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Caenorhabditis elegans Teneurin, *ten-1*, Is Required for Gonadal and Pharyngeal Basement Membrane Integrity and Acts Redundantly with Integrin *ina-1* and Dystroglycan *dgn-1*

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The *Caenorhabditis elegans* teneurin ortholog, *ten-1*, plays an important role in gonad and pharynx development. We found that lack of TEN-1 does not affect germline proliferation but leads to local basement membrane deficiency and early gonad disruption. Teneurin is expressed in the somatic precursor cells of the gonad that appear to be crucial for gonad epithelialization and basement membrane integrity. *Ten-1* null mutants also arrest as L1 larvae with malformed pharynges and disorganized pharyngeal basement membranes. The pleiotropic phenotype of *ten-1* mutant worms is similar to defects found in basement membrane receptor mutants *ina-1* and *dgn-1* as well as in the mutants of the extracellular matrix component laminin, *epi-1*. We show that the *ten-1* mutation is synthetic lethal with mutations of genes encoding basement membrane components and receptors due to pharyngeal or hypodermal defects. This indicates that TEN-1 could act redundantly with integrin INA-1, dystroglycan DGN-1, and laminin EPI-1 in *C. elegans* development. Moreover, *ten-1* deletion sensitizes worms to loss of nidogen *nid-1* causing a pharynx unattached phenotype in *ten-1;nid-1* double mutants. We conclude that TEN-1 is important for basement membrane maintenance and/or adhesion in particular organs and affects the function of somatic gonad precursor cells.

INTRODUCTION

Teneurins are large transmembrane proteins that play important roles in cell signaling and cell adhesion (Tucker and Chiquet-Ehrismann, 2006; Tucker *et al.*, 2007). Teneurins are phylogenetically conserved among metazoans and they were described in several species, including *ten-1* in *Caenorhabditis elegans* (Drabikowski *et al.*, 2005), *ten-m/odz* and *ten-a* in *Drosophila* (Baumgartner *et al.*, 1994; Levine *et al.*, 1994; Fascetti and Baumgartner, 2002; Rakovitsky *et al.*, 2007), zebrafish (Mieda *et al.*, 1999), and in chicken (Minet *et al.*, 1999; Tucker *et al.*, 2000; Tucker *et al.*, 2001; Rubin *et al.*, 2002) and mouse (Oohashi *et al.*, 1999; Ben-Zur *et al.*, 2000; Zhou *et al.*, 2003). In vertebrates, the four teneurin paralogs were named teneurin-1 to -4, ten-m1 to -m4, or odz-1 to -4.

The extracellular domain of all teneurins is composed of eight tenascin-type EGF-like repeats, a region of conserved cysteines, and YD repeats that are also found in a few bacterial proteins (Minet and Chiquet-Ehrismann, 2000). The intracellular domain contains proline-rich stretches and putative tyrosine phosphorylation sites but is less conserved than the extracellular part and cannot be aligned in a linear way between the phyla. Teneurins are thought to interact in a homophilic manner (Oohashi *et al.*, 1999; Rubin *et al.*, 2002; Bagutti *et al.*, 2003; Leamey *et al.*, 2008) and to date, no other ligand has been identified.

The name "teneurins" refers to their high expression in the developing and adult nervous system (Mieda *et al.*, 1999; Oohashi *et al.*, 1999; Otaki and Firestein, 1999; Ben-Zur *et al.*, 2000; Tucker *et al.*, 2000; Rubin *et al.*, 2002; Zhou *et al.*, 2003). In the developing mouse cortex, all teneurins are expressed in distinctive gradients and may be required for neocortical patterning (Li *et al.*, 2006). Several reports point out their role in the development of visual pathways. Leamey *et al.* (2008) have found that teneurins are up-regulated in visual versus somatosensory areas of the neocortex. Moreover, expression of different teneurins is largely nonoverlapping and can be found in interconnected regions of the developing visual system (Rubin *et al.*, 1999, 2002; Kenzelmann *et al.*, 2008; Leamey *et al.*, 2008). For instance, teneurin-1 staining is found in the tectofugal pathway, and teneurin-2 is primarily expressed in the thalamofugal pathway. In addition, teneurins were shown to promote neurite outgrowth in vitro (Minet *et al.*, 1999; Rubin *et al.*, 1999) and in vivo (Leamey *et al.*, 2008), suggesting an important function for teneurins in axon guidance and target recognition. Recently, the first vertebrate teneurin knockout was described (Leamey *et al.*, 2007). Teneurin-3 regulates eye-specific patterning in the

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Abbreviations used: BM, basement membrane; DIC, differential interference contrast; L1, first larval stage; L2, second larval stage; L3, third larval stage; L4, fourth larval stage; Pun, pharynx unattached; SGP, somatic gonad precursor cells.

visual system, and the knockout mice show impaired binocular vision.

Beside prominent expression in the nervous system, teneurins are also found in nonneuronal tissues. They are expressed in alternating parasegments in the fly embryo, as well as in cardiac cells, muscle attachment sites, and the tracheal system in *Drosophila* (Baumgartner and Chiquet-Ehrismann, 1993; Baumgartner *et al.*, 1994). In the chicken teneurins are found in limb buds, branchial arches, and somites (Tucker *et al.*, 2000, 2001), and in *C. elegans ten-1* is expressed in gonadal somatic cells, pharynx, and muscles (Drabikowski *et al.*, 2005). Teneurin expression in each of these tissues is often associated with pattern formation and cell migration.

The in vivo function of teneurins is mainly inferred from studies of *C. elegans* and *Drosophila* mutants. Mutation of the fly *ten-m* gene causes embryonic lethality due to the fusion of adjacent denticle belts (Baumgartner *et al.*, 1994; Levine *et al.*, 1994). Moreover, defects in the ventral nerve cord, cardiac cells and eye patterning are found in late *ten-m* mutant embryos (Levine *et al.*, 1994; Kinel-Tahan *et al.*, 2007). Similar defects in cuticle and eye development have been observed for the second *Drosophila* teneurin gene, *ten-a* (Rakovitsky *et al.*, 2007). In *C. elegans*, deletion in the *ten-1* gene causes a pleiotropic phenotype, including gonad disorganization, nerve cord defasciculation, and defects in distal tip cell migration and axonal pathfinding (Drabikowski *et al.*, 2005).

The single teneurin ortholog in *C. elegans*, *ten-1*, is under control of alternative promoters giving rise to two protein variants. The isoforms differ only in their intracellular domains. Their expression patterns are complex but mostly nonoverlapping: TEN-1 long (TEN-1L) is found mainly in the mesoderm, including pharynx, somatic gonad, and various muscles and neurons, and TEN-1 short (TEN-1S) is predominantly expressed in some hypodermal cells and in a subset of neurons (Drabikowski *et al.*, 2005).

We report here the role of TEN-1 in gonadal basement membrane maintenance, as well as in epidermal and pharyngeal development. Mutation of the *ten-1* gene leads to gonad rupture and sterility. Germ cell leakage from the gonads has also been reported for basement membrane mutants, e.g., integrin α *ina-1*, dystroglycan *dgn-1*, and laminin αB *epi-1* (Baum and Garriga, 1997; Huang *et al.*, 2003; Johnson *et al.*, 2006). Furthermore, the genetic interactions between *ten-1*, *ina-1*, *dgn-1*, *epi-1*, and *nid-1* suggest that teneurin, integrin, and dystroglycan have related and partly redundant functions in *C. elegans* development.

MATERIALS AND METHODS

General Methods and C. elegans Strains

C. elegans strains were maintained at 20°C as described (Brenner, 1974). The following strains were used in this study: wild-type N2, variety Bristol, CH120: *cle-1(cg120) I*, CB444: *unc-52(e444) II*, VC518: *ten-1(ok641) III*; TM0651: *ten-1(tm651) III*; NG39: *ina-1(gm39) III*; NG144: *ina-1(gm144) III*; CB189: *unc-32(e189) III*; CX2914: nDf16/*dpy-17(e164) unc-32(e189) III*; CH119: *nid-1(cg119) V*; CH121: *dgn-1(cg121)/dpy-6(e14) unc-115(mn481) X*. The *tm651* deletion removes nucleotides R13F6: 3661-4550 of the *ten-1* coding sequence.

The following GFP marker strains were used: RU7: *kdEx7* [*ten-1a::gfp*]; RU97: *ten-1(ok641) kdEx45* [*F36A3, III*]; JK2049: *qIs19* [*lag-2::gfp*]; SS0747: *bnIs1* [*pie-1::GFP::PGL-1*] (gift of Susan Strome, University of California, Santa Cruz, CA); IM253: *urEx131* [*lam-1::gfp*] (gift of William Wadsworth, Robert Wood Johnson Medical School, Piscataway, NJ), CH1878: *dgn-2(ok209) dgn-3(tm1092) dgn-1(cg121)*; *cgEx308* [*DGN-1::GFP*] (gift of James Kramer, Northwestern University Medical School, Chicago, IL).

Double mutant worms were maintained as [*ten-1(ok641);ina-1(gm144); kdEx45*], [*ten-1(ok641/+);nid-1(cg119)*], [*ten-1(ok641);dgn-1(cg121/+); kdEx45*] or [*ten-1(ok641/+);dgn-1(cg121); cgEx308*] strains and genotyped by PCR for the phenotypic analysis.

Constructs and Plasmids

The translational *Pten-1a::GFP::TEN-1L* minigene reporter construct was generated by cloning SpeI-HindIII cDNA fragment and HindIII-XhoI genomic fragment of TEN-1 long variant into p123T vector (Mo Bi Tec, Goettingen, Germany). The following restriction sites were introduced into the primers: SpeI and XhoI flanking the *ten-1* coding sequence, SacII at the 5′ end of the *ten-1a* promoter, and ApaI downstream of the 3′ UTR.

The long intracellular domain, transmembrane domain, and a short fragment of the extracellular part were amplified using 5′-AACAGTCTACCGAATCCCAACC-3′ and 5′-ATAACTAGTATGTTCCAGCACAGGTAAACTACCACG-3′ primers and cDNA from mixed stage N2 worms as a template. For the extracellular domain of *ten-1* we used 5′-GCTGAAATACCCACTCGCCAGC-3′ and 5′-ATCTCGAGCTATTCAGATTTTCGGAACTTCC-3′ primers and R06H12 cosmid as a template. The sequence encoding green fluorescent protein (GFP) was amplified from pPD117.01 vector and its NcoI site was mutated to CCTTGG. GFP was fused by PCR to the N-terminus of the *ten-1* cDNA fragment, which was cloned into SpeI-NcoI sites of *ten-1* minigene. Hemagglutinin (HA) tag was added at the C-terminus of *ten-1* coding sequence by PCR and cloned into HpaI-XhoI sites. The *Pten-1a::GFP::TEN-1L* construct contained 4235 base pairs of the *ten-1a* promoter and a 512-base pair sequence downstream of the stop codon. PCR fragments were generated with Pfu Turbo DNA polymerase (Stratagene, La Jolla, CA).

Transgenic Animals

Transgenic lines were generated as previously described (Mello *et al.*, 1991). The *Pten-1a::GFP::TEN-1L* plasmid was injected into *ten-1(ok641)* mutant worms. Injections of *GFP::TEN-1* minigene at low concentration (5 ng/μl) resulted in a very weak GFP fluorescence, mainly in the nervous system. Therefore, we injected the worms with high concentrations of the transgene (40 ng/μl) and obtained several lines giving stronger GFP fluorescence. We used pRF4 [*rol-6*] as a coinjection marker. This resulted in the line RU152: *kdEx121* [*Pten-1a::GFP::TEN-1L*] used in this study.

RNA Interference

RNA-mediated interference (RNAi) was performed as described (Kamath and Ahringer, 2003). The K08C7.3 RNAi clone was obtained from the Ahringer feeding library. Wild-type and *ten-1(ok641)* synchronized L4 hermaphrodites were placed on RNAi plates and grown at 15°C for 72 h. Single adult worms were placed on fresh RNAi plates and allowed to lay eggs for 24 h. These plates were examined for 3 d to determine embryonic lethality and postembryonic phenotypes.

Immunostaining of C. elegans Larvae

C. elegans larvae were prepared as previously described (Finney and Ruvkun, 1990). Fixed animals were blocked overnight at 4°C in PBS containing 0.1% Triton X-100 (Triton) and 10% goat serum. Samples were incubated with an antibody against collagen IV LET-2 (NW68, kind gift of James Kramer) overnight at 4°C, washed in PBS containing Triton, and incubated with fluorescein conjugated goat anti-rabbit secondary antibody overnight at room temperature. Finally, fixed larvae were washed in PBS containing Triton and Hoechst, followed by PBS alone.

Electron Microscopy

Worms were washed in M9 and anesthetized in 8% ethanol in M9 for 5 min. They were placed in a fixative (2.5% glutaraldehyde, 1% paraformaldehyde in 0.1M sucrose, and 10 mM PBS, pH 7.4), cut open with a needle at both anterior and posterior ends, and fixed for 2 h. Worms were embedded in 2% agarose, cut into small blocks, and washed three times in PBS. Subsequently, pieces were fixed with a second solution (1% osmium tetroxide, 1.5% potassium ferrocyanide in PBS) for 2 h and washed three times in water. Worms were stained with 1% uranyl acetate for 1 h. Samples were dehydrated in ethanol (10 min in 50% ethanol, 10 min in 70% ethanol, 10 min in 90% ethanol, and 10 min in 100% ethanol) and acetone (10 min). Blocks with worms were embedded in Epon resin (Fluka, Buchs, Switzerland): first in Epon-acetone (1:1) for 1–2 h and then in pure resin for 2–4 h. Samples polymerized for 24–48 h at 60°C and in 60-nm sections were prepared with Ultracut E. Sections were stained in uranyl acetate for 60 min and then 2 min in Millonig's lead acetate stain. Pictures were taken on Philips Morgagni 80 KV microscope (Eindhoven, The Netherlands).

Phenotypic Analysis

Young adult hermaphrodites were placed on separate plates and allowed to lay eggs for 24 h. The progeny were analyzed for embryonic and postembryonic phenotypes: lethality, larval arrest, sterility, and bursting at the vulva.

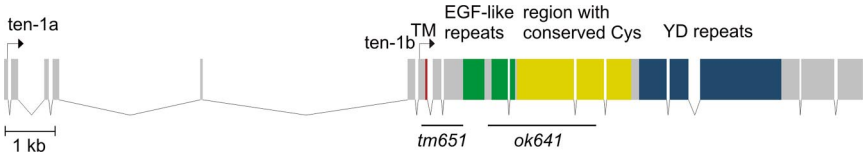

Figure 1. Genomic organization of *ten-1* gene and location of *tm651* and *ok641* deletions. Exons are depicted as boxes and introns are shown as lines. Expression of *ten-1* is regulated by alternative promoters: *ten-1a* and *ten-1b*, resulting in two type II transmembrane protein variants differing in the length of their intracellular domain. Fragments of exons encoding different protein domains are labeled as follows: red, single transmembrane domain, green, EGF-like repeats in two groups, yellow, region of conserved cysteines, and blue, stretch of YD repeats. Black horizontal lines show the regions deleted in two *ten-1* mutants: *tm651* and *ok641*.

Time Course of Germline Development and Basement Membrane Breakdown

Synchronized, starved L1 larvae carrying the *GFP::PGL-1* marker were placed on bacteria plates. We scored the number of germ cells in 20 worms for each genotype at 0, 8, 12, 16, and 20 h. For the study of basement membrane integrity, we used synchronized worms carrying the *LAM-1::GFP* marker and analyzed 50–62 worms for each developmental stage.

Microscopy

Animals were mounted on 2% agarose pads in a drop of M9 buffer containing 25 mM sodium azide. Differential interference contrast (DIC) and fluorescence images were acquired with Z1 microscope (Zeiss, Jena, Germany) and AxioCam Mrm camera (Zeiss) using 63×/1.4 NA Plan-Apochromat objective (Zeiss) and AxioVision software.

RESULTS

Both ten-1(ok641) and ten-1(tm651) Are Functional Null Alleles

In our previous study we described the *ten-1* mutation, *ok641*, that carries an in-frame 2130-base pair deletion removing four EGF-like repeats and a large part of the conserved cysteines region (Drabikowski *et al.*, 2005). We now obtained another allele, *tm651*, lacking 890 base pairs and introducing a frameshift into the *ten-1* coding sequence (Figure 1). This deletion results in a loss of the transmembrane domain and the entire extracellular part. Therefore, *tm651* is most likely a null allele. Because phenotypes of both *ten-1* mutants show similar penetrance (Table 1), we assume that *ok641* represents a functional null allele as well.

To confirm this hypothesis, we created heterozygous worms carrying nDf16 deficiency in trans to *tm651* or *ok641* and investigated whether the mutant phenotypes became aggravated after complete removal of one copy of the *ten-1* gene. The *ok641*/nDf16 and *tm651*/nDf16 worms displayed a similar range of defects to *ok641* and *tm651* homozygous animals, and the values observed were very close to those calculated under the assumption of *ten-1* mutants being null alleles (Table 2).

These data and the fact that *ok641* and *tm651* deletions affected protein regions that are common to both TEN-1 isoforms, suggested that there was no functional TEN-1 present in any of the *ten-1* mutants.

Gonads of ten-1 Mutant Worms Burst Early in Development

Previous studies demonstrated that TEN-1 plays an important role in gonad development and function (Drabikowski *et al.*, 2005). Homozygous *ten-1(ok641)* worms are viable, but 15–20% are sterile or burst-through-the-vulva due to germ cell leakage in the middle of the gonad. Occasionally, gonads disintegrate completely and germ cells float in the pseudocoelom. We could rescue gonadal and vulval defects by expression of the *kdEx121* transgene encoding the long teneurin isoform under its own promoter (Table 1).

To determine the basis and the developmental stage of gonad bursting, we performed a time course experiment of germ cell proliferation in the early gonads of *ten-1(ok641)* mutants. We used worms carrying a P-granule GFP marker to distinguish between germ cells and somatic gonad precursor cells. Interestingly, we found that germ cells were released from the gonads of *ten-1* mutant already at the early L3 stage (Figure 2B). At the same time point, there were no germ cells present around the developing somatic gonad primordium in the wild-type worms (Figure 2A). A sharp DIC boundary surrounding the gonad was visible in the

Table 1. Phenotypes of *ten-1* deletion mutants

Genotype	Embryonic lethality (%)	Larval arrest (%)	Sterile and/or vulva defects (%)	Fertile adults (%)	n
Wild type	0.9	0	0	99.1	321
ten-1(tm651)	5.7	31.9	17.4	45.1	386
ten-1(ok641)	6.4	32.1	16.7	44.8	346
ten-1(ok641), kdEx121	1.2	5.5	7.2	86.1	165

Table 2. Embryonic lethality and larval arrest phenotypes appearing in the progeny of nDf16/*ten-1 unc-32* transheterozygotes

Genotype	Embryonic lethality (%)	Larval arrest (%)	Adults: total (%)	% Unc in adult worms	n
nDf16/*ten-1(tm651) unc-32(e189)*	29.7	27.2	43.1	32.1	492
nDf16/*ten-1(ok641) unc-32(e189)*	33.5	22.6	43.9	30.1	310
Expected value for nDf16/*ten-1*[a]	29.5	24.0	46.5	33.3	

[a] The calculated ratio of phenotypes expected if the *ten-1* mutants are null mutants.

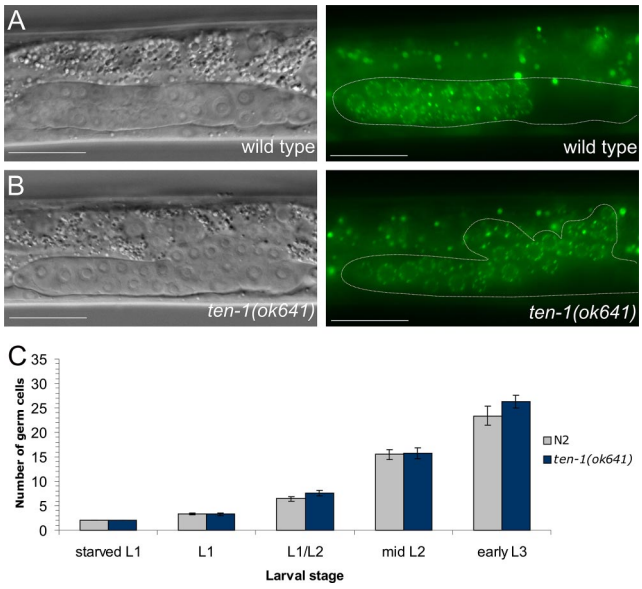

Figure 2. Germ cells are released from the early gonad of *ten-1(ok641)* mutant through the central break. Germ cell number and localization were evaluated using the P-granule marker *pie-1::GFP::PGL-1*. (A) Wild-type L3 gonad. The somatic gonadal primordium forms in the middle of the gonad, and germ cells fill the two gonad arms (only one arm is shown). (B) Ruptured gonadal primordium of a *ten-1(ok641)* L3 larva. Germ cells are released into the body cavity and localize in the vicinity of the developing somatic gonad primordium. (C) Time course of germline development in wild-type animals and *ten-1(ok641)* mutants. There is no germline overproliferation in the early gonads of the *ten-1(ok641)* mutant. Scale bar, 20 μm.

wild type as well as a large part of *ten-1(ok641)* gonad (Figure 2, A and B) but absent on the dorsal side of the mutant gonad, where the germ cells leaked out into the pseudocoelom. Gonad bursting was not the result of germline overproliferation causing increased pressure on the gonadal basement membrane (BM), because we did not find any difference in the number of germ cells between wild-type and *ten-1* mutants at this stage (Figure 2C).

Gonadal Basement Membrane Is Not Maintained in the ten-1 Mutant

Bursting of the early gonads in the *ten-1* mutant suggested that mutant worms have defects in BM formation or maintenance. Therefore, we examined the organization of the BMs in the *ten-1(ok641)* worms using a laminin-β *LAM-1::GFP* marker and an anti-collagen IV antibody that label most BMs in worms.

At hatching, wild-type and the majority of *ten-1* mutant gonad primordia were compact and completely surrounded by laminin (Figure 3, A and B). At the L2 stage the laminin layer surrounding the developing gonad of the mutants appeared to get thinner at the dorsal side, but germ cells did not lose contacts and gonads kept their tubular shape, similarly to wild-type (Figure 3, C and D). As the gonadal precursor cells divided, a discontinuity appeared in the *ten-1(ok641)* gonadal BM that could be seen by a lack of laminin as well as of collagen IV LET-2 (Figure 3, C and E). In L3 larvae germ cells were released in the center of the mutant gonad, where there was no laminin-GFP detectable. Gonad disruption appeared always on the dorsal side, whereas gonad arms were normally covered with BM (Figure 3F). In wild-type animals the gonads re-

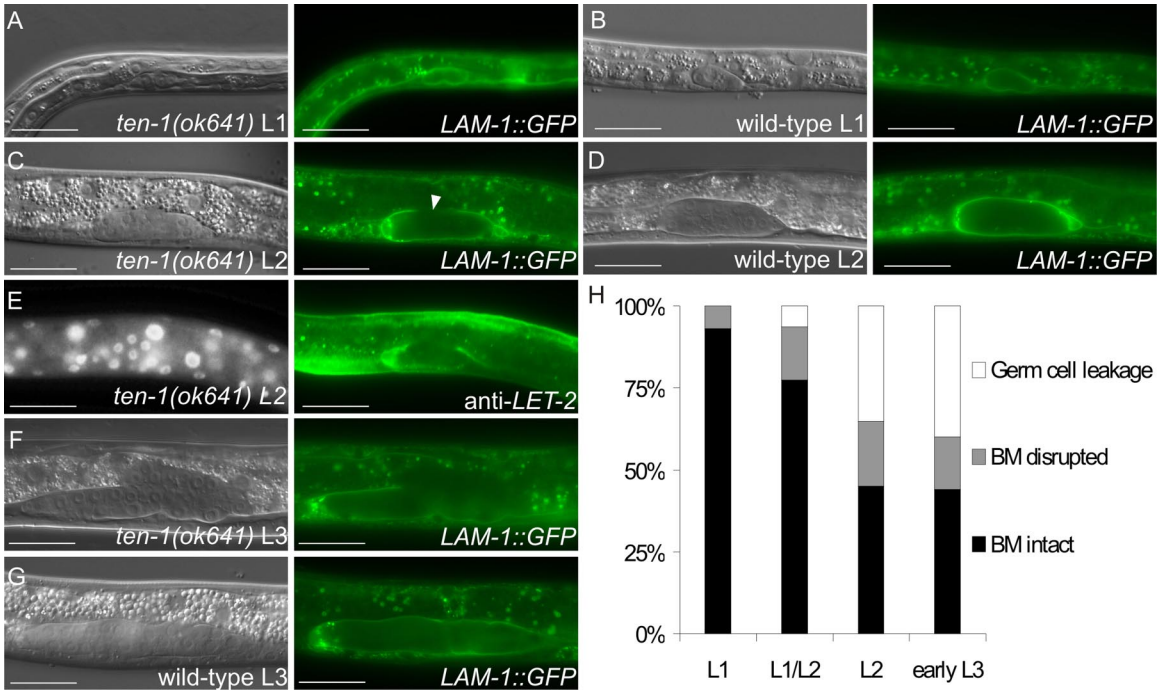

Figure 3. The basement membrane breaks on the dorsal side of the *ten-1(ok641)* gonads. Basement membranes were visualized by the *LAM-1::GFP* marker (A–D and F–G) and the anti-LET-2 immunostaining (E). The *ten-1(ok641)* L1 gonad (A), wild-type L1 (B), and L2 (D) gonads are uniformly covered by laminin. (C) In the *ten-1(ok641)* mutant, the gonadal basement membrane becomes thinner or fails to assemble correctly (arrowhead) at the L2 stage. (E) Lack of gonadal BM on the dorsal side of *ten-1(ok641)* L2 gonad is visualized by collagen IV immunostaining with anti-LET-2. (F) There is no laminin present in the center of the *ten-1(ok641)* L3 gonad. The basement membrane is absent completely, and germ cells are released. (G) The wild-type L3 gonad is entirely covered by laminin. (H) Time-course analysis of gonadal BM integrity in *ten-1(ok641)* worms carrying the *LAM-1::GFP* marker. Scale bar, 20 μm.

mained completely ensheathed by a BM (Figure 3G). A time course of the appearance and the penetrance of BM defects in young *ten-1(ok641)* larvae is summarized in Figure 3H.

Furthermore, we compared the BM ultrastructure in wild-type and *ten-1* mutant worms using transmission electron microscopy of thin sections of L3 larvae. Wild-type gonads were completely ensheathed by BM, which appeared as a thin mesh of extracellular material along the plasma membranes (Figure 4, B and B1). In *ten-1(ok641)* worms, gonads had a round shape and were entirely covered by BM in sections localized distally from the break (Figure 4C and C1). However, in the midbody region no BM was present on the dorsal side of the broken gonad and germ cells invaded the intestine (Figure 4, D and D1). In contrast, the BMs on the ventral side of the burst gonad as well as the BMs between the intestine and the hypodermis showed a wild-type ultrastructure (Figure 4, D and D2). Moreover, we did not find any whorls or clumps of extracellular material that might suggest a general defect in BM organization. Such a phenotype was described for some BM mutants such as *epi-1*, *lam-1*, or *dig-1* (Huang *et al.*, 2003; Benard *et al.*, 2006; Kao *et al.*, 2006).

In summary, the gonadal BM in the *ten-1(ok641)* hermaphrodites was properly assembled at hatching but was not maintained later in development. The localized BM deficiency could result from defects in BM assembly, stability, or protein expression.

Gonadal Defects of ten-1 Mutants Are Similar to Those Found in the Dystroglycan dgn-1, Integrin ina-1, and Laminin epi-1 Mutants

Laminins are secreted proteins that play fundamental roles in BM formation and function (Previtali *et al.*, 2003; Miner and Yurchenco, 2004). EPI-1 is one of two laminin α chains found in the *C. elegans* genome. Both *C. elegans* laminin isoforms are broadly distributed among BMs, but the gonadal BM contains the EPI-1 isoform only (Huang *et al.*, 2003). Dystroglycan and integrins, two cell surface receptors interacting with laminin, are required for BM assembly, adhesion, and signal transduction (Bokel and Brown, 2002; Higginson and Winder, 2005). In *C. elegans*, gonadal epithelialization defects were reported for the dystroglycan *dgn-1(cg121)* worms and laminin α chain *epi-1* mutants (Huang *et al.*, 2003; Johnson *et al.*, 2006). Gonads of integrin α chain *ina-1* mutants are oddly sized and show germ cell leakage, but the cause of the defects remains unknown (Baum and Garriga, 1997).

Gonads of *dgn-1* mutants and *epi-1(RNAi)* worms were variably misshapen (Figure 5, C and E), burst during development, and led to worm sterility. Early gonads of *ina-1(gm39)* worms hardly ever burst (Figure 5F) and rather seemed to be swollen in the center. However, at the L4 stage *ina-1* mutant gonads were clearly ruptured, and the germ cells clustered around the developing vulva (Figure 5G), similarly to *ten-1(ok641)* gonads (Figure 5H). Gonads of adult worms carrying the weaker *ina-1* allele, *gm144*, had enlarged arms but we did not observe any germ cell leakage (unpublished data).

We analyzed the organization of the laminin network surrounding the developing gonad in *dgn-1* mutants using the *LAM-1::GFP* marker. Although the DIC pictures of *ten-1* and *dgn-1* mutants appeared similar, *dgn-1(cg121)* hermaphrodite gonads did not have any localized breaks as did the *ten-1(ok641)* gonads. In contrast, the *dgn-1* mutant gonads were generally disorganized, and *LAM-1::GFP* seemed to be more diffuse throughout the gonadal surface in comparison to *ten-1* mutant gonads (Figure 5D). Nevertheless, gonadal defects described for *dgn-1(cg121)*, *ina-1(gm39)*, and *epi-1(RNAi)* worms resembled the defects that we observed in the *ten-1* mutants (Figure 5B), suggesting that TEN-1 could be involved in gonadal BM maintenance, together with laminin receptors INA-1 and DGN-1.

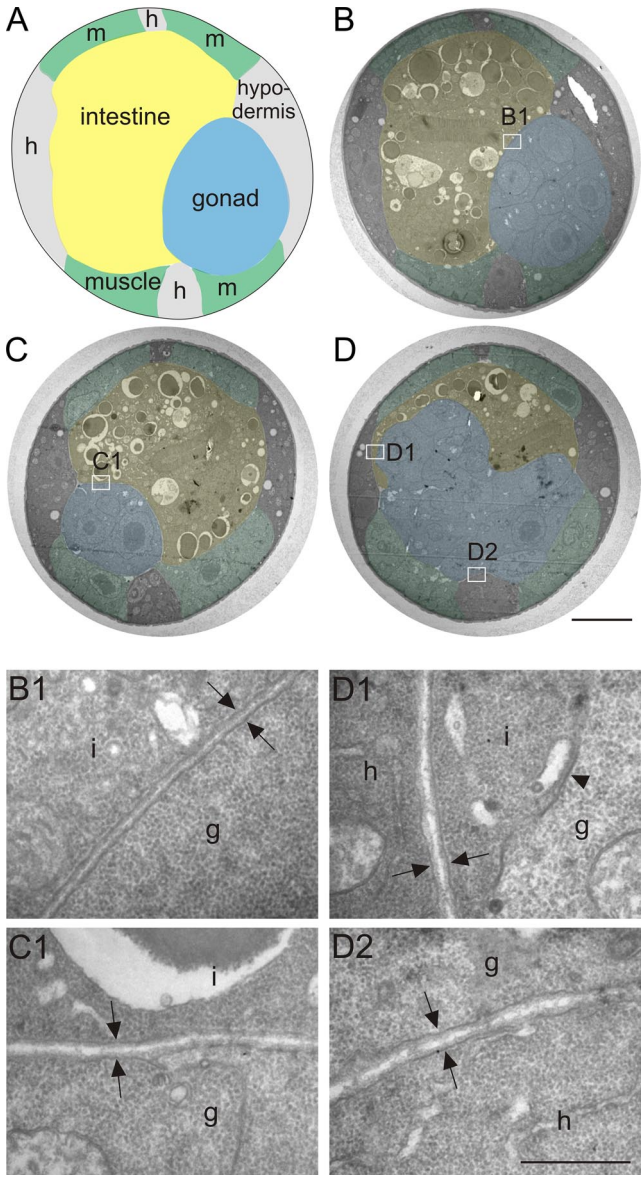

Figure 4. Basement membrane ultrastructure in *ten-1* mutant worms. Schematic cross-section through the midbody of wild-type worm (A). Transmission electron microscopy sections of a wild-type L3 (B) and a *ten-1(ok641)* mutant (C and D). Tissues are labeled as follows: blue, gonad; yellow, intestine; green, muscles; and gray/unlabeled, hypodermis . Enlargements (B1–D2) are marked on the cross-sections (B–D) with white rectangles. Morphology of wild-type BMs at the boundaries between gonad and intestine (B1, arrows). The gonadal and intestinal BM of the *ten-1* mutant appears wild-type in a section 2 μm distant from the central break (C1, arrows). In the midbody region, the mutant gonad breaks on its dorsal side, and there is no BM present between germ cells and intestine (D1, arrowhead). However, BMs between intestine and hypodermis (D1, arrows) or ventral gonad and hypodermal ridge (D2, arrows) have a normal ultrastructure. Scale bar, 5 μm (A–D) and 500 nm (B1–D2).

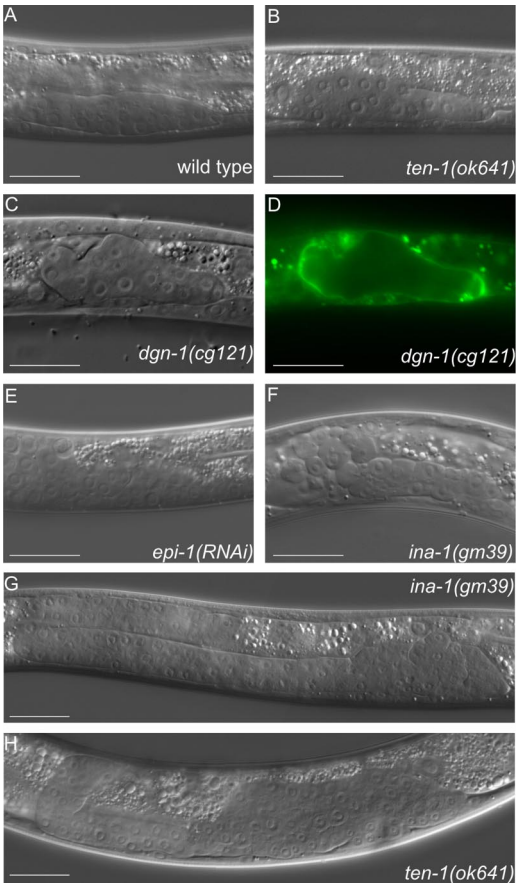

Figure 5. Misshapen gonadal primordia are found in several basement membrane mutants, i.e., dystroglycan *dgn-1*, integrin *ina-1*, and laminin *epi-1(RNAi)* worms. DIC pictures of early gonads in wild type (A), *ten-1(ok641)* (B), *dgn-1(cg121)* (C), and the corresponding *LAM-1::GFP* pattern (D), *epi-1(RNAi)* (E), *ina-1(gm39)* L2 larva (F), *ina-1(gm39)* L4 larva (G), and *ten-1(ok641)* L4 larva (H). Mutant gonads do not form a tube-like structure but grow into a disorganized mass. Scale bar, 20 μm.

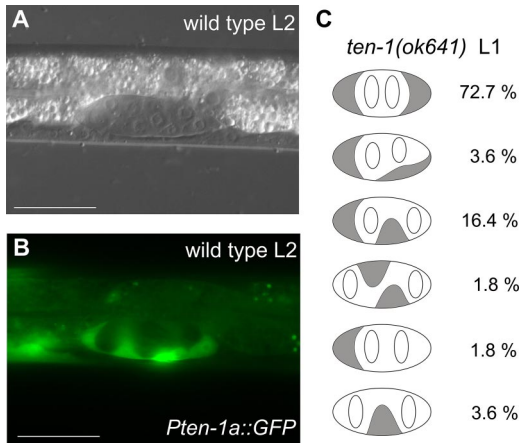

Figure 6. Teneurin is expressed in somatic cells of the early gonads and SGPs are mislocalized in the L1 gonads of *ten-1* mutants. Expression from the upstream promoter of *ten-1* is found in the SGPs of L2 gonads in wild-type worms (A and B). In C we present a schematic representation of the position of the Z1 and Z4 (gray shading) and Z2 and Z3 (white) cells in gonads of *ten-1(ok641)* L1 larvae carrying the *lag-2::gfp* marker (n = 55). We found that Z1 and Z4 cells are often mispositioned, and the percentage of animals showing the observed patterns is indicated to the right. Scale bar, 20 μm.

In early gonads TEN-1 was found to be expressed in the somatic gonad founder cells Z1 and Z4 (Drabikowski *et al.*, 2005). Also their descendants, the somatic gonad precursor cells (SGPs) during the L2 stage express TEN-1 (Figure 6, A and B). We found that Z1 and Z4 cells were often displaced from the tips of the early L1 gonads in *ten-1(ok641)* worms (Figure 6C). In almost 20% of the mutants, Z1 and/or Z4 cells interdigitated between germ cell precursors Z2 and Z3, or sometimes one of the SGPs was lost. Because the SGPs are required for the deposition of the gonad BM, the BM defects observed in the *ten-1* mutant worms might be due to the inability of the SGPs to form an intact epithelial layer around the gonad primordium. Interestingly, similar gonad epithelialization defects were reported for *dgn-1* and *epi-1* mutant worms (Johnson *et al.*, 2006).

ten-1 Is Synthetic Lethal with dgn-1, ina-1, epi-1, and nid-1

The similar gonadal phenotypes of *ten-1*, *dgn-1*, and *ina-1* mutants and *epi-1(RNAi)* worms suggested that TEN-1 could act in a parallel pathway and have a partly redundant function to dystroglycan and/or integrin receptors. To asses the interaction between *ten-1* and genes encoding various BM components, we constructed double mutant combinations. In the crosses we used *ten-1(ok641)* and *dgn-1(cg121)* null alleles, the weak *ina-1* hypomorphic allele *gm144*, and an RNAi approach in the case of *epi-1*.

To analyze the genetic interaction network of *ten-1* further, we included additional genes encoding BM proteins, namely nidogen *nid-1*, perlecan *unc-52*, and collagen XVIII *cle-1*. *C. elegans* nidogen deletion does not affect BM assembly (Kang and Kramer, 2000), but *nid-1* mutants show defects in neuromuscular junction organization (Ackley *et al.*, 2003) and axonal tract positioning (Kim and Wadsworth, 2000). Interestingly, the *nid-1(cg119)* null mutant was found to be synthetic lethal with *dgn-1* as a result of pharyngeal defects (J. M. Kramer, personal communication). Mutation *e444* in the perlecan *unc-52* gene causes progressive paralysis in worms as well as gonad disorganization and germ cell release into the body cavity (Gilchrist and Moerman, 1992). Loss-of-function mutation in the collagen *cle-1* gene leads to cell migration and axon guidance defects. Some *cle-1(cg120)* mutant larvae are unable to pump and arrest at the L1 stage with misshapen pharynges (Ackley *et al.*, 2001).

Interestingly, we observed more severe phenotypes in several double mutants than in any single mutant alone (Table 3). Synthetic lethality was found in *ten-1(ok641);dgn-1(cg121)*, *ten-1(ok641);ina-1(gm144)*, *ten-1(ok641);nid-1(cg119)* double mutants, and *ten-1(ok641);epi-1(RNAi)* worms. Lack of dystroglycan or nidogen in the *ten-1* mutant background led to developmental arrest during late embryogenesis or L1 larval stage in almost 100% of worms. Double-mutant larvae were translucent suggesting a feeding defect. Morphological defects found in *epi-1* deficient worms (Figure 7C) were enhanced by *ten-1* deletion. More than 90% of *ten-1(ok641); epi-1(RNAi)* animals arrested during embryogenesis or as early larvae and showed dramatic disorganization of developing tissues (Figure 7E). Moreover, *ten-1;ina-1* mutants showed severe morphological defects not found in any single mutant alone (Figure 7, B and D), and nearly 100% of double mutant worms arrested as disorganized embryos or L1 larvae (Figure 7F).

Table 3. *ten-1* is synthetic lethal with *dgn-1, ina-1, epi-1,* and *nid-1*

Genotype	Embryonic lethality	Larval arrest	Sterile and/or vulva defects[a]	Fertile adults	n
Wild type	0.9	0	0	99.1	321
Ten-1(ok641)	6.4	32.1	16.7	44.8	346
dgn-1(cg121)	5.4	2.2	92.4	0	92
Ten-1(ok641);dgn-1(cg121)	14.0	84.2	1.8	0	57
Ina-1(gm144)	10.0	30.6	23.5	35.9	170
ten-1(ok641);ina-1(gm144)	12.9	85.7	1.4	0	70
nid-1(cg119)	4.2	7.3	0.3	88.2	765
ten-1(ok641);nid-1(cg119)	34.7	65.2	0	0	88
epi-1(RNAi)	17.1	29.5	53.4	0	442
ten-1(ok641);epi-1 (RNAi)	48.5	44.2	7.3	0	293
cle-1(cg120)	0.7	0.7	1.1	97.5	283
ten-1(ok641);cle-1(cg120)	3.4	24.5	23.1	49.0	147
unc-52(e444)	3.5	1.5	5.6	89.4	198
ten-1(ok641);unc-52(e444)	4.8	21.5	36.5	37.2	293

Percentage of wild-type and mutant worms (single and double mutants) showing the following phenotypes: embryonic lethality, larval arrest, sterility or vulval defects, and wild-type fertile adults.

[a] The "Vulva defects" category includes protruding vulva and bursting-at-the-vulva phenotypes.

In contrast, mutations in *unc-52* or *cle-1* did not cause synthetic lethality in the *ten-1* mutant background. These two mutations did not enhance embryonic lethality, larval arrest, or sterility of the *ten-1(ok641)* worms. However, we cannot exclude that *unc-52* and *cle-1* interact genetically with *ten-1* in other processes, such as axon guidance or distal tip cell migration.

Teneurin Functions with Nidogen and Dystroglycan in Pharynx Development

Because larval arrest was significantly increased in several double mutants, we decided to investigate the phenotypes of the starved L1 larvae of *ten-1;dgn-1* and *ten-1;nid-1* double mutants, suspecting that these three proteins could have an important role in pharyngeal morphogenesis. This hypothesis was supported by the fact that the expression of the long TEN-1 isoform rescued the larval arrest phenotype of *ten-1* mutants (Table 1) and *GFP::TEN-1L* was detectable in the developing pharynx (Figure 8, A and B) until adulthood (Figure 8, C and D). As 30% of *ten-1* single mutant worms arrest as L1 translucent larvae, we examined their pharyngeal defects. The wild-type foregut is a short tube, with two bulbs, surrounded by a thick BM (Figure 9, A and B). As viewed by DIC microscopy, *ten-1*–arrested larvae had variably misshapen pharynges, and the outline of the pharynx was often barely visible (Figure 9C). In addition, we examined the pharyngeal BM organization with the *LAM-1::GFP* marker and found that it seemed to be disordered and missing in some parts of the pharynx (Figure 9D).

In contrast to *ten-1* mutant worms, only a low percentage of *dgn-1* and *nid-1* single mutants arrested during larval stages. Pharynges of *dgn-1* larvae showed mostly wild-type appearance (Figure 9E), whereas the few *nid-1* arrested larvae had a bend in the anterior-most part of their foregut (Figure 9F). Another phenotype found at low penetrance in the *nid-1* single mutant was pharynx unattached (Pun), where the pharyngeal epithelium did not connect to the arcade cells of the hypoderm.

Removal of *dgn-1* in the *ten-1* mutant background enhanced the defects found in the *ten-1* single mutant and double mutants of *ten-1;dgn-1* arrested as larvae with their pharynges variably misshapen (Figure 9G). Interestingly,

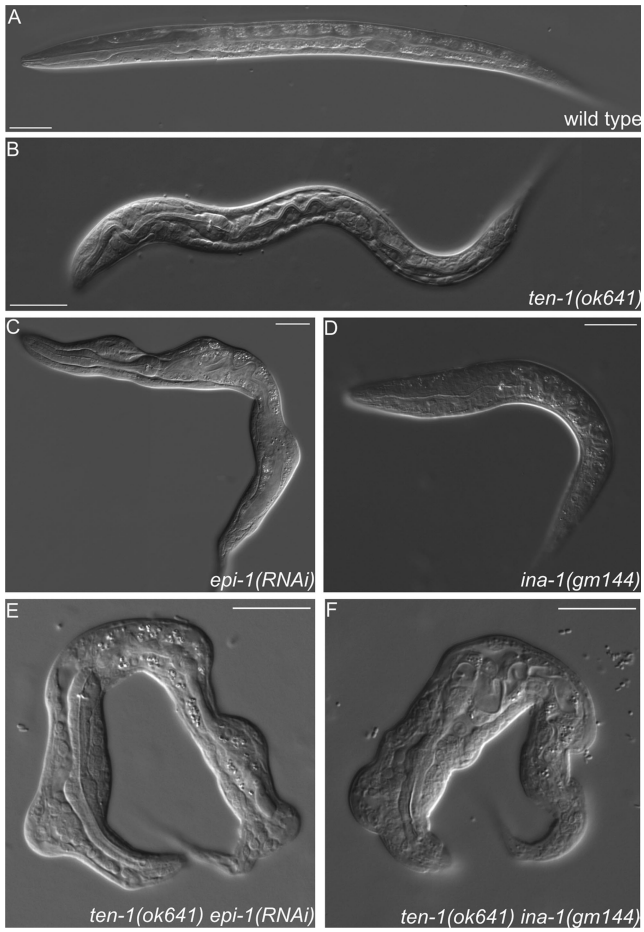

Figure 7. Morphological defects found in *epi-1(RNAi)* worms, *ten-1(ok641); epi-1(RNAi)* animals, and *ten-1; ina-1* double mutants. Wild-type (A) and *ten-1(ok641)* L1 larvae (B). *epi-1*-depleted worms are often misshapen, but defects are relatively mild (C). Arrested larva of *ina-1(gm144)* mutant (D). Morphological defects of *epi-1(RNAi)* worms were enhanced by *ten-1(ok641)* deletion and caused deformation of the entire body in the arrested larvae (E). Similar defects were found in *ten-1(ok641);ina-1(gm144)* double mutants (F). Severity and penetrance of the defects were greatly enhanced in the double mutants compared with single mutants. Scale bar, 20 μm.

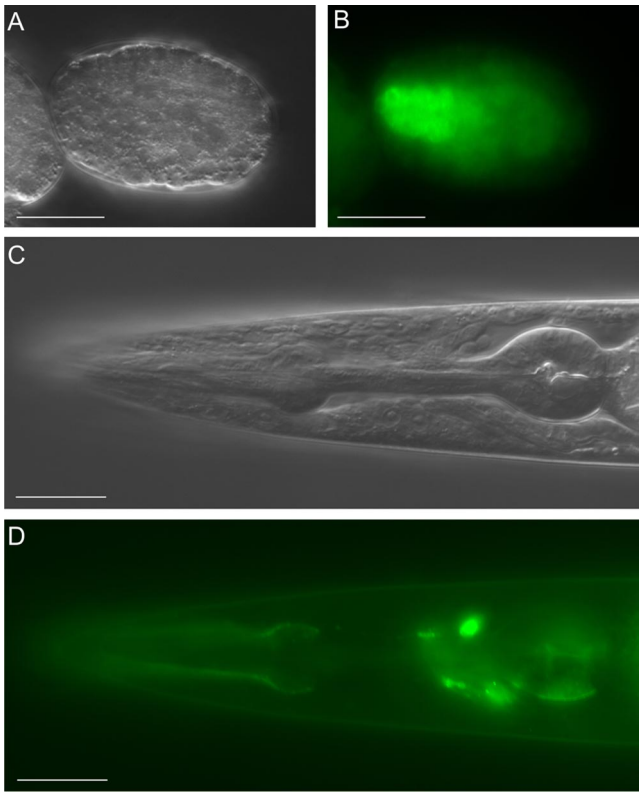

Figure 8. The long TEN-1 isoform is expressed in the developing and adult pharynx. The GFP::TEN-1 transgene (*kdEx121)* is expressed in the developing pharynx of the early embryo (A and B) and outlines the adult pharynx (C and D). Expression of the *kdEx121* is also found in some head neurons (D). Scale bar, 20 μm.

ten-1;nid-1 double mutants arrested as larvae that were unable to feed because their pharynges were not attached to the lips (Figure 9H).

In summary, our data suggest that *ten-1* and *dgn-1* act redundantly in pharyngeal morphogenesis and/or function. Moreover, both *ten-1* and *dgn-1* caused synthetic lethality in the *nid-1* mutant background, implying an important role for these two receptors in the process of pharyngeal attachment.

DISCUSSION

Function of TEN-1 in Somatic Gonad Precursor Cells

We found that TEN-1 is essential for the maintenance of the BM early in development of the gonads in *C. elegans*. The BM surrounding the gonad was formed properly at hatching but during larval development ruptured at a very specific location on the dorsal side in the middle of the gonad. The *ten-1a* promoter is active in the SGPs of L1-L2 larvae and RNAi specific for the TEN-1 long variant is known to cause gonadal disorganization (Drabikowski *et al.,* 2005). Therefore, SGPs may play an important role in extracellular matrix production or BM assembly by expression of specific receptors that organize extracellular matrix proteins provided by adjacent tissue. Such a mechanism has been described for several BM proteins. Graham *et al.* (1997) showed that type IV collagen is assembled on tissues that do not express it themselves, and they postulated that these tissues express receptors that facilitate collagen IV assembly. Another example is fibulin-1, which is secreted by the intestine and deposited on the gonadal surface (Muriel *et al.,* 2005). Also in the case of laminin isoforms it was suggested that their differential distribution is at least partly based on differential assembly mediated by cell surface receptors (Huang *et al.,* 2003). TEN-1 could be a novel receptor promoting BM assembly in the gonad. Another possibility is that teneurin is essential for SGPs polarization, adhesion, or migration. We showed that the position of Z1 and Z4 cells is often altered in the early gonads of *ten-1* mutants. This could indicate that TEN-1 plays an important role in SGPs at the early stages of gonad epithelialization and that gonadal BM discontinuity is the consequence of somatic cell mispositioning.

Teneurin Acts Redundantly with BM Receptors Integrin and Dystroglycan

Mutants in the *ten-1*, *ina-1* and *dgn-1* genes share several phenotypic features, including gonad disorganization, protruding vulva, defasciculation of the ventral nerve cord, distal tip cell migration, and axonal guidance defects (Baum and Garriga, 1997; Drabikowski *et al.,* 2005; Johnson *et al.,* 2006; Meighan and Schwarzbauer, 2007). Double mutants between *ten-1*, *ina-1,* and *dgn-1* showed synergistic genetic interaction implying that these three genes act in similar developmental processes and have partly redundant function. However, the mechanism of teneurin signaling remains unclear. Related roles of these receptors in gonad development could not be directly assessed because of functional redundancy in other developmental processes, i.e., pharyngeal or hypodermal morphogenesis.

Although *dgn-1* mutants do not show any obvious pharyngeal defects, arrested larvae of *ten-1;dgn-1* worms were translucent with misshapen pharynges. This suggests that there is compensation between *ten-1* and *dgn-1* in pharynx development and function. Interestingly, lack of the *ten-1* gene in *nid-1* mutant worms had the same effect as the removal of *dgn-1* in the *nid-1* mutant background (J. M. Kramer, personal communication), and both double mutants show a Pun phenotype. Therefore, loss of teneurin or dystroglycan sensitizes the worms strongly to loss of nidogen, which confirms the functional redundancy between *ten-1* and *dgn-1*.

Furthermore, *ten-1;ina-1* mutants were synthetic lethal and arrested as embryos or early larvae, frequently with severe morphological defects. Integrin loss-of-function mutants *ina-1(gm39)* show malformation of the anterior hypoderm, manifesting as a notched-head phenotype (Baum and Garriga, 1997), whereas *ten-1* mutants have low penetrance morphological defects in the posterior body (Drabikowski *et al.,* 2005). Combination of mutations in both genes resulted in worms arrested as L1 larvae with the entire body deformed. Mosaic analysis revealed that INA-1 is important in hypodermis (Baum and Garriga, 1997), and TEN-1S is known to be expressed in hypodermal cells of the developing embryo (Drabikowski *et al.,* 2005). Therefore, mild defects found in single mutants may be due to compensation by activity in a parallel pathway. This strongly suggests that *ina-1* and *ten-1* could act together in several developmental processes, including hypodermal morphogenesis.

In summary, TEN-1, INA-1, and DGN-1 are not required for BM function in general, but they are crucial in particular tissues and organs such as the gonad, pharynx, or hypodermis. The lack of a phenotype in all BMs could also reflect redundancy between these three receptors, where deletion

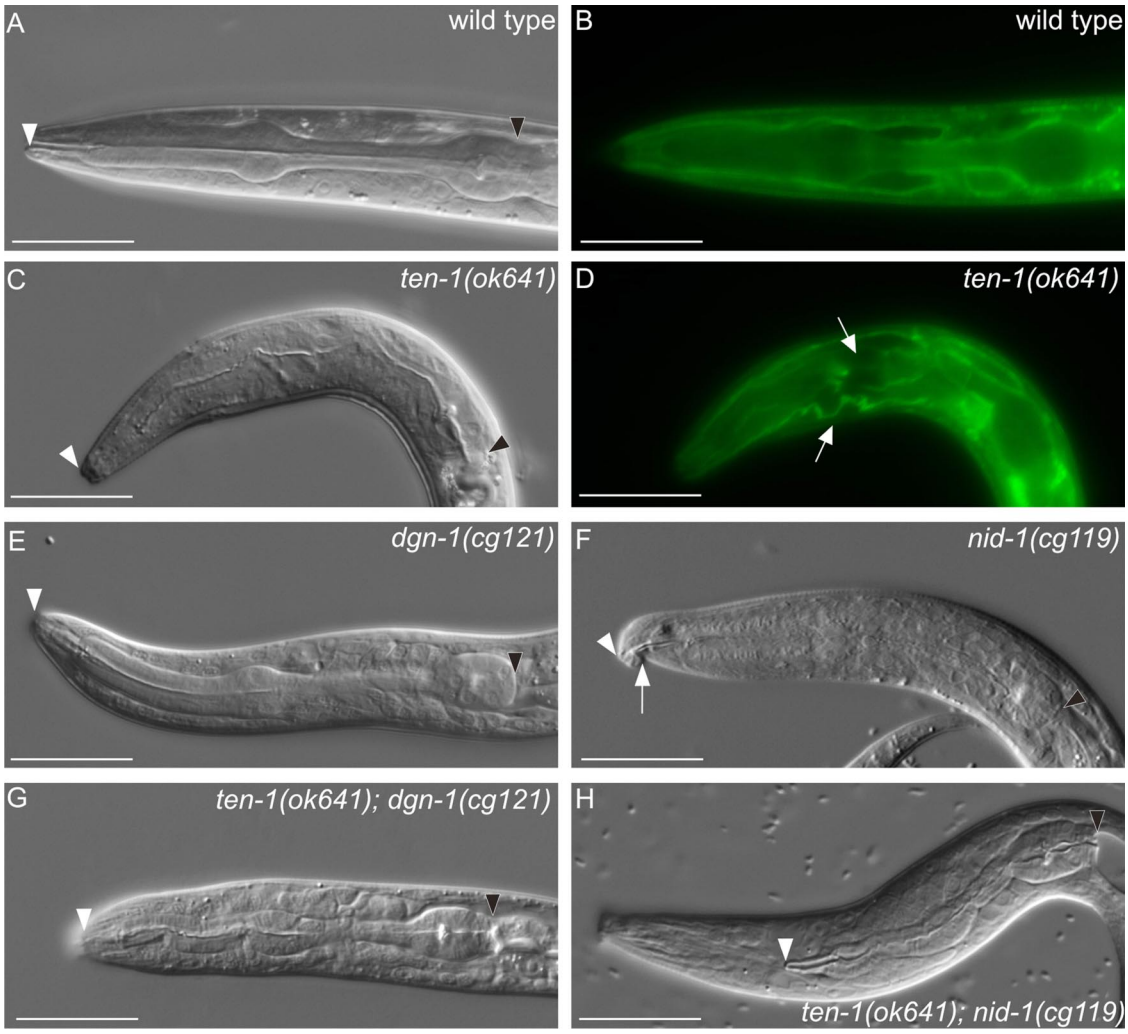

Figure 9. Pharyngeal defects in *ten-1*, *nid-1*, and *dgn-1* single and double mutants. Pharynx morphology of L1 larva is shown. *LAM-1::GFP* marker labels the pharyngeal basement membrane. Wild-type pharynx is outlined by a sharp DIC boundary visible by DIC microscopy (A). Basement membrane organization in the wild-type larva visualized by *LAM-1::GFP* (B). Arrested larvae of *ten-1(ok641)* mutant have misshapen pharynges and the pharyngeal outline is invisible on DIC pictures (C). In the *ten-1* mutant, the basement membrane around the pharynx is disorganized or missing in some parts (arrows) (D). The pharynx of the *dgn-1* mutant worms shows no obvious defects (E). Arrested larvae of *nid-1* mutants have sometimes bent pharynges (arrow) (F) or their pharynges do not attach to the hypoderm (similar to the double mutant shown in H). Variably misshapen pharynges were found in the *ten-1;dgn-1* double mutants (G). An unattached pharynx (Pun) phenotype observed in *ten-1;nid-1* double mutants (H). White arrowheads mark the anterior and black arrowheads posterior ends of the pharynges. Scale bar, 20 μm.

of a single gene can be compensated for by the presence of other receptors.

ten-1 Is Synthetic Lethal with Genes Encoding Two BM Proteins: Laminin and Nidogen

The *ten-1* mutant phenotype resembled in many aspects the phenotypes of *epi-1* as well as the laminin binding receptors *ina-1* and *dgn-1*. Laminin *epi-1* mutants are generally sick and show cell polarization defects, tissue disorganization, and physical disruption of BMs (Huang *et al.*, 2003). Similar phenotypes have been described for laminin β loss-of-function mutants, *lam-1(rh219)* (Kao *et al.*, 2006). Thus, mutations in the laminin genes cause more severe defects than *dgn-1* or *ina-1* single mutants, suggesting that these two receptors might be functionally redundant or that additional laminin receptors exist. Mutation in the *ten-1* gene strongly enhanced the effects of *epi-1* depletion by RNAi, leading to almost complete lethality of *ten-1(ok641);epi-1(RNAi)* worms. This result suggests that EPI-1 could be a ligand for TEN-1; however, direct interaction between TEN-1 and EPI-1 needs confirmation by further biochemical studies.

C. elegans nidogen is found in most BMs (Kang and Kramer, 2000), but loss of *nid-1* alone causes very mild defects, mainly in the nervous system (Kim and Wadsworth, 2000; Ackley *et al.*, 2003). The defects are, however, dramatically enhanced, if a *nid-1* deletion is combined with a mutation in teneurin, BM receptor *dgn-1,* or axon guidance molecules such as the *sax-3* Robo receptor or the *unc-40* netrin receptor (J. M. Kramer, personal communication). In such sensitized backgrounds, lack of *nid-1* causes a highly penetrant Pun phenotype. It appears that correct attachment of pharyngeal epithelium to arcade cells requires several receptors and guidance molecules as well as nidogen. Currently, NID-1 is considered more as a regulatory molecule

(Kim and Wadsworth, 2000; Hobert and Bulow, 2003) rather than being a purely structural component linking laminin and collagen networks (Fox *et al.*, 1991).

Conservation in Higher Organisms

Our data provide the first indication of a link between TEN-1 and BM function. There is little evidence from previous studies in vertebrates suggesting that teneurins could be BM receptors. However, in retrospect, the finding that induction of filopodia formation in neuroblastoma cells by teneurin-2 depends on the substrate and is more prominent on laminin than on poly-L-lysine (Rubin *et al.*, 1999) may reflect a direct interaction between these proteins. Furthermore, chicken teneurin-2 was found to colocalize with laminin in BMs of the optic cup and the heart endocardium (Tucker *et al.*, 2001).

Teneurin-3 knockout mice show defects in the positioning of specific visual circuits, leading to impaired binocular vision (Leamey *et al.*, 2007). Such a mild phenotype in the single mutant might be due to functional redundancy with other teneurins or, in the context of the present study, with BM receptors like integrins and dystroglycan. Our studies in *C. elegans* could be instructive for further analyses of teneurins, integrins, or dystroglycan function in vertebrates, because they point out redundancy not only between several receptors of the same family but also between structurally distinct receptor families.

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