates contained CED-3 protease activity. First, bacterial lysates contained a 31-kDa CED-3 fragment and the mature 13-kDa caspase subunit (Fig. 4a). Second, bacterial lysate could cleave ^{35}S-labeled CED-3 and ^{35}S-labeled CED-3(C358S) (Fig. 4b and Fig. 5).

Drosophila lamin Dm_0 (Gruenbaum *et al.*, 1988) has two potential cleavage sites for CED-3 (E/DXXD) at residues D387 and D457. Incubation of ^{35}S-labeled *Drosophila* lamin Dm_0 with CED-3 lysate produced a 45-kDa fragment corresponding to cleavage at D387 (arrow in Fig. 4b, lane 11). The size of that fragment and the efficiency of cleavage are in agreement with that reported in lamin DM_0 cleav-



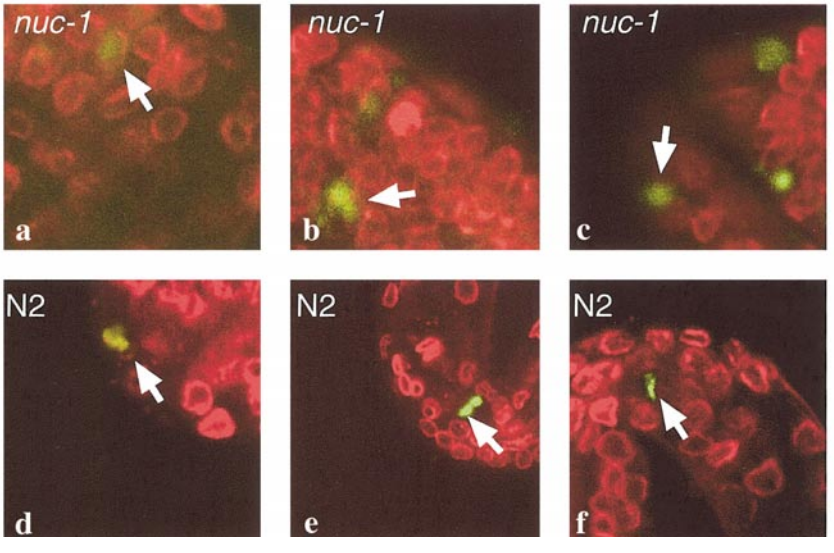


FIG. 2. CED-4 and Ce-lamin colocalize in P_{hsp}-*egl-1* embryos. P_{hsp}-*egl-1* (a and b) and wild-type (c and d) embryos were stained with antibodies against CED-4 (left panels) and either the Ce-lamin N-terminus (middle panels in a and c) or the Ce-lamin C terminus (middle panels in b and d). Merged images are shown in the right panels. Embryos in panels a and b were induced to undergo apoptosis by heat-shock for 1 h at 33°C and recovery for 45 min at 20°C.

FIG. 3. Localization of Ce-lamin in TUNEL-positive nuclei. *nuc-1(e1392am)* (upper panels) or wild-type (lower panels) embryos double labeled with TUNEL (green) and Ce-lamin antibodies (red) and viewed by confocal microscopy. An arrow in (a) shows a TUNEL-positive nucleus with normal levels of Ce-lamin staining. Arrows in (b) and (d) show TUNEL-positive nuclei with residual Ce-lamin staining and the arrows in (c), (e) and (f) show TUNEL-positive nuclei with no detectable Ce-lamin staining.

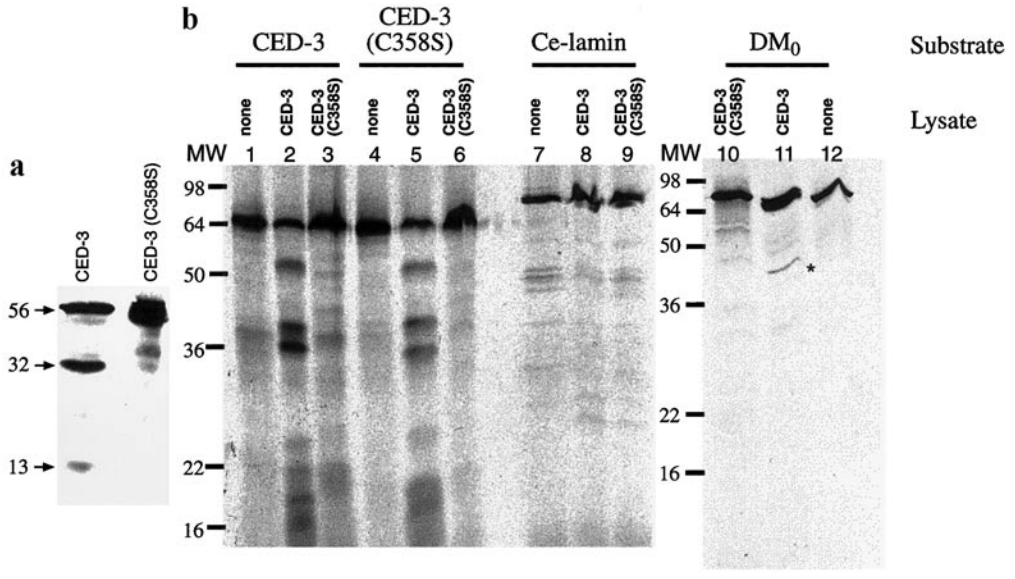


FIG. 4. Ce-lamin is not cleaved by CED-3. (a) CED-3-FLAG, either wild type (left) or C358S (right) was expressed in bacteria and detected with anti-FLAG antibodies. The expected positions of uncleaved CED-3 (56 kDa), partially cleaved CED-3 (32 kDa), and mature CED-3 (13 kDa) are shown on the left. (b) ^{35}S-labeled CED-3, CED-3(C358S), Ce-lamin or *Drosophila* lamin Dm_0 incubated with ddH_2O (none), CED-3 lysate, or CED-3(C358S) lysate. Molecular weight markers (MW) are shown on the left of each panel. The asterisk marks the position of the cleaved fragment of lamin Dm_0.

age by the *Drosophila* caspase drICE (Fraser and Evan, 1997; Fraser *et al.*, 1997). Although cleavage was inefficient, this result showed that CED-3 lysates were capable of cleaving lamin Dm_0 at a correct site. By contrast, although Ce-lamin has potential CED-3 cleavage sites at residues D178, D339, D342, D361, D364, D429, D453, D555, D558, and D561, ^{35}S-labeled Ce-lamin was not cleaved by the CED-3 lysate (Fig. 4b, lane 8). Addition of three times the amount of the CED-3 extract to the reaction had no effect on Ce-lamin cleavage (data not shown). Likewise, bacterially expressed and purified Ce-lamin was not cleaved by CED-3 lysates (data not shown). Together, these results suggest that Ce-lamin is not a substrate for CED-3 cleavage *in vivo* or *in vitro*.

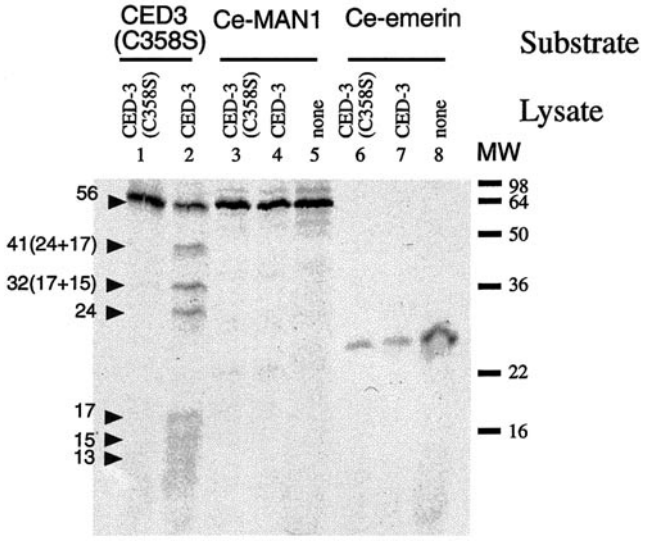


FIG. 5. Ce-emerin and Ce-MAN1 are not cleaved by CED-3. ^{35}S-labeled CED-3(C358S), Ce-MAN1, or Ce-emerin incubated with either CED-3 lysate or CED-3(C358S) lysate. The CED-3(C358S) cleavage fragments are marked with arrows. Molecular weights markers (MW) are shown on the right.

Ce-Emerin and Ce-MAN1 Are Not Substrates for CED-3 Cleavage in Vitro

In vertebrates, at least two LEM-domain proteins, LAP2α and LAP2β, are early targets for caspases (Gotzmann *et al.*, 2000). Ce-emerin and Ce-MAN1 are the only integral membrane lamina proteins with a LEM domain in *C. elegans* (Lee *et al.*, 2000). The ability of CED-3 to cleave these lamin-binding proteins was analyzed *in vitro*. ^{35}S-labeled Ce-emerin and Ce-MAN1 were incubated with the recombinant CED-3, but neither Ce-emerin nor Ce-MAN1 were cleaved by CED-3 (Fig. 5).

DISCUSSION

In vertebrates and in *Drosophila* the lamin proteins are early targets for caspase degradation (Gruenbaum *et al.*, 2000). Moreover, in mammalian cells

the cleavage of lamins by caspase-6 is an early step in programmed cell death that occurs prior to DNA laddering (Rao *et al.*, 1996). The results presented in this manuscript show that the single *C. elegans* lamin, Ce-lamin, is likely not a direct target of the CED-3 caspase, which is the *C. elegans* caspase involved in apoptosis (Yuan, 1995; Shaham *et al.*, 1999). This conclusion is based on four lines of evidence. First, Ce-lamin was not cleaved following the induction of apoptosis by expression of EGL-1. Second, both N- and C-regions of Ce-lamin remained associated with the nuclear envelope in nuclei induced to undergo apoptosis and in which CED-4 has already translocated to the nuclear envelope. Third, TUNEL-positive nuclei can retain a rim staining of Ce-lamin. Finally, Ce-lamin was not cleaved by CED-3 *in vitro*. The only evidence for lamin degradation was the loss of Ce-lamin immunofluorescence in some TUNEL-positive nuclei. Thus, our results suggest that lamin might be cleaved by a different protease, and that lamin remains associated with the nuclear envelope. Also, if complete degradation of Ce-lamin occurs rapidly after cleavage, these fragments could have been missed in the Western blot analyses.

We conclude that lamin cleavage may not be essential for apoptosis in *C. elegans*. There are several possible explanations for a requirement for lamin degradation in vertebrates but not in *C. elegans*. Vertebrates contain A-type lamins encoded by the LMNA gene, in addition to at least one B-type lamin (Stuurman *et al.*, 1988). Perhaps the more complex lamina structure interferes with apoptotic events in the nucleus and must be eliminated. Alternatively, lamins and their associated proteins may have additional roles that affect the apoptotic process in vertebrates (Cohen *et al.*, 2001; Stuurman *et al.*, 1998). Vertebrates contain additional nuclear envelope proteins not conserved in *C. elegans* including LBR, LAP1, LAP2, and Nurim that are targeted by caspases during apoptosis. Other proteins, such as emerin, may require intact lamin for their nuclear envelope localization (Lee *et al.*, 2002; Olins *et al.*, 2001; Sullivan *et al.*, 1999). Cleavage of lamins in vertebrate cells may release these lamin-binding proteins from the nuclear envelope, and this release may be important for apoptosis. Perhaps in *C. elegans*, the translocation of CED-4 to the nuclear envelope is an important step in apoptosis that requires either Ce-lamin or lamin-associated proteins. In vertebrates, nuclear translocation of the CED-4 homolog, Apaf-1 (*a*poptotic *p*rotease-*a*ctivating *f*actor-1) does not appear to occur and thus may not require lamin for activity. Two additional *C. elegans* nuclear lamina proteins, Ce-MAN1 and Ce-emerin, also failed to be cleaved *in vitro* by CED-3. Since Ce-emerin and Ce-MAN1 are the only integral membrane LEM-domain proteins in *C. elegans* and since the LEM-domain proteins LAP2α and LAP2β are cleaved by caspases during vertebrate apoptosis, the lack of their cleavage suggests differences in the involvement of nuclear lamina proteins in apoptosis in vertebrates and in *C. elegans*. It would be interesting now to go back and follow the nuclear architecture in worms and in human cells at different stages of apoptosis and to determine which features of apoptosis are shared and which are distinct in the two types of cells.

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