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SHORT COMMUNICATION

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Evolutionarily conserved nuclear migration genes required for early embryonic development in *Caenorhabditis elegans*

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Abstract The *nudF* and *nudC* genes of the fungus *Aspergillus nidulans* encode proteins that are members of two evolutionarily conserved families. In *A. nidulans* these proteins mediate nuclear migration along the hyphae. The human ortholog of *nudF* is *Lis1*, a gene essential for neuronal migration in the developing cerebral cortex. The mammalian ortholog of *nudC* encodes a protein that interacts with Lis1. We have identified orthologs of *nudC* and *Lis1* from the nematode *Caenorhabditis elegans*. Heterologous expression of the *C. elegans nudC* ortholog, *nud-1*, complements the *A. nidulans nudC3* mutant, demonstrating evolutionary conservation of function. A *C. elegans nud-1*::GFP fusion produces sustained fluorescence in sensory neurons and embryos, and transient fluorescence in the gonad, gut, vulva, ventral cord, and hypodermal seam cells. Fusion of GFP to *C. elegans lis-1* revealed expression in all major neuronal processes of the animal as well as the multinucleate spermathecal valves and adult seam cells. Phenotypic analysis of either *nud-1* and *lis-1* by RNA interference yielded similar phenotypes, including embryonic lethality, sterility, altered vulval morphology, and uncoordinated movement. Digital time-lapse video microscopy was used to determine that RNAi-treated embryos exhibited nuclear positioning defects in early embryonic cell division similar to those reported for dynein/dynactin depletion. These results demonstrate that the LIS-1/NUDC-like proteins of *C. elegans* represent a link between nuclear positioning, cell division, and neuronal function.

Keywords *nudF* · *nud-1* · Lissencephaly · Nuclear positioning

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Introduction

Miller-Dieker lissencephaly is a malformation of the brain in which the improper migration of neurons in the developing cortex yields a smooth cerebral surface instead of the characteristic folds (Walsh and Goffinet 2000). This syndrome has been shown to be the result of an insufficiency of the LIS-1 protein arising from heterozygous deletions (Reiner et al. 1993) or mutations (Lo Nigro et al. 1997). The human LIS-1 protein has seven WD-40 repeats and is similar to the β-subunits of heterotrimeric G proteins (Reiner et al. 1993). LIS-1 binds tubulin, affects the frequency of microtubule catastrophe in vitro (Sapir et al. 1997), and interacts with the murine homolog of an *A. nidulans* nuclear movement protein, NUDC, both in vitro and in vivo (Morris et al. 1998b).

Microtubule-dependent nuclear positioning is a requirement for proper growth, development, and cellular function in both lower and higher eukaryotes. Mutants of *A. nidulans* defective in *nu*clear *d*istribution (*nud*) have enabled the identification of 13 genes that are required for nuclear migration, including the heavy and light chains of cytoplasmic dynein and a homolog of Arp1 (actin-related protein 1), while others, including *nudC*,

nudE, and *nudF*, are not part of any previously defined motor complex (Morris 2000).

NUDF shares 42% identity with human LIS-1, functions as a dimer (Ahn and Morris 2001), and has reduced protein level in a *nudC3* mutant strain (Xiang et al. 1995). Recent observations by Han et al. (2001) have demonstrated a co-localization of NUDF and NUDA, the dynein heavy chain, to microtubule ends. A gene encoding a protein similar to *A. nidulans* NUDC has been predicted in the genomes of both *Schizosaccharomyces pombe* and *Arabidopsis thaliana*, and additional homologs are known to be present in *Drosophila melanogaster* (Cunniff et al. 1997), rat (Morris et al. 1997), mouse (Morris et al. 1998b), and human (Miller et al. 1999). All are approximately 60% identical at the C-terminus, suggesting a conservation of function. Although the exact role of these proteins is unclear, they represent a link between the molecular components of nuclear migration and neuronal migration. It has been suggested that the lack of neuronal migration observed in lissencephaly patients and Lis-1-deficient mice could be explained by the failure of nuclear movement analogous to the *nud* mutants of *A. nidulans* (Morris 2000; Morris et al. 1998a).

The anatomical, genetic, and genomic advantages of *C. elegans* make it an ideal system for analysis of these proteins in a cellular and developmental context. In this study, we examine the consequences of disrupting the activity of the NUDC and LIS-1 proteins during *C. elegans* development and establish this nematode as a model for studying highly conserved proteins and cytoskeletal mechanisms related to nuclear migration.

Materials and methods

General methods

Wild-type *C. elegans* strain N2 (Bristol variety) was cultured under standard conditions. *Aspergillus nidulans* strains used were GR5 (*pyrG89, wA2, pyroA4*) and AO1 (*nudC3, wA2, nicA2, pabaA1, pyrG89, chaA1*) (Osmani et al. 1990). Microscopy was performed using either an Olympus IX70 microscope and a Princeton Instruments MicroMax camera with IPLab software (Scanalytics), or a Nikon E800 microscope with a Spot RT camera (Diagnostic Instruments) controlled by MetaMorph software (Universal Imaging Systems). *A. nidulans* germlings were observed at 1,000× after fixing and staining with 4, 6-diamidino-2-phenylindole (DAPI) using the method of Chiu et al. (1997). Filters used were 330–385 nm UV for DAPI or 480 nm excitation, 535 nm emission for GFP. All PCR primers used in this study are listed in Supplementary Table 1 (Electronic Supplementary Material).

Isolation of cDNA clones and construction of *A. nidulans* expression vectors

Primers CEM148-U and CEM148-L were used to amplify a C-terminal fragment of *nud-1* from *C. elegans* genomic DNA, since the region of the *nud-1* gene that corresponds to the shorter *A. nidulans nudC* gene is contained in a single exon. This product was subcloned into the pAL5 expression vector to create pAL5-CEM148 that allowed control of expression of the C-terminal 173 amino acids of the NUD-1 protein from methionine 148.

Partial cDNAs for *nud-1* (yk391a2) and *lis-1* (yk97h8) were obtained from the laboratory of Yuji Kohara (DNA DataBank of Japan). Primers CNUDC-KPN-5 and CNUDC-BAM-3 were used to amplify a full-length *nud-1* cDNA from clone yk391a2 before subcloning into *A. nidulans* expression vector pAL5 to create pAL5-NC. Primer CNUDC-KPN-5 contained 11 bp of 5' sequence that was missing from clone yk391a2, but present at the start of the *nud-1* gene predicted by the *C. elegans* genome project (see Electronic Supplementary Material). The *C. elegans lis-1* cDNA was amplified from clone yk97h8 using primers CNUDF-KPN-5-A and CNUDF-KPN-3-A, then subcloned into pAL5 to create pAL5-NF. Transformation of *A. nidulans* was performed as previously described (Dawe et al. 2000).

Reporter gene fusions and microinjection

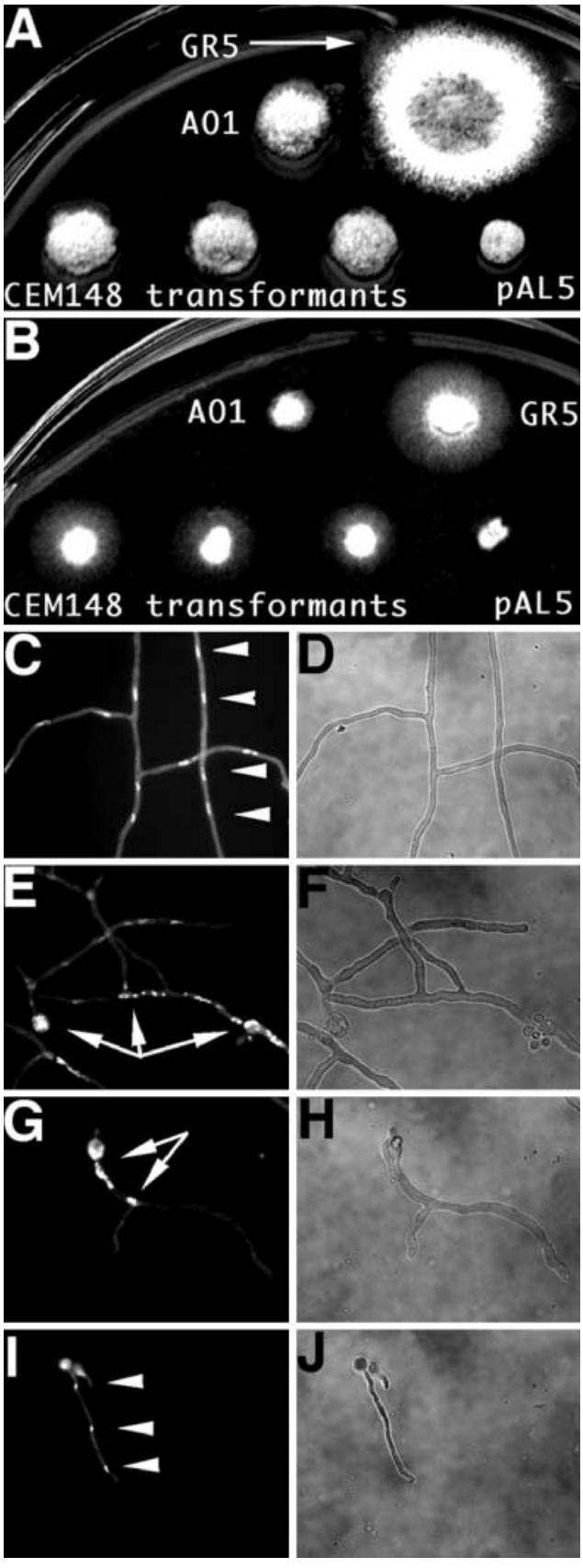
GenBank accession numbers for the *C. elegans* cosmids are Z81113 (T03F6 containing *lis-1*) and Z81546 (F53A2 containing *nud-1*). There were no predicted ORFs upstream of *nud-1* for more than 5 kb or upstream of *lis-1* for more than 9 kb. Cosmid F53A2 was used with primers NCP-U and NCP-L2 to generate a genomic fragment containing a portion of the second exon of the *nud-1* gene and 4.5 kb of upstream sequence. This was cloned into *C. elegans* GFP expression vector pPD95.77 (a gift from Andy Fire, Carnegie Institute) to create pPD95.77-NCP. Cosmid T03F6 was used with primers CENF-SPH-5 and CENF-PST-3 to amplify a genomic fragment containing approximately 3.0 kb of sequence 5' to the start of translation for *lis-1*, prior to cloning into pPD95.79 to create pPD95.79-NFP. The 3'-UTR on all of these *C. elegans* expression vectors is that of the *unc-54* gene. These reporter constructs and a dominant marker DNA (*rol-6*) were microinjected into wild-type N2 young adults and four stable lines of each construct were propagated at 20°C. The strains were named UA1 (*baEx1*) for the NUD-1::GFP fusion and UA2 *(baEx2)* for the LIS-1::GFP fusion.

RNAi

RNA-mediated interference was performed according to Fire et al. (1998). cDNA templates for in vitro transcription (Ambion Megashortscript kit) were produced by PCR (primers T7NUDC and T3NUDC for *nud-1* and T7NUDF and T3NUDF for *lis-1*), resuspended at approximately 5 mg/ml, then annealed and injected. Eighty worms (in duplicate 40 worm experiments) were injected with each preparation, and then placed at 20°C for 12 h before being transferred to individual plates, returned to 20°C, and scored for phenotypes at 12-h intervals. For the embryo analyses, animals were injected with dsRNA and young adult hermaphrodites recovered at 15°C for between 18 and 36 h. Embryos were dissected out in M9 medium and placed on a 2% agarose pad with a coverslip mounted on top. Early development was typically analyzed in wild-type embryos from shortly after fertilization to the four-cell stage (approximately 40 min) while RNAi-treated embryos were examined for approximately 1 h.

Results and discussion

To investigate the role of nuclear migration proteins in *C. elegans*, we searched the available sequence databases and identified two predicted proteins that shared distinct similarity to the NUDC and NUDF proteins of *A. nidulans*. NUD-1, the *nudC* homolog, shows 47% identity and 67% similarity to *A. nidulans* NUDC protein. The second protein, *C. elegans* LIS-1, is 59% identical/69% similar to human LIS-1 and 42% identical/52% similar to *A. nidulans* NUDF. Sequence comparisons are available in the Electronic Supplementary Material.



To address the functional homology of the NUD-1 protein to *A. nidulans* NUDC, we cloned cDNAs for both the full-coding region of *nud-1* and the C-terminal region corresponding to NUDC, into the *A. nidulans* expression vector pAL5 and transformed the constructs into the fungal *nudC3* mutant. At the restrictive temperature, the mutant strain forms a small colony with fatter, highly branched, hyphae and defective nuclear distribution (Osmani et al. 1990). However, expression of the C-terminal (CEM148) fragment was able to restore hyphal growth under conditions non-permissive for the mutant (Fig. 1 A, B) demonstrating that the C-terminal 173 amino acids of NUD-1 are functionally homologous to *A. nidulans* NUDC. Spores germinated under identical conditions and stained with DAPI to visualize nuclei showed a restoration of nuclear migration when the C-terminal portion of the NUD-1 gene was expressed (Fig. 1C–J). The small difference in growth represents a slower germination of the complemented transformants and may reflect a reduced level of NUDC protein in the germling because of the non-native promoter sequences, or a reduced "dowry" of NUDC as suggested by Chiu et al. (1997). The full-length *nud-1* gene also rescued the fungal nuclear migration defect, but growth restoration was less complete (data not shown). We similarly tested whether the *C. elegans* LIS-1 protein could complement mutations in the *nudF* gene. No complemented transformants were recovered, suggesting that the *C. elegans* LIS-1 protein was unable to functionally replace *A. nidulans* NUDF, consistent with similar attempts using human and *Drosophila Lis1* genes (X. Xiang, Y.-H. Chiu, N.R. Morris, unpublished observations).

To further explore the similarity between the NUDC-like proteins, we performed parsimony analysis on members of this family. This analysis yielded three equally parsimonious trees of 602 steps (CI=0.944, RC=0.644; Electronic Supplementary Material). Two basic clades were resolved: a Eumetazoan clade (62% bootstrap replication support) and an unresolved trichotomy of yeast, plant, and fungal NUDC protein sequences (<50%

Fig. 1A–J NUD-1 can complement the temperature sensitive *nudC3* mutation of *A. nidulans*. **A, B** Restoration of growth by the C-terminal 173 amino acids of NUD-1 at 42°C (restrictive temperature). A01 (*nudC3* t.s. mutant) and GR5 (WT for nuclear migration) are controls. Three independent A01 transformants carrying the pAL5-CEM148 construct are shown in the *lower row*, while the *rightmost colony* carries the pAL5 vector only. **A** Glucose (repressing) medium, **B** ethanol (inducing) medium. The colonies carrying the CEM148 fragment show hyphal development on inducing medium. **C–J** Restoration of nuclear migration by the C-terminal 173 amino acids of NUD-1 at 42°C. *Left panels* are DAPI-stained to show nuclei with *arrows* indicating improperly distributed nuclei and *arrowheads* showing correctly migrating nuclei. *Right panels* are phase images of the same hyphae. **C, D** GR5 on glucose medium showing normal nuclear distribution. **E, F** A01 on glucose showing aberrantly distributed nuclei**,** increased girth, and greater branching of the *nudC3* mutant. **G, H** A01 transformant carrying the pAL5-CEM148 construct and grown on glucose (repressing) medium showing clustered nuclei. **I, J** The same A01 transformant grown on ethanol (inducing) medium showing restoration of nuclear migration

bootstrap support). Within the Eumetazoa, *C. elegans* NUDC protein was the basal-most member; *D. melanogaster* was sister to a clade of vertebrate NUDC-like proteins (62% bootstrap support); human + mouse NUDC-like proteins were supported in 100% of bootstrap replicates. The basic dichotomy between Eumetazoan NUDC-like proteins and yeast, plant, and fungal NUDC-like proteins may be due to the either altered composition or complete lack of an N-terminal amino acid extension among the latter proteins (Electronic Supplementary Material); further taxon sampling is required to support this potential character. The functional significance of this domain remains unclear, but is likely to be involved in protein-protein interactions (M. Shiffler, and G.A. Caldwell, unpublished observations).

Phylogenetic analysis of LIS-1 protein family members yielded four equally parsimonious trees of 475 steps (CI=0.971, RC=0.799; Electronic Supplementary Material). Two basic clades were also resolved in this analysis: a *C. elegans* + fungus clade (88% bootstrap support) and a *D. melanogaster* + vertebrate LIS-1 clade (100% bootstrap support). Few differences (ca. 1% mean difference in amino acid residues) were found among vertebrate LIS-1 protein family members. A possible explanation for these relationships is in the evolution of functional differences between these proteins. While LIS-1 clearly retains a conserved and essential cellular function in all eukaryotes, the vertebrate and insect gene products may be utilized for additional activities related to a role as a subunit of the heterotrimeric enzyme platelet-activating factor acetyl hydrolase (PAFAH). LIS-1 was initially shown to be a non-catalytic subunit of this enzyme, although mammalian isoforms of PAFAH do not absolutely require LIS-1 for catalytic activity (Hattori et al. 1994). Additionally, the *C. elegans* genome does not contain the two catalytic subunits of PAFAH. While the *D. melanogaster* genome contains homologues of both LIS-1 and the catalytic subunits of PAFAH, this complex was catalytically inactive against PAFAH substrates (Sheffield et al. 2000). Therefore, although LIS-1 may operate as part of the PAFAH complex in mammals, its function in other organisms appears independent of such an interaction, suggesting multiple (PAFAH and non-PAFAH) roles in mammalian systems as well.

We used RNAi to examine the consequences of the absence of either NUD-1 or LIS-1 on *C. elegans* development. The majority of the worms injected (94% for *nud-1*, 96% for *lis-1*) proceeded to lay eggs that displayed mutant phenotypes with 73% (*nud-1*) and 85% (*lis-1*) producing only dead embryos. Closer inspection of the *nud-1* RNAi-treated progeny laid 24–30 h after injection revealed normal pronuclear migration when compared with wild-type embryos (Fig. 2A, F) by digital time-lapse video microscopy (video Figures SV1.mov and SV2.mov, Electronic Supplementary Material). Following initial migration (Fig. 2B, G), the centrosome pair and associated pronuclei moved to the center of the embryo with concomitant rotation in wild-type embryos only (Fig. 2C). In contrast, the centrosome-pronuclear complex in *nud-1* (RNAi) embryos migrated toward the center of the embryo but failed to rotate (Fig. 2H). The failure in rotation of the centrosome-pronuclear complex resulted in skewed nuclear envelope breakdown (Fig. 2I). Consequently, the spindle was initially assembled perpendicular to the longitudinal axis. By the end of anaphase, however, the spindle was reoriented along the longitudinal axis and daughter blastomeres were generated (Fig. 2J). Embryos laid approximately 30 h after injection displayed aberrant pronuclear migration in addition to rotation defects. Egg and sperm pronuclei usually migrated towards each other but conjoined at variable regions of the embryo, instead of approximately 70% egg length, as is characteristic of wild-type (data not shown). Most embryos laid up to 30 h after injection were capable of forming four-cell staged embryos. The *nud-1* RNAi defect reached a threshold of severity at 30 h after injection. At this time eggs did not possess the standard signposts for fertilization, such as an eggshell or extruded polar bodies.

In contrast, *lis-1* RNAi-treated embryos typically displayed a consistent phenotype across the time periods analyzed (24–36 h). These embryos usually contained one or more egg pronuclei located near the anterior cortex and one sperm pronucleus abutting the posterior cortex (Fig. 2K and video figure SV3.mov, Electronic Supplementary Material). Pronuclear migration did not take place in these mutants (Fig. 2L), rather the sperm pronucleus broke down first (Fig. 2M) followed by dissolution of egg pronuclei 2 min later (Fig. 2N). *lis-1* RNAi-treated embryos did not display a bipolar spindle after undergoing asynchronous pronuclear envelope breakdown. At this stage, an area lacking yolk granules extended from the posterior toward the anterior of the embryo but did not contain an aster (Fig. 2M, N). Irregular cytoplasmic furrowing ensued, but was unproductive and did not yield a conventional two-celled embryo (Fig. 2O). Instead, the successive emergence of several small nuclei suggests the formation of nuclear envelopes around non-segregated chromosomes in the absence of LIS-1 protein (Fig. 2O). Examination of worm embryos following *lis-1* RNAi indicates that these typically arrest at the 50-to 100-cell stage and contain asymmetric aggregates of nuclei by DAPI staining (data not shown), whereas *nud-1* embryos appear to die between the comma to one-fold stage of development.

The described defects in *lis-1* and *nud-1* RNAi-affected embryos are strikingly similar to those attributed to dynein and dynactin, respectively, during pronuclear migration (Gonczy et al. 1999; Skop and White 1998). This was especially true for *lis-1*-treated embryos whose cytoplasmic events completely mimicked the RNAi phenotype of the gene encoding *C. elegans* cytoplasmic dynein heavy chain (Gonczy et al. 1999) and agrees with results independently obtained for *lis-1* in a large-scale RNAi screen of *C. elegans* chromosome III (Gonczy et al. 2000). Gonczy et al. (1999) noted that *dhc-1* (RNAi) embryos exhibited multiple egg pronuclei, as was observed in approximately 15% of *lis-1* RNAi embryos.

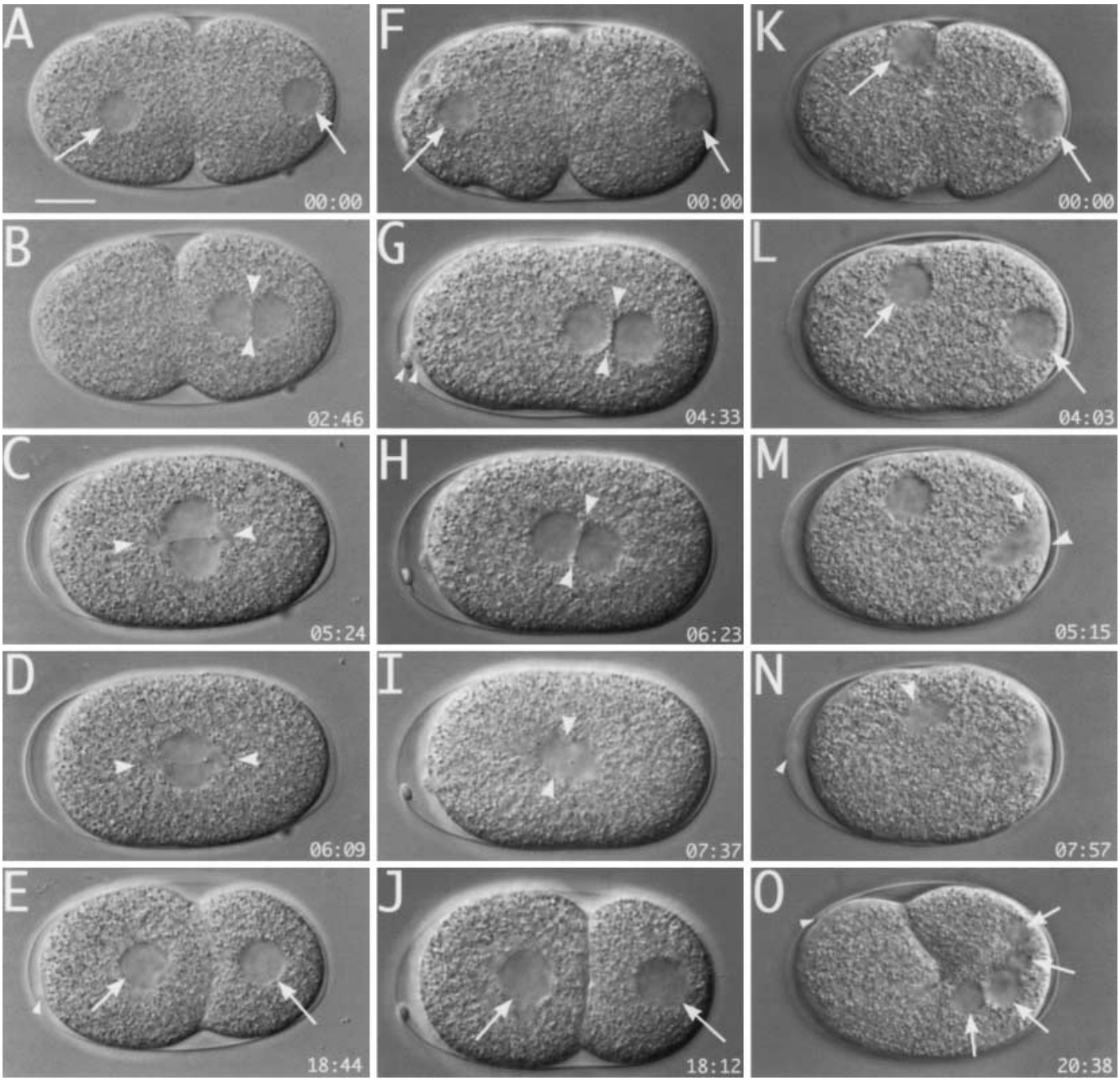


Fig. 2A–O Pronuclear migration and rotation defects in *nud-1* and *lis-1* RNAi-treated embryos 24–30 h after injection of parent. Nomarski images demonstrating progression through the two-cell embryo stage in wild-type embryos (**A–E**), in *nud-1* RNAi-treated (**F–J**), and *lis-1* RNAi-treated embryos (**K–O**). Each image displays the time elapsed (min:sec) since initiating sequence capture, approximately 20 min after fertilization. Embryos are aligned with the anterior to the left, as determined by the presence of the polar body and denoted by *small arrowheads* (some polar bodies are out of the focal plane). The scale bar represents 10 μm in all panels. (**A, F, K**) In wild-type, *nud-1*, and *lis-1* RNAi-treated embryos, the pronuclei are positioned appropriately with the sperm pronuclei (*right arrows*) apposed to the posterior cortex and the egg pronuclei (*left arrows*) located slightly off the anterior cortex. A pseudocleavage furrow is present in all embryos. (**B, G, L**) The egg and sperm pronuclei of wild-type and *nud-1* RNAi-treated embryos migrate towards each other from their initial positions and meet at approximately 70% egg length. The juxtaposed pronuclear membranes can be visualized between centrosomes (*arrowheads*). In *lis-1* RNAi-treated embryos pronuclei (*arrows*) do not migrate towards each other. (**C, H, M**) The centrosomes and associated pronuclei in wild-type move toward the center of the embryo and rotate onto the a-p axis (*arrowheads*). *nud-1* pronuclei never rotate onto the a-p axis (*arrowheads*). In *lis-1* RNAi-treated embryos, the sperm pronucleus undergoes premature nuclear envelope breakdown. A bipolar spindle is not evident, however a granule-free zone is present (*arrowheads*) and extends toward the anterior. (**D, I, N**) Nuclear envelope breakdown and spindle assembly occurs in the cell center along the longitudinal axis in wild-type embryos (*arrowheads*). In the absence of rotation onto the a-p axis, *nud-1* RNAi-treated embryos undergo nuclear envelope breakdown along the dorsal-ventral axis (*arrowheads*). As the spindles begin to separate, they straighten out along the a-p axis, likely due to the constraints of the eggshell. In *lis-1* RNAi-treated embryos, the intact female pronucleus breaks down (*arrowhead*); spindle formation is absent. (**E, J, O**) Following completion of the first cell division, wild-type and *nud-1* RNAi two-cell stage embryos are comprised of the anterior, AB, and the posterior, P1, blastomeres. Both cells contain centrally located nuclei (*arrows*). The *lis-1* RNAi-treated embryos exhibit unconventional furrowing activity and many small nuclei reorganize, possibly around non-segregated chromosomes (*arrows*), without proper cell division occurring. Digtial time-lapse videos of these embryos are in the Electronic Supplementary Materials http://dx.doi.org/10.1007/s004270100176

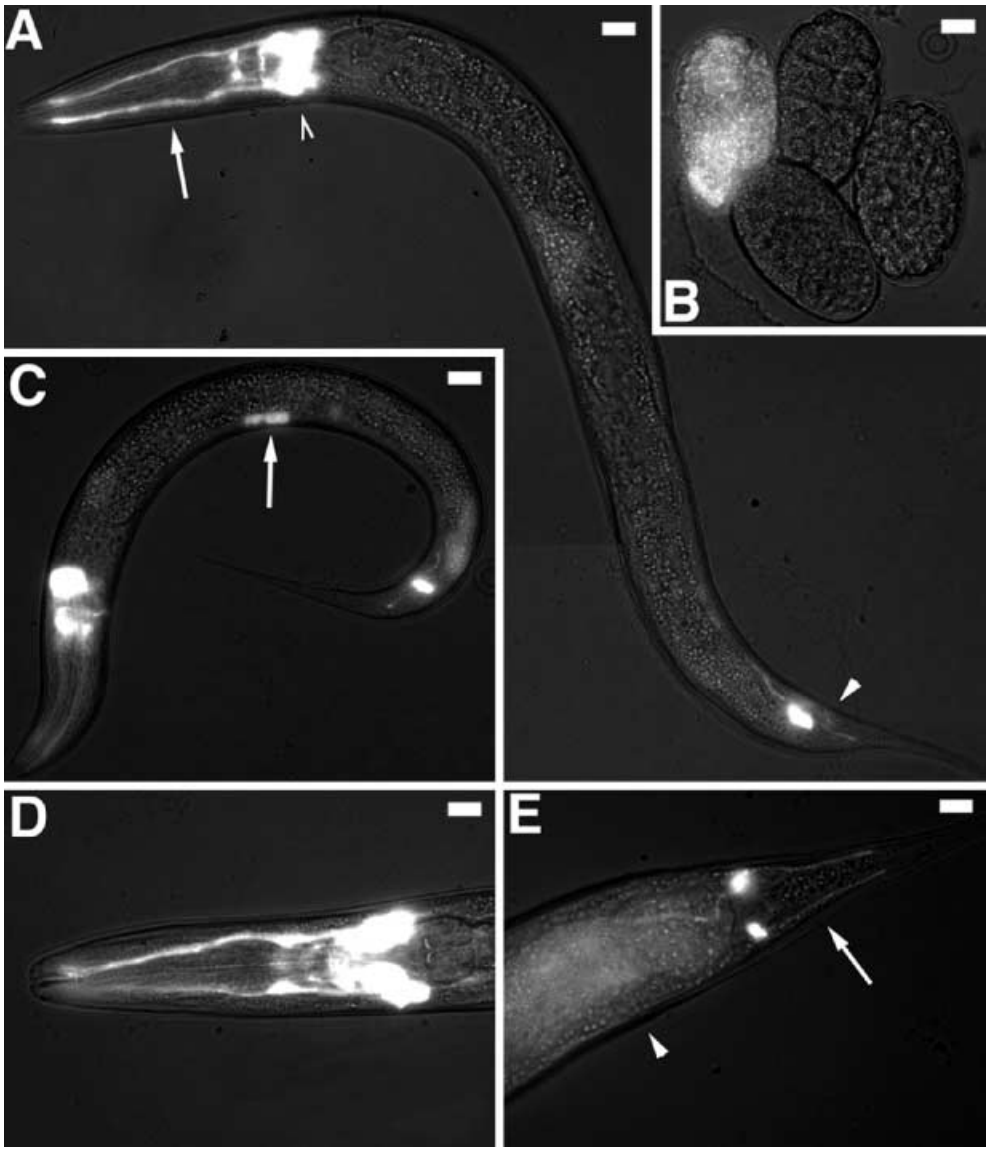


Fig. 3A–E Expression pattern of NUD-1::GFP observed in embryonic, larval, and adult stages of *C. elegans* carrying the pPD95.77-NCP construct. Anterior is to the left and ventral is down in all images. The *scale bar* represents 10 µm in all panels. **A** A two-image composite of an L2 larva showing expression in the amphid (*head, arrows*) and phasmid neurons (*tail, arrowhead*) as well as the nerve ring (*open arrowhead*). **B** An early embryo showing diffuse expression with three untransformed eggs as controls. **C** An L1 larva with head and tail expression, as for **A,** and additional mid-ventral fluorescence in the region of the gonadal primordium (*arrow*). **D** A close-up of a young adult head showing amphids. **E** The tail of an adult showing phasmids (*arrow*) and diffuse intestinal cell expression (*arrowhead*)

The presence of multiple pronuclei is suggestive of a defect during oogenesis and may correspond to problems in meiotic divisions, since the number of pronuclei increased as the number of polar bodies extruded decreased. These effects on embryonic development are also consistent with results from other organisms. Mice homozygous for a null *Lis1* mutation exhibited early embryonic lethality (Hirotsune et al. 1998). In *Drosophila*, *DNudC* is expressed in embryos as well as adult flies (Cunniff et al. 1997), while *DLis1* is essential for oogenesis and embryonic viability and has genetic interactions with cytoplasmic dynein (Lei and Warrior 2000). These data provide further evidence that a role of LIS-1 is to modulate the function of the cytoplasmic dynein complex and represents evolutionary conservation of function with that of *A. nidulans nudF* and *Drosophila DLis-1* (Lei and Warrior 2000; Xiang et al. 1995).

Parents injected with either *nud-1* or *lis-1* dsRNA also tended to lay fewer eggs than uninjected worms. Eggs did not appear to accumulate in the uterus of these animals, perhaps reflecting a disruption of germline development or gonadal function. A limited number of embryos obtained from a small group (less than 25%) of the injected mothers were able to escape lethality. These viable progeny allowed observations of the effect of interruption of NUD-1 or LIS-1 function in subsequent developmental stages. More than 50% of these “escaper” progeny displayed an everted vulva phenotype (Evl) and more than 75% exhibited uncoordinated movement (Unc) in the case of either *nud-1* or *lis-1* dsRNA. Interestingly, all F1 escaper animals were sterile and many exhibited lack of cuticle integrity, in that this protective outer covering of the nematode was physically pliable when touched with a platinum wire and had a cellophane-like appearance, indicative of a hypodermal defect. Photographs depicting these post-embryonic defects are available in the Electronic Supplementary Material.

To determine sites of NUD-1 and LIS-1 function in *C. elegans*, we created fusions of each gene to the coding

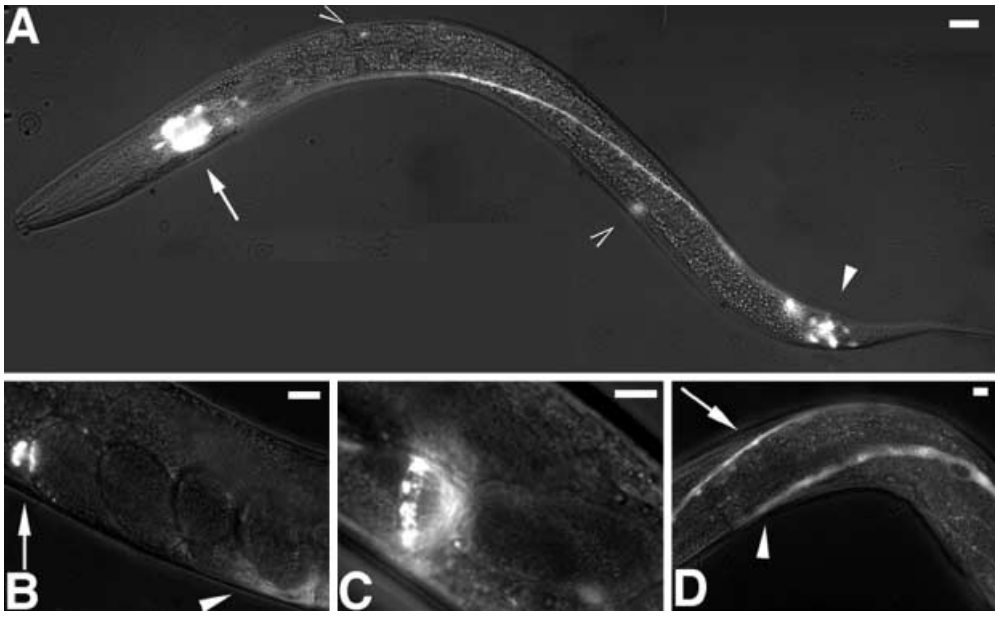


Fig. 4A–D Expression pattern of LIS-1::GFP as observed in larval and adult stages of *C. elegans* carrying the pPD95.79-NFP construct. Anterior is to the left and ventral is down in all images. The *scale bar* represents 10 μm in all panels. **A** A three-image composite of an L2/L3 larva showing neuronal expression in the head (including the nerve ring, *solid arrow*) and tail (*filled arrowhead*), as well as ventral nerve cord running the length of the animal. The points of fluorescence in the mid-ventral posterior and mid-dorsal anterior (*open arrowheads*) represent an unidentified pair of neurons in which expression is observed. Note the absence of expression in the seam cells at this larval stage. **B** A young adult worm showing expression in the resting spermathecal valve (*arrow*) and uterine wall (*arrowhead*). **C** An egg passing through the distended spermathecal valve of an adult worm. **D** A mid-section close-up of an adult worm showing expression in the ventral nerve cord (*upper stripe, arrow*) and the now-fused seam cell syncytium (*lower stripe, arrowhead*)

sequence of green fluorescent protein (GFP). Representative expression of the NUD-1::GFP fusion is shown in Fig. 3. It should be noted that all reporter gene expression patterns may only represent a subset of tissue expression, and these studies await future confirmation of protein localization by immunocytological analysis. Nevertheless, in all adult and larval stages fluorescence was observed in the amphid and phasmid sensory neurons, as well as the neuronal processes extending to the nerve ring and preanal ganglion (Fig. 3C–E). Transient expression was also observed in the mid-ventral region of younger animals up to L2 stage (Fig. 3C) corresponding to the location of the gonadal primordium. Weak fluorescence was also seen in intestinal and hypodermal regions of larval and adult stages (Fig. 3C, E) and uv cells connecting the uterus and vulva (not shown). In addition, diffuse early embryonic expression was seen in all lines (Fig. 3B). The LIS-1::GFP fusion was observed in all major neural processes: the ventral nerve cord (Fig. 4A, D) as well as dorsal and lateral cords and connecting commissures (not shown). Expression was noted in the region of the nerve ring and the preanal ganglion (Fig. 4A). Young adult stages showed strong expression, in particular non-neural cells including the spermathecal valve (Fig. 4B, C) and the seam cell syncytium (Fig. 4D), as well as weaker fluorescence in the uterine wall (Fig. 4B), hypodermal and intestinal cells (not shown).

The *C. elegans* gonad exists as a syncytium in which the germ cell nuclei progress from gametogenesis to fertilization. Several cells required for the function of the egg-laying apparatus in *C. elegans* form attachments to the overlying lateral seam syncytium on each side of the worm. The sterility and improper vulval morphogenesis seen with *lis-1* and *nud-1* RNAi-treated worms may stem from gonad abnormalities or incorrect maturation of the hypodermal seam and its connection to the somatic gonad. Interestingly, the hypodermal seam cells form a multinucleate syncytium during the larval to adult transition. This correlates with the advent of LIS-1::GFP expression in the newly fused cells. Transient expression of both NUD-1 and LIS-1 GFP fusions was observed in cells of the main hypodermis, which are also syncytial and undergo dramatic and rapid cytoskeletal rearrangements at each larval molt (Costa et al. 1997). We also observed strong expression of the LIS-1::GFP fusion in the spermathecal valve, a large cell that contains four nuclei and is situated between the spermatheca and the uterus. This multinucleated valve undergoes striking cytoskeletal expansions and contractions in which nuclear positioning may be regulated as eggs traverse through it (Figure 4C). We hypothesize that LIS-1 activity is necessary for the control of nuclear positioning in these cells, analogous to the role of NUDF in regulating dynein-mediated nuclear spacing in *A. nidulans*.

Expression of LIS-1::GFP in the principal neural network of worms, coupled with the uncoordinated movement observed in surviving *lis-1* RNAi-affected progeny, is consistent with a requirement for LIS-1 during neuronal development. While the precise cause of

the associated Unc phenotype requires further investigation, we attempted to discern if this was due to improper nuclear migrations of ventral cord precursor cells. To determine if such migrations were disturbed in *lis-1* escaper progeny, we stained these animals with DAPI, but did not observe any change in numbers or positioning of ventral cord nuclei in RNAi escapers. Alternatively, the observation that both the adult F1 *nud-1* and *lis-1* RNAi survivors appeared to have an improperly formed cuticle suggests that the hypodermal syncytium that secretes this protective layer was incorrectly developed. Thus, the Unc phenotype could be a function of an absence of proper body rigidity caused by defective cytoskeletal restructuring during cuticle formation at molting (Costa et al. 1997) or may be a result of combined neuronal and hypodermal defects.

Further studies will define the nature of both the coordinate and independent interactions of NUD-1 and LIS-1 in various cell types. Discerning their respective functions as part of the overall cytoskeletal network will provide additional insights into the relationship between the conserved developmental mechanisms controlling nuclear positioning, cell division, and neuronal migration.

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