

Matefin, a *Caenorhabditis elegans* germ line-specific SUN-domain nuclear membrane protein, is essential for early embryonic and germ cell development

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Caenorhabditis elegans mtf-1 encodes matefin, which has a predicted SUN domain, a coiled-coil region, an anti-erbB-2 IgG domain, and two hydrophobic regions. We show that matefin is a nuclear membrane protein that colocalizes *in vivo* with Ce-lamin, the single nuclear lamin protein in *C. elegans*, and binds Ce-lamin *in vitro* but does not require Ce-lamin for its localization. Matefin is detected in all embryonic cells until midembryogenesis and thereafter only in germ-line cells. Embryonic matefin is maternally deposited, and matefin is the first nuclear membrane protein known to have germ line-restricted expression. Animals homozygous for an *mtf-1* deletion allele show that matefin is essential for germ line maturation and survival. However, matefin is also required for embryogenesis because *mtf-1* (RNAi) embryos die around the ≈300-cell stage with defects in nuclear structure, DNA content, and chromatin morphology. Down-regulating matefin in *mes-3* animals only slightly enhances embryonic lethality, and elimination of UNC-84, the only other SUN-domain gene in *C. elegans*, has no effect on *mtf-1* (RNAi) animals. Thus, *mtf-1* mediates a previously uncharacterized pathway(s) required for embryogenesis as well as germ line proliferation or survival.

Lamins are nuclear intermediate filament proteins found in metazoan cells at the nuclear periphery and in the nucleoplasm (1). Lamins interact with most known inner nuclear membrane proteins as well as with several nucleoplasmic proteins (2). Nuclear architecture, cell cycle progression, DNA replication, and RNA transcription and splicing all depend on lamins (3, 4). Consistent with such roles, many of these lamin-binding proteins also bind transcription repressors and chromatin proteins.

To understand nuclear lamins and lamin-associated protein functions *in vivo* we turned to *Caenorhabditis elegans*, studying its single lamin protein, Ce-lamin (5), and three inner nuclear membrane proteins, Ce-emerin, Ce-MAN1 (5, 6), and UNC-84 (7). UNC-84 contains an ≈120-residue SUN (Sad1p-UNC-84 homology) domain (8) with an unknown function. The SUN domain is also found in four human proteins, two of which localize at the nuclear envelope (9). UNC-84 is expressed in most *C. elegans* cells, and it depends on Ce-lamin for its nuclear-envelope localization (7). However, mutations in *unc-84* cause nuclear migration or nuclear anchoring defects in only a subset of cells, leading to uncoordinated movement (8). At least two nuclear-envelope proteins, UNC-83 and ANC-1, require the SUN domain of UNC-84 for their nuclear-envelope localization and ability to regulate nuclear position (10). To explain their nuclear-envelope anchoring, a “bridging model” was proposed in which the transmembrane domains of UNC-83 and ANC-1 cross the outer nuclear membrane (ONM), and their luminal domains interact with the luminal domain of UNC-84 embedded in the inner nuclear membrane (7, 10).

Based on the hypothesis that the SUN domain defined a new family of nuclear-envelope proteins, we searched the *C. elegans* genome for other SUN domains, and found one: an ORF on

chromosome V, designated F57B1.2. We report that this gene (*mtf-1*) encodes matefin, a nuclear membrane protein that binds Ce-lamin and has essential embryonic and germ line-specific functions.

Materials and Methods

C. elegans Strains. *C. elegans* strains N2, PD4793, *ced-3(n717)*, and *unc-84(n369)* were obtained from the *C. elegans* genetic center. Strain PD4793 has an integrated multiconstruct array comprising *myo-2::GFP*, *pes-10::GFP*, and *gut::GFP*, which lead to GFP expression in four-cell embryos, pharyngeal muscle, and gut (11). Strain VC292 containing the *gk199* deletion allele of *mtf-1* [+/*nT1 IV*; *mtf-1(gk99)/nT1 V*] was prepared by the *C. elegans* Gene Knockout Consortium by using TMP/UV as mutagen. This strain was outcrossed once before balancing. The *gk199* allele on chromosome V was outcrossed again with strain PD4793 (11). *mes-2* (ss186), *mes-3* (ss313), and *mes-6* (ss553) were kindly provided by S. Strome (Indiana University, Bloomington). The AZ212 strain expressing H2B::GFP was kindly provided by J. Austin (University of Chicago, Chicago).

Antibodies, Indirect Immunofluorescence Staining of *C. elegans*, and Time-Lapse Microscopy.

C. elegans embryos, larva, and adults were fixed and prepared for indirect immunofluorescence exactly as described (12, 13). Covance (Princeton) produced all antisera against matefin. Rat 3663 was immunized with KLH-conjugated peptide CRHTISPOQFSNRHSP (matefin residues 3–16 plus one Cys for conjugation). Rat 3665 and mouse 3666 were immunized with KLH-conjugated peptide PMTDNGTESKLESAC (matefin residues 451–465 plus one Cys for conjugation). Rabbit anti-PGL-1, Rat anti-Mes-2, rabbit anti-Mes-6, and rat anti-Mes-3 were kindly provided by S. Strome. Immunogold transmission electron microscopy (TEM) with matefin serum 3663 (diluted 1:30) and thin-section TEM were performed as described (14). For time-lapse microscopy, F₁ progeny of hermaphrodites fed with *mtf-1* (RNAi) (see below) were mounted on an agar pad with egg salt buffer, sealed with vacuum grease, and viewed by using a Zeiss Axioplan II microscope equipped for fluorescence. An Axiocam CCD camera and the AxioVision Image Analysis package were used to record time-lapse data every 2 min.

RNA Interference (RNAi) Experiments. *mtf-1* cDNA nucleotides 311–731 (exon 3) were subcloned into feeding vector L4440 (15) and used for RNAi feeding as described (6). Control animals were fed with bacteria carrying an empty L4440 construct.

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Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; RNAi, RNA interference; TEM, transmission electron microscopy.

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Worms were either examined live to determine viability or fixed and prepared for indirect immunofluorescence staining.

Synthesis of ³⁵S-Met-Labeled Proteins and Blot Overlay Assays. cDNAs encoding Ce-emerin (residues 1–130; lacking only the short C-terminal transmembrane domain), Ce-lamin globular domain (residues 440–566), Ce-*mtf-1-N* (residues 1–111), and full-length Ce-lamin were cloned into pET20b expression vector. cDNAs encoding Ce-*mtf-1-C* (residues 129–473) and full-length matefin were cloned into the pET28a expression vector. Ce-MAN1 was cloned into the pET15b expression vector. *Mtf-1* cDNAs yk703g5 and yk729e11 were kindly provided by Yuji Kohara (Genome Biology Laboratory, Mishima, Japan). Constructs were transformed into *Escherichia coli* BL21 (DE3) and proteins were purified via Ni-NTA agarose (Qiagen, Valencia, CA). Equal amounts of protein (determined by a Bradford assay) were resolved by 15% SDS/PAGE and transferred to nitrocellulose membrane. Blot overlay assays were done essentially as described (13).

Results

Matefin Is a Previously Uncharacterized *C. elegans* Nuclear Envelope Protein with a SUN Domain and Two Putative Transmembrane Domains. Homologs for UNC-84 are known to exist in the nuclear envelope of a wide variety of species and are thought to control the position of the nucleus in the cell (10). UNC-84 contains an evolutionarily conserved SUN domain, which we hypothesized might define a new family of nuclear-envelope proteins. We therefore searched for new SUN-domain genes in *C. elegans* and found one ORF on chromosome V (F57B1.2). We named this gene *mtf-1* and its product matefin (“envelope” in Hebrew), based on its localization. Matefin was predicted to have a 20-residue hydrophobic region, a 36-residue coiled-coil region, a second 21-residue hydrophobic region, one SUN domain, and a 101-residue “anti-erbB-2 Ig” domain overlapping both the second hydrophobic region and the SUN domain (Fig. 1A). Hydrophathy programs suggested that the first and possibly both hydrophobic regions were transmembrane domains. Matefin is conserved (57% identical) in the nematode *Caenorhabditis briggsae*. The SUN domain of matefin is 31%, 28%, and 29% identical and 44%, 37%, and 39% similar to the SUN domains in human UNC-84, UNC-84a, and SUN2, respectively (8, 9). The anti-erbB-2 Ig domain is 29% identical and 45% similar to the human anti-erbB-2 Ig domain (16). However, outside these domains matefin appears to be unique, with no detectable homology to vertebrate, *Drosophila*, yeast, or plant genes.

We generated rat polyclonal sera against an N-terminal peptide (serum 3663) and a C-terminal peptide (serum 3665; Fig. 1A; also see *Materials and Methods*). Western blot analysis of whole worm lysate revealed a major band of 55 kDa, the predicted mass of matefin (Fig. 1B). By indirect immunofluorescence, both antisera specifically stained the nuclear envelope of *C. elegans* embryos (Fig. 1C). Thin-section immunogold TEM analysis of preembedded stained embryos further showed that the N-terminal matefin epitope (serum 3663) remained associated with peripheral chromatin after cells were extracted with Triton X-100 (Fig. 1D), consistent with the behavior of nuclear membrane proteins that bind lamins (13). Double immunofluorescent labeling of *C. elegans* embryos with anti-lamin and anti-matefin antibodies showed that endogenous Ce-lamin and matefin colocalized at the nuclear envelope (Fig. 1C).

To determine whether matefin bound directly to Ce-lamin, we used ³⁵S-matefin to probe various recombinant proteins immobilized on blots (Fig. 1E Left, lanes 1–6). There was a strong signal for ³⁵S-matefin binding to the complete Ce-lamin, but not to its isolated tail domain. Matefin also bound weakly to itself (Fig. 1E, lanes 5 and 6). The converse experiment with ³⁵S-Ce-lamin confirmed direct binding to matefin, and further showed

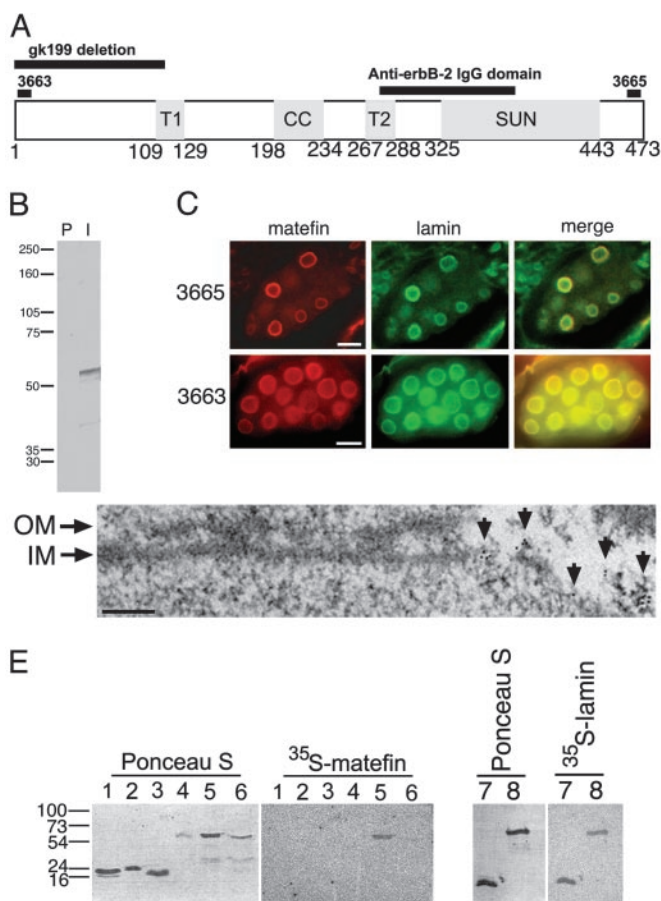


Fig. 1. Matefin in *C. elegans* and its interaction with Ce-lamin. (A) A schematic diagram of full-length matefin, drawn approximately to scale. Bars on top indicate the positions of the *gk199* deletion, the peptides used to prepare serum 3663 and 3665 antibodies against matefin, and the anti-erbB-2 IgG domain. T1 and T2, putative transmembrane regions 1 and 2, respectively; CC, coiled-coil domain; SUN, SUN domain. (B) Western blot of crude protein extract of mixed stage *C. elegans* animals probed with preimmune (P) or immune (I) serum 3665. (C) Indirect immunofluorescence double-staining of embryos by using antibodies specific for Ce-lamin plus either the N terminus (serum 3663, Lower) or C terminus (serum 3665, Upper) of endogenous matefin. Please note that although both embryonic and adult nuclei are stained with Ce-lamin, only the embryonic nuclei are stained with matefin (Upper). (Bars, 10 μ m.) (D) Immunogold TEM localization of the matefin N terminus (serum 3663) on peripheral chromatin in detergent-extracted nuclei. Fragments of remaining nuclear outer membrane (OM) and inner membrane (IM) are indicated. Arrows indicate the position of the 6-nm gold labeling. (Bar, 100 nm.) (E) Equal amounts of protein, as determined by Bradford assay, were resolved by SDS/PAGE, transferred to nitrocellulose, stained with Ponceau S, and probed with either ³⁵S-matefin or ³⁵S-Ce-lamin. Please note that Ponceau S staining does not always correspond to Bradford assay results. Numbered gel lanes contain the following polypeptides: 1, Ce-emerin (residues 1–130); 2, the C-terminal globular domain of Ce-lamin (residues 440–566); 3, Ce-MAN1 (residues 400–500); 4, BSA; 5, Ce-lamin; 6, matefin; 7, matefin N-terminal domain (residues 1–111); 8, matefin C-terminal domain (residues 129–473).

that Ce-lamin binds two regions of matefin: residues 1–111 (up to the first putative transmembrane domain) and residues 129–473, comprising all residues after this first transmembrane domain (Fig. 1E, lanes 7 and 8). Pull-down experiments verified the blot overlay results (data not shown). We concluded that matefin is a lamin-binding protein of the inner nuclear membrane. Furthermore, Ce-lamin binding to both “halves” of matefin suggests (but does not prove) that matefin is anchored by two transmembrane domains, with its N and C termini both available to bind Ce-lamin in the nucleoplasm.

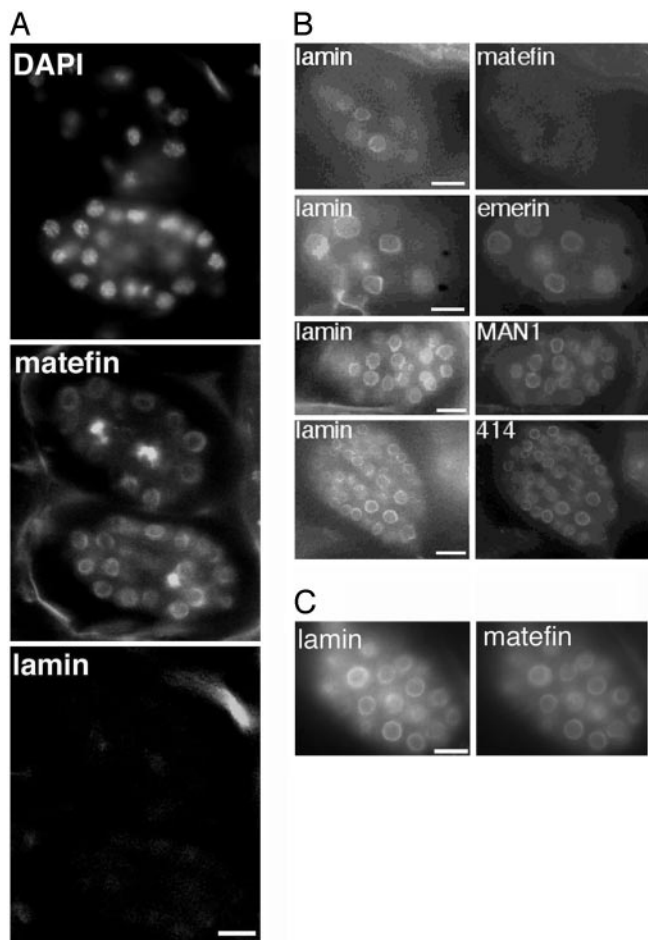


Fig. 2. Nuclear-envelope localization of matefin does not require Ce-lamin or UNC-84, and matefin is not required to localize other known nuclear-envelope proteins. (A) Ce-lamin-knockdown [*lmn-1* (*RNAi*)] embryos triple-stained for DNA [with 4',6-diamidino-2-phenylindole (DAPI)] and for endogenous matefin and Ce-lamin by using specific antibodies and indirect immunofluorescence. (B) Double-staining of *mtf-1* (*RNAi*) embryos by indirect immunofluorescence for endogenous Ce-lamin (Left), plus endogenous matefin, Ce-emerin, Ce-MAN1, or FG-nucleoporins (mAb414). (C) Double-staining of *unc-84*(*n369*) null embryos by indirect immunofluorescence for endogenous Ce-lamin and endogenous matefin. (Bars, 10 μ m.) Wild-type embryos (100–300 cells) were stained with DAPI for DNA and with rat anti-matefin antibodies (serum 3663) by indirect immunofluorescence (IF).

To our surprise, RNAi-mediated down-regulation of Ce-lamin had no obvious effect on the nuclear-envelope localization of matefin (Fig. 2A). Matefin is thus the first known *C. elegans* nuclear membrane protein that remains nuclear-envelope-localized in the absence of Ce-lamin. Matefin localization was also normal in *unc-84*(*n369*) null embryos and gonads (Figs. 2C and 6, which is published as supporting information on the PNAS web site). Similarly, RNAi-mediated down-regulation of matefin had no effect on the embryonic nuclear-envelope localization of Ce-lamin, Ce-emerin, Ce-MAN1, FG-repeat nucleoporins (Fig. 2B), or UNC-84 (data not shown).

Immunofluorescence studies of early embryos also showed that matefin remained nuclear-envelope-localized through early anaphase and then completely disassembled during mid/late anaphase, similar to Ce-lamin and other known nuclear membrane proteins (refs. 7 and 12 and data not shown).

Matefin Is Present in all Early Embryonic Nuclei but Is Restricted to Germ Cells from Midembryogenesis to Adulthood. To address when and where matefin functions, we used the anti-matefin antibodies

to investigate its subcellular localization during development. To control for antibody penetration into *C. elegans* tissues, we always double-labeled the samples with rabbit antibodies against Ce-lamin, which is expressed in all *C. elegans* cells except mature sperm (5), and used DAPI to label DNA. Matefin was detected at the nuclear envelope of all early embryonic cells (Fig. 1C). In late embryos (after the comma stage) matefin staining decreased in all somatic cells but intensified in the nuclear envelopes of the two primordial germ cells, Z2 and Z3 (Fig. 3A, arrow). The identity of Z2 and Z3 cells was verified by double labeling with antibodies against PGL-1, which is specific to germ cells (data not shown). Throughout larva stages L1–L4 and in adults, matefin was present only in germ cells (Fig. 3B shows staining in L2 larva, and Fig. 3C and D shows staining in adults). Matefin signal declined during spermatogenesis and was undetectable in sperm (Fig. 3C, arrowhead). These results suggest that matefin functions in the germ cell lineage.

To test the possibility that matefin protein in early embryos is deposited maternally, we double-stained F₁ embryos of strain VC292 by using antibodies against endogenous matefin and Ce-lamin. Strain VC292 contains the *gk199* allele, a 435-bp deletion in *mtf-1* that removes 105 bp 5' of the ORF plus the first 110 residues of matefin, including the epitope recognized by serum 3663 (Fig. 1A).

Among self-fertilizing hermaphrodites heterozygous for the *gk199* allele, 25% of the embryos should be homozygous for *gk199* and 50% heterozygous for the *mtf-1* deletion and the nT1 balancer chromosome V, which expresses wild-type matefin (see below). The remaining 25% were predicted to be homozygous for the nT1 balancer chromosome. We found that \approx 97% of embryos ($n = 103$) stained positively for both matefin (serum 3663) and Ce-lamin antibodies, with roughly similar fluorescence signals for matefin (data not shown). The remaining 3% of embryos ($n = 3$) stained weakly for both matefin and Ce-lamin, probably because of inefficient antibody penetration into these embryos. In contrast, 100% of L4 larva or adult hermaphrodites stained positive for Ce-lamin, whereas only \approx 75% were positive for matefin and had normal germ line (see below). We concluded that matefin is maternally deposited in the egg and persists in somatic cells through early embryonic development. However, in later embryos, larvae and adults matefin expression is limited to germ cells.

Matefin Is Essential for Viability of the Germ Line. Matefin function was analyzed in animals heterozygous or homozygous for the *gk199* allele. Heterozygote worms were indistinguishable from wild-type worms, with similar brood sizes. Homozygous *gk199/gk199* progeny all developed into adult animals that were sterile but otherwise healthy. We conclude that the maternally supplied matefin is sufficient for embryos to survive development (see below) but insufficient for its role in germ-line cells. Thus, matefin gene expression in embryonic germ cells appeared to be specifically required for the germ line.

The sterile phenotype of *gk199/gk199* animals was further analyzed by staining with DAPI and PGL-1 antibodies, as a germ cell marker. During the L3 stage, *gk199/gk199* animals contained an average total of 30 germ cells ($n = 20$), similar to heterozygote and wild-type animals. During early L4 the average number of germ cells in *gk199/gk199* gonads was <80 ($n = 20$), compared to >250 germ cells in *gk199/GFP* heterozygous and wild-type animals ($n = 20$). Both the number of germ cells and gonad size were significantly reduced in adult *gk199/gk199* animals (Fig. 4A), relative to heterozygous or wild-type animals. Interestingly, the two gonads in each animal sometimes had nonequal numbers of germ cells (Fig. 4A, arrows indicate gonads of different size), and in many cases germ cells were clustered in separate places in the same gonad (Fig. 4A, arrowhead). The reduced number of germ cells in adult *gk199/gk199* gonads

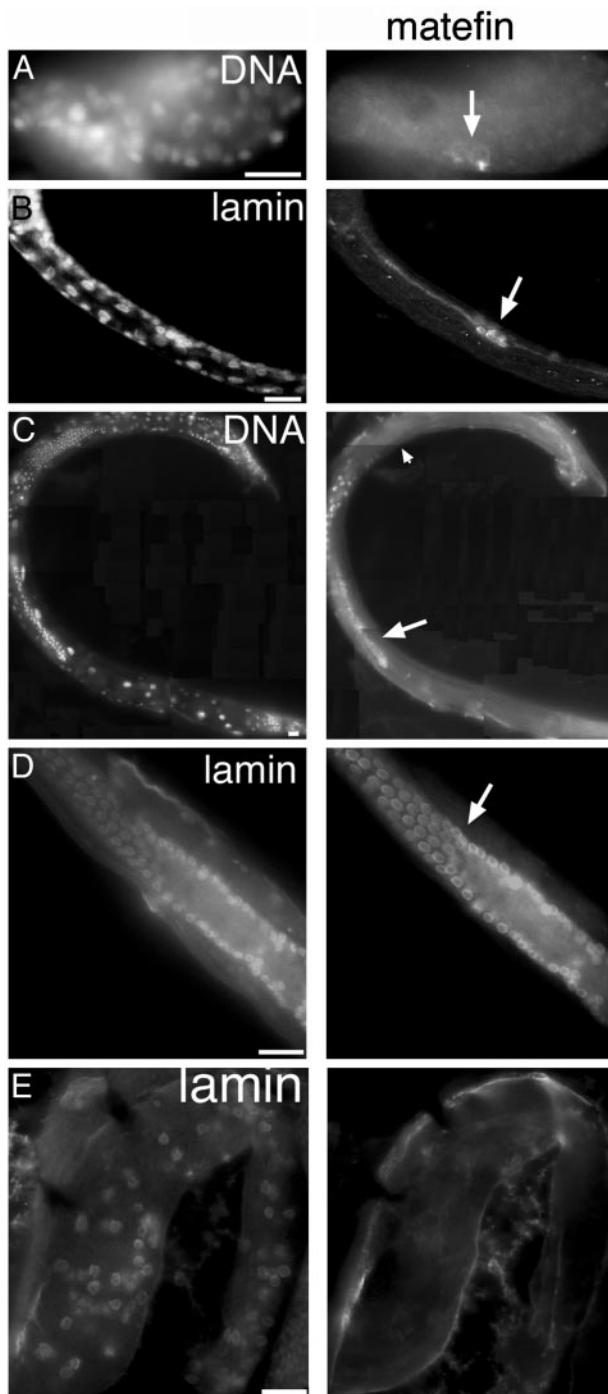


Fig. 3. Matefin expression is confined to the germ line from midembryogenesis to adulthood. Indirect immunofluorescence images of WT late embryo (A), WT early L2 larva (B), WT adult male (montage shown in C), WT adult hermaphrodites (D), and gonad of an adult hermaphrodite homozygous for the *gk199* allele (E), all tripled-stained with DAPI (for DNA), antibodies specific for matefin (serum 3665 for A–C or 3663 for D–E), and Ce-lamin. (A–D) The arrow indicates germ cells. Matefin did not stain mature sperm (arrowhead in C) or *gk199* homozygous gonads. Left shows DAPI staining (A and C) or Ce-lamin staining (B, D, and E). Right shows staining results for endogenous matefin. (Bars, 10 μm .)

suggested germ-line cell proliferation defects, germ cell degeneration, or both. Gametes were never seen in *gk199/gk199* animals (Fig. 4). The *gk199/gk199* germ cells stained positively for both SYTO 11 and SYTO 12 (Molecular Probes), markers for

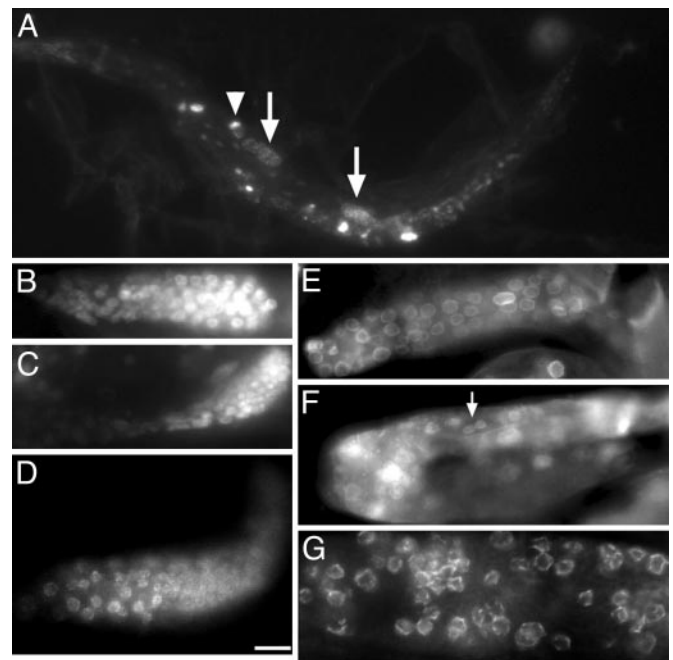


Fig. 4. *Mtf-1* deletion (*gk199/gk199*) homozygous animals have reduced numbers of germ cells and lack mature gametes. (A) *gk199/gk199* animals, which are homozygous for the *mtf-1* deletion allele, have germ cells, but their gonads were often of unequal size; arrows in A show a pair of gonads of different size. The arrowhead shows a group of germ cells separated from the main cluster of germ cells. (B and C) Two *gk199* homozygous gonads at higher resolution. No gametes were seen in any *gk199* gonads. (D–G) Indirect immunofluorescent staining of *gk199/gk199* gonads showed positive staining for PGL-1 (D) and normal nuclear-envelope localizations of endogenous Ce-lamin (E), endogenous UNC-84 (F), and endogenous FG-nucleoporins (G). (Bar: 50 μm for A; 10 μm for C; 7.5 μm for B and D–F; and 5 μm for G.)

apoptosis, but staining was detected only when there were already reduced numbers of germ cells (data not shown). We conclude that germ cell reduction and lack of mature gametes probably results from both reduced proliferation and apoptosis. We speculate that apoptosis might be secondary to a primary defect in proliferation. Indirect immunofluorescence staining of adult *gk199/gk199* animals showed that surviving germ cells did express the germ-line marker protein PGL-1 (Fig. 3D). We concluded that matefin is not needed to establish germ line fate, but is required for germ line proliferation or survival.

Other inner nuclear membrane/lamina proteins including Ce-lamin, Ce-emerin, Ce-MAN1, UNC-84, and nucleoporins are all normally expressed in the nuclear envelope of germ cells (6, 7, 13). Loss of matefin, verified by indirect immunofluorescence and immunoblotting with 3663 and 3666 sera (Fig. 3E and data not shown), had no effect on the localization of these proteins in germ-line cells, as shown by immunostaining *gk199/gk199* gonads with antibodies specific for Ce-lamin (Fig. 3E), UNC-84 (Fig. 4F, arrow), FG-nucleoporins (Fig. 4G), Ce-emerin, or Ce-MAN1 (data not shown), consistent with our RNAi findings in embryonic somatic cells (Fig. 2B).

***mtf-1* Is Essential for Early Embryos.** To understand the embryonic functions of *mtf-1* *in vivo*, we further examined embryos down-regulated for *mtf-1* expression by feeding wild-type (Bristol N2) worms with bacteria expressing *mtf-1* dsRNA [*mtf-1* (RNAi)]. As noted above, matefin protein was significantly reduced in most *mtf-1* (RNAi) embryos (Fig. 2B). Loss of matefin was lethal to embryos, because $59 \pm 10\%$ of *mtf-1* (RNAi) embryos died ($n > 10$ independent experiments). Differential interference contrast

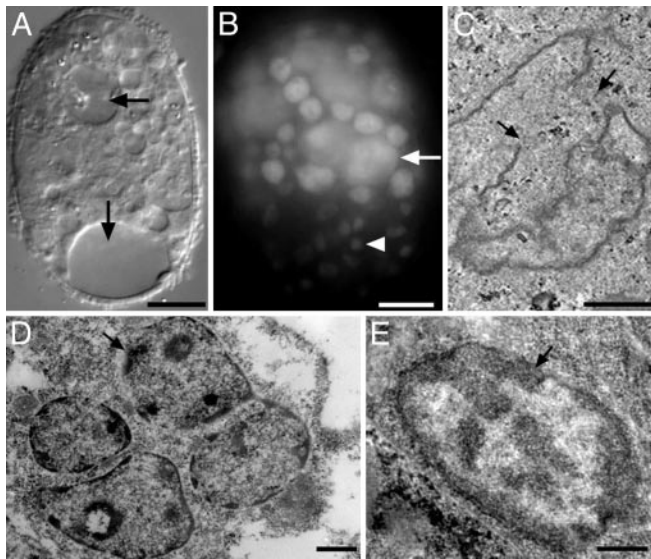


Fig. 5. Phenotypes of *mtf-1* (*RNAi*) embryos, which became arrested by the ≈ 300 -cell stage. Arrested embryos were viewed by differential interference contrast microscopy (A), stained for DNA with DAPI (B), or viewed by thin section transmission EM (C–E). Arrows in A indicate vacuoles. The arrow in B indicates a large nucleus, and the arrowhead indicates a condensed nucleus. Nuclei in C and D have abnormal shapes. The nucleus in C also has a discontinuous nuclear envelope (arrows) and membrane blebs. The cell shown in D contains four nuclei. Cells in D and E have abnormally condensed chromatin (arrows). (Bars: A and B, 10 μm ; C–E, 1 μm .)

microscopy and DNA staining showed that most *mtf-1* (*RNAi*) embryos died before the ≈ 300 -cell stage (Fig. 5A and B). All arrested *mtf-1* (*RNAi*) embryos were abnormal with nuclei that varied in size and DNA content (Fig. 5B). We conclude that matefin is an essential component of the nuclear envelope in early embryos. Embryos that escaped lethality had no visible phenotypes; they developed into normal larva and fertile adults with brood sizes similar to wild-type worms (data not shown). The gonads of these *mtf-1* (*RNAi*) “escapers” stained positive for matefin, indicating that the *mtf-1* dsRNA had not affected these animals (data not shown).

The cellular phenotypes of *mtf-1* (*RNAi*) embryos were examined by differential interference contrast, DAPI staining, and thin-section TEM. Arrested embryos had large vacuoles, which are commonly observed in dying embryos (Fig. 5A, arrows), aberrant nuclei with aneuploid DNA content (Fig. 5B, arrow shows a giant nucleus), or abnormally condensed chromatin (Fig. 5B, arrowhead). To further analyze these nuclear and chromatin phenotypes, *mtf-1* (*RNAi*) embryos were fixed, embedded, sectioned, and viewed by TEM. Control embryos were derived from hermaphrodites fed an empty L4440 vector. We saw abnormally shaped nuclei, discontinuous and “blebbed” nuclear envelopes (Fig. 4C), and clumped chromatin (arrows in Fig. 5D and E) in $\approx 60\%$ of embryos ($n = 24$), corresponding to the percent embryonic lethality. A subset of *mtf-1* (*RNAi*) embryos had cells with multiple (2–4) nuclei (Fig. 5D), a phenomenon never seen in control embryos.

To determine when these nuclear and chromatin abnormalities appeared, we analyzed living *mtf-1* (*RNAi*) embryos by time-lapse microscopy in wild-type worms or strain AZ212, which expresses GFP-fused histone H2B (H2B-GFP) (17). The *mtf-1* (*RNAi*) nuclei had morphologically normal structure and chromosome segregation for most cell divisions and showed the above defects late, just before embryonic arrest [Movie 1, which is published as supporting information on the PNAS web site, shows a normal mitosis in *mtf-1* (*RNAi*) embryos].

The *mes* Mutant Backgrounds Do Not Significantly Affect Embryonic Lethality of *mtf-1* (*RNAi*). The above expression pattern for matefin overlapped with maternal effect sterile (*Mes*) proteins (18, 19), suggesting that the *Mes* protein complex and matefin might interact. *Mes* proteins are required for germ line survival, and their absence causes the germ line to degenerate starting at the L3/L4 larva stages (20). *Mes* proteins are also important for chromatin silencing in the germ line (21) and for regulating *Hox* gene expression in the soma of *C. elegans* (22). To determine whether *mes* mutations affect matefin expression, we immunostained for endogenous matefin in F₁ embryos and gonads of *mes-2* (ss186), *mes-3* (ss313), or *mes-6* (ss553) homozygous animals. Matefin expression was normal in all cases (see Fig. 7A, which is published as supporting information on the PNAS web site, for staining in *mes-3* and *mes-6* embryos). Similarly, immunostaining showed that *Mes-2*, *Mes-3*, and *Mes-6* proteins localized normally in *mtf-1* (*RNAi*) embryos (data not shown).

The lethality of *mtf-1* (*RNAi*) embryos was only slightly increased ($P < 0.01$) in the *mes-3* (ss313) or *mes-6* (ss553) genetic background (Fig. 7B). If *mtf-1* and *mes* genes directly interacted or were components of the same pathway, we would expect more extensive (“synthetic”) lethality. Thus, the slight increase in lethality probably reflects the additive effects of two independently essential genes.

UNC-84 Activity Does Not Overlap with Matefin. The only other gene in *C. elegans* with a SUN domain is UNC-84, which like matefin is localized to the nuclear envelope (7, 8). Animals homozygous for *unc-84* mutations are viable (8). We therefore tested the possibility that matefin and UNC-84 have overlapping functions by feeding *unc-84* homozygous null animals (*n369* allele) with *mtf-1* dsRNA. The average percentage of dead embryos (58.6%) was similar to that of wild-type embryos fed with *mtf-1* dsRNA (60.5%; $n = 20$). Furthermore, UNC-84 did not affect matefin expression or localization, because matefin expression was normal in *unc-84*(*n369*) animals (Figs. 2C and 6).

Discussion

We characterized matefin, a previously uncharacterized SUN-domain protein and the second of two SUN-domain proteins in *C. elegans*. Outside its SUN and “anti-erbB-2 Ig” domains, matefin is not related to other SUN-domain proteins or other proteins in the database. Our findings suggest that matefin is anchored at the nuclear inner membrane by two putative transmembrane domains, with exposed N- and C-terminal domains that both bind Ce-lamin *in vitro*. These findings predicted lamin-dependent localization of matefin at the nuclear envelope. However, we found no requirement for Ce-lamin *in vivo*, suggesting a lamin-independent mechanism for matefin at the nuclear membrane. This mechanism might involve binding to chromatin or other stable (lamin-independent) partners at the inner membrane. The lack of binding of matefin to the isolated globular tail domain of Ce-lamin suggests that matefin might instead bind the rod domain, as seen previously for LAP2, otefin, and DNA (23). It is unlikely that matefin binds the globular head domain of Ce-lamin because the head domain mediates polymerization (1), and no other lamin-binding proteins are known to bind there.

Matefin and UNC-84 are both SUN-domain proteins at the nuclear envelope, but their specific localizations and functions appear to differ significantly. Much evidence localizes matefin to the nuclear inner membrane: its direct binding to Ce-lamin, colocalization with Ce-lamin in embryos, and association with peripheral chromatin in detergent-extracted nuclei (shown in this work). In contrast, UNC-84 requires Ce-lamin for its nuclear localization but may not bind directly to lamins (7). SUN domains are likely to have a conserved folded backbone structure. However, this structure is unknown, and whether different

SUN domains have conserved surface residues or partners remains open to question.

Matefin is maternally deposited in eggs, but the *mtf-1* gene is expressed exclusively in germ-line cells. All other characterized *C. elegans* nuclear membrane proteins are expressed in both the soma and germ-line cells (5–7, 12). Matefin and UNC-83 in *C. elegans*, as well as Young Arrest in *Drosophila* (24) and various vertebrate lamin isoforms (25) comprise a growing list of nuclear-envelope proteins with cell- or tissue-specific expression and function. This theme is likely to resound in human cells, with implications for tissue-specific diseases (23).

Matefin is required for germ line and embryonic development. Animals homozygous for an *mtf-1* deletion had normal somatic development and produced germ cells and gonads until larval L3 stage, when the number of germ cells in *mtf-1* deletion animals was still similar to wild type. After L3/early L4, the number of germ cells declined, the remaining germ cells made no gametes, and adult animals were sterile. It remains open to question whether matefin is required for germ line proliferation at late stages, germ line maintenance, or both. This germ-line phenotype *per se* was not surprising, because matefin gene expression is restricted to germ cells. Matefin is not required to specify germ cell fate, but its actual role(s) in germ cells versus embryonic cells remain unclear.

The *mtf-1* and *mes* genes have similar germ-line expression patterns (20). However, the *mtf-1* phenotype is seen in homozygous F₁ animals, whereas the *mes* phenotype is seen only in F₂ homozygous animals (grandchildless) (26). The number of germ cells present at each stage of development is similar in *gk199/gk199* homozygote and *mes* F₂ animals. Mes proteins regulate gene expression, and so might nuclear-envelope proteins (3). However, the nature of matefin's function is unknown, except that matefin and Mes proteins do not regulate each other's expression or localization. Further work is needed to determine whether *mtf-1* functions in a known germ line pathway [e.g., *nos*, *puf*, *glh*, *pgl*, *glp-1*, or *glp-4* (27)] or whether matefin defines a new nuclear-membrane-dependent pathway.

Matefin also appears to be functionally independent of UNC-84, the other SUN-domain protein in *C. elegans* (7), because

UNC-84 and matefin did not affect each other's expression and down-regulating matefin in *unc-84* null embryos did not increase lethality. These results suggest that matefin has unique biological role(s) in embryos and germ cells.

In addition to its presence in the germ line, matefin was present in all nuclei of early embryos. The role of matefin in early embryonic soma could not be analyzed in *mtf-1* homozygous animals, because they are sterile and *mtf-1* heterozygous animals provided sufficient matefin to their embryos. RNAi-induced down-regulation of matefin in early embryos was lethal to embryos, which became arrested before the comma stage with <300 cells. However, in marked contrast to the loss of Ce-lamin, LEM-domain proteins, or BAF (28), which cause anaphase-bridged chromatin and nuclear structure defects at very early stages, the cell cycle in *mtf-1* (RNAi) embryos looked normal. Thus, matefin is unlikely to have fundamental roles in nuclear assembly *per se*, and we can only speculate that it has a previously uncharacterized role late in embryogenesis and/or a specialized role in the germ line or meiosis. The idea of novel specialized roles is supported by the large number (>70) of proposed nuclear membrane proteins identified in a proteomic analysis of rat liver (29).

It was recently suggested that matefin bind ZYG-12, a protein required for centrosome attachment to the nucleus (30). Our data showing that matefin is present only in early embryos and germ cells would limit this hypothetical interaction to a small number of cell types and would further suggest that in most cell types, centrosome positioning by ZYG-12 would require partners other than matefin.

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