

Talin loss-of-function uncovers roles in cell contractility and migration in *C. elegans*

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Summary

Integrin receptors for extracellular matrix transmit mechanical and biochemical information through molecular connections to the actin cytoskeleton and to several intracellular signaling pathways. In *Caenorhabditis elegans*, integrins are essential for embryonic development, muscle cell adhesion and contraction, and migration of nerve cell axons and gonadal distal tip cells. To identify key components involved in distal tip cell migration, we are using an RNA interference (RNAi)-based genetic screen for deformities in gonad morphogenesis. We have found that talin, a cytoskeletal-associated protein and focal adhesion component, is expressed in the distal tip cell and plays a central role in regulating its migration. Reduction of talin expression caused severe defects in gonad formation

because of aberrant distal tip cell migration and also disrupted oocyte maturation and gonad sheath cell structure. Contractile muscle cells showed disorganization of the actin cytoskeleton leading to complete paralysis, a phenotype that was also observed with depletion of *pat-2* and *pat-3* integrins. These in vivo analyses show that talin is required not only for strong adhesion and cytoskeletal organization by contractile cells, but also for dynamic regulation of integrin signals during cell migration. In addition, induction of distal tip cell migration defects by bacterial RNAi in *C. elegans* provides an effective screen to identify genes involved in integrin signaling and function.

Key words: *C. elegans*, Talin, β integrin, Focal adhesion

Introduction

Connections between the extracellular matrix (ECM) and the actin cytoskeleton occur through integrin receptor clustering at focal adhesions and play a critical role in cell adhesion, spreading, migration and changes in cell morphology. Integrin receptors, cytoskeletal proteins such as α -actinin, vinculin and talin, and signaling/adaptor molecules such as focal adhesion kinase (FAK), integrin linked kinase (ILK) and paxillin are major components of these structures (Hynes, 2002; Petit and Thiery, 2000; Schoenwaelder and Burridge, 1999). Many members of the mammalian focal adhesion complex have closely related *C. elegans* counterparts that localize to muscle adhesion complexes called dense bodies (Barstead and Waterston, 1991; Moulder et al., 1996; Rogalski et al., 2000). These structures play an essential role during *C. elegans* embryogenesis. Disruption of dense body components such as *pat-2*/ α integrin, *pat-3*/ β integrin, *deb-1*/vinculin, or *pat-4*/ILK leads to embryonic lethality (Gettner et al., 1995; Mackinnon et al., 2002; Riddle et al., 1997; Williams and Waterston, 1994). The Pat (paralyzed arrested-at-twofold) phenotype results from defective adhesion and lack of cytoskeletal organization in muscle cells in the embryo. Furthermore, expression of a dominant negative inhibitor of PAT-3 integrin function causes defects in muscle function in post-embryonic animals (Lee et al., 2001).

An overt post-embryonic phenotype caused by inactivation of integrins is malformation of the gonad arms (Baum and Garriga, 1997; Lee et al., 2001). Severe gonad morphological defects are

easily visible with low-power microscopy, and we used this phenotype as the basis for an RNA interference (RNAi)-based genetic screen for molecules involved in integrin-related pathways. RNAi is an effective method for analyzing gene function in *C. elegans* as it phenocopies loss-of-function phenotypes for the gene in question (Fire et al., 1998; Timmons et al., 2001). In RNAi, double stranded RNA (dsRNA) introduced into larvae or adults activates an enzymatic pathway that eliminates endogenous RNAs homologous to the dsRNA (Hannon, 2002). In this screen, we isolated the gene encoding talin, a protein that localizes to dense bodies and focal adhesions (Critchley, 2000). At these sites, talin binds to integrin β cytoplasmic tails, vinculin and actin filaments (Calderwood et al., 1999; Critchley, 2000). The vital role of talin in embryogenesis is illustrated by the talin mutant mouse, in which embryos die at day E8.5 from a failure in embryonic mesoderm formation (Monkley et al., 2000). In *Drosophila*, clones of talin-negative cells in the wing fail to adhere between layers causing a wing blister and embryos lacking talin exhibit muscle detachment and failure of germband retraction (Brown et al., 2002). In *C. elegans*, talin is found in muscle dense bodies along with integrins, and PAT-3/ β integrin is required for talin localization to these structures (Moulder et al., 1996). The functions of talin in muscle and other nematode tissues have not been determined.

Gonad morphogenesis is dependent on migration of a specialized gonadal leader cell, the distal tip cell (DTC) (Blelloch et al., 1999; Hubbard and Greenstein, 2000; Lehmann, 2001). Our analysis shows that loss of talin caused

several gonad defects, including inappropriate migration of the hermaphrodite DTCs, disruption of the actin cytoskeleton in gonad sheath cells, and oocyte maturation defects in the proximal gonad. Reduction of talin also resulted in progressive uncoordination and paralysis, a phenotype that was also observed with reduction of integrin *pat-2* and *pat-3* RNA levels. Immunofluorescence staining showed perturbation of muscle filament structure in the paralyzed adults. Thus, talin is an essential gene that participates in cell migration and contractile functions in *C. elegans* larval and adult tissues.

Materials and Methods

Nematode culture and egg collection

Nematodes were cultivated on NGM agar plates with OP50 bacteria according to standard techniques (Brenner, 1974). Nematode culture and observations were performed at room temperature. The following *C. elegans* strains were used: N2 (wild-type reference strain, Bristol) and *talin::GFP(zdIs15)*.

In order to generate *talin::GFP(zdIs15)* nematodes, nucleotide sequences upstream of the talin gene (−2070 to +6, where +1 is first base of ATG) were amplified from N2 genomic DNA using PCR and cloned into the *SphI* and *BamHI* sites of the GFP expression vector pPD95.77 (provided by A. Fire) to generate the *talin::gfp* reporter. Extrachromosomal arrays were generated by germline transformation of *lin-15(n765)* animals with *talin::gfp* DNA (50 µg/ml) and *lin-15(+)* gene (pSK1) (50 µg/ml), and stable chromosomal integration was induced by treatment with TMP and UV. *lin-15(n765)* mutants exhibit a temperature-dependent multivulva phenotype that can be rescued by pSK1 (Clark et al., 1994). The *talin::gfp(zdIs15)* integrant (MT4015) was backcrossed three times with N2.

Starved nematodes were allowed to recover on fresh *E. coli* OP50-seeded NGM plates for two days. This procedure partially synchronizes the nematode cultures, and guarantees gravid animals for egg collection. Eggs were released from gravid hermaphrodites using alkaline hypochlorite solution (Hope, 1999). Following washes in M9 buffer, eggs were then transferred to plates seeded with RNAi HT115(DE3) bacteria expressing dsRNA.

RNAi and analysis of phenotypes

The RNAi feeding protocol was essentially as described (Timmons et al., 2001). Briefly, bacteria were cultured overnight in LB supplemented with 40 µg/ml ampicillin, and seeded onto NGM agar supplemented with carbenicillin (25 µg/ml) and IPTG (1 mM). Double-stranded RNA expression was induced overnight at room temperature on the IPTG plates. Eggs were then transferred onto the plates and RNAi phenotypes were monitored at varying times.

The talin clone from well 8M01 of the *C. elegans* Chromosome I library is a genomic fragment of talin (Gene pairs name: Y71G12A_195.e) cloned into the RNAi feeding vector pPD129.36 (Fraser et al., 2000) and transformed into HT115(DE3), an RNase III-deficient *E. coli* strain with IPTG-inducible T7 polymerase activity (Timmons et al., 2001). The empty pPD129.36 vector in HT115(DE3) was used as a control.

To construct the *pat-2* (F54F2.1) RNAi clone, primers at base positions 5 and 386 relative to the ATG in the predicted cDNA sequence were used for RT-PCR amplification with N2 total RNA as the template. Primers included *NheI* and *HindIII* sites at the 5' and 3' ends, respectively, and these sites were used to clone the PCR product into pPD129.36 vector. For the *pat-3* (ZK1058.2) RNAi clone, primers at positions 2073 and 2403 in the gene were used for amplification. *NcoI* and *PstI* sites subsequently introduced at the 5' and 3' ends were then used to clone the PCR product into pPD129.36. Primers used were:

pat-2 5F 5'-AGGCTAGCGAGAGGGTAGTTTCCGCG-3'
pat-2 386R 5'-AGAAGCTTGATCGAACAGTTGCACC-3'

pat-3 2073F 5'-CTCAACGAAACTACACCCTGCC-3'

pat-3 2403R 5'-TTAGTTGGCTTTCCAGCGTATACTGG-3'

To characterize phenotypes, eggs were hatched on bacteria expressing dsRNA and larvae and adults were examined using a dissecting microscope. Observed phenotypes included Unc (uncoordinated) movement, paralysis, large clear patches in the body cavity, Egl (egg-laying defective) and sterile.

To analyze gonad morphology, young adult or L4 hermaphrodites were mounted in a drop of M9 buffer containing 0.1 M sodium azide on a cover slip coated with 2% agarose and examined using a Nikon Eclipse TE 2000-U microscope with DIC optics. Defects such as inappropriate or extra turns, migration in the wrong direction, or aberrant stops were counted as DTC migration anomalies. Oocyte accumulation was scored when oocytes were present in a non-linear arrangement in the proximal gonad. Paralysis was scored when animals were able only to move their heads.

Standard errors for the proportions of defective DTC migration, oocyte stacking and paralysis in a population were calculated using the observed frequency and sample size, assuming a binomial distribution as previously described (Hedgecock et al., 1990).

Fluorescence microscopy

Fluorescence microscopy was performed with a Nikon Eclipse TE 2000-U microscope equipped for epifluorescence. Images were captured with a Cooke SensiCam High Performance camera using IP lab software (Scanalytics).

DNA organization in the gonads was monitored in animals stained with DAPI (0.01 µg/ml). To visualize muscle structure, RNAi-treated and control whole nematodes were frozen on poly-L-lysine-coated slides and fixed with methanol and acetone as described (Lee et al., 2001). Fixed animals were stained at room temperature with MH25 anti-PAT-3 antibodies (1:250 dilution) for 4 hours at room temperature, followed by rhodamine-conjugated goat anti-mouse IgG (Molecular Probes) for 2 hours at room temperature. Actin cytoskeletal organization of muscle cells was examined by staining fixed animals with 0.4 U/ml of rhodamine-conjugated phalloidin (Molecular Probes) for 2 hours at room temperature.

Nematode gonads were dissected, fixed and stained as described (McCarter et al., 1997; Strome, 1986). Briefly, animals in PBS containing 0.2 mM levamisole were decapitated with syringe needles resulting in gonad extrusion. For visualization of actin, dissected gonads were fixed in formaldehyde for 2 hours, extracted with ice-cold acetone for 3 minutes, washed in PBS and incubated in 2 U/ml rhodamine-phalloidin (Molecular Probes) for 1 hour. Dissected gonads were then transferred to slides coated with 2% agarose and visualized as described above.

Real time RT-PCR

RNA from mixed-stage populations of nematodes was isolated. Briefly, animals were collected in M9 buffer, and pelleted through a 4% sucrose gradient to remove bacteria. After grinding with 3.5 g glass beads (Sigma) in 2.5 ml of Trizol (Invitrogen), the pellet was extracted twice in Trizol and 1/5 volumes of chloroform. RNA was precipitated with isopropanol, rinsed with ethanol and treated with RQ1 DNase (Promega). RNA (1 µg total) was used to synthesize cDNA using Superscript reverse transcriptase (Invitrogen) primed with random hexamers in a 20 µL reaction. For PCR amplification, 1 µL of cDNA reaction product was used in each reaction with iQ SYBRgreen Supermix (BioRad) in a 96-well plate. Primer concentrations were optimized according to the manufacturer's recommendations. Amplification of *C. elegans* gene T09F3.3 (GAPDH) was used as a loading and amplification control. All assays were amplified and evaluated in real time using the iCycler iQ detection system (BioRad), and the relative quantitation of talin, *pat-2* and *pat-3* mRNA expression was calculated by the comparative Ct method (Livak and Schmittgen,

2001). Primers used for amplification are listed below. Numbers refer to base positions within the cDNA relative to the ATG.

Talin 2714F 5'-AGCAAGCAGAATCACAGC-3'
 Talin 2908R 5'-CGATCTGCTTTCAATGACATC-3'
pat-2 2149F 5'-ACCATGAGCAGAAGGGAATG-3'
pat-2 240R 5'-ACGGAGCAAGCATAAACTGC-3'
pat-3 347F 5'-CTGAGGAAGAAGCCGTTTCAG-3'
pat-3 429R 5'-AATCAACGGCTTGACGGTAG-3'
 GAPDH 827F 5'-TGAAGGGAATTCTCGCTTACACC-3'
 GAPDH 980R 5'-GAGTATCCGAACTCGTTATCGTAC-3'

Results

Talin is required for gonad development

An RNAi-based screen was used to identify *C. elegans* genes involved in gonad formation, a process that requires integrin activity. Wild-type N2 eggs were plated onto bacteria expressing dsRNA and nematodes were monitored during all larval stages and as adults. Continuous feeding of *C. elegans* with bacteria expressing dsRNA results in specific and ongoing inactivation of the homologous gene (Timmons et al., 2001). RNAi does not efficiently affect nerve cells, and therefore neural RNAi phenotypes might not be apparent (Tavernarakis et al., 2000). With reduction of talin by bacterial RNAi, adult hermaphrodites displayed abnormal clear patches not apparent in animals fed bacteria containing the empty RNAi vector (Fig. 1A,B). This clearing within the body cavity results from displacement of the intestine because of inappropriate turns of the gonad arms, gonad distension or other defects in gonad morphology (Nishiwaki, 1999).

During normal *C. elegans* development, migration of specialized gonadal leader cells, the DTCs, forms the two arms of the U-shaped hermaphrodite gonad. Defects in interaction of the DTC with extracellular substrates and pathfinding cues result in malformed gonad arms (Hubbard and Greenstein, 2000). Comparison of gonads from N2 nematodes with talin RNAi animals demonstrated that loss of talin expression induced significant defects in gonad morphology. Misdirected gonad migration was observed in 61% ($\pm 3\%$, $n=236$) of talin RNAi hermaphrodite gonad arms after 48-50 hours of exposure to RNAi bacteria. The most typical defects were failure to turn and migrate along the dorsal side of the animal. Other defects included inappropriate turns and premature stops (Fig. 1C,D). Gonad elongation along the ventral body wall appeared normal. Defects were rarely observed in animals fed bacteria containing an empty RNAi vector ($6\% \pm 2\%$, $n=154$).

Disruption of talin function within the DTC could generate these migration and gonad morphological defects. In order to determine whether talin is expressed in the DTC, transgenic nematode lines that express a *talin::gfp* transcriptional fusion were prepared. Fluorescence microscopy of transgenic animals showed that GFP was expressed in body wall muscles, sex muscles, DTCs and sheath cells of the somatic gonad (Fig. 2). These results show that talin is appropriately distributed to affect DTC migration.

Oocyte maturation is defective in the proximal gonad

In wild-type nematodes, oocytes line up at the spermatheca, mature, and are ovulated in an assembly line fashion. Fertilization occurs as oocytes are moved through this organ in response to complex signals from the spermatheca, the oocyte

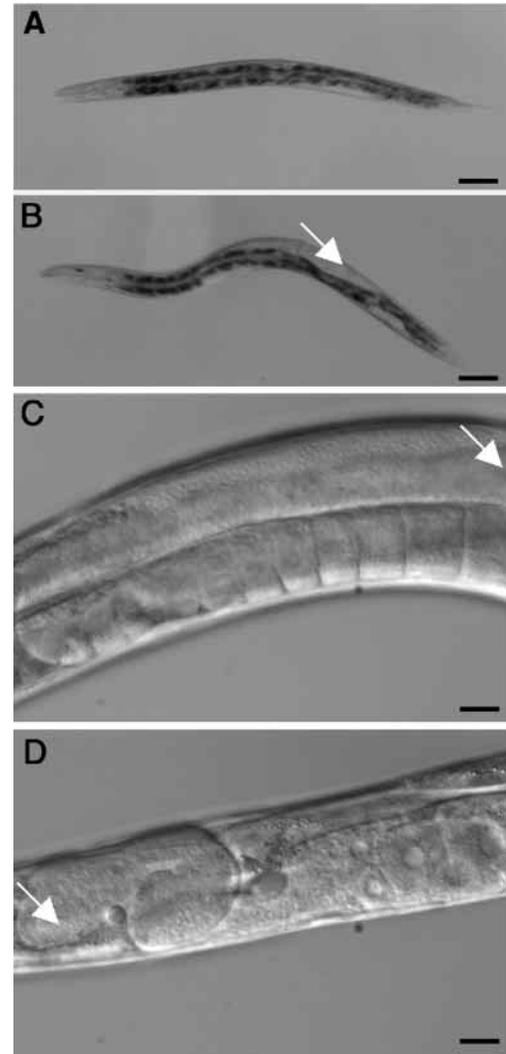


Fig. 1. Talin RNAi results in DTC migration defects. N2 hermaphrodites were grown on *E. coli* HT115(DE3) carrying empty vector (A) or pPD129.36-talin plasmid (B). Arrow in B points to clearing in the body cavity from displacement of the dark gray intestines. Both animals are anterior to the left, ventral up. DIC images show wild-type (C) and 52-hour talin RNAi (D) hermaphrodite gonad arms. Arrows indicate locations of DTCs. Anterior is to the left, ventral down in both images. Scale bars: 100 μ m (A,B), 25 μ m (C,D).

and the sheath cells that surround the gonad (Schedl, 1997). Stacking of oocytes occurs when oocytes are unable to move through the spermatheca because of a failure in proper signaling, or to defective spermatheca or sheath cell expansion and contraction (Hubbard and Greenstein, 2000; McCarter et al., 1997; McCarter et al., 1999).

We found that oocytes commonly accumulated in the proximal gonad arms of animals with reduced talin levels (Fig. 3B). Most of these animals had at least one gonad arm affected ($85\% \pm 3\%$, $n=114$). This arrangement is in contrast to wild-type oocytes, which are organized in a linear array (Fig. 3A). Germ cell DNA, visualized by DAPI staining, appeared normal in the distal gonad arm and sperm were visible proximal to the oocytes (Fig. 3C). In contrast to control hermaphrodites, germ

line nuclei were frequently observed throughout the gonad arms of talin RNAi animals ($55\% \pm 7\%$, $n=49$) (Fig. 3D), perhaps because of a failure to mature into oocytes. By 72 hours of exposure to talin RNAi, the gonads usually ruptured, leading to severely compromised gonad function and sterility of the talin RNAi hermaphrodites.

Because talin is expressed in the sheath cells (see Fig. 2), it seemed probable that defective sheath cell cytoskeletal connections to the plasma membrane led to gonad distension by oocyte stacking. In order to observe the actin cytoskeleton in sheath cells, dissected gonads were stained with rhodamine-phalloidin. Wild-type gonads showed the typical actin filament pattern demarcating the edges of oocytes as well as extending length-wise throughout the gonad sheath cells (Fig. 4A). Talin RNAi animals had abundant actin filaments surrounding the germ cells but very little organized actin in the proximal gonad (Fig. 4B). Thus, loss of talin leads to a severe reduction of actin cytoskeletal organization in the proximal gonad.

Loss of talin leads to total body muscle paralysis

In addition to the gonad-related phenotypes, talin RNAi resulted in progressive and ultimately complete paralysis. By

58 hours post-hatch, the majority of the nematodes became paralyzed (Fig. 5A), able only to move their heads (Fig. 5B). By 72 hours, almost 100% of the animals were paralyzed.

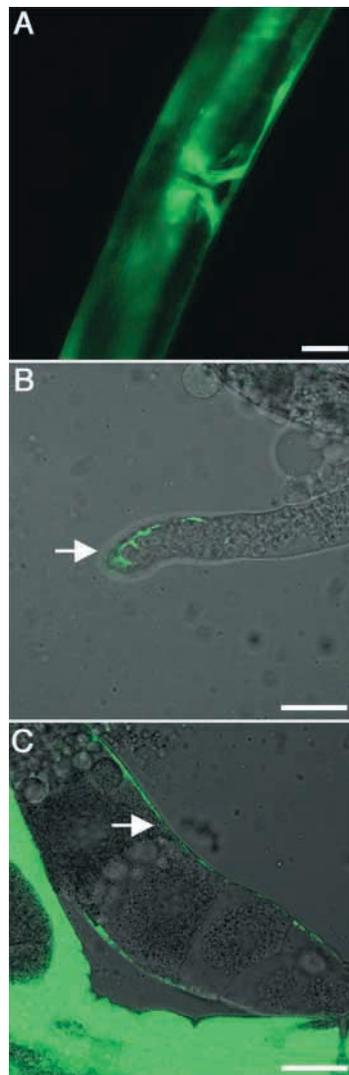


Fig. 2. Expression pattern of talin in adult hermaphrodites. The GFP expression pattern was examined in the *talin::GFP(zdIs15)* line by fluorescence microscopy. In these animals, GFP is expressed under the control of the talin promoter. (A) GFP is seen throughout the body wall and sex muscle cells. Confocal microscopy of dissected gonad arms shows GFP expression in the DTC (B, arrow) and in the gonad sheath cells (C, arrow). Scale bars: 25 μm .

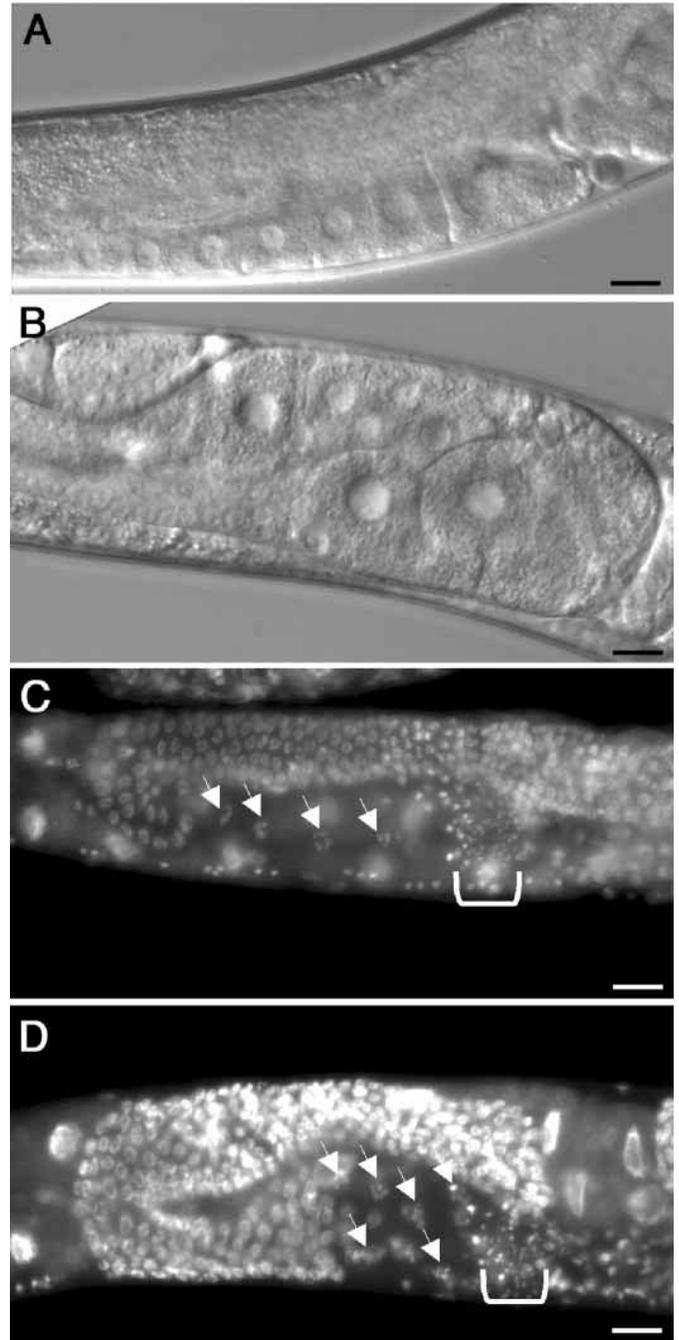


Fig. 3. Ovulation defects in talin RNAi hermaphrodites. Nematodes were grown on HT115(DE3) with empty vector (A,C) or with pPD129.36-talin (B,D) for 52 hours. (A) DIC image shows normal linear arrangement of oocytes in the proximal gonad arm. (B) This arrangement contrasts with stacking of oocytes in a distended gonad arm of talin RNAi animals. (C) Normal DNA organization in the germ cells and oocytes (arrows) was visualized by DAPI staining. (D) Talin RNAi animals had stacked oocytes in the proximal gonad arm (arrows) and germ line nuclei were observed throughout the gonad arm. Brackets in C and D indicate location of sperm. All animals are anterior to the left, ventral down. Scale bars: 25 μm .

C. elegans body wall muscles consist of rows of cells that extend along the body and form multiple attachments to the basement membrane (Moerman and Fire, 1997). Talin is localized to muscle dense body complexes that form a regularly repeated dotted pattern along the length of muscle cells (Moulder et al., 1996). Indirect immunofluorescence staining of the integrin β subunit PAT-3 was used to visualize dense body organization. By 72 hours of talin RNAi, PAT-3 organization appeared very irregular and disordered and the muscle structure was significantly disrupted (Fig. 5C,D). Contraction of muscles with weakened cytoskeletal attachments probably results in the observed irregular patches in the muscle cells.

Talin functions with PAT-2 integrins in muscle

C. elegans has two integrin α subunits, INA-1 and PAT-2, which

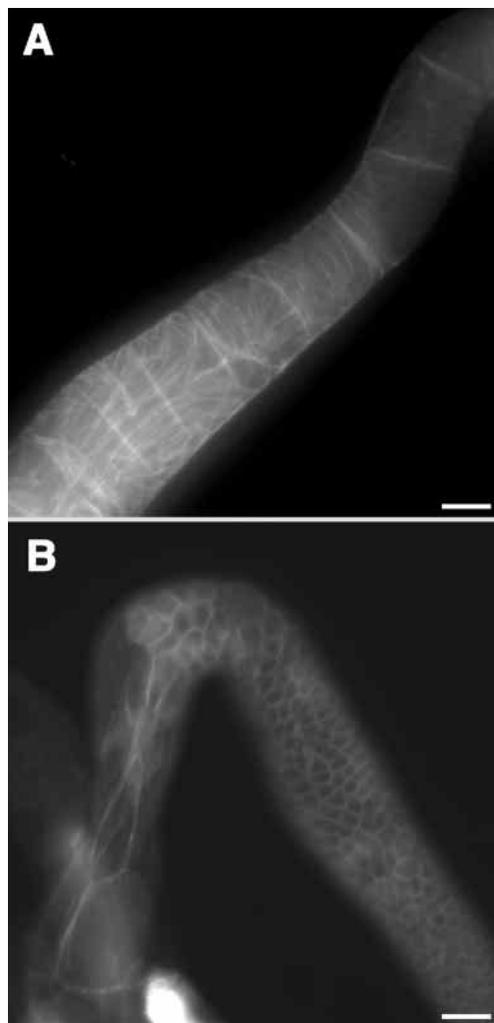


Fig. 4. Actin filament organization is disrupted in the gonad sheath cells. Rhodamine-conjugated phalloidin was used to stain actin filaments in dissected gonad arms of (A) animals grown on bacteria carrying the empty RNAi vector or (B) talin RNAi animals for 48 hours. (A) Proximal gonad arm has longitudinal actin filaments in the sheath cells. This pattern is absent from the proximal arm of a talin RNAi gonad (B, left), but actin filaments appear normal in distal gonad arm (B, right). Scale bars: 25 μ m.

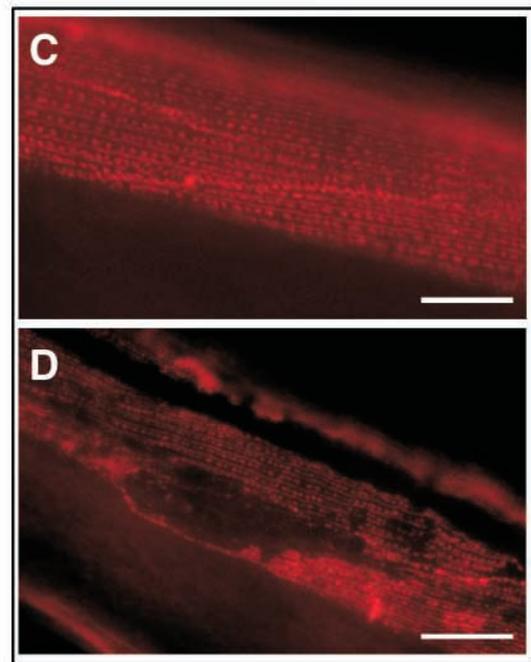
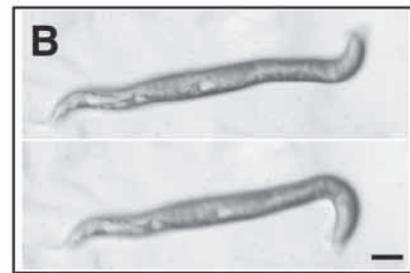
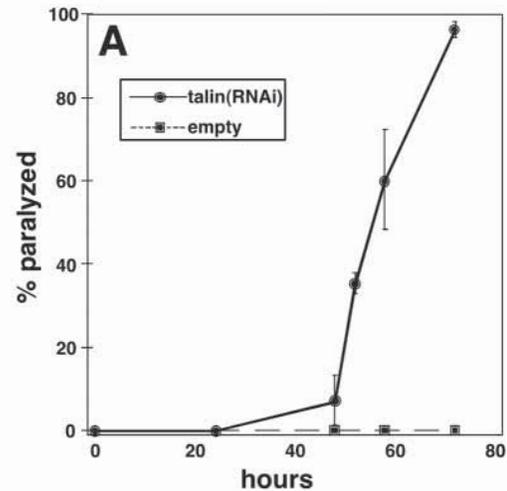


Fig. 5. Progressive paralysis and loss of muscle filament organization with talin RNAi. (A) Time course of paralysis in talin-depleted animals (circles) or animals grown on bacteria carrying the empty RNAi vector (squares). Paralyzed animals were counted at 24, 48, 52, 58 and 72 hours. The error bars represent the standard deviation of at least 5 independent experiments. (B) At 72 hours, paralyzed animals are immobile except for occasional movement of the head. Staining with MH25 anti-PAT-3 monoclonal antibody was used to visualize dense bodies in body wall muscle cells of animals grown on bacteria with empty vector (C) and with talin RNAi (D) for 72 hours. Scale bars: 100 μ m (B), 25 μ m (C,D).

primarily function in distinct tissues. INA-1 is expressed in many non-muscle cell types including DTCs, uterine, vulval and neuronal cells (Baum and Garriga, 1997). Several viable *ina-1* mutant alleles have similar misdirected or misshapen gonad arms and mislocalized germ cells as seen in talin-depleted animals, suggesting that talin works in concert with INA-1/PAT-3 $\alpha\beta$ heterodimers in these tissues. Although the tissue distribution and mutant phenotypes of *pat-2* have not been published, *pat-2* has been shown to be essential for muscle development (Williams and Waterston, 1994). Therefore, we predicted that depletion of PAT-2 would result in muscle phenotypes similar to those of talin-depleted animals. Reduction of either PAT-2 or PAT-3 by RNAi caused muscle-related phenotypes identical to talin RNAi. Within 72 hours, paralysis was accompanied by severe disorganization of body muscle actin filaments (Fig. 6). Reduction of PAT-2 levels also led to an egg-laying defect, with animals developing the bag-of-worms

phenotype by 72 hours ($75\pm 4\%$ $n=95$). Unlike talin loss-of-function, however, gonad morphology was not visibly perturbed by *pat-2* RNAi. Real time reverse transcriptase polymerase chain reaction (RT-PCR) analysis confirmed that defective animals had significantly reduced levels of talin, *pat-2* and *pat-3* RNA by 48 hours (Fig. 7). Talin RNA levels were reduced by 48 hours and remained low throughout the experimental time course. In addition, we verified that levels of *pat-2* and *pat-3* RNA do not change in response to talin RNAi at 48 hours (data not shown). These results indicate that PAT-2/PAT-3 integrins are working in concert with talin in *C. elegans* muscle whereas INA-1/PAT-3 integrins function with talin during DTC migration.

Discussion

Our analyses of talin loss of function in *C. elegans* show that this protein is essential for cell contractility and motility *in vivo*. Talin is required for (1) DTC migration and pathfinding on the dorsal ECM, (2) body wall muscle cell and gonad myoepithelial sheath cell contraction, and (3) oocyte maturation and ovulation. These phenotypes show significant roles for talin in stabilizing strong adhesive contacts such as muscle dense bodies as well as in contributing to more dynamic cell-ECM interactions as seen during cell migration. One common feature of these phenotypes is a requirement for connections between actin cytoskeletal structures and integrin-ECM contacts. A critical role for talin in linking integrins to the actin cytoskeleton has recently been demonstrated in *Drosophila* (Brown et al., 2002).

In talin RNAi animals, we commonly observed DTCs that had failed to turn and migrate toward the midpoint on the dorsal ECM. This phenotype is similar to the abnormal gonad morphology we observed with dominant negative inhibition of integrins in the DTC (Lee et al., 2001). Talin binding to the β integrin cytoplasmic tail allows integrin activation and ligand binding in cell culture systems, perhaps by relieving an inhibitory interaction between the α and β integrin subunits (Calderwood et al., 2002; Calderwood et al., 1999). Thus, talin reduction by RNAi will probably affect the activity of integrins. Failure of the DTCs to complete the appropriate migratory path could be due to ineffective cell adhesion, or to incorrect detection or interpretation of directional cues positioned within the dorsal ECM. Talin may also be required for initial DTC migration along the ventral ECM, but this might not be evident by talin RNAi because larvae may contain residual talin protein synthesized during embryogenesis. Proper formation of hermaphrodite gonad arms depends on directional cues from proteins such as UNC-6/netrin and UNC-40/netrin receptor for ventral to dorsal migration (Ishii et al., 1992), and UNC-129/TGF- β on the dorsal side (Colavita et al.,

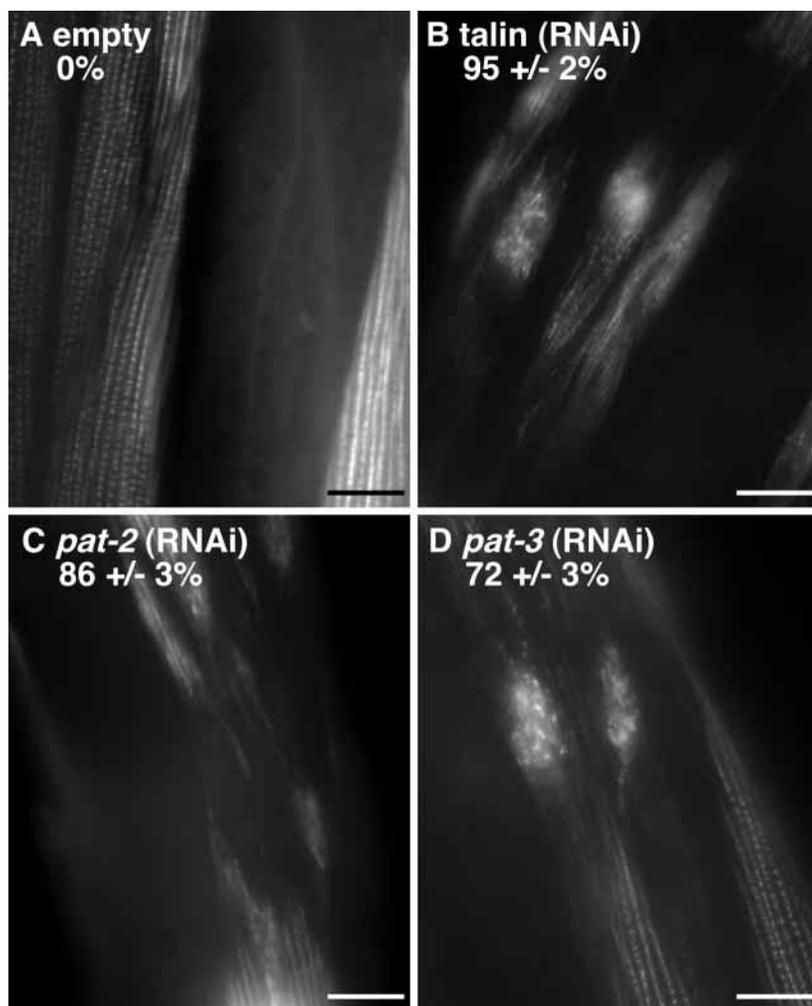


Fig. 6. Depletion of PAT-2/ α or PAT-3/ β integrins disrupts muscle structure. Rhodamine-conjugated phalloidin was used to stain actin filaments in body wall muscle cells of animals grown on bacteria carrying the empty vector (A), or bacteria expressing dsRNA for talin (B), *pat-2* (C) and *pat-3* (D) for 72 hours. Compared with wild type, actin filaments were disorganized in all of the RNAi-treated animals. Percentages in each panel represent proportion of paralyzed animals at 72 hours post-hatch (\pm s.d.). Scale bars: 25 μ m.

1998). Coordination between these signals and integrin-talin connections to the cytoskeleton appear to control directed movement during gonad morphogenesis.

Our results support the hypothesis that the integrin $\alpha\beta$ PAT-2/PAT-3 heterodimer is the functional integrin required in nematode muscles. Furthermore, we have shown that this integrin pair acts through talin to organize and maintain the muscle actinomyosin contractile structure. Depletion of talin, PAT-2 or PAT-3 resulted in paralysis and breakdown of the muscle actin structure by 72 hours. Embryos homozygous for loss of function mutations in various genes of the *C. elegans* dense body including *deb-1/vinculin*, *pat-2/ α -integrin*, *pat-3/ β -integrin*, *pat-4/ILK* and *pat-6/ α -parvin* exhibit similar Pat phenotypes and embryonic lethality (Williams and Waterston, 1994). In these animals, muscle cell actin and myosin are not organized into sarcomeres, and dense body and M-line components fail to assemble. Animals depleted of talin by RNAi show similar muscle phenotypes, suggesting the null phenotype of talin will probably be Pat.

Oocyte maturation, ovulation and fertilization must occur in the proper sequence and in coordination with the somatic gonad in order to produce viable progeny. Ablation of sheath cells results in an impairment of germ cell meiotic progression and gametogenesis (Schedl, 1997), a process that involves MAP kinase signaling (Church et al., 1995; Miller et al., 2003). Ovulation is dependent on sheath cell contractions that move mature oocytes through the spermatheca (Schedl, 1997). We found that the sheath cell actin cytoskeleton of talin RNAi animals was severely disorganized. Compromised sheath cell function blocked germ cell maturation and allowed gonad distension and oocyte accumulation in the proximal gonad arms. These results demonstrate that talin plays essential roles in multiple sheath cell functions during oocyte development and ovulation.

Talin loss of function phenotypes show considerable similarities to previously reported *pat-3* integrin defects (Gettner et al., 1995; Lee et al., 2001; Williams and Waterston, 1994). In addition to talin, integrins interact with many intracellular proteins that play key roles in integrin function

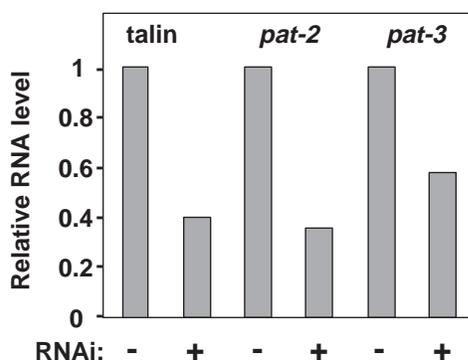


Fig. 7. Bacterial RNAi decreases mRNA levels for talin, *pat-2* and *pat-3*. Real-time RT-PCR analysis was applied after RNAi treatment. Shown are relative levels of RT-PCR products amplified from total RNA isolated from control animals fed bacteria containing empty vector (-) or bacteria expressing the indicated dsRNA (+) for 48 hours. Data were normalized to GAPDH and (-) cDNA samples were set to 1.

(Liu et al., 2000), and we suspected loss of some of these proteins might result in phenotypes similar to those of talin RNAi. The *C. elegans* genomic database of RNAi results was searched for those genes giving a paralyzed (Prz) RNAi phenotype (Kamath et al., 2003). Of the 49 genes that had a Prz phenotype, known cell architecture and cell signaling genes were over-represented with 38% compared with 21% of all genes with RNAi phenotypes. Among these, loss of *pat-4/ILK* resulted in cell contraction phenotypes that most closely resembled talin RNAi, including uncoordination, paralysis and lethality. Whether ILK and other Prz genes participate in cell migration in addition to their roles in contractile cell functions remains to be determined.

Our results show that talin plays important roles in post-embryonic organogenesis and tissue functions. Furthermore, talin functions with the $\alpha\beta$ PAT-2/PAT-3 heterodimer, and probably the $\alpha\beta$ INA-1/PAT-3 heterodimer, for critical tissue- and stage-specific functions. In addition, this work demonstrates that screening for defects in DTC migration using bacterial RNAi can rapidly identify gene products that affect integrin signaling and function.

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