The expression, lamin-dependent localization and RNAi depletion phenotype for emerin in *C. elegans*

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Summary

Emerin belongs to the LEM-domain family of nuclear membrane proteins, which are conserved in metazoans from *C. elegans* to humans. Loss of emerin in humans causes the X-linked form of Emery-Dreifuss muscular dystrophy (EDMD), but the disease mechanism is not understood. We have begun to address the function of emerin in *C. elegans*, a genetically tractable nematode. The emerin gene (*emr-1*) is conserved in *C. elegans*. We detect Ce-emerin protein in the nuclear envelopes of all cell types except sperm, and find that Ce-emerin co-immunoprecipitates with Ce-lamin from embryo lysates. We show for the first time in any organism that nuclear lamins are essential for the nuclear envelope localization of emerin during early development. We further show that four other types of nuclear envelope proteins, including fellow LEM-domain protein Ce-MAN1, as well as Ce-lamin, UNC-84 and nucleoporins do not depend on Ce-emerin for their localization. This result suggests that emerin is not essential to organize or localize the only lamin (B-type) expressed in *C. elegans*. We also analyzed the RNAi phenotype resulting from the loss of emerin function in *C. elegans* under laboratory growth conditions, and found no detectable phenotype throughout development. We propose that *C. elegans* is an appropriate system in which to study the molecular mechanisms of emerin function in vivo.

Key words: Lamin, Lap2, LEM-domain, Nuclear envelope, Emery-Dreifuss muscular dystrophy

Introduction

X-linked Emery-Dreifuss muscular dystrophy (EDMD) is a recessive disorder characterized by early contractures of the Achilles, elbow and neck tendons, progressive muscle wasting, and conduction defects in the heart (Emery, 1989; Morris and Manilal, 1999). X-linked EDMD is caused by the loss of emerin (Bione et al., 1994), an integral membrane protein localized at the nuclear inner membrane (Manilal et al., 1996; Nagano et al., 1996; Yorifuji et al., 1997). There is also an autosomal dominant form of EDMD caused by mutations in *LMNA*, the gene encoding A-type lamins (Bonne et al., 1999; Bonne et al., 2000). At least two additional diseases also map to *LMNA*: dilated cardiomyopathy with conduction system disease, and Dunnigan-type familial partial lipodystrophy (reviewed by Bonne et al., 2000; Cohen et al., 2000; Wilson et al., 2001). A new term, laminopathy, has been coined to encompass the growing number of disorders caused by mutations in nuclear lamins and lamin-binding proteins.

The nuclear envelope encloses and attaches to the chromosomes. The envelope consists of the outer and inner membranes and their enclosed lumenal space, nuclear pore complexes, and an underlying lamina (Gant and Wilson, 1997). The shape and structure of the nucleus depend on the nuclear lamina. The lamina consists of a network of polymerized lamins (type V intermediate filament proteins) (Moir et al., 2000; Stuurman et al., 1998) plus a growing number of lamin-binding proteins (Dechat et al., 2000). Humans express three B-type lamins (encoded by two genes) and four A-type lamins, which arise by alternative splicing of *LMNA* (Stuurman et al., 1998; Erber et al., 1999). The expression of A-type lamins is generally correlated with cell differentiation, since all cells express at least one B-type lamin, but not all cells express A-type lamins (Riemer et al., 1995).

Emerin belongs to the LEM family of nuclear proteins, most of which are integral membrane proteins. This family includes emerin, found in *C. elegans* to humans; LAP2, found only in vertebrates (Foisner and Gerace, 1993); MAN1, found in *C. elegans* to humans (Lin et al., 2000); otefin, found in *Drosophila* (Goldberg et al., 1998); and Lem-3, found in *C. elegans* to mammals (Lee et al., 2000) (see IMAGE clone number 1243400 for mouse Lem-3 ortholog). LEM proteins all share a ~40-residue motif known as the LEM-domain (Lin et al., 2000). Emerin and the β-isoform of LAP2 are also similar outside the LEM-domain, suggesting that their functions might be related. In biochemical experiments, both LAP2 and emerin bind directly to lamins; emerin in particular can interact with both A- and B-type lamins (Fairley et al., 1999; Clements et al., 2000). In addition to binding lamins, both LAP2 (Furukawa, 1999; Shumaker et al., 2001) and emerin (Lee et al., 2001) interact with a small DNA-bridging protein named BAF, whose in vivo function is unknown (barrier-to-autointegration factor) (Lee and Craigie, 1998; Chen and Engelman, 1998; Zheng et al., 2000). Emerin localization at the nuclear envelope is proposed to be essential for its function,

since mutations that mislocalize emerin to the ER cause EDMD (Ellis et al., 1998). A-type lamins contribute to localizing emerin at the nuclear inner membrane, since emerin becomes localized to both the nuclear envelope and endoplasmic reticulum (ER) network in *LMNA*-null mice (Sullivan et al., 1999). However, the continued localization of emerin at the nuclear envelope in *LMNA*-null mice suggested that other proteins, such as B-type lamins, BAF, or other nuclear membrane proteins, might also help retain emerin at the nuclear envelope.

Many models have been proposed to explain the symptoms of EDMD, ranging from defects in mechanical instability or muscle cell regeneration, to defects in gene expression, lamina structure or nuclear signaling (Morris and Manilal, 1999; Östlund et al., 1999; Gruenbaum et al., 2000; Wilson, 2000). Testing such models will require a model organism with powerful genetics. This model organism must also have nuclear envelope proteins and dynamics that parallel the human nucleus. These criteria rule out the use of single-celled eukaryotes such as *S. cerevisiae*, which do not express lamins, LEM-domain proteins or BAF (Cohen et al., 2000). We have chosen *C. elegans* as a possible model system to study the function of emerin and the molecular mechanisms underlying EDMD. *C. elegans* is a genetically tractable nematode with differentiated cells and tissues including muscle (Wood, 1988; Culetto and Sattelle, 2000). The *C. elegans* nuclear envelope is a sophisticated yet simple version of the human nuclear envelope. Sophisticated, because the *C. elegans* nucleus shares many conserved nuclear envelope proteins with vertebrates, and also breaks down completely during mitosis (Lee et al., 2000), with nuclear structural dynamics similar to human nuclei. However, *C. elegans* is comparatively simple because it encodes only a single B-type lamin gene (*lmn-1*) (Riemer et al., 1993) that is essential for viability (Liu et al., 2000). By contrast, humans have two B-type lamin genes and one A-type gene (Stuurman et al., 1998). There are only three genes encoding LEM proteins in *C. elegans*: *emr-1, lem-2* and *lem-3*, encoding Ce-emerin, Ce-MAN1 and Ce-Lem3, respectively (Lee et al., 2000), all of which are conserved in mammals (Lin et al., 2000; Lee et al., 2000) (IMAGE clone 1243400 for mouse Lem-3). However, humans have additional proteins at the nuclear inner membrane that are unique to vertebrates, including a group of alternatively spliced LEM-domain proteins named lamin associated polypeptide 2 (LAP2) (Foisner and Gerace, 1993; Berger et al., 1996; Dechat et al., 2000). The conservation and relatively small number of LEM-domain proteins in *C. elegans* means that it will ultimately be feasible to investigate the function(s) of an entire family of LEM proteins in this single organism.

Here we have investigated the localization, nuclear envelope interactions and loss-of-function phenotype for Ce-emerin. We show that Ce-emerin co-localizes at the nuclear envelope with Ce-lamin in *C. elegans* embryos, co-immunoprecipitates with Ce-lamin from embryonic lysates, and requires Ce-lamin for its localization at the envelope in vivo. However, no other nuclear envelope proteins tested depend on emerin for their envelope localization, including Ce-lamin. Our results also show that Ce-emerin is widely expressed, like human emerin, and similarly dispensible during embryonic development, suggesting that *C. elegans* is an appropriate genetic model for emerin function.

Materials and Methods

Antibodies and indirect immunofluorescence staining of *C. elegans*

Adult N2 *C. elegans* nematodes were fixed and prepared for indirect immunofluorescence staining as described (Lee et al., 2000), using the following polyclonal antisera. Rat anti-emerin serum 3598 and mouse anti-emerin serum 3272 were used at 1:100 dilution; both sera were raised against the same C-terminal peptide of Ce-emerin (Lee et al., 2000). Affinity-purified rabbit anti-lamin antibodies were used at 1:400 dilution (Liu et al., 2000). Rat anti-MAN1 serum 3597 was raised against KLH-conjugated C-terminal Ce-MAN1 peptide CAVWKWIGNQSQKRW-COOH (the same antigen as serum 3268) (Lee et al., 2000) and used at 1:100 dilution. Rat anti-UNC-84 serum 3595 was used at 1:100 dilution; this serum was raised against a KLH-conjugated N-terminal peptide of Ce-UNC-84 (residues 5-18: TEADNNFDTHEWKSC-COOH). Two independent rabbit anti-lamin sera used for immunoprecipitations were raised against a KLH-conjugated N-terminal peptide of Ce-lamin (CRKGTRSSRIVTLERSAN; serum 3930) and a KLH-conjugated C-terminal peptide of Ce-lamin (VEFSESSDPSDPADRC; serum 3932). Peptides and KLH-conjugates were synthesized by Boston BioMolecules (Woburn, MA). Immunization and serum production were done by Covance (Denver, PA). Monoclonal antibody mAb414, which recognizes a subset of nucleoporins in *C. elegans* (Lee et al., 2000; Davis and Blobel, 1986; Browning and Strome, 1996), was purchased from BAbCo (Richmond, CA). Our secondary antibodies were affinity-purified Cy3-conjugated goat anti-rat, affinity-purified Cy3-conjugated goat anti-mouse, and affinity-purified FITC-conjugated goat anti-rabbit. All secondary antibodies were purchased from Jackson Laboratories (West Grove, PA) and used at 1:200 dilution.

Embryo lysate preparation and immunoprecipitation

Gravid wild-type N2 hermaphrodites were recovered from four 10 cm plates by washing with 15 ml of M9 buffer (Lewis and Fleming, 1995). The collected worms were pelleted by centrifugation at 450 ***g*** for 1 minute, resuspended in 15 ml of bleach solution (0.5% hypochlorite, 1N NaOH), and incubated at 22-24°C for 3 minutes with occasional shaking to dissolve the adult nematodes. The released embryos were then pelleted at 450 ***g*** for 1 minute, resuspended in 15 ml of bleach solution, repelleted, and washed twice with M9 buffer. The final pellet of isolated embryos was resuspended in 250 μl homogenization buffer (HB; 15 mM Hepes pH 7.6, 10 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM EGTA, 44 mM sucrose) containing 100 μg/μl protease inhibitors (aprotinin, leupeptin and PMSF) and 1 mM DTT. The embryos were then crushed using about 25 strokes of a dounce homogenizer (pestle A). After recovering the crushed embryos, the homogenizer was rinsed with 250 μl of HB, and both this and the crushed embryos (total volume ~500 μl) were combined in an eppendorf tube and centrifuged for 20 minutes at 14,000 ***g***. The supernatant (embryo lysate) was recovered, supplemented with 50 μl of cold buffer (15 mM Hepes, pH 7.6, 1 M NaCl), and used as described below for immunoprecipitation reactions.

Co-immunoprecipitation was done as follows (modified from Lee and Schedl, 2002). Isolated N2 embryonic extracts (500 μl) were pre-cleared by incubating for 30 minutes at 22-24°C with 100 μl uncoupled Protein A Sepharose (Amersham Pharmacia Biotech AB, Uppsala Sweden), followed by centrifugation at 4000 ***g*** for 2 minutes. The cleared extract supernatant was then incubated for 1 hour at 4°C (constant mixing) with 5 μl of either immune or preimmune rabbit antiserum against an N-terminal (serum 3930) or C-terminal (serum 3932) peptide of Ce-lamin. Samples were then supplemented with Protein A Sepharose beads (50 μl), incubated for 4 hours at 4°C, and centrifuged at 4000 ***g*** for 2 minutes. The beads were washed four times with HBS (HB containing 250 mM NaCl and protease

inhibitors), resuspended in 25 μl of 2× SDS gel-sample buffer and boiled for 5 minutes. Samples were loaded onto a 4-12% SDS-PAGE gradient gel, electrophoresed for 45 minutes at 200 volts, and blotted to nitrocellulose membrane. Membranes were then blocked for 2 hours in TBS-T containing 5% (weight/volume) nonfat dry milk. Blots were probed with rat anti-Ce-emerin serum 3598 (1:500 dilution) for 2 hours at 22-24°C, washed four times (5 minutes each) in TBS-T, and incubated for 1 hour with HRP-conjugated goat anti-rat secondary antibody (1:10,000 dilution; Jackson Immunoresearch, West Grove, PA). Blots were then washed four times (5 minutes each) in TBS-T, incubated with ECL reagents (Amersham) and exposed to film.

cDNA clones for Ce-emerin and Ce-lamin

The full-length cDNA encoding Ce-lamin has been described (Liu et al., 2000). An EST containing the entire open reading frame of Ce-emerin was obtained from Y. Kohara (National Institute of Genetics, Japan; yk258d11) and confirmed by DNA sequence analysis (not shown).

RNA-mediated interference (RNAi) experiments

Double-stranded RNA (dsRNA) corresponding to Ce-emerin was synthesized from plasmid yk258d11 (Bluescript vector) using the Ambion Megascript T7 and T3 kits to synthesize single stranded RNAs. These RNAs were combined to form dsRNA as previously described (Liu et al., 2000). The dsRNA for Ce-lamin was synthesized as described (Liu et al., 2000). For injection experiments, dsRNA (0.1-1 μg/μl) was injected into both gonads of at least 10 adult hermaphrodites per construct as described (Fire et al., 1998; Montgomery et al., 1998). From 12 to 60 hours after injection, the adults and embryos were either examined for viability as described (Liu et al., 2000), or fixed and stained by indirect immunofluorescence as described above. For the *lmn-1(RNAi)* feeding experiments, pJKL483.1, which contains the full length lamin cDNA subcloned into feeding vector L4440, was used to transform *E. coli* HT115(DE3) cells. The transformed bacteria were used to feed N2 nematodes as described (Timmons et al., 2001).

Results

Wild-type *C. elegans* embryos were stained for endogenous Ce-emerin and Ce-lamin by indirect immunofluorescence. Ce-emerin

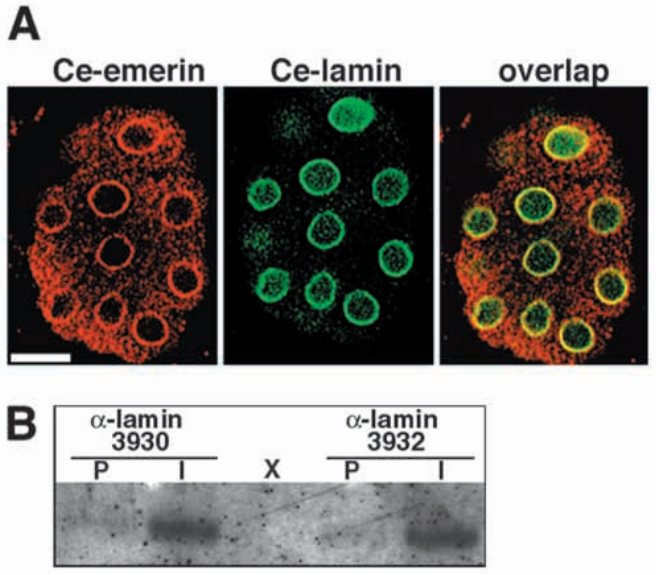

Fig. 1. Colocalization and co-immunoprecipitation of endogenous Ce-emerin and Ce-lamin in wild-type *C. elegans* embryos. (A) Panels show indirect immunofluorescence staining of wild-type *C. elegans* embryos with antibodies against endogenous Ce-emerin (red; serum 3272), endogenous Ce-lamin (green), and the overlap of both signals (yellow). Bar, 10 μm. (B) Ce-emerin and Ce-lamin are co-immunoprecipitated from *C. elegans* embryonic lysates by immune (I) but not preimmune (P) antibodies raised against an N-terminal peptide (serum 3930) or a C-terminal peptide (serum 3932) of Ce-lamin. Shown is a western blot of immunoprecipitates probed with antibodies against Ce-emerin (see Materials and Methods). *X* indicates a blank lane.

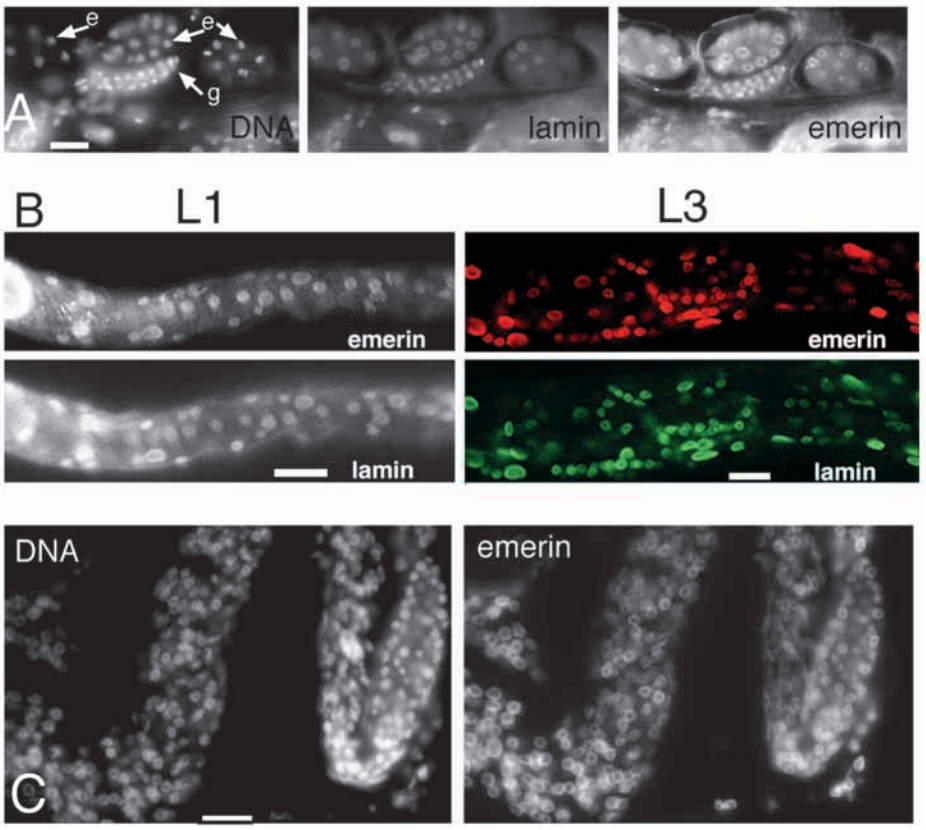

Fig. 2. Ce-emerin is expressed throughout development and is ubiquitous in adult *C. elegans*. Shown are samples stained for DNA and stained by indirect immunofluorescence for endogenous Ce-emerin (serum 3272) and endogenous Ce-lamin. (A) Triple-staining of early embryos ('e'), plus adjacent adult tissue including the gonad ('g'). (B) Double-staining of L1 (left) and L3 (right) larvae for endogenous Ce-lamin and Ce-emerin, as indicated; anterior regions are oriented on the left, and posterior on the right. (C) Adult gonad double-stained for DNA and endogenous Ce-emerin, showing Ce-emerin-positive eggs and embryos. Bars, 10 μm.

colocalized with Ce-lamin at the nuclear envelope of embryonic cells (Fig. 1A). Ce-emerin also co-immunoprecipitated with endogenous Ce-lamin from lysates of *C. elegans* embryos, using antibodies directed against N- or C-terminal peptides of Ce-lamin, as shown in blots probed for Ce-emerin (Fig. 1B). Ce-emerin is extracted from purified nuclei by combined treatment with high salt plus detergent, but not by salt or detergent alone (Lee et al., 2000), similar to other nuclear integral membrane proteins (Foisner and Gerace, 1993), including Ce-MAN1 (Lee et al., 2000). The biochemical properties, colocalization and co-immunoprecipitation results confirmed that Ce-emerin is a lamin-binding nuclear membrane protein, like human emerin.

The expression pattern of endogenous Ce-emerin protein was determined by indirect immunofluorescence in embryos, larvae and adult cells. To control for antibody penetration into nematode tissues, we double-labeled with antibodies against Ce-lamin, which is expressed in all *C. elegans* cells except sperm (Liu et al., 2000). Ce-emerin was detected at the nuclear envelope in all embryonic cells (Fig. 2A), and was detected in all cells examined throughout larval development, as shown for L1 and L3 larvae (Fig. 2B) and in adult N2 hermaphrodites, including the gonad (Fig. 2C), with one possible exception. Ce-emerin was not detected in cells undergoing spermiogenesis that had reached the stage of having condensed chromatin (data not shown). However, we could not definitively conclude that emerin was absent from sperm, because sperm lack Ce-lamin (Liu et al., 2000) and we therefore had no positive control for antibody access into these cells during staining. Nevertheless, the nearly ubiquitous positive staining seen for Ce-emerin throughout development and in adult animals was similar to that seen for human emerin, which is found in most cell types examined (Manilal et al., 1999).

Nuclear localization of other nuclear envelope proteins does not depend on Ce-emerin

To determine whether lamin or other nuclear envelope proteins depend on Ce-emerin for their localization, we created embryos deficient in Ce-emerin. This was done by the RNA interference (RNAi) method, in which mRNA production from a specific gene is disrupted by injecting adult hermaphrodites with double-stranded RNA (dsRNA) corresponding to the targeted gene (Fire et al., 1998; Montgomery et al., 1998). We used a 500 base pair dsRNA, corresponding to the entire open reading frame of the Ce-emerin gene, *emr-1* (Lee et al., 2000). Emerin protein was not detected by indirect immunofluorescence in *emr-1(RNAi)* embryos (Fig. 3A), relative to uninjected controls (Fig. 3A, 'WT'). In these experiments, embryos were double-stained for endogenous Ce-lamin as a positive control for antibody access, and to rule out the possibility that loss of Ce-emerin might affect Ce-lamin localization (Fig. 3, right panels). Ce-lamin localized normally in the absence of Ce-emerin, demonstrating that emerin is not structurally required for lamin assembly in *C. elegans*. We also localized two other inner membrane proteins: fellow LEM protein Ce-MAN1 (Lee et al., 2000), and a conserved non-LEM protein named UNC-84 (Malone et al., 1999; Dreger et al., 2001) (K.K.L., D. Starr, M.C. et al., unpublished). Both Ce-MAN1 and UNC-84 were localized at the nuclear envelope in *emr-1(RNAi)* embryos (Fig. 3B), as were nuclear pore complexes detected using monoclonal antibody mAb414 (Fig. 3B). Thus, of the nuclear proteins tested

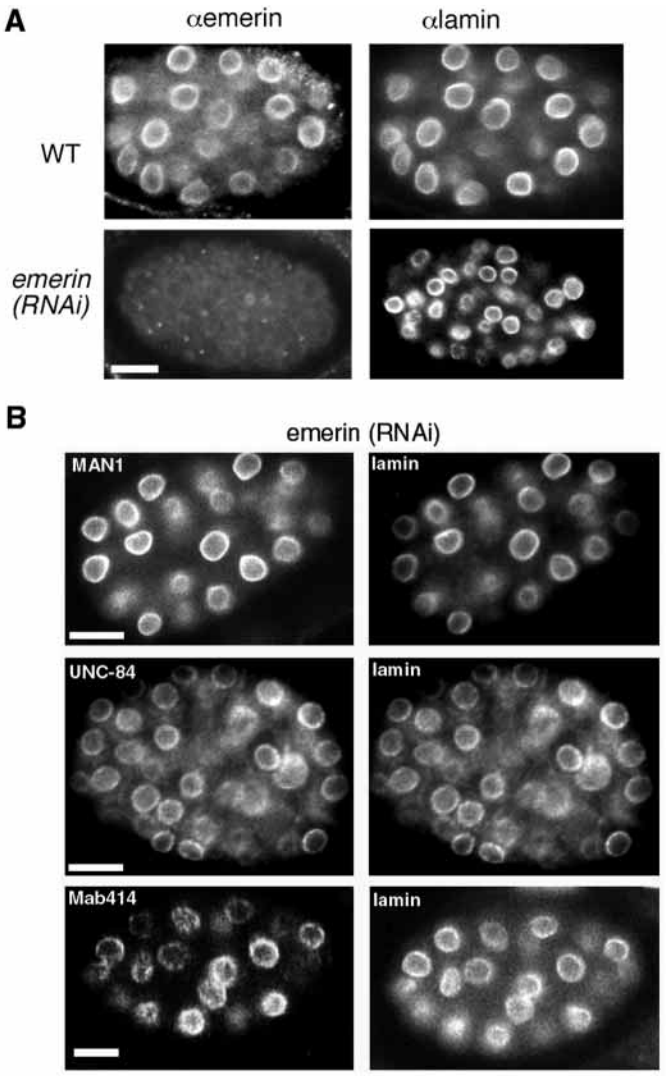

Fig. 3. Ce-emerin is not required to localize Ce-lamin, Ce-MAN1, UNC-84 or nuclear pore complexes. The panels show double-staining by indirect immunofluorescence for endogenous Ce-lamin (right panels), plus either Ce-emerin, Ce-MAN1, UNC-84 or nuclear pore complex proteins (NPCs). (A) Staining for Ce-emerin (αemerin) and Ce-lamin (αlamin) in uninjected wild-type embryos (WT; top row) and in emerin*(RNAi)* embryos (bottom row). (B) Double staining for each indicated marker on the left, and Ce-lamin on the right, in emerin*(RNAi)* embryos. Bars, 10 μm.

(Ce-lamin, Ce-MAN1, UNC-84 and nucleoporins), none depended on Ce-emerin. The RNAi method was quite effective at removing Ce-emerin. Thus, even though we cannot rule out the possible activity of undetectable residual Ce-emerin, we concluded that emerin is not structurally (stoichiometrically) required for the localization or retention of the B-type lamin in *C. elegans*, fellow LEM protein Ce-MAN1, or unrelated nuclear envelope protein, UNC-84.

Ce-emerin is not required for viability in embryos or adults

We then examined animals depleted of Ce-emerin for any phenotype during development. We found that the *emr-*

1(RNAi) embryos with no detectable Ce-emerin developed at normal rates into fertile adult nematodes. Because the *emr-1*(*RNAi*) embryos remained emerin-depleted through adulthood, we also examined the Ce-emerin depletion phenotype in adult nematodes. Gonad cells, which usually gave the brightest staining for Ce-emerin in adults, had undetectable Ce-emerin as shown by double-staining for Ce-lamin (Fig. 4, green) and Ce-emerin (Fig. 4, red; note the nonspecific red staining of the cuticle). These emerin-depleted adults had no detectable phenotype: they displayed normal movement and feeding behavior, and produced viable fertile offspring: *emr-1(RNAi)* animals and control N2 animals had similar brood sizes (averaging 206 for *emr-1(RNAi)* and 208 for N2; n=5), and aged at similar rates (50-58% not moving well and 35% dead by day 23, and all dead by day 26 at 20ºC; n=20 each). This result was expected, since emerin loss in humans has no detectable effect until childhood, and then selectively affects a few specific tissues (Emery, 1989). We concluded that Ce-emerin is not essential in *C. elegans*.

The nuclear localization of Ce-emerin is dependent on Ce-lamin

In mice that lack A-type lamins, emerin is found both in the nuclear envelope and ER. To test definitively for dependence on lamins, we made lamin-deficient *lmn-1(RNAi)* embryos (Liu et al., 2000; see Materials and Methods). These *lmn-1(RNAi)* embryos were then triple-stained for DNA, Ce-lamin, and Ce-emerin (Fig. 5). Ce-emerin staining at the nuclear envelope was not detectable in lamin-deficient embryos (Fig. 5A, 'injection'). This result showed that either the expression or nuclear envelope localization of Ce-emerin requires Ce-lamin. The background cytoplasmic staining for Ce-emerin appeared to increase in lamin-deficient cells. However, this signal was too weak to rigorously conclude that Ce-emerin dispersed into the ER in the absence of Ce-lamin. Lamin-dependent nuclear localization

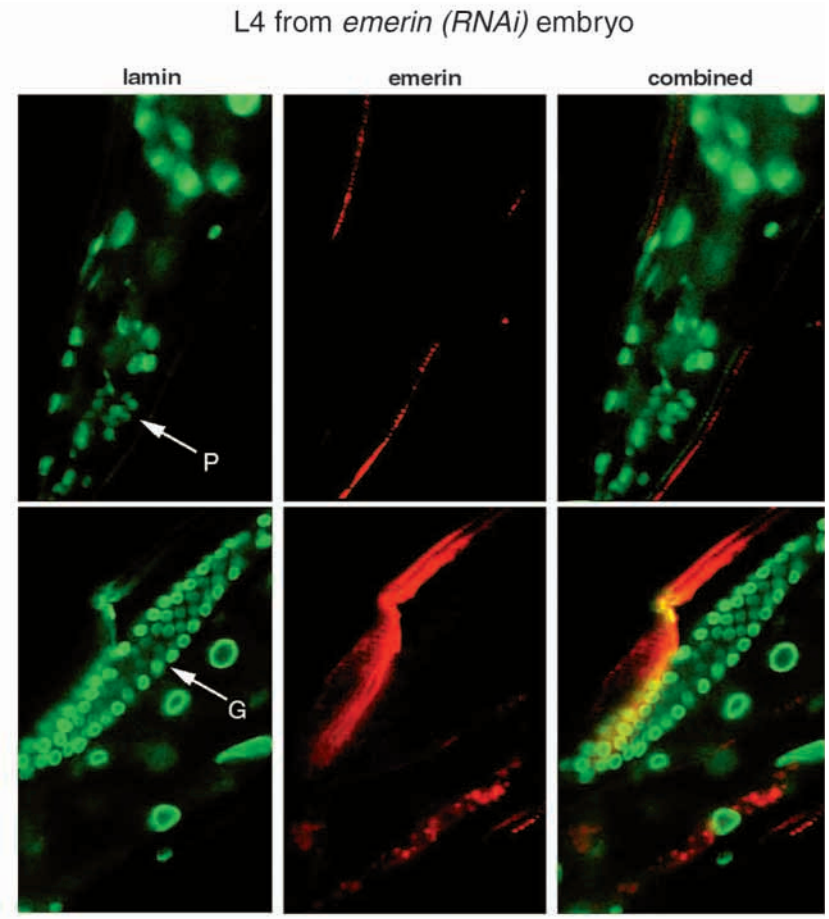

Fig. 4. RNAi-induced loss of Ce-emerin protein persists into adulthood. L1 larvae that developed from emerin*(RNAi)* embryos were double-stained by indirect immunofluorescence for endogenous Ce-lamin (green), endogenous Ce-emerin (red), or both (combined). The cuticle, a non-cellular structure, stained red nonspecifically. Ce-emerin was not detected in any cells or tissues examined, including the pharynx (P; upper row) and gonad (G; bottom row). The pharynx has 20 muscle cells, 20 neurons, 9 epithelial cells, and 9 specialized epithelial cells named marginal cells (White, 1988). Ce-lamin served as the positive control for antibody penetration during staining. Bar, 10 μm.

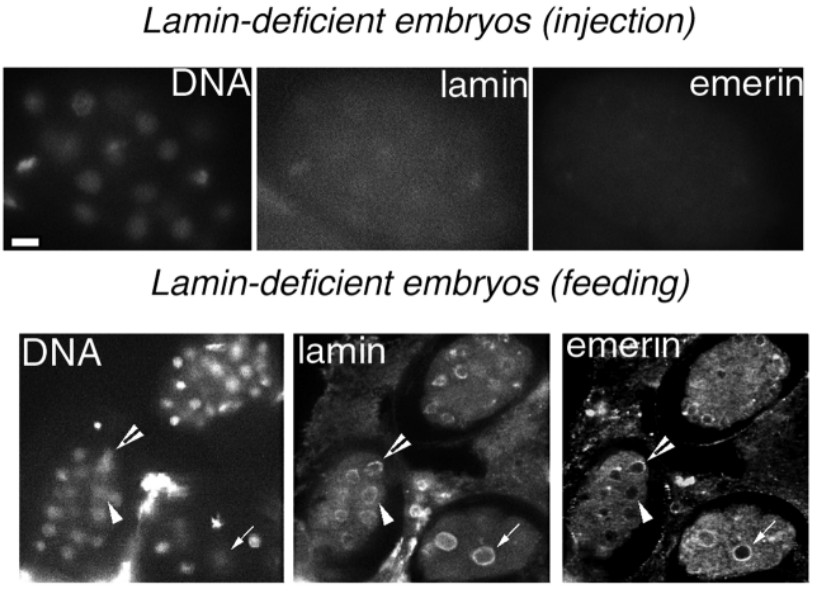

Fig. 5. Nuclear envelope localization of Ce-emerin depends on Ce-lamin. Ce-lamin-deficient *(lmn-1(RNAi)*) embryos were stained for DNA and double-stained by indirect immunofluorescence for endogenous Ce-lamin and Ce-emerin. (Top panels) Triple staining of an embryo depleted of Ce-lamin by injecting *lmn-1* dsRNA ('injection'). (Bottom panels) Triple-stained embryos were inefficiently depleted of Ce-lamin by the 'feeding' method (see Materials and Methods), producing nuclei with nearly normal amounts of Ce-lamin (arrow), or reduced levels of Ce-lamin plus mildly reduced (barred arrowhead) or severely reduced (arrowhead) levels of Ce-emerin at the nuclear envelope. Nuclear envelope rim-staining for Ce-emerin decreased in parallel with Ce-lamin. Bars, 10 μm.

of Ce-emerin was also seen in animals fed with bacteria that expressed *lmn-1* dsRNA; with this method, individual embryonic nuclei became lamin-depleted at different times (Liu et al., 2000). With the feeding method, Ce-emerin was detected only in nuclear envelopes with high residual levels of Ce-lamin (Fig. 5, 'feeding', arrow), and was reduced in nuclei with reduced or low staining for Ce-lamin (Fig. 5, 'feeding', arrowheads). Together these results clearly showed that Ce-emerin depends on lamins for stable localization at the nuclear inner membrane in *C. elegans* embryos.

Discussion

In *LMNA*-null mouse cells, emerin is localized both in nuclear and ER membranes (Sullivan et al., 1999), suggesting that A-type lamins are important but leaving open the possibility that other proposed binding partners, such as BAF or B-type lamins, might also promote emerin retention at the nuclear envelope. Our results show that in *C. elegans* embryos, Ce-emerin depends on nuclear lamin for its localization at the nuclear inner membrane. This result does not rule out the possibility that other proteins also contribute to emerin localization or retention. For example, emerin localization is also likely to depend on any protein that is essential for lamin organization or stability. To our knowledge, our results also provide the first evidence that emerin itself is not required to localize any other nuclear proteins tested, including Ce-MAN1, UNC-84 and nucleoporins. We cannot rule out the possibility that residual traces of Ce-emerin protein remained in *emr-1(RNAi)* animals, which were undetectable by immunofluorescence, although it seems highly unlikely that traces of emerin would be sufficient to physically mediate localization. Importantly, depletion of Ce-emerin had no effect on the localization of Ce-lamin. Consistent with our findings, lamins localize normally in X-linked EDMD (emerin-null) human cells (G. Morris, personal communication). The only published report on this question suggested that lamins might localize abnormally in a minority (18%) of emerin-null cells (Ognibene et al., 1999). We examined *emr-1(RNAi)* animals for defects similar to those induced by lamin deficiency (e.g. chromosome segregation defects, and abnormal spacing of nuclear pore complexes) (Liu et al., 2000), and found no evidence for lamin phenotypes in our emerin-deficient *C. elegans* cells.

The physiological effects of emerin deficiency on tendon growth and cardiac function in people cannot be directly studied in nematodes, since nematodes lack tendons and heart. However, nematodes do have differentiated and specialized muscle cells, and highly conserved muscle proteins (Waterston, 1988). Our RNAi-depletion results suggest that emerin is not required for *C. elegans* development, and might also be non-essential in adults, with the caveat that trace amounts of Ce-emerin in *emr-1(RNAi)* adults might have covered a putative essential function. It is also possible that a phenotype for Ce-emerin depletion will only appear when worms are physically stressed by growth conditions found in nature. Although these RNAi results are negative, they are consistent with the human null phenotype for emerin; human emerin is not essential for development, and its loss causes no known phenotype in most human tissues that express emerin, except for heart, skeletal muscle and tendons. One potentially critical difference between nematodes and humans is that the disease progresses very slowly (years) in humans, whereas nematodes live less than 4 weeks. Thus, if the disease mechanism is strictly linked to physical trauma or long-term mechanical disruption of muscle cell function (Morris and Manilal, 1999; Fairley et al., 1999), which cannot currently be ruled out, then nematodes will not provide a model for disease. Alternatively, disease may arise from the disruption of specific emerin-dependent interactions required for gene expression (Wilson, 2000). Supporting this model, the emerin-related protein LAP2β can directly repress the expression of a reporter gene in mammalian cells (Nili et al., 2001).

Our present work shows that the nuclear envelope localization of Ce-emerin is lamin-dependent during development, and that emerin is expressed in nearly all cell types in *C. elegans*, similar to human emerin. The only cells we detected in *C. elegans* that might lack emerin are the amoeba-like sperm cells, which also fail to stain for Ce-lamin (Liu et al., 2000). Emerin mRNA is present in human testis (Small et al., 1997), but it is not known whether emerin is present in human sperm. In humans, the non-myocyte cells of the heart are among the few cell types known to lack emerin (Manilal et al., 1999). Thus, the presence of emerin at the nuclear envelope in a wide range of cells and tissues is a conserved feature of emerin from humans to nematodes. Given the complexity of nuclear envelope structure and function, the possible functional overlap between emerin and other LEM-domain proteins, and the possibility that emerin might have multiple roles (e.g. lamin-related, BAF-related and novel), *C. elegans* will be a useful genetic system for dissecting the functional inter-relationships among LEM proteins, lamins and other nuclear envelope proteins, as well as the mechanisms of Emery-Dreifuss muscular dystrophy.

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