Ce-emerin and LEM-2 : essential roles in *Caenorhabditis elegans* development, muscle function, and mitosis

Rachel Barkan[a], Adam J. Zahand[b], Kfir Sharabi[a], Ayelet T. Lamm[a], Naomi Feinstein[a], Erin Haithcock[b], Katherine L. Wilson[c], Jun Liu[b], and Yosef Gruenbaum[a]

[a]Department of Genetics, Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem 91904, Israel; [b]Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853; [c]Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

ABSTRACT **Emerin and LEM2 are ubiquitous inner nuclear membrane proteins conserved from humans to *Caenorhabditis elegans*. Loss of human emerin causes Emery-Dreifuss muscular dystrophy (EDMD). To test the roles of emerin and LEM2 in somatic cells, we used null alleles of both genes to generate *C. elegans* animals that were either hypomorphic (LEM-2–null and heterozygous for Ce-emerin) or null for both proteins. Single-null and hypomorphic animals were viable and fertile. Double-null animals used the maternal pool of Ce-emerin to develop to the larval L2 stage, then arrested. Nondividing somatic cell nuclei appeared normal, whereas dividing cells had abnormal nuclear envelope and chromatin organization and severe defects in postembryonic cell divisions, including the mesodermal lineage. Life span was unaffected by loss of Ce-emerin alone but was significantly reduced in LEM-2–null animals, and double-null animals had an even shorter life span. In addition to striated muscle defects, double-null animals and LEM-2–null animals showed unexpected defects in smooth muscle activity. These findings implicate human LEM2 mutations as a potential cause of EDMD and further suggest human LEM2 mutations might cause distinct disorders of greater severity, since *C. elegans* lacking only LEM-2 had significantly reduced life span and smooth muscle activity.**

Monitoring Editor
Martin Hetzer
Salk Institute for Biological Studies

Received: Jun 13, 2011
Revised: Nov 2, 2011
Accepted: Dec 8, 2011

INTRODUCTION

The nuclear envelope (NE) has two membranes (inner and outer nuclear membranes [INM and ONM, respectively]) and nuclear pore complexes (NPCs) that mediate traffic between the nucleus and cytoplasm (Gruenbaum *et al.*, 2005; Stewart *et al.*, 2007). The NE and its lumen are continuous with the endoplasmic reticulum (ER) and share many ER functions. However, the INM and ONM are also specifically enriched for distinct integral membrane proteins (Schirmer and Gerace, 2005; Malik *et al.*, 2010). The mammalian INM has more than 50 distinct membrane proteins (Wilson and Berk, 2010), most of which are uncharacterized. Among the characterized INM proteins, most can bind directly to nuclear intermediate filaments formed by A- or B-type lamin proteins (Dechat *et al.*, 2008). Lamins are major components of the nucleoskeleton (Simon and Wilson, 2011).

One prominent family of lamin-binding proteins shares the LAP2, emerin, MAN1 (LEM) domain, an ~45-residue folded motif (Laguri *et al.*, 2001). Most LEM-domain proteins are located at the INM. The LEM domain binds a conserved metazoan chromatin protein named barrier-to-autointegration factor (BAF). BAF is a mobile lamin-binding protein that can "bridge" double-stranded DNA (dsDNA); BAF also binds histones (Montes de Oca *et al.*, 2005, 2009; Margalit *et al.*, 2007a) and influences many specific histone posttranslational modifications (Montes de Oca *et al.*, 2011). Mammals have four

This article was published online ahead of print in MBoC in Press (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E11-06-0505) on December 14, 2011.

Address correspondence to: Yosef Gruenbaum (gru@vms.huji.ac.il).

Abbreviations used: BAF, barrier-to-autointegration factor; BWM, body wall muscles; CC, coelomocyte; DIC, differential interference contrast; dsDNA, double-stranded DNA; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FO, fibrous organelle; GFP, green fluorescent protein; IFB-1, intermediate filament protein B; INM, inner nuclear membrane; LEM domain, LAP2, Emerin, MAN1 domain; M, mesodermal; NE, nuclear envelope; NGM, nematode growth medium; NPC, nuclear pore complex; ONM, outer nuclear membrane; RNAi, RNA interference; TEM, transmission electron microscopy; ums, uterine muscles; vms, vulval muscles; X-EDMD, X-linked Emery-Dreifuss muscular dystrophy.

characterized LEM genes encoding LAP2 (α, β, and other isoforms), MAN1, emerin, and LEM2/NET25, and three uncharacterized genes (*ANKLE1*, *ANKLE2*, *LEMD1*) encoding predicted proteins also known as LEM3, LEM4, and LEM5, respectively (Wagner and Krohne, 2007; Wilson and Foisner, 2010).

Mutations in emerin cause recessive X-linked Emery-Dreifuss muscular dystrophy (X-EDMD; Bione *et al.*, 1994), which is characterized by weakening of specific skeletal muscles, contractures, cardiomyopathy, and cardiac conduction system defects that can cause sudden death (Muchir and Worman, 2007). EDMD can also be caused by dominant mutations in the genes encoding A-type lamins (*LMNA*), nesprin-1 proteins (*SYNE-1*), or nesprin-2 proteins (*SYNE-2*; Haque *et al.*, 2010; Wheeler and Ellis, 2010). Emerin binds directly to products of all three genes, and its localization at the INM in somatic mammalian cells requires A-type lamins (Mislow *et al.*, 2002; Zhang *et al.*, 2005; D'Angelo and Hetzer, 2006). Emerin also associates directly or indirectly with the conserved INM protein LUMA, encoded by a fifth gene linked to EDMD (*TMEM43*; Liang *et al.*, 2011). Specific mutations in a sixth gene (four-and-a-half LIM domains 1 [*FHL1*]) encoding a transcription factor relevant to muscle mass also cause muscular disorders, including EDMD (Gueneau *et al.*, 2009).

The *C. elegans* genome has only three LEM-domain genes: *emr-1* (encoding Ce-emerin protein), *lem-2* (encoding LEM-2, previously termed Ce-MAN1), and *lem-3*. The protein encoded by *lem-3* has no transmembrane domain and is uncharacterized. Ce-emerin and LEM-2 are INM proteins expressed in essentially all cells during *C. elegans* development (Lee *et al.*, 2000). They have similar biochemical properties: both bind Ce-lamin, both require Ce-lamin for their nuclear envelope localization (Gruenbaum *et al.*, 2002; Liu *et al.*, 2003), and both bind directly to BAF-1 (Liu *et al.*, 2003). Thus Ce-emerin and LEM-2 are the only membrane-integral LEM-domain proteins in *C. elegans*, and their known properties are similar to human emerin and LEM2.

Ce-emerin and LEM-2 have overlapping functions in *C. elegans* early embryos, since neither gene (*emr-1* or *lem-2*) alone is essential (Gruenbaum *et al.*, 2002; Liu *et al.*, 2003), but RNA interference (RNAi)-mediated down-regulation of both genes is "synthetically lethal" to early embryos, with death at the ~100-cell stage (Liu *et al.*, 2003). Mammalian emerin and LEM2 also have overlapping functions in the regulation of extracellular, signal-regulated kinase signaling in developing myoblasts (Huber *et al.*, 2009), suggesting that genetic analysis of both proteins in *C. elegans* might yield important insights into their somatic roles.

We therefore used genetic-null alleles of *emr-1* and *lem-2* to create double-null animals that survived embryogenesis due to the maternal contribution of proteins from the heterozygous mothers. Animals homozygous null for either gene alone and hypomorphic (*lem-2*–null and *emr-1* heterozygous; one copy of *emr-1*) worms were all viable and fertile. In contrast, L2 animals homozygous for both deletions (double-null) stopped developing and showed several prominent developmental and physiological phenotypes in both dividing and nondividing somatic cells.

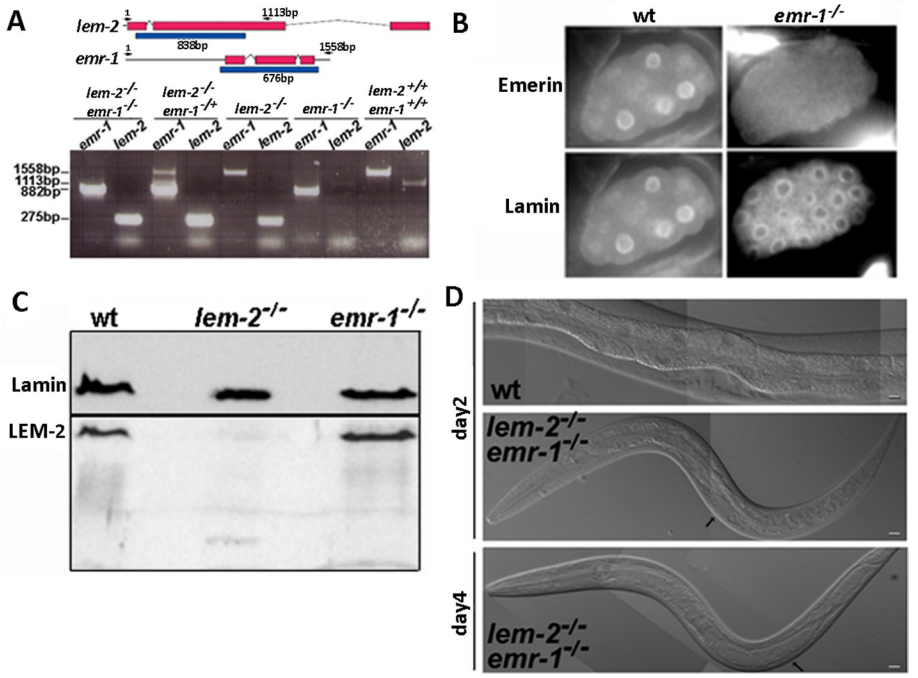

FIGURE 1: Gene deletions of *emr-1* or *lem-2*, strain verification, and crosses. (A) Top, structure of the *emr-1* and *lem-2* genes and their mutant alleles. Exons are red; deleted regions are blue. Positions of primers (arrows) and expected PCR product size are shown above genes; the deletion is indicated below each gene. Bottom, ethidium bromide-stained gel of genomic PCR analysis verifying the LEM-domain strains used in this study. The PCR-queried gene is slanted. (B)Wild-type (wt) and *gk119/gk119* (*emr-1*$^{-/-}$) embryos double-stained by indirect immunofluorescence with antibodies against Ce-emerin (Emerin) or Ce-lamin (Lamin). Ce-emerin was undetectable at the nuclear envelope of *emr-1*$^{-/-}$ embryos. (C) Western blot of protein lysates from either wild-type (wt), *lem-2*–null, or *emr-1*–null animals. The blot was cut horizontally; the top was probed for Ce-lamin (66 kDa) and the bottom was probed for LEM-2 (55 kDa). (D) Nomarski images of wild-type (wt) animals at day 2 (L4) of development, and double-null animals at day 2 (L2) and day 4 (L2/L3-arrested). Arrows mark the position of the gonads. Scale bar: 10 μm.

RESULTS

Ce-emerin and LEM-2 have overlapping functions essential for early embryogenesis (Liu *et al.*, 2003). To bypass this essential phase and examine their roles in germ line and somatic tissues, we used two knockout lines, *emr-1* (*gk119* allele; Haithcock *et al.*, 2005) and *lem-2* (*tm1582* allele; obtained from the National Bioresource Project for the Nematode in Japan) to generate the strain (Supplemental Figure 1). The *tm1582* allele is a deletion in the promoter and first and second exons of *lem-2* (Figure 1A). The *gk119* allele is a deletion of the entire open reading frame of *emr-1* (Figure 1A; Haithcock *et al.*, 2005). Under normal growth conditions, animals homozygous for each deletion were viable and indistinguishable from wild-type (N2) animals (unpublished data).

We confirmed these alleles are null for *lem-2* or *emr-1* by showing that each protein was undetectable by indirect immunofluorescence staining (shown for *emr-1*$^{-/-}$ worms in Figure 1B) and in Western blots of homozygous adult animals (shown for *lem-2*$^{-/-}$ worms in Figure 1C). Furthermore, when we fed the *lem-2*$^{-/-}$ (*tm1582/tm1582*) animals with *emr-1* dsRNA to down-regulate Ce-emerin or, conversely, fed *emr-1*$^{-/-}$ (*gk119/gk119*) animals with *lem-2* dsRNA to down-regulate LEM-2, both treatments resulted in embryonic-lethal

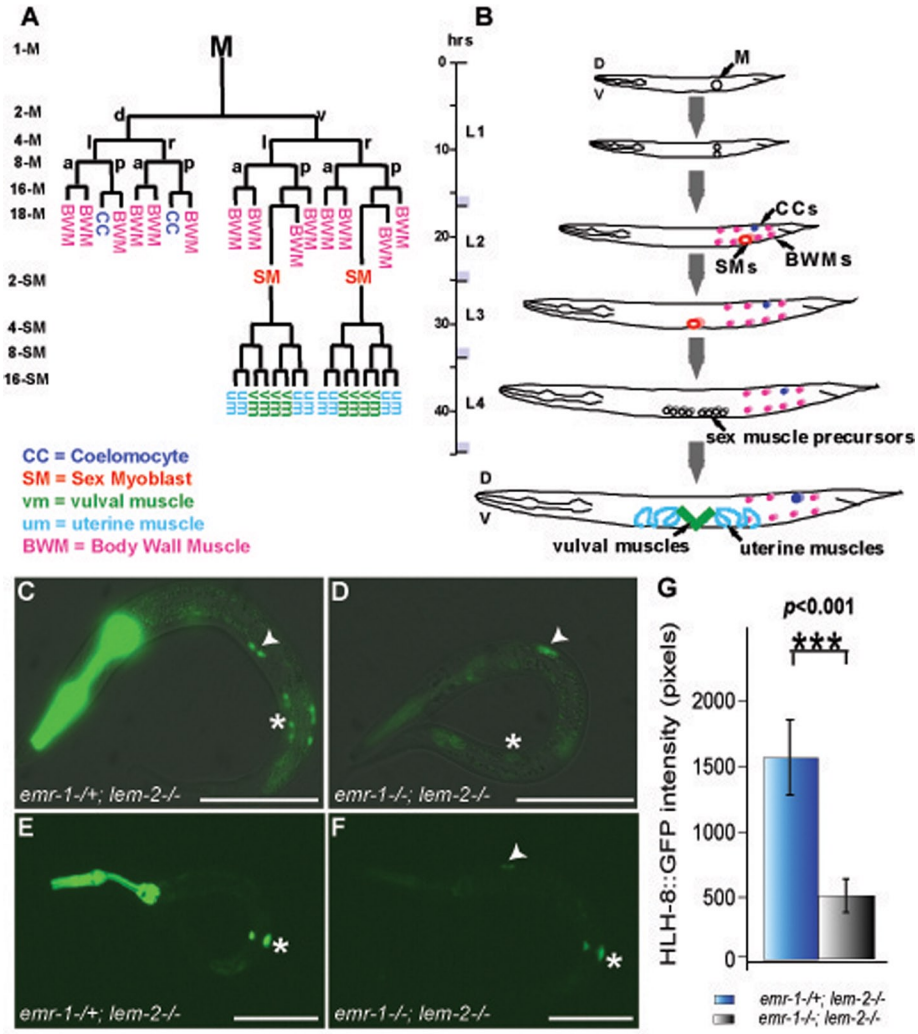

FIGURE 2: Double-null animals show defects in cell proliferation and gene expression in the postembryonic mesoderm. (A–B) The *C. elegans* hermaphrodite postembryonic M lineage (modified from Jiang *et al.*, 2005). Times indicated are hours posthatching at 25°C. (A) The M lineage with all the differentiated cell types; corresponding stages referred to in the text are indicated at left. (B) A schematic lateral view of the M lineage through larval development. D, dorsal; V, ventral; L, left; R, right; A, anterior; P, posterior. (C and D) Twenty-four hours after plating synchronized L1s, hypomorphic animals (C, with bright pharyngeal GFP) reached the 14-M stage, whereas double-null animals (D) were still at the 2-M stage. M lineage cells are marked by *hlh-8::gfp* (*). White arrowheads point to embryonically derived CCs. (E and F) *hlh-8::gfp* expression level at the 2-M stage is higher in a hypomorphic animal (E) than in a double-null animal (F). (G) Quantification of *hlh-8::gfp* signals at the 2-M stage. For each genotype, the pixel intensities of GFP signals from two M lineage cells in 10 different animals (20 cells total per genotype) were measured using Openlab software. Error bars represent 95% confidence intervals for the GFP intensity. Statistical significance was analyzed by Student's *t* test. ***, $p < 0.001$. Scale bars: 50 μm.

phenotypes that recapitulated the double-RNAi experiment (Liu *et al.*, 2003; unpublished data). We concluded that these alleles are null.

We generated (Supplemental Figure S1) and verified with PCR (Figure 1A) a *C. elegans* strain that is homozygous for *tm1582 (lem-2$^{-/-}$)* and heterozygous for *gk119 (emr-1$^{-/+}$)*; this strain *hT2[qIs48]/emr-1(gk119); lem-2(tm1582)*, known as *emr-1$^{+/-}$; lem-2$^{-/-}$*, was balanced and viable. Thus one copy of *emr-1* is sufficient to maintain the viability of *lem-2$^{-/-}$* animals.

Self-fertilization of the hypomorphic strain (*hT2[qIs48]/emr-1^{-}; lem-2$^{-/-}$*) yielded three types of progeny: *hT2[qIs48]/hT2[qIs48]* animals that died; hypomorphic *emr-1$^{+/-}$; lem-2$^{-/-}$* animals that expressed green fluorescent protein (GFP) in the pharynx (due to the GFP insertion in the balancer hT2); and double-null *emr-1$^{-/-}$; lem-2$^{-/-}$* animals that lacked GFP expression. The maternally contributed Ce-emerin sustained normal development of the double-null animals until the larval L2 stage. In early L2, the double-null animals appeared normal and had the same width, but were 8.5% shorter ($p < 4 \times 10^{-5}$) than wild-type L2 controls. At late L2/early L3, double-null animals had abnormal gonads and stopped developing (Figure 1D shows animals at developmental days 2 and 4), but remained viable for 10–11 d.

Dual developmental roles of Ce-emerin and LEM-2: cell division and the expression of a lineage regulator

To understand how Ce-emerin and LEM-2 influence development, we followed in real time the development of the postembryonic mesodermal (M) lineage in the double-null mutants. The M lineage is derived from a single, embryonically derived, pluripotent mesoblast cell, the M cell. During postembryonic development, the M cell undergoes several rounds of cell divisions to produce 14 postembryonically derived body wall muscles (BWMs), two nonmuscle coelomocytes (CCs), and 16 nonstriated egg-laying vulval and uterine muscles (vms and ums) (Sulston and Horvitz, 1977; Sulston *et al.*, 1983; Figure 2, A and B). The M cell is present and correctly specified in the double-null mutant based on the unique position and cell shape observed using differential interference contrast (DIC) optics and the expression of the M lineage–specific reporter, *hlh-8::gfp*, which labels all undifferentiated M lineage cells (Harfe *et al.*, 1998). All hypomorphic mutant and wild-type control animals completed three to four rounds of cell division to produce 12–16 M lineage descendants that were labeled by *hlh-8::gfp* (Figure 2C) during the time frame of our experiment (24 h after plating synchronized L1s onto nematode growth medium (NGM) plates at 20°C). By contrast, 22 of the 24 double-null animals examined completed only one round of cell division (2-M stage), and two animals underwent only two rounds of cell division (4-M stage) in the M lineage (Figure 2D). Consequently, the double-null animals failed to produce any of the differentiated M lineage cells that were seen in the wild-type or hypomorphic animals, including the 14 BWMs, two CCs and 16 vms and ums, as evidenced by both DIC and cell type-specific GFP markers, including *egl-15::gfp* that labels type I vulval muscles and a *CC::gfp* that labels all CCs (Jiang *et al.*, 2005; unpublished data). Surprisingly, we also found that the expression level of the M lineage–specific *hlh-8::gfp* marker was significantly (threefold) lower in double-null animals than in hypomorphic control animals

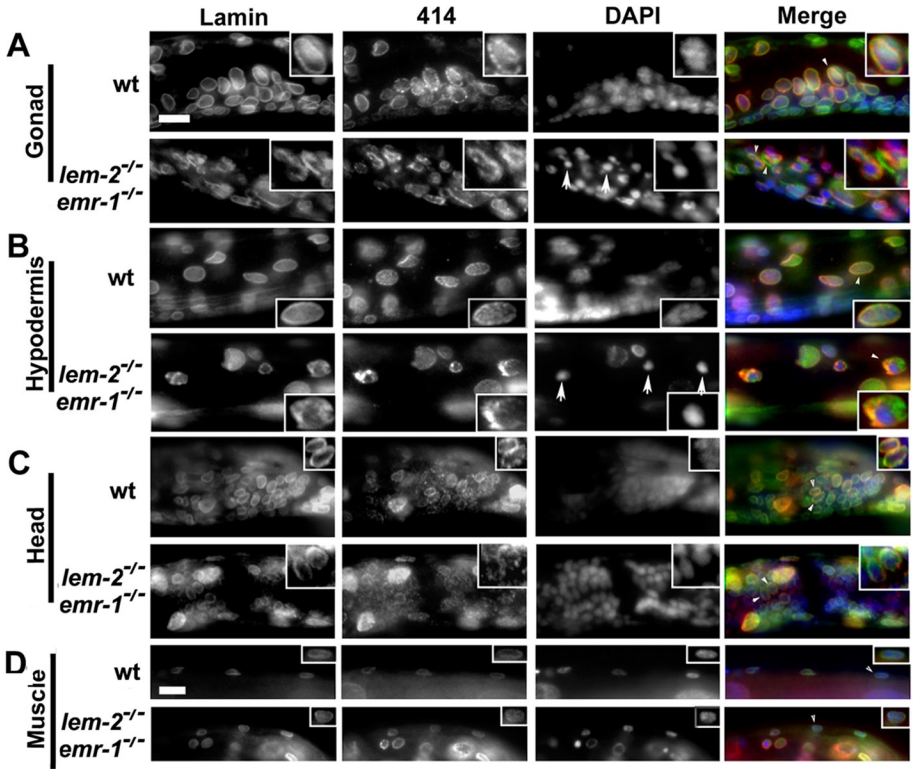

FIGURE 3: Nuclear and chromatin structure defects in dividing double-null cells. Indirect immunofluorescence images of the gonads (L2), hypodermis (L2), head (L2), and muscle cells (day 3) of animals grown at 20°C and stained with antibodies specific for Ce-lamin (Lamin) or FG-repeat–containing NPC proteins (414). Chromatin was stained with 4′,6-diamidino-2-phenylindole (DAPI). Arrows indicate abnormally condensed chromatin. Merged images shown at right; arrowheads indicate region shown at higher magnification (inset). Scale bars: 10 μm (applies to all panels).

(Figure 2, E–G) and was later undetectable in the L2-arrested, double-null animals (unpublished data). Thus Ce-emerin and LEM-2 are required for cell proliferation of the postembryonic M lineage and for maintaining the expression of *hlh-8*, a bHLH transcription factor required for the proper patterning of the M lineage (Harfe *et al.*, 1998; Corsi *et al.*, 2000). The role of Ce-emerin and LEM-2 in regulating cell proliferation is not limited to the M lineage, as cell division in other postembryonic lineages is also affected in the double-null animals, as shown in the next section.

Ce-emerin and LEM-2 are required to organize chromatin, the nucleoskeleton, and the nuclear envelope during postembryonic development

Cell division defects were consistent with previous evidence that LEM-domain proteins are required for mitosis and nuclear assembly in early embryos (Liu *et al.*, 2003). To determine whether LEM proteins had these roles after embryogenesis, we stained the double-null animals with antibodies against Ce-lamin (Liu *et al.*, 2000) and NPCs (mAb414). The double-null animals had defects in the organization of chromatin, nuclear intermediate filaments, and NPCs, and defects in mitosis in cells that continue to divide after embryogenesis. In the gonad mitotic zone, where cells actively divide, Ce-lamin and NPCs localized normally and nuclei appeared normal in single-null and hypomorphic animals (Figure S2A) and wild-type animals (Figure 3A), suggesting that one copy of *emr-1* was sufficient to maintain normal nuclear organization in germline cells of lem2$^{-/-}$ animals. However, in the gonad mitotic zone of double-null animals, Ce-lamin and NPCs were mislocalized, nuclei were misshapen, and many had condensed chromatin (Figure 3A, arrows). These phenotypes resembled those seen in early embryos down-regulated for both *emr-1* and *lem-2* (Liu *et al.*, 2003).

Somatic hypodermal cells, which are also actively dividing, showed similar double-null phenotypes. Nuclear shape, Ce-lamin localization, and NPC distribution appeared normal in single-null, hypomorphic (Figure S2C), and wild-type animals (Figure 3B), whereas many double-null hypodermal cells had misshapen nuclei with large Ce-lamin aggregates, clustered NPCs (Figure 3B), and condensed chromatin (Figure 3B, arrows). Misshapen hypodermal nuclei were also observed with transmission electron microscopy (TEM) at day 3 of development (Figure S3).

By contrast, in all somatic cells that do not undergo postembryonic cell divisions, Ce-lamin and NPCs localized normally (see examples in the head [Figure 3C] and body muscle [Figure 3D]; pharynx and neurons not shown). Ce-lamin also localized normally in all somatic nuclei of single-null and hypomorphic animals (Figure S2, B and D), suggesting the maternal contribution of Ce-emerin was sufficient to organize nuclear structure in the nondividing somatic cells of the double-null animals. Thus Ce-emerin and LEM-2 are required to organize nuclear intermediate filaments (lamins), NPCs, and chromatin, not only during embryogenesis (Liu *et al.*, 2003), but also during postembryonic development in mitotically active cells. Importantly, as shown in the next section, Ce-emerin and LEM-2 also appear to function in nondividing somatic cells.

Double-null animals have defects in motility, sarcomere organization, and muscle attachment to hypodermis

Human emerin mutations cause EDMD or similar disorders (Wheeler and Ellis, 2010), and emerin and LEM2 have overlapping functions required for myogenic differentiation of mouse C2C12 cells (Huber *et al.*, 2009). We therefore tested motility and muscle organization in our LEM protein–deficient strains. We compared the number of head movements per minute for each genotype placed on agar plates. Measurements were taken daily from day 1 (L2 stage) to day 9 at 20°C (Figure 4). The motility of double-null animals was significantly lower than that of wild-type (N2) controls on day 1 ($p < 1.7 \times 10^{-04}$) and continued to decrease; by day 6, the double-null animals were nearly paralyzed and could move only their heads (Figure 4). The motility of *emr-1*$^{-/-}$ animals was lower than that of wild-type (N2) on day 1 ($p < 0.008$), but did not differ significantly on days 2–6, and to our surprise, the motility of emerin-null animals deteriorated more slowly than that of wild-type controls on days 7–9 (Figure 4; $p < 0.005$ and $p < 0.015$, respectively). The *lem-2*$^{-/-}$ and hypomorphic animals had very similar motility on days 2–6 and did not differ significantly from wild-type through day 7; however, by day 9, the motility of hypomorphic animals had deteriorated significantly (Figure 4; $p < 7.8 \times 10^{-06}$).

To test whether these motility phenotypes were specifically due to the loss of LEM-domain gene activity, we generated a strain that

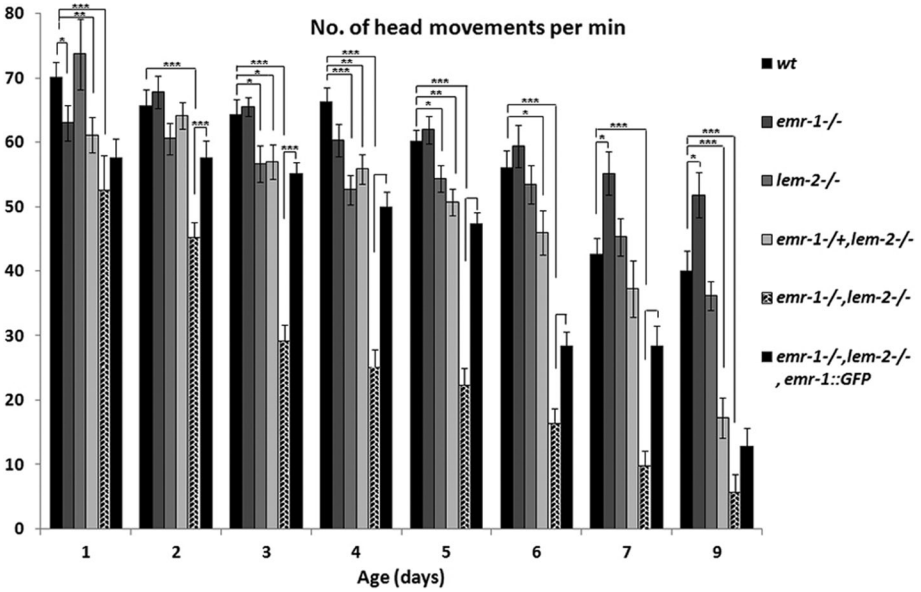

FIGURE 4: Effects of LEM-domain mutations on *C. elegans* motility. Motility was measured as the number of head movements per minute on agar plates as a function of age (days 1, 2, 3, 4, 5, 6, 7, and 9 of development at 20°C), for wild-type (wt) animals, each indicated mutant strain, and double-null animals that expressed wild-type *emr-1::GFP*. Error bars indicate SEM. Significance was assessed by Student's *t* test (*, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$).

expresses Ce-emerin fused to GFP, and crossed it into the hypomorphic background (*emr-1*$^{-}$*/hT2[qIs48]*; *lem-2*$^{-/-}$; *emr-1::gfp)*. In the double-null animals, the Ce-emerin::GFP protein was expressed and localized normally at the nuclear envelopes of all tissues except for the gonads (Figure S4). Transgenic expression of Ce-emerin::GFP allowed double-null animals (*emr-1*$^{-}$*/*$^{-}$; *lem-2*$^{-/-}$; *emr-1::gfp)* to develop to adulthood, but there was no gonadal expression of the *emr-1::gfp* transgene due to transgene silencing (Kelly *et al.*, 1997), so the rescued double-null animals were sterile. The expression of Ce-emerin::GFP in double-null animals rescued motility to almost the same extent as the hypomorphs (no statistically significant difference between hypomorphs and rescue strain, except for day 6; Figure 4). To further test whether the motility phenotypes were specifically due to loss of LEM-domain gene activity, we down-regulated *lem-2* using RNAi (*lem-2 (RNAi)*) in Ce-emerin null animals and tested their motility. Ce-emerin–null animals down-regulated for *lem-2* showed significantly reduced motility on the second day on RNAi plates, compared with Ce-emerin–null animals fed with an empty vector ($p < 0.001$; Figure S5A; "emr-1 (lem-2)" vs. "emr-1 (EV)," respectively). These controls verified that the motility phenotypes were specifically due to loss of LEM-domain gene activity.

The motility phenotypes suggested potential muscle defects. We used thin-section TEM to view sarcomere structure in wild-type and double-null animals and double-null animals that expressed *emr-1::gfp*. Wild-type (N2) animals had organized sarcomeres, as visualized in transverse sections at days 3 and 6 (Figure 5, A and B respectively) and in cross-sections at day 6 (Figure 5C), as expected. However, in transverse sections of body wall muscle, cells from double-null animals at developmental days 3 and 6 (Figure 5, D and E, respectively), and in cross-sections at developmental day 6 (Figure 5F), we observed regions in which thin and thick filaments were "wavy" and disorganized (Figure 5, D and E, arrowheads). Indeed, it was sometimes hard to recognize thick filaments in cross-sections (Figure 5F). In double-null animals, we also occasionally found muscle cells that were abnormally positioned with respect to one another at day 6 (Figure 5G, asterisk) and day 9 (Figure 5, H and I, asterisks). The sarcomeres visualized in double-null animals that expressed *emr-1::gfp* had overall normal filament organization (Figure 5, J and K) and muscle cell positioning appeared normal (unpublished data).

Abnormal body wall muscle organization was independently suggested in older (day 6) double-null animals that expressed GFP-fused myosin heavy chain, MYO-3, compared with L2 animals with wild-type background (Figure 6A); when the lengths of individual muscle cells were normalized to the length of the entire animal, double-null muscle cells were 15% shorter than wild-type (Figure 6B; $p < 3 \times 10^{-11}$). Double-null muscle fibers also appeared misoriented with respect to body axis. While these defects might be due, at least in part, to the missing M lineage–derived body wall muscles (Figure 2), they also suggest potential defects in muscle cell attachment to the overlying hypodermal cells.

To test potential attachment defects, we generated double-null animals that expressed intermediate filament protein B (IFB-1) fused to GFP. IFB-1::GFP resides in hemidesmosome-like structures ("fibrous organelles") within hypodermal cells that couple muscles to the cuticle and are required for *C. elegans* hypodermal mechanical strength (Chisholm and Hardin, 2005). At day 3 of development, the double-null animals showed abnormal organization of IFB-1::GFP compared with wild-type background at the L2 stage; specifically, the circumferential banding pattern that characterizes wild-type was disorganized, mainly from the center to the tail region, in 10 of 11 animals examined (Figure 6C). This might be due, at least in part, to the structural abnormality of hypodermal nuclei (Figure S3). We concluded that muscle cell attachment to hypodermal cells, as well as muscle cell location and sarcomere organization, all require the activity of LEM-domain proteins. These striated muscle phenotypes were not surprising, given the emerin-null phenotype in human males (EDMD) and the known overlap between emerin and LEM2 in mouse myogenic signaling (Huber *et al.*, 2009). These findings establish *C. elegans* as a model in which to dissect the overlapping versus unique contributions of two different LEM-domain proteins to striated muscle development and function. The double-null and LEM-2–null animals had additional phenotypes, as shown in the next section.

Ce-emerin and LEM-2 contribute distinctly to life span

Worms down-regulated for *lem-2* by RNAi showed reduced life span, which was further reduced by *lem-2(RNAi)* in the *emr-1*$^{-/-}$ background (Haithcock *et al.*, 2005). To determine genetically how Ce-emerin and LEM-2 each contribute to life span, we measured the average life span of animals of each LEM-domain mutant strain (Figure 7). At 20°C, *emr-1*$^{-/-}$ and wild-type (N2) animals had similar average life spans of 23.3 d (SEM = 0.49 d) and 22 d (SEM = 0.22 d), respectively. The life spans of *lem-2*$^{-/-}$ and hypomorphic animals were significantly reduced to 18.6 d (SEM = 0.25 d) and 17.3 d (SEM = 0.18 d), respectively ($p < 0.0001$; Figure 7, A and B). The average life span of double-null animals was only 11.2 d (SEM = 0.12 d; $p < 0.0001$). Ce-emerin loss was completely rescued by expressing Ce-emerin::GFP in the double-null animals; average life span became similar to that of *lem-2*$^{-/-}$ animals (Figure 7, A and B). We

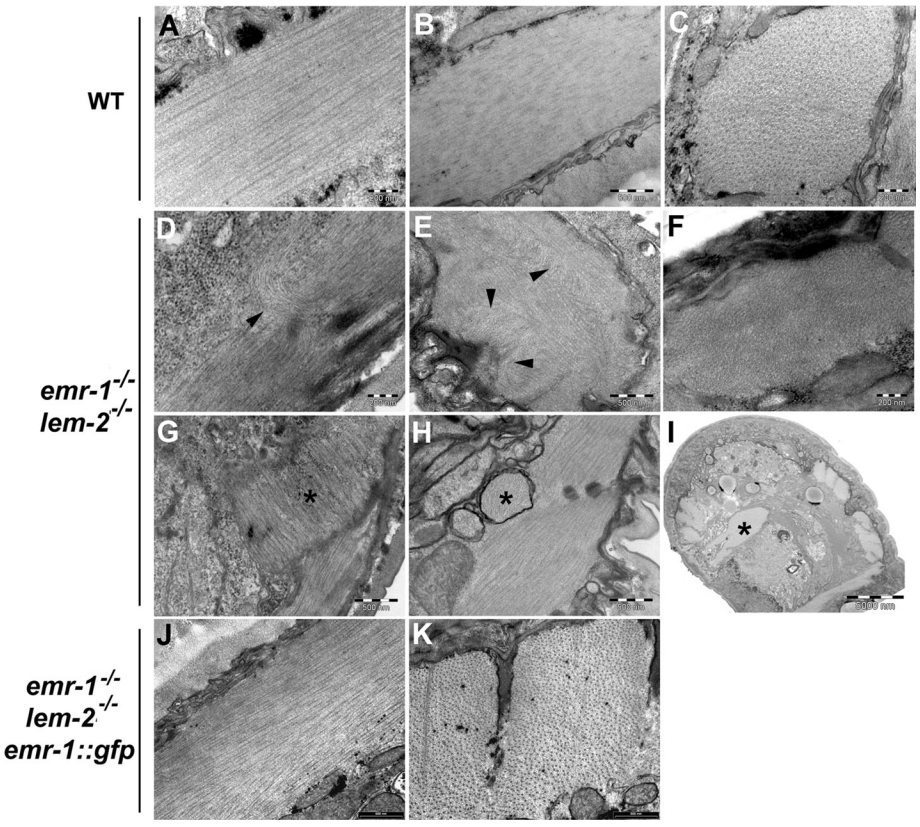

FIGURE 5: Thin-section TEM micrographs reveal abnormal muscle organization in double-null animals. Images of longitudinal sections from the midbody region at day 3 (A, D, and J) or day 6 (B and E), and transverse sections from a midbody region at day 6 (C, F, and K) of development at 20°C. Muscle organization appeared normal on days 3 and 6 in wild-type (WT) (A,B,C) and double-null animals expressing Ce-emerin::GFP on day 6 (J and K) but was abnormal in double-null animals (arrows in D and E). Also shown are longitudinal head sections (G and H) and transverse tail sections (I) from day 6 (G) or day 9 (H and I), in which sarcomeres were sometimes misoriented (*). Scale bars: 200 nm (A, D, C, and F), 500 nm (B, E, G, H, J, and K) or 5 μm (I).

concluded that LEM-2 has unique positive roles in maintaining life span that do not overlap with Ce-emerin.

Novel roles for Ce-emerin and LEM-2 in the contraction of smooth muscles

To explore potentially novel physiological roles for LEM-domain proteins, we tested the pumping rate of the pharynx, which is regulated by nonstriated (smooth) muscle cells. At day 1 (L2 stage) the pumping rates of *emr-1*$^{-/-}$, *lem-2*$^{-/-}$, and hypomorph worms were slightly reduced, compared with wild-type animals (~5%; Figure S6); this slight reduction might not be accurate, due to difficulty in counting pumping at the L2 stage. However, the double-null animals showed ~15% reduced pumping rate with greater statistical significance ($p < 5.6 \times 10^{-06}$; Figure S6). Ce-emerin::GFP expression rescued the pumping of double-null animals (Figure S6). At day 3, *emr-1*–null animals had pumping rates similar to wild-type animals (Figure 8), whereas *lem-2*$^{-/-}$ and hypomorphic worms had mildly but significantly reduced pumping rates (~12% less; $p < 0.003$ and $p < 0.007$, respectively; Figure 8). Remarkably, the pumping rate of double-null animals was 80% lower than that of wild-type animals ($p < 4.2 \times 10^{-22}$; Figure 8). This pumping defect was rescued to the level seen in *lem-2*$^{-/-}$ animals by expressing Ce-emerin::GFP in the double-null animals (Figure 8). Further controls also suggested LEM-2 functions specifically in the pharynx; down-regulating lem-2 in *emr-1*–null animals significantly reduced the pumping rate, compared with *emr-1*–null animals fed with empty vector ($p < 0.0005$ on day 1; $p < 5.14 \times 10^{-06}$ on day 2; Figure S5B). We conclude that Ce-emerin and LEM-2 are required for the contraction of nonstriated pharyngeal muscle (smooth muscle) cells in *C. elegans*, and that LEM-2 has additional roles in smooth muscle that cannot be provided by Ce-emerin.

The above findings indicated aging-dependent deterioration of pharynx activity (Figure 8) in the double-null animals. However, this pumping defect raised the possibility that the reduced motility of double-null animals (Figure 4) might be a consequence of reduced food intake. We therefore tested the movement of *eat-2* animals, which pump less food (Raizen *et al.*, 1995; Figure S5B) at a similar stage of development. Interestingly the *eat-2* mutants moved faster than wild-type animals (Figure S5A). We concluded that the reduced movement of double-null animals was probably not a consequence of reduced pumping rate.

DISCUSSION

Study of INM LEM-domain proteins in *C. elegans*

Ce-emerin and LEM-2 were known to have overlapping functions required for mitosis and nuclear organization in early embryos (Liu *et al.*, 2003). Our new results with genetic null alleles for both genes demonstrates they share redundant functions (e.g., in mitosis and striated muscle) but also have distinct roles during postembryonic development. Notably, we uncovered a novel shared role for Ce-emerin and LEM-2 in smooth muscle. Both proteins are required for nuclear organization and cell proliferation in dividing germ line and somatic cells, and to maintain the expression of at least one gene (*hlh-8*). They are also required for the development, maintenance, and/or function of specific differentiated cells, including striated muscle, hypodermis, and smooth muscle. Our genetic analysis confirmed our previous data (Haithcock *et al.*, 2005) that both genes contribute to life span, since the life span of double-nulls was shorter than either single-null alone. This analysis also showed that LEM-2 contributes uniquely to life span, since life span was unaffected in animals lacking Ce-emerin but decreased significantly in LEM-2–null animals. One copy of Ce-emerin was sufficient to complement most, but importantly not all, LEM-domain activities required for normal life span. The requirement for a specific LEM-domain protein in a specific biological process is not unique to LEM-2. A previous study of pharynx cells showed emerin inhibits the binding of PHA-4 (a FoxA transcription activator) to a PHA-4–regulated pax-1 promoter and is required for chromatin condensation (Fakhouri *et al.*, 2010).

While RNAi-induced down-regulation of Ce-emerin plus LEM-2 causes early embryonic lethality (Liu *et al.*, 2003), the phenotypes of double-null animals appeared after the larval L1 stage, suggesting the maternal provision from one copy of *emr-1* was sufficient for embryonic development. Phenotypes that emerged at the larval L1 stage and beyond were presumably due to cells with insufficient

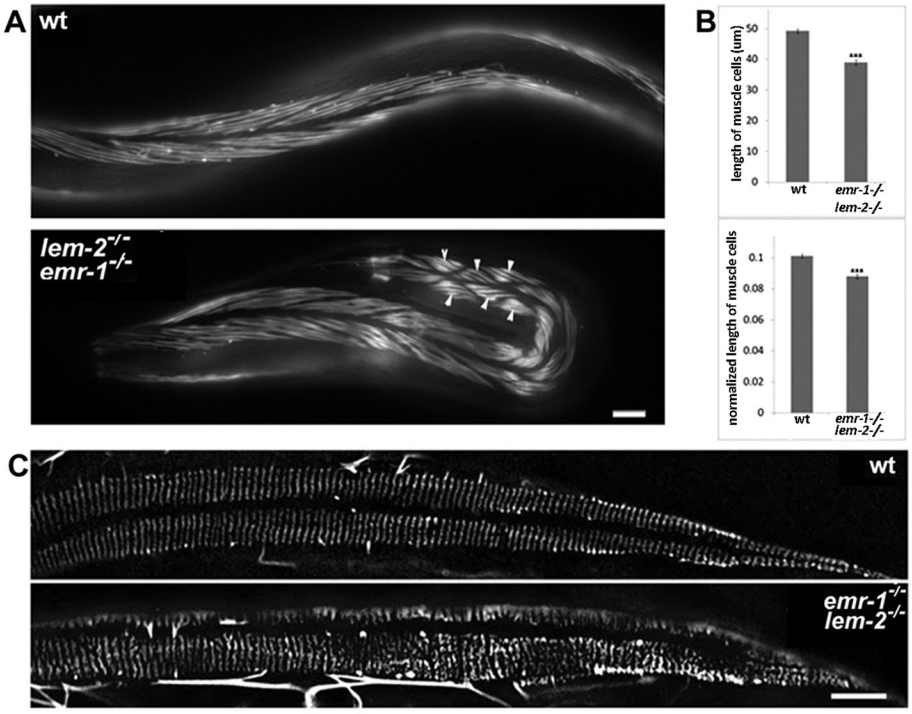

FIGURE 6: Abnormal muscle cell and FO organization in double-null animals. (A) MYO-3::GFP expression in wild-type (wt) L2 stage animals (day 1), and double-null (*emr-1*$^{-/-}$*; lem-2*$^{-/-}$) animals at day 6 of development. Double-null muscle cells from the midbody to the tail were shorter (arrowheads). Scale bar: 10 µm. (B) Quantification of absolute muscle cell length (top), or after muscle cell length was normalized to animal length (bottom). (C) IFB-1::GFP expression in wild-type (wt) L2 stage animals on day 1 and double-null (*emr-1*$^{-/-}$*; lem-2*$^{-/-}$) animals on day 3 of development. The figure shows the posterior half of the animal. Double-null animals showed abnormal distribution of IFB-1::GFP in all the hypodermal cells in this region. Scale bar: 10 µm.

Ce-emerin to cover the combined roles of both Ce-emerin and LEM-2.

LEM-domain proteins are required for muscle organization and maintenance

This work establishes *C. elegans* as a genetic model for understanding the diverse molecular mechanisms of EDMD and related laminopathies. Our *C. elegans* strains strongly support both individual and combined roles for Ce-emerin and LEM-2 in the development, organization, and maintenance of striated muscle. While motility was normal in animals null for either Ce-emerin or LEM-2 throughout the tested period, hypomorphic animals showed significant motility defects by day 9 of development; thus, despite their good copy of *emr-1*, the hypomorphic animals fell below a threshold level of LEM-domain activity required to maintain muscle integrity and motility. The double-null animals had much earlier (day 2) motility defects, consistent with other phenotypes, including misplaced muscle cells, poor attachment to hypodermis, and "wavy," deteriorated sarcomeres. We conclude striated muscle function requires both Ce-emerin and LEM-2.

The motility and muscle phenotypes of double-null animals could also be due to defective muscle cell attachment to hypodermal cells. In *C. elegans*, muscle cells are mechanically coupled to the cuticle by attachments in the hypodermal cells. These attachments, called fibrous organelles (FOs), are hemidesmosome-like structures. Down-regulating FO proteins in *C. elegans* causes muscle cells to detach when embryonic muscle contraction begins. Later in development, FOs become delocalized and are no longer restricted to regions contacted by muscle (Chisholm and Hardin, 2005). IFB-1, which was mislocalized in double-null animals, is a component of the FO, and its maldistribution might therefore explain, at least in part, why muscle cells were misplaced and appeared shorter in the double-null animals. Future work is needed to determine whether Ce-emerin and LEM-2 are specifically required in the hypodermis (e.g., to regulate expression of IFB-1 or other genes), and whether these phenotypes are due to gross defects in nuclear structure.

These *C. elegans* strains will enable future studies undertaken to understand LEM-domain protein functions in striated muscle, particularly in the area of signaling. Emerin negatively regulates extracellular signal-regulated kinase (ERK) signaling in the mouse heart (Muchir *et al.*, 2007a, 2007b, 2008) and directly binds, and somehow attenuates signaling by, at least two specific signaling transcription factors: b-catenin (Markiewicz *et al.*, 2006; Tilgner *et al.*, 2009) and Lmo7 (Holaska, 2008). Emerin and LEM2/NET25 together regulate ERK signaling during mouse myoblast differentiation, with LEM2 being slightly more important (Huber *et al.*, 2009). Our findings also suggest LEM2 is slightly more "important," since the *lem-2*$^{-/-}$ animals had reduced lifespan and pumping rate.

LEM-domain proteins have essential roles in nuclear structure in somatic cells

Our new findings demonstrate that two LEM-domain proteins are essential for organizing the intermediate filament nucleoskeleton and NPCs, which is consistent with their roles in embryogenesis (Liu *et al.*, 2003; Margalit *et al.*, 2007b), and for organizing chromatin in dividing somatic cells. These roles were detected in mitotically active gonadal and hypodermal cells of double-null animals, which had misshapen nuclei, lamin and NPC assembly defects, and condensed chromatin. These structural defects might explain, at least in part, why these animals also had reduced cell proliferation and gene expression in the postembryonic mesodermal lineage. We therefore propose that LEM-domain proteins have essential roles in organizing the structure of both the nucleus and the genome during mitosis in all dividing cells in the animal. We hypothesize that a threshold amount of INM-localized LEM-domain proteins is required to progress through mitosis and reassemble nuclei.

Our results also clearly show that Ce-emerin and LEM-2 have additional roles in specific tissues, including striated muscle (as discussed in the preceding sections) and smooth muscle (as discussed in the following section). In contrast with dividing cells, which showed gross defects in nuclear structure, the nondividing somatic cells of double-null animals had apparently normal nuclear structure. However, this does not mean these cells had normal chromatin organization. Indeed, we propose the phenotypes of our double-mutant animals might reveal a subset of nondividing somatic cells (including hypodermis, striated muscle, and smooth muscle) that require

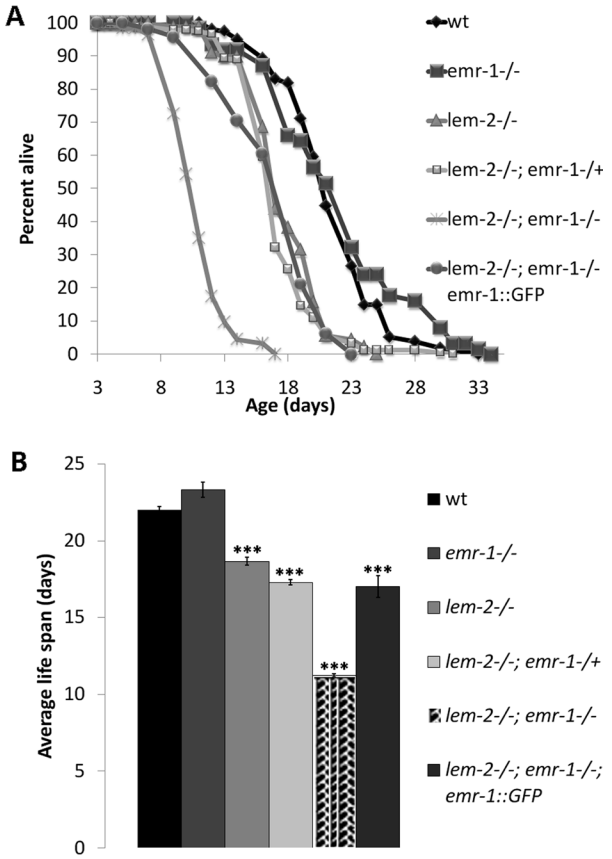

FIGURE 7: Shorter life span in animals lacking Ce-emerin and LEM-2. (A) Survival plots of wild-type (N2) animals (wt), and animals with each mutant genotype, at 20°C (see *Materials and Methods*). (B) Data from (A) were used to calculate average life span. Exogenous Ce-emerin::GFP expression restored the average life span of double-null animals to that of animals with one copy of *emr-1* (*emr-1*$^{-/+}$; *lem-2*$^{-/-}$). (In a comparison with wt, *** represents $p < 0.0001$.)

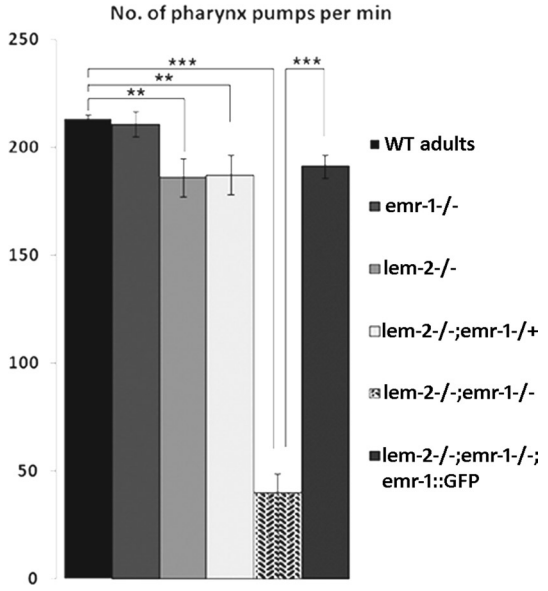

FIGURE 8: Effects of LEM-domain mutations on *C. elegans* smooth muscle activity. Pharynx pumping activity (pumps/min) was counted for 1 min in animals ($n > 30$ for each strain) at day 3 of development at 20°C when all animals were young adults, except the double-nulls, which arrested at L2. Expression of Ce-emerin::GFP rescued the pumping defect in double-null animals. Error bars indicate SEM. Significance was assessed by Student's *t* test (*, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$).

both Ce-emerin and LEM-2 to regulate specific developmental or tissue-specific genes, perhaps at the level of tethering these genes to the nuclear envelope and nucleoskeleton (Guelen *et al.*, 2008; Jiang *et al.*, 2008; Meister *et al.*, 2010; Dedeic *et al.*, 2011; Mattout *et al.*, 2011. reviewed by Geyer *et al.*, 2011).

Smooth muscle biology: novel functions for Ce-emerin and LEM-2

This genetic analysis revealed roles for Ce-emerin and LEM-2 in several new cell types, suggesting there is still much to learn about their functions in somatic cells. The unexpected discovery that double-null pharynx (smooth) muscles had significantly reduced pumping activity is, to our knowledge, the first evidence that LEM-domain proteins are required in smooth muscle. Whether LEM-domain proteins are required in human smooth muscle is unknown and warrants testing, since it suggests genetic links to human smooth muscle disease and might provide molecular insight into the vascular smooth muscle pathology of Hutchinson-Gilford progeria syndrome, which is caused by severe mutations in *LMNA* (Rodríguez and Eriksson, 2010). Collectively, our findings support the possibility that human LEM2 mutations might influence or cause EDMD. Importantly, they further suggest mutations in human LEM2 might cause more severe or novel diseases. In stark contrast to loss of Ce-emerin, which caused relatively benign phenotypes, the loss of LEM-2 alone significantly reduced life span and smooth muscle activity in living animals.

MATERIALS AND METHODS

C. elegans strains

C. elegans strains were handled as previously described (Brenner, 1974). Strains N2 (wild-type), VC237[*emr-1(gk119)*] and RW1596 *myo-3(st386)* V; stEx30 [*Pmyo-3::myo-3::gfp + rol-6(su1006)*], DA1116 [*eat-2(ad1116)*] were obtained from the *C. elegans* Genome Center at the University of Minnesota. Strain *lem-2(tm1582)* was obtained from the *C. elegans* National BioResource Project in Japan. Strains *emr-1(gk119)* and *lem-2(tm1582)* were outcrossed three times and six times, respectively.

The following strains were generated in this study. LW1073: *emr-1(gk119)/hT2[qIs48]; lem-2(tm1582/tm1582).* YG2102: *emr-1(gk119)/hT2::gfp; lem-2(tm1582/tm1582); Plmn-1::emr-1::gfp.* YG2120: stEx30 [*Pmyo-3::myo-3::GFP + rol-6(su1006)*], which was generated by crossing RW1596 (Herndon *et al.*, 2002), myo-*3(st386) V; stEx30 [Pmyo-3::myo-3::GFP + rol-6(su1006)]* with wild-type N2 animals. YG2123: *emr-1(gk119)/hT2[qIs48]; lem-2(tm1582/tm1582); stEx30 [Pmyo-3::myo-3::GFP + rol-6(su1006)*]. YG2110: *emr-1(gk119)/hT2[qIs48]; lem-2(tm1582/tm1582); juIs176(ifb-1::gfp* (Woo *et al.*, 2004) LW2711 *emr-1(gk119)/hT2[qIs48]; lem-2(tm1582/tm1582); ccIs4438(intrinsic CC::gfp); ayIs2(egl-15::gfp); ayIs6(hlh-8::gfp).* The GFP markers *ccIs4438(intrinsic CC::gfp)* (labeling CCs), *ayIs2(egl-15::gfp)* (labeling M lineage–derived vms), and *ayIs6(hlh-8::gfp)* (labeling all undifferentiated M lineage descendants) were described previously (Harfe *et al.*, 1998).

Microscopy and live-cell imaging

TEM analysis of *C. elegans* was done as described previously (Cohen *et al.*, 2002). DIC and immunofluorescence images were taken with either an Axiocam CCD camera (Zeiss) or a Hamamatsu Orca ER/AG camera mounted on a Zeiss Axioplan II microscope (Zeiss, Oberkochen, Germany) or a Leica DMRA2 microscope (Leica Microsystems, Wetzlar, Germany) equipped for fluorescence and

DIC. Stack images of *myo-3::gfp*–expressing worms were taken by Micro-Manager (version 1.3) and processed using AutoQuantX (version X2.1.3; Media Cybernetics, Bethesda, MD).

Maximal muscle cell length and worm length were measured in wild-type (N2) larval L2 animals and in L2 double-null animals using Image J. At least 15 cells from the center to the tail region were measured in each animal. Since animals differed in size, and correlation curves showed that animal length correlated with muscle cell length (unpublished results), we also plotted results after normalizing muscle cell length to the length of each animal.

To follow the development of the M lineage, hermaphrodite animals at the gravid adult stage were collected and treated with hypochlorite. The resulting embryos were allowed to hatch in M9 buffer at 16°C. Synchronized L1s were plated onto NGM plates and their development was monitored using a Nikon fluorescence stereomicroscope and confirmed using the Leila DMRA2 compound microscope. *hlh-8::gfp* fluorescence pixel intensities were measured using Openlab software.

Antibodies, indirect immunofluorescence staining, and immunoblots

C. elegans were fixed and stained by indirect immunofluorescence as previously described (Fridkin *et al.*, 2004). Primary antibodies were diluted as follows: rabbit Ce-lamin antibody (Liu *et al.*, 2000) and mAb414 at 1:400, rat anti-emerin serum 3598 and rabbit anti-lamin serum 3932 (Gruenbaum *et al.*, 2002) at 1:100. For immunoblot analysis, animals were collected in 25 µl M9 buffer and then mixed with 15 µl 2% SDS sample buffer, boiled 10 min, and resolved as described (Margalit *et al.*, 2005). Primary antibodies mouse anti–LEM-2 serum 3268 (Lee *et al.*, 2000) and rabbit anti–Ce-lamin serum 3932 (Gruenbaum *et al.*, 2002) were diluted at 1:500.

PCR analyses

Single-worm PCR analysis with primers 5′-GCGTTGTTGTCAGCGCGTAGGCG-3′ and 5′-GCGCTGCAGATGGTCGACGTTGAGAAAATGTCG-3′ was used to distinguish between wild-type and *lem-2(tm1582/tm1582)* worms, and 5′-CTGAGAGAGAGATTCATAGC-3′ and 5′-CATCAAGCTAACCTCGAAATC-3′ were used to distinguish between wild-type or *emr-1(gk119)/hT2[qIs48]* or *emr-1(gk119)/(gk119)* worms (Figure 1A), essentially as previously described (Williams *et al.*, 1992).

Movement and pumping assays

Animals were collected, washed with M9 buffer, treated for 5 min with hypochlorite solution (1.1% hypochlorite, 0.62 M NaOH), and washed with M9, and embryos were collected and grown on NGM plates or plates seeded with feeding bacteria containing dsRNA to down-regulate *lem-2* at 20°C. For movement assays, *C. elegans* were transferred to NGM plates without bacteria, and the number of head movements counted for 1 min at days 1, 2, 3, 4, 5, 6, 7, and 9 of development or at day 1 and 2 for the RNAi experiments. For pumping assays, L2 animals, *emr-1*$^{-/-}$ animals down-regulated for *lem-2* and for control empty vector, and young adult animals of each genotype (day 3 of development), plus L2-arrested double-null [*emr-1(gk119/gk119)*; *lem-2(tm1582/tm1582)]* animals at day 3 were scored under Olympus MVX-ZB10 MacroView Microscope (maximal magnification: 126×) for the number of pharynx pumping events in 1 min. Both assays were repeated three times using at least 10 worms per genotype each time.

Life span assays

Life span was assayed by slight modification of a published protocol (Lee *et al.*, 2003). Synchronized animals were grown on NGM plates at 20°C to the young adult stage. Fifty to 100 animals (≈30 animals per plate) were then transferred to NGM plates containing 0.05 mg/ml 5-fluoro 2′-deoxyuridine to prevent growth of progeny; they were allowed to grow on these plates at 20°C unless noted. Animals were scored every day until they no longer responded to gentle prodding with a platinum wire; this is the point at which they are considered to be dead. Animals that exploded were censored from the experiments. For all life span experiments, assays were repeated at least twice. Results for each genotype from all independent assays were united and analyzed statistically using the Kaplan–Meier Survival Analysis of SPSS software (version 11; SPSS, Chicago, IL).

ACKNOWLEDGMENTS

We are grateful to the *C. elegans* Gene Knockout Consortium and *C. elegans* Reverse Genetics Core Facility at the University of British Columbia for providing the *emr-1* deletion allele (*gk119*) and the *C. elegans* National BioResource Project in Japan for providing the *lem-2* deletion allele (*tm1582*). We also thank the *C. elegans* Genetic Center (which is funded by the National Center for Research Resources) for strains. We gratefully acknowledge funding from the Morasha Legacy 1798/10, Muscular Dystrophy Association (MDA 4329) and Israel Ministry of Health (MOH 2965) to Y.G., funding from the National Institutes of Health (NIH; RO1 GM048646) and the Binational Israel–USA Science Foundation (BSF 2007215) to K.L.W. and Y.G., and funding from the Muscular Dystrophy Association (MDA 3894) and the NIH (R01 GM066953) to J.L.

REFERENCES

Bione S, Maestrini E, Rivella S, Mancini M, Regis S, Romeo G, Toniolo D (1994). Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. Nat Genet 8, 323–327.

Brenner S (1974). The genetics of *Caenorhabditis elegans*. Genetics 77, 71–94.

Chisholm AD, Hardin J (2005). Epidermal morphogenesis. In: WormBook: The Online Review of *C. elegans* Biology, ed. Martin Chalfie. DOI: 10.1895/wormbook.1.35.1.

Cohen M, Tzur YB, Neufeld E, Feinstein N, Delannoy MR, Wilson KL, Gruenbaum Y (2002). Transmission electron microscope studies of the nuclear envelope in *Caenorhabditis elegans* embryos. J Struct Biol 140, 232–240.

Corsi AK, Kostas SA, Fire A, Krause M (2000). *Caenorhabditis elegans* Twist plays an essential role in non-striated muscle development. Development 127, 2041–2051.

D'Angelo MA, Hetzer MW (2006). The role of the nuclear envelope in cellular organization. Cell Mol Life Sci 63, 316–332.

Dechat T, Pfleghaar K, Sengupta K, Shimi T, Shumaker DK, Solimando L, Goldman RD (2008). Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. Genes Dev 22, 832–853.

Dedeic Z, Cetera M, Cohen TV, Holaska JM (2011). Emerin inhibits Lmo7 binding to the Pax3 and MyoD promoters and expression of myoblast proliferation genes. J Cell Sci 124, 1691–1702.

Fakhouri TH, Stevenson J, Chisholm AD, Mango SE (2010). Dynamic chromatin organization during foregut development mediated by the organ selector gene PHA-4/FoxA. PLoS Genet 12, e1001060.

Fridkin A, Mills E, Margalit A, Neufeld E, Lee KK, Feinstein N, Cohen M, Wilson KL, Gruenbaum Y (2004). Matefin, a *Caenorhabditis elegans* germ-line specific SUN-domain nuclear membrane protein, is essential for early embryonic and germ cell development. Proc Natl Acad Sci USA 101, 6987–6992.

Geyer PK, Vitalini MW, Wallrath LL (2011). Nuclear organization: taking a position on gene expression. Curr Opin Cell Biol 23, 354–359.

Gruenbaum Y, Lee KK, Liu J, Cohen M, Wilson KL (2002). The expression, lamin-dependent localization and RNAi depletion phenotype for emerin in *C. elegans*. J Cell Sci 115, 923–929.

Gruenbaum Y, Margalit A, Goldman RD, Shumaker DK, Wilson KL (2005). The nuclear lamina comes of age. Nat Rev Mol Cell Biol 6, 21–31.
Guelen L *et al.* (2008). Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature 453, 948–951.
Gueneau L *et al.* (2009). Mutations of the FHL1 gene cause Emery-Dreifuss muscular dystrophy. Am J Hum Genet 85, 338–353.
Haithcock E, Dayani Y, Neufeld E, Zahand AJ, Feinstein N, Mattout N, Gruenbaum Y, Liu J (2005). Age-related changes of nuclear architecture in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 102, 16690–16695.
Haque F, Mazzeo D, Patel JT, Smallwood DT, Ellis JA, Shannahan CM, Shackleton S (2010). Mammalian SUN protein networks at the inner nuclear membrane and their role in laminopathy disease processes. J Biol Chem 285, 3487–3498.
Harfe BD, Vaz Gomes A, Kenyon C, Liu J, Krause M, Fire A (1998). Analysis of a *Caenorhabditis elegans* Twist homolog identifies conserved and divergent aspects of mesodermal patterning. Genes Dev 12, 2623–2635.
Herndon LA, Schmeissner PJ, Dudaronek JM, Brown PA, Listner KM, Sakano Y, Paupard MC, Hall DH, Driscoll M (2002). Stochastic and genetic factors influence tissue-specific decline in ageing *C. elegans*. Nature 419, 808–814.
Holaska JM (2008). Emerin and the nuclear lamina in muscle and cardiac disease. Circ Res 103, 16–23.
Huber MD, Guan T, Gerace L (2009). Overlapping functions of nuclear envelope proteins NET25 (Lem2) and emerin in regulation of extracellular signal-regulated kinase signaling in myoblast differentiation. Mol Cell Biol 29, 5718–5728.
Jiang X, Xia L, Chen D, Yang Y, Huang H, Yang L, Zhao Q, Shen L, Wang J, Chen D (2008). Otefin, a nuclear membrane protein, determines the fate of germline stem cells in *Drosophila* via interaction with Smad complexes. Dev Cell 14, 494–506.
Jiang Y, Horner V, Liu J (2005). The HMX homeodomain protein MLS-2 regulates cleavage orientation, cell proliferation and cell fate specification in the *C. elegans* postembryonic mesoderm. Development 132, 4119–4130.
Kelly WG, Xu S, Montgomery MK, Fire A (1997). Distinct requirements for somatic and germline expression of a generally expressed *Caenorhabditis elegans* gene. Genetics 146, 227–238.
Laguri C, Gilquin B, Wolff N, Romi-Lebrun R, Courchay K, Callebaut I, Worman HJ, Zinn-Justin S (2001). Structural characterization of the LEM motif common to three human inner nuclear membrane proteins. Structure 9, 503–511.
Lee KK, Gruenbaum Y, Spann P, Liu J, Wilson KL (2000). *C. elegans* nuclear envelope proteins emerin, MAN1, lamin, and nucleoporins reveal unique timing of nuclear envelope breakdown during mitosis. Mol Biol Cell 11, 3089–3099.
Lee SS, Lee RY, Fraser AG, Kamath RS, Ahringer J, Ruvkun G (2003). A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. Nat Genet 33, 40–48.
Liang WC, Mitsuhashi H, Keduka E, Nonaka I, Noguchi S, Nishino I, Hayashi YK (2011). TMEM43 mutations in Emery-Dreifuss muscular dystrophy-related myopathy. Ann Neurol 69, 1005–1013.
Liu J, Lee KK, Segura-Totten M, Neufeld E, Wilson KL, Gruenbaum Y (2003). MAN1 and emerin have overlapping function(s) essential for chromosome segregation and cell division in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 100, 4598–4603.
Liu J, Rolef-Ben Shahar T, Riemer D, Spann P, Treinin M, Weber K, Fire A, Gruenbaum Y (2000). Essential roles for *Caenorhabditis elegans* lamin gene in nuclear organization, cell cycle progression, and spatial organization of nuclear pore complexes. Mol Biol Cell 11, 3937–3947.
Malik P, Korfali N, Srsen V, Lazou V, Batrakou DG, Zuleger N, Kavanagh DM, Wilkie GS, Goldberg MW, Schirmer EC (2010). Cell-specific and lamin-dependent targeting of novel transmembrane proteins in the nuclear envelope. Cell Mol Life Sci 67, 1353–1369.
Margalit A, Brachner A, Gotzmann J, Foisner R, Gruenbaum Y (2007a). Barrier-to-autointegration factor—a BAFfling little protein. Trends Cell Biol 17, 202–208.
Margalit A, Neufeld E, Feinstein N, Wilson KL, Podbilewicz B, Gruenbaum Y (2007b). Barrier-to-autointegration factor (BAF) is required for blocking premature cell fusion, vulva formation, germ cell development and survival, DTC migration and adult muscle integrity in *C. elegans*. J Cell Biol 178, 661–673.
Margalit A, Segura-Totten M, Gruenbaum Y, Wilson KL (2005). Barrier-to-autointegration factor is required to segregate and enclose chromosomes within the nuclear envelope and assemble the nuclear lamina. Proc Natl Acad Sci USA 102, 3290–3295.
Markiewicz E *et al.* (2006). The inner nuclear membrane protein emerin regulates β-catenin activity by restricting its accumulation in the nucleus. EMBO J 25, 3275–3285.
Mattout A, Pike BL, Towbin BD, Bank EM, Gonzalez-Sandoval A, Stadler MB, Meister P, Gruenbaum Y, Gasser SM (2011). An EDMD mutation in *C. elegans* lamin blocks muscle-specific gene relocation and compromises muscle integrity. Curr Biol 21, 1603–1614.
Meister P, Towbin BD, Pike BL, Ponti A, Gasser SM (2010). The spatial dynamics of tissue-specific promoters during *C. elegans* development. Genes Dev 24, 766–782.
Mislow MKJ, Holaska JM, Kim MS, Lee KK, Segura-Totten M, Wilson KL, McNally EM (2002). Nesprin-1a self-associates and binds directly to emerin and lamin A in vitro. FEBS Lett 525, 135–140.
Montes de Oca R, Shoemaker CJ, Gucek M, Cole RN, Wilson KL (2009). Barrier-to-autointegration factor proteome reveals chromatin-regulatory partners. PLoS One 4, e7050.
Montes de Oca RM, Lee KK, Wilson KL (2005). Binding of barrier to autointegration factor (BAF) to histone H3 and selected linker histones including H1.1. J Biol Chem 280, 42252–42262.
Montes de Oca RM, Andreassen PR, Wilson KL (2011). Barrier-to-autointegration factor influences specific histone modifications. Nucleus 2 (*in press*).
Muchir A, Pavlidis P, Bonne G, Hayashi YK, Worman HJ (2007a). Activation of MAPK in hearts of EMD null mice: similarities between mouse models of X-linked and autosomal dominant Emery Dreifuss muscular dystrophy. Hum Mol Genet 16, 1884–1895.
Muchir A, Pavlidis P, Decostre V, Herron AJ, Arimura T, Bonne G, Worman HJ (2007b). Activation of MAPK pathways links LMNA mutations to cardiomyopathy in Emery-Dreifuss muscular dystrophy. J Clin Invest 117, 1282–1293.
Muchir A, Shan J, Bonne G, Lehnart SE, Worman HJ (2008). Inhibition of extracellular signal-regulated kinase signaling to prevent cardiomyopathy caused by mutation in the gene encoding A-type lamins. Hum Mol Genet 18, 241–247.
Muchir A, Worman HJ (2007). Emery-Dreifuss muscular dystrophy. Curr Neurol Neurosci Rep 7, 78–83.
Raizen DM, Lee RY, Avery L (1995). Interacting genes required for pharyngeal excitation by motor neuron MC in *Caenorhabditis elegans*. Genetics 141, 1365–1382.
Rodríguez S, Eriksson M (2010). Evidence for the involvement of lamins in aging. Curr Aging Sci 3, 81–89.
Schirmer EC, Gerace L (2005). The nuclear membrane proteome: extending the envelope. Trends Biochem Sci 30, 551–558.
Simon DN, Wilson KL (2011). The nucleoskeleton as a genome-associated dynamic "network of networks." Nat Rev Mol Cell Biol 12, 171–181.
Stewart CL, Roux KJ, Burke B (2007). Blurring the boundary: the nuclear envelope extends its reach. Science 318, 1408–1412.
Sulston JE, Horvitz HR (1977). Postembryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev Biol 56, 110–156.
Sulston JE, Schierenberg E, White JG, Thomson JN (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev Biol 100, 64–119.
Tilgner K, Wojciechowicz K, Jahoda C, Hutchison C, Markiewicz E (2009). Dynamic complexes of A-type lamins and emerin influence adipogenic capacity of the cell via nucleocytoplasmic distribution of β-catenin. J Cell Sci 122, 401–413.
Wagner N, Krohne G (2007). LEM-domain proteins: new insights into lamin-interacting proteins. Int Rev Cytol 261, 1–46.
Wheeler MA, Ellis JA (2010). Molecular signatures of Emery-Dreifuss muscular dystrophy. Biochem Soc Trans 36, 1354–1358.
Williams BD, Schrank B, Huynh C, Shownkeen R, Waterston RH (1992). A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. Genetics 131, 609–624.
Wilson KL, Berk JM (2010). The nuclear envelope at a glance. J Cell Sci 123, 1973–1978.
Wilson KL, Foisner R (2010). Lamin-binding proteins. Cold Spring Harb Perspect Biol 2, a000554.
Woo WM, Goncharov A, Jin Y, Chisholm AD (2004). Intermediate filaments are required for *C. elegans* epidermal elongation. Dev Biol 267, 216–229.
Zhang Q, Ragnauth CD, Skepper JN, Worth NF, Warren DT, Roberts RG, Weissberg PL, Ellis JA, Shanahan CM (2005). Nesprin-2 is a multi-isomeric protein that binds lamin and emerin at the nuclear envelope and forms a subcellular network in skeletal muscle. J Cell Sci 118, 673–687.