

MIG-17/ADAMTS controls cell migration by recruiting nidogen to the basement membrane in *C. elegans*

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Mutations in the *a* disintegrin and metalloprotease with thrombospondin motifs (ADAMTS) family of secreted proteases cause diseases linked to ECM abnormalities. However, the mechanisms by which these enzymes modulate the ECM during development are mostly unexplored. The *Caenorhabditis elegans* MIG-17/ADAMTS protein is secreted from body wall muscle cells and localizes to the basement membrane (BM) of the developing gonad where it controls directional migration of gonadal leader cells. Here we show that specific amino acid changes in the ECM proteins fibulin-1C (FBL-1C) and type IV collagen (LET-2) result in bypass of the requirement for MIG-17 activity in gonadal leader cell migration in a nidogen (NID-1)-dependent and -independent manner, respectively. The MIG-17, FBL-1C and LET-2 activities are required for proper accumulation of NID-1 at the gonadal BM. However, mutant FBL-1C or LET-2 in the absence of MIG-17 promotes NID-1 localization. Furthermore, overexpression of NID-1 in *mig-17* mutants substantially rescues leader cell migration defects. These results suggest that functional interactions among BM molecules are important for MIG-17 control of gonadal leader cell migration. We propose that FBL-1C and LET-2 act downstream of MIG-17-dependent proteolysis to recruit NID-1 and that LET-2 also activates a NID-1-independent pathway, thereby inducing the remodeling of the BM required for directional control of leader cell migration.

ECM | fibulin-1 | organogenesis | type IV collagen

The interaction between basement membranes (BMs) and migrating cells or the epithelial layer is a complicated but carefully controlled process that involves remodeling of the ECM. For example, the migration of border cells required for oogenesis and patterning of the early embryo in *Drosophila melanogaster* is accompanied by precise regulation of the synthesis and degradation of type IV collagen, laminin, and perlecan (1, 2). Fibronectin expression is required for branching morphogenesis of the submandibular salivary gland, lung, and kidney in mouse (3). However, the mechanisms of cell-ECM interactions and the roles of BMs in cell migration remain largely unknown.

Gonadogenesis in the nematode *Caenorhabditis elegans* serves as a simple model system for elucidating the function of BMs in organ morphogenesis. The development of hermaphrodite gonads is regulated by the migration of gonadal distal tip cells (DTCs), which promote directional elongation of the gonad arms during the larval stages to form the U-shaped gonads found in adult animals (4, 5). Two secreted *a* disintegrin and metalloprotease with thrombospondin motifs (ADAMTS) family metalloproteases, MIG-17 and GON-1, are involved in this process. GON-1 is required for DTC motility, whereas MIG-17 controls the direction of DTC movement but does not control DTC motility per se (6, 7). MIG-17 is expressed in the body wall muscles and is localized to the BM of the gonad surface, where it is required for DTC migration (7, 8). It has been proposed that MIG-17 and GON-1 remodel BMs via proteolysis (6, 7). Dominant mutations in *fbl-1*, which encodes fibulin-1 (FBL-1), an ECM protein, can bypass the requirement for MIG-17 activity in DTC migration (9). Furthermore, *fbl-1* deletion

suppresses the gonadal elongation defects of *gon-1* null mutants (10). These observations suggest that mutation of FBL-1 or depletion of FBL-1 may give rise to alterations in BM ECM architecture that mimic the downstream events normally elicited by MIG-17 or GON-1 activities, respectively.

In this study, we analyzed a novel genetic locus, *saf-2* [suppressor of *a* disintegrin and metalloprotease (ADAM) family defect], in which mutations act as dominant suppressors of DTC migration defects in *mig-17* mutants. *saf-2* was found to be equivalent to *let-2*, which was previously identified by lethal mutations and encodes the type IV collagen $\alpha 2$ chain (11). Two suppressor *let-2* mutations were identified in the C-terminal region of LET-2, one in the noncollagenous (NC1) domain and the other in a Gly-X-Y repeat just upstream of the NC1 domain. *let-2*; *fbl-1* double mutants exhibited severe gonadal defects that were not observed in either single mutant, revealing a synthetic interaction between these independently identified suppressor genes. Interestingly, *fbl-1* mutants suppressed *mig-17* in a *nid-1*-dependent manner, whereas *let-2* mutants suppressed *mig-17* in a *nid-1*-independent manner. Genetic analysis of protein localization to the gonadal BM revealed that MIG-17 is required for active accumulation of FBL-1C, which contributes to NID-1 localization. Our findings suggest that intermolecular interactions among FBL-1C, NID-1, and LET-2 are involved in MIG-17/ADAMTS-dependent regulation of gonadal leader cell migration.

Results

Mutations in the Type IV Collagen $\alpha 2$ Chain Suppress DTC Migration Defects in *mig-17* Mutants. Mutations in *mig-17* resulted in misshapen gonads due to misdirected migration of the gonadal DTCs (Fig. 1 *A* and *B*). To identify molecules that interact with MIG-17 to regulate DTC migration, we used ethylmethane sulfonate mutagenesis to isolate suppressors of *mig-17(k174)*, a null allele containing a nonsense mutation. The suppressor mutations *let-2(k193)* and *let-2(k196)* were dominant and strongly suppressed the DTC migration defects in homozygotes and heterozygotes (Fig. 1 *C*, *D*, and *F*). *mig-17(k174)* single mutants show gonad morphogenesis defects, whereas *mig-17*; *let-2(k193)* and *mig-17*; *let-2(k196)* double mutants mostly had U-shaped gonad arms similar to those in WT animals, although the gonads were slightly thicker and shorter than in the WT (Fig. 1 *C*, *D*, and *F*). *let-2* encodes LET-2A

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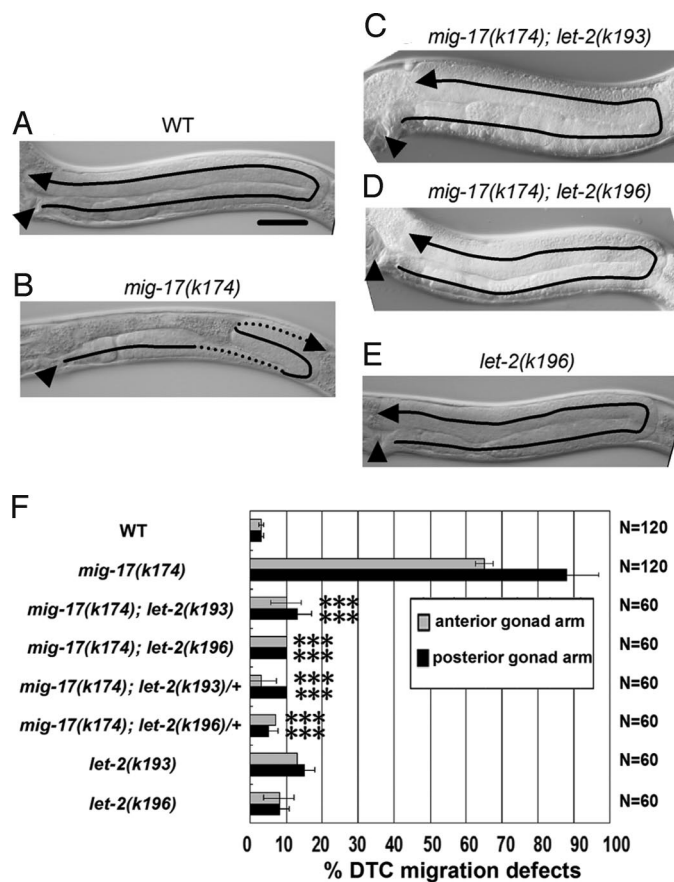


Fig. 1. Gonadal phenotypes of the WT and mutants. Anterior is left and dorsal is up. Posterior gonads of WT (A), *mig-17(k174)* (B), *mig-17(k174); let-2(k193)* (C), *mig-17(k174); let-2(k196)* (D) and *let-2(k196)* (E) hermaphrodites are shown. The predicted migratory routes of DTCs are indicated by arrows. The stippled lines in (B) indicate that the corresponding regions of the gonad arm are out of focus. Arrowheads point to the vulvae. (Scale bar, 50 μ m.) (F) Percentages of anterior and posterior gonad arms with abnormal DTC migration. Error bars represent the mean SD. Results for Fisher's exact test against *mig-17(k174)* are indicated: ***, $P < 0.001$; NS, not significant.

and LET-2B, two spliced isoforms of the $\alpha 2$ chain of type IV collagen (12). The *k193* and *k196* mutants contained amino acid substitutions in the NC1 domain and Gly-X-Y repeats of LET-2, respectively [supporting information (SI) Fig. S1]. The serine mutated in *k193* is evolutionarily conserved between the *C. elegans* and human $\alpha 2$ chains, suggesting its functional importance in type IV collagen (Fig. S1A).

Because the conventional *let-2* mutations are temperature-sensitive (ts) embryonic or larval lethal (12), we examined these phenotypes in the suppressor *let-2* mutants. We found that *k193* has weak nonconditional embryonic lethality and *k196* has ts embryonic and larval lethality (Table S1). The suppressor *let-2* mutants showed minimal DTC migration defects by themselves (Fig. 1 E and F). To analyze the nature of the mutant LET-2 proteins, we examined the effect of transgenic extrachromosomal arrays containing multiple *let-2* genes on the suppression of DTC migration defects when introduced into *mig-17(k174)* mutants (Fig. S1B). Although WT *let-2* arrays weakly suppressed *mig-17* defects in the anterior gonads, mutant *let-2* transgene arrays comprised of the *k193* and *k196* mutations strongly suppressed *mig-17* defects (Fig. S1B). These results suggest that the suppressor *let-2* mutations are gain-of-function mutations that bypass the MIG-17 requirement for control of DTC migration.

We also investigated the effects of known *let-2* mutations on

gonad morphogenesis in *mig-17* mutants. Two ts lethal alleles, *g25* and *b246* (12), suppressed the DTC migration defects very weakly at 20 $^{\circ}$ C (Table S2). When animals were shifted from 20 $^{\circ}$ C to 22.5 $^{\circ}$ C at the L2 stage, *b246* and *g25* partially suppressed the posterior gonadal defects of *mig-17*. Very weak suppression was observed in L3 stage shifted animals (Table S3). Shifting to 25 $^{\circ}$ C resulted in severe gonadal defects, which precluded the analysis of *mig-17* suppression. To examine whether reduction of type IV collagen in the BM affects the suppression, we depleted a single copy of *emb-9/* $\alpha 1$ chain of type IV collagen using the null allele *emb-9(g23cg46)* (13). We found that *g23cg46/+* partially suppressed *mig-17(k174)* (Fig. S1C). These results suggest that a defect in or reduction of type IV collagen suppresses the DTC migration defect of *mig-17* mutants.

Expression of Suppressor LET-2 Proteins. The LET-2 protein is secreted from the body wall muscle cells and localizes to the BM. We examined whether suppressor LET-2 mutant proteins localize to the BM. An Ab against LET-2 clearly stained cross-sections of gonadal and intestinal BMs of WT and *mig-17* hermaphrodites (Fig. 2 A and B). The Ab similarly stained BMs of *let-2(k193)* and *let-2(k196)* mutants at 20 $^{\circ}$ C although weak accumulation of LET-2 proteins was detectable in the cytoplasm of the muscle cells (Fig. 2 C and D). BM localization was also observed in *let-2(b246)* mutants (13), but less LET-2 protein localized in *let-2(b246)* mutants than in the suppressor *let-2* mutants grown at 20 $^{\circ}$ C (Fig. 2 C, D, and F) or 25 $^{\circ}$ C (Fig. 2 E and G). In contrast, accumulation of LET-2 proteins in the muscle cells in *let-2(b246)* mutants (13) was much greater than in the suppressor mutants (compare Fig. 2 C, D, and F with E and G). Because LET-2 is highly expressed in DTCs (14), we also examined LET-2 expression in DTCs in animals shifted to 25 $^{\circ}$ C. As in the case for the muscle cells, some accumulation of mutant LET-2(*k193*) and LET-2(*k196*) proteins was observed, although no LET-2 accumulation was detected in WT DTCs (Fig. S2).

Genetic Interactions Between Suppressor Alleles of *let-2* and *fbl-1*. We previously showed that specific amino acid substitutions in the BM protein FBL-1C can suppress *mig-17* mutations in a dominant gain-of-function manner (9)—a mode of suppression reminiscent of that observed in the *let-2* suppressors. Therefore, we tested potential genetic interactions among *let-2*, *fbl-1*, and *mig-17* mutants by constructing double and triple mutants. We used two *fbl-1* alleles, *k201* and *k206*. When combined with *let-2* or *let-2*; *mig-17* double mutants, they showed strong gonad morphogenesis defects that were not observed in either the *fbl-1* or the *let-2* single mutants. The gonad arms of the double and triple mutants were about 1.5- to 2-fold thicker than those of WT. In particular, the DTCs of the anterior gonad arms often ceased migration shortly after the first or second turn, which resulted in severe swelling of the proximal part of the gonad arms (Fig. S3).

We next examined whether *fbl-1(k201)* and *let-2(k196)* interacted genetically with mutations in other BM proteins. *fbl-1(k201)* and *let-2(k196)* enhanced the DTC migration defect of *unc-52(e1421)/perlecan* but not of *cle-1(cg120)/typeXVIII collagen* (Fig. S4). A weak allele of *epi-1/laminina*, *gm57* was also enhanced by *let-2(k196)* (Fig. S4). These results suggest that the gonadal BMs in *let-2* and *fbl-1* mutants are partially compromised and that LET-2 and FBL-1 act together in the gonadal BM to achieve proper morphogenesis of the gonad.

NID-1 Dependency of Suppression. Mammalian nidogen is known to specifically bind to type IV collagen in vitro (15). We therefore examined whether suppression of *mig-17* mutations by mutant LET-2 proteins depends on NID-1. *C. elegans* has a single nidogen gene and a null mutation in this gene, *nid-1(cg119)* (16), showed only weak DTC migration defects by itself (Fig. 3 A and B and Fig. S5A). *nid-1(cg119)* enhanced the DTC migration

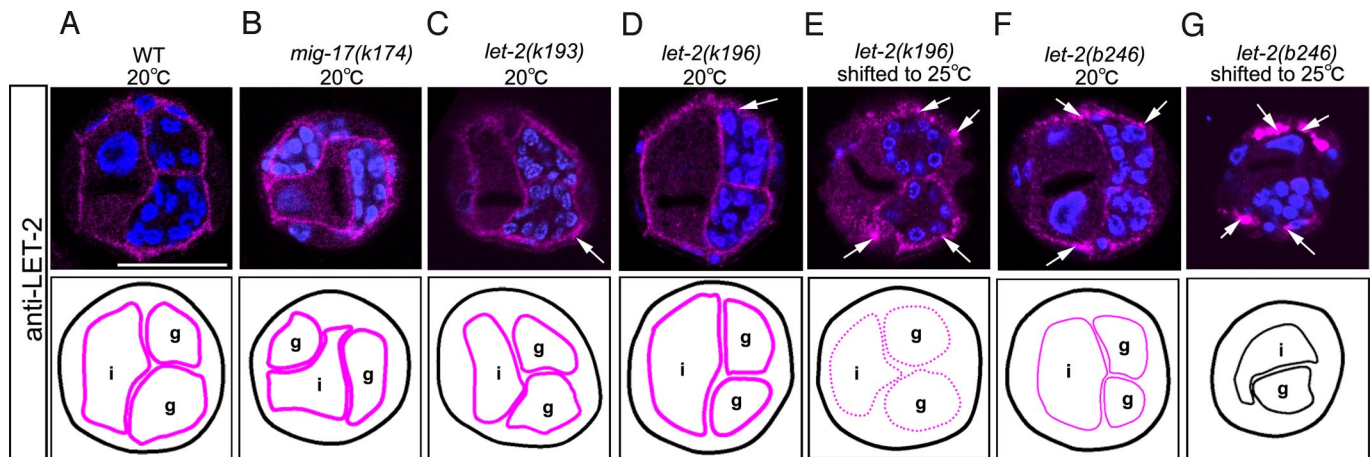


Fig. 2. LET-2 localization to the gonadal BM. Localization of LET-2 proteins in WT (A), *mig-17* (B) and *let-2* (C–G) animals. Cross-sections of L4 hermaphrodites were stained with anti-LET-2 (magenta) and DAPI (blue). Arrows indicate accumulation of LET-2 proteins in the muscle cells. Borders of the gonads and intestines are illustrated below the photos. Levels of LET-2 localization to the BM are indicated in magenta by thick lines (normal levels), thin lines (low levels), and dotted lines (very low levels). Black lines indicate undetectable levels of protein. g, gonad; i, intestine. (Scale bar, 25 μ m.) The accumulation of LET-2(k196) in the muscle cells was higher at 25 $^{\circ}$ C than at 20 $^{\circ}$ C. The result of *let-2*(k193) shifted to 25 $^{\circ}$ C was similar to (C).

defect in *mig-17*(k174) mutants, suggesting that the functions of *mig-17* and *nid-1* are partially redundant with respect to control of DTC migration (Fig. S5A). *nid-1*(cg119) did not enhance the weak DTC migration defects of the *let-2* mutants (Fig. S5B). The introduction of *nid-1*(cg119) into the *mig-17*(k174); *let-2*(k193) or *mig-17*(k174); *let-2*(k196) backgrounds did not influence the suppressive activity of the *let-2* mutations, indicating that the suppression by mutant LET-2 proteins does not depend on NID-1 (Fig. 3 D and E and Fig. S5B).

Because human fibulin-1 has also been reported to physically interact with nidogen in vitro (17), we examined whether *mig-17* suppression by the FBL-1C mutant proteins requires NID-1. We introduced *nid-1*(cg119) into the *mig-17*(k174); *fbl-1*(k201) or *mig-17*(k174); *fbl-1*(k206) backgrounds. Interestingly, we observed that suppression of DTC migration defects by the *fbl-1* mutations was severely impaired in the absence of NID-1, in contrast to that observed for the *let-2* mutations (Fig. 3 F and G and Fig. S5C). The triple mutants exhibited meandering DTC migration phenotypes similar to those observed in *mig-17* (Fig. 3C). To examine whether the intact NID-1 is required for suppression of *mig-17* by *fbl-1*, an in-frame deletion allele *nid-1*(cg118) lacking the G2 domain was introduced into the *fbl-1*(k201); *mig-17*(k174) or *fbl-1*(k206); *mig-17*(k174) backgrounds (16). The suppression was partially weakened in *fbl-1*(k206); *mig-17*(k174), and was not affected in *fbl-1*(k201); *mig-17*(k174) (Fig. S5C). These results indicate that the G2 domain of NID-1 is not essential for suppression of *mig-17* by mutant FBL-1 proteins.

NID-1 Accumulation Is Increased in *mig-17*; *fbl-1* and *mig-17*; *let-2* Double Mutants. To examine localization of NID-1, we generated a specific Ab that recognizes the C-terminal G3 domain of NID-1. Using immunohistochemistry, we demonstrated that this Ab stained the gonadal and intestinal BMs in WT but not in *nid-1*(cg119) animals (Fig. 4 A and B). The signal was fainter in *mig-17*(k174) and *let-2*(b246) and was very faint in *fbl-1*(tk45) (Fig. 4 C–E). These results suggest that NID-1 localization is strongly dependent on FBL-1C and partially dependent on MIG-17 and LET-2. We therefore examined whether NID-1 localization is affected by the *fbl-1* and *let-2* suppressors. Interestingly, NID-1 accumulation in the gonadal BM was increased in both *mig-17*(k174); *fbl-1* and *mig-17*(k174); *let-2* double mutants (Fig. 5A–D). Because the G2 domain of nidogen binds type IV collagen in mammals (18, 19), we examined NID-1(cg118) localization in the

mig-17(k174), *mig-17*(k174); *let-2*(k193) and *mig-17*(k174); *let-2*(k196) mutants. In contrast to the weak localization of NID-1(cg118) observed in the *mig-17*(k174) mutants, NID-1(cg118) localization was increased in both *mig-17*(k174); *let-2*(k193) and *mig-17*(k174); *let-2*(k196) mutants (Fig. S6), indicating that mutant LET-2 proteins do not require the G2 domain to recruit NID-1. Thus, the recruitment of NID-1 to the gonadal BM by LET-2 may be indirect. These results indicate that one of the molecular consequences of the suppressor *let-2* and *fbl-1* mutations is enhancement of the activity to localize NID-1 in the gonadal BMs which is weakened in the absence of MIG-17.

These observations raise the possibility that reduction of NID-1 in the gonadal BM is the cause of misdirected DTC migration in *mig-17* mutants. We therefore examined whether overexpression of NID-1 in *mig-17* mutants suppresses the DTC migration defects. We used HA epitope-tagged NID-1, which can restore suppression when expressed in *mig-17*(k174) *nid-1*(g119); *fbl-1*, in this experiment. Surprisingly, the DTC migra-

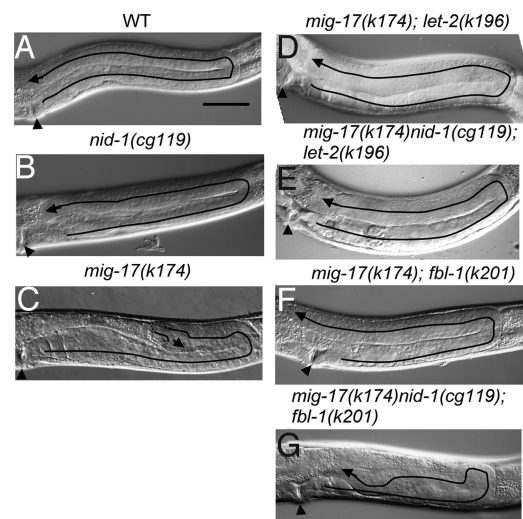


Fig. 3. NID-1-independent and -dependent suppression of *mig-17*. (A–G) Phenotypes of WT and mutant gonads. (Scale bar, 50 μ m.) The predicted migratory routes of DTCs are depicted by lines, and the distal tips of the gonad arms are indicated by arrows. Arrowheads point to the vulvae.

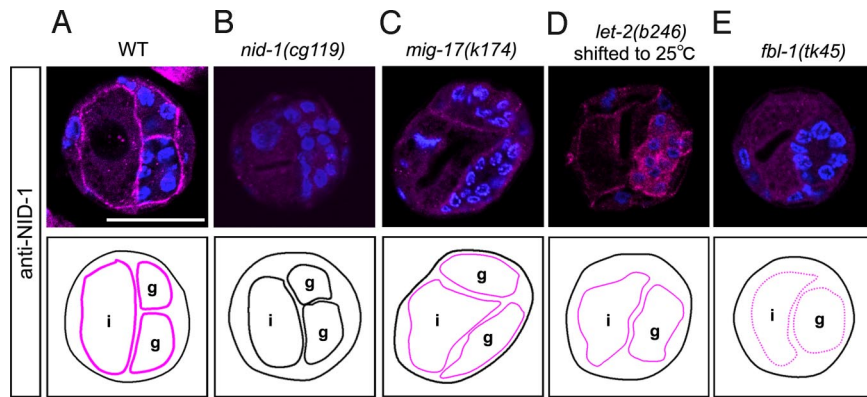


Fig. 4. Genetic analysis of NID-1 localization. Localization of NID-1 in (A) WT, (B) *nid-1(cg119)*, (C) *mig-17(k174)*, (D) *let-2(b246)* shifted to 25°C, and (E) *fbl-1(tk45)* animals. Cross-sections of L4 hermaphrodites were stained with anti-NID-1 (magenta) and DAPI (blue). The Ab recognizes an unknown antigen in the gonad of *let-2(b246)* from animals reared at 25°C (D). Levels of NID-1 localization to the BM are indicated by magenta thick lines (normal levels), thin lines (low levels) and dotted lines (very low levels). g, gonad; i, intestine. (Scale bar, 25 μ m.)

tion in *mig-17* animals was markedly ameliorated by NID-1-HA overexpression (Fig. 5 E and F). Thus, it is likely that NID-1 accumulation in the gonadal BM plays an important role in MIG-17-dependent control of DTC migration.

We previously showed that the gonadal localization of FBL-1C is reduced in *mig-17* mutants (9). Therefore, it is possible that the

decreased localization of NID-1 in *mig-17* might reflect the reduction of FBL-1C. We examined whether overexpression of *fbl-1C::3HA*, which rescues the *fbl-1(tk45)* null mutant, can enhance NID-1 accumulation in the gonadal BM and rescue the DTC migration defects of *mig-17* mutants. FBL-1C-3HA weakly localized to the BM (Fig. S7C). However, accumulation of NID-1

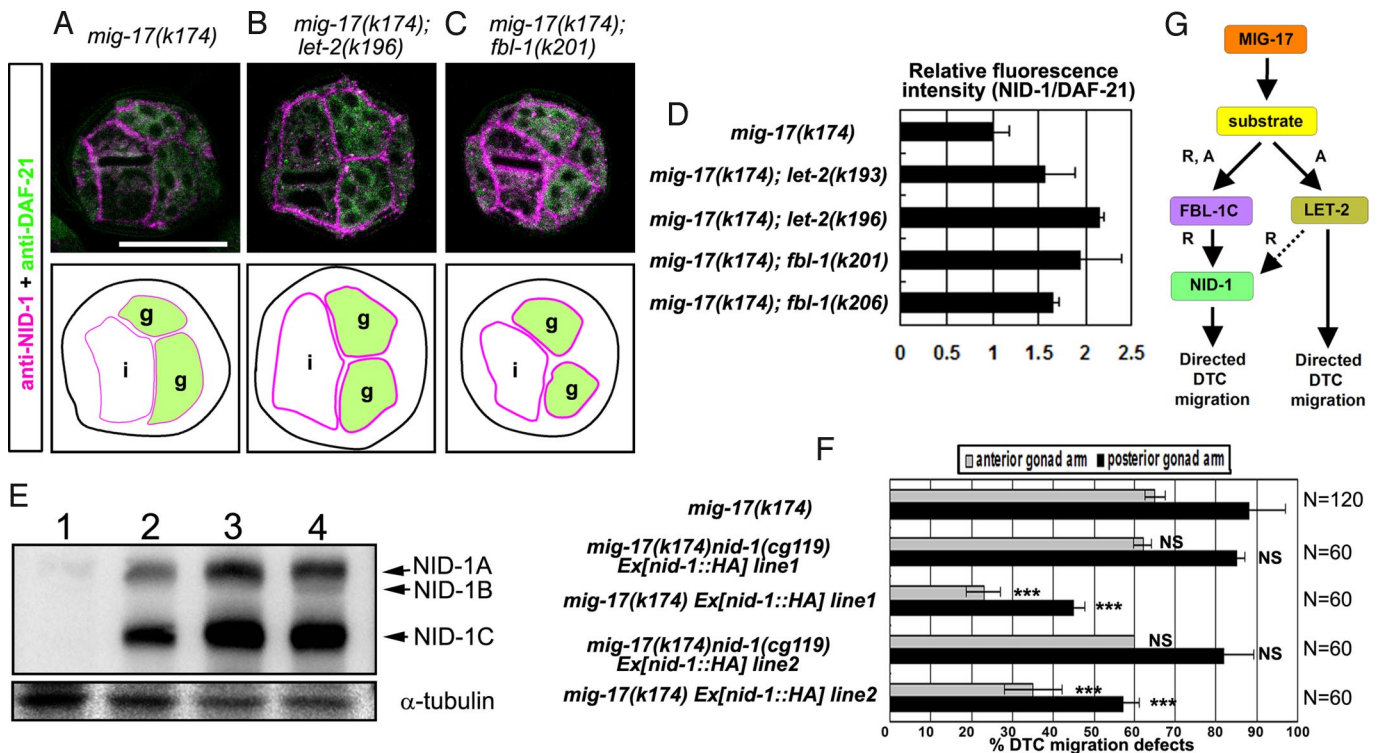


Fig. 5. NID-1 overexpression suppresses *mig-17*. (A–C) Cross-sections of L4 hermaphrodites stained with anti-NID-1 (magenta) and anti-DAF-21 (HSP90) (green). DAF-21 was used as an internal standard for quantification of NID-1. (A) *mig-17*, (B) *mig-17; let-2(k196)*, (C) *mig-17; fbl-1(k201)*. Borders of gonads and intestines are illustrated below the photos. Levels of NID-1 localization to the BM are indicated by magenta thick lines (normal levels) and thin lines (low levels). g: gonads; i: intestines. (Scale bar, 25 μ m.) (D) Fluorescence intensity of anti-NID-1 relative to that of anti-DAF-21. The relative fluorescence intensity for each sample was normalized to that of *mig-17*. See Fig. S6E legend for methods. (E) Extracts from *nid-1(cg119)* (lane 1), *mig-17(k174)* (lane 2), *mig-17 Ex[nid-1::HA] line1* (lane 3) or *mig-17 Ex[nid-1::HA] line2* (lane 4) hermaphrodites were analyzed. *nid-1* expresses three spliced isoforms, NID-1A, B, and C (arrows). Expression of NID-1 proteins are 2.5-fold (line 3) and 2.8-fold (line 4) higher in these transgenic animals compared to *mig-17* animals ($n = 3$). (F) Quantification of DTC migration defects in *mig-17* and *mig-17 Ex[nid-1::HA]* (a NID-1-HA overexpression strain). Results for Fisher's exact test against *mig-17(k174)* are indicated: ***, $P < 0.001$; NS, not significant. Error bars represent the mean SD. (G) Model for the protein cascade downstream of MIG-17. MIG-17-dependent proteolysis of an unknown substrate recruits and activates FBL-1C, which then recruits NID-1 to the BM to control DTC migration. MIG-17-dependent proteolysis also activates LET-2 to induce NID-1-dependent and -independent MIG-17 pathways. Dashed arrow, potentially indirect interaction. R, recruitment; A, activation.

was not affected and was similar to that observed in *mig-17* single mutants (Fig. S7 B and D). Consistent with these results, overexpression of FBL-1C-3HA did not rescue the DTC migration defects in *mig-17* mutants (Fig. S7E).

Discussion

MIG-17 is an ADAMTS protease localized to the gonadal BM, where it is likely required for proper remodeling of the BM to promote gonad morphogenesis. Using *C. elegans* as a model system, we isolated two suppressor mutations of the gonadogenesis defects of *mig-17* mutants and found that they occur in the C-terminal region of the $\alpha 2$ chain of type IV collagen, which is encoded by *let-2*. The suppressor *let-2* mutations showed synergistic effects when combined with suppressor *fbl-1* mutations. The suppression by *fbl-1* was dependent on *nid-1*, whereas that by *let-2* was not. Analysis of protein localization revealed that NID-1 localization to the gonadal BM was reduced in *mig-17* mutants, but suppressor *let-2* or *fbl-1* mutations promoted NID-1 localization in *mig-17* mutants.

In this study, we analyzed protein localization to the BM of gonad arms rather than to the DTCs because the latter analysis is technically difficult. However, we have previously shown that MIG-17 localizes throughout, and recruits FBL-1C to, the gonadal BM (8, 9). We also demonstrated that MIG-17 is activated in the BM of gonad arms specifically during directional migration of DTCs (20). These results are consistent with our observation that NID-1 localization to the BM of gonad arms correlates with MIG-17-dependent control of DTC migration, suggesting that MIG-17 recruitment of FBL-1C and NID-1 in the BM of gonad arms reflects its activity in DTC migration.

Partial Dysfunction of BMs in Suppressor Mutants. The *let-2(b246)* animals showed strong accumulation of type IV collagen in muscle cells even at 20 °C and had weak suppressor activity, whereas the secretion of type IV collagens was mostly normal in suppressor *let-2(k196)* and *let-2(k193)* mutants and *mig-17* mutant suppression was substantial at 20 °C, implying that the latter *let-2* mutants exhibited strong suppression due to defects in the BM that are induced by the secreted mutant LET-2 proteins. The *k193* mutation is in the C-terminal NC1 domain that is important for initiating the assembly of three α chains (two $\alpha 1$ and one $\alpha 2$), as well as for the covalent linkage of NC1 domains of two type IV collagen trimers (21). Because the *k193* and *k196* mutants are viable and fertile, the gonadal BMs are presumably not as seriously impaired as in the lethal *let-2* mutants. Thus, it may be that the α chain trimer is partially malformed in *k193* mutants. Alternatively, the type IV collagen network that is formed in *k193* mutants could be partially disorganized due to aberrant formation of intermolecular bonds involving NC1 domains. Because the *k196* mutation is an amino acid substitution that can perturb triple helix formation near the NC1 domain, it may also affect the function of the NC1 domain.

The dysfunction of BMs in the *let-2* and *fbl-1* suppressor mutants is supported by the fact that the *let-2(k196)* mutation genetically interacts with *fbl-1* mutations *k201* and *k206* and that the *let-2; fbl-1* double mutants exhibit a strong swollen gonad phenotype. The enhancement of DTC migration defects of *unc-52* and *epi-1* by *let-2* and *fbl-1* suppressors also support this idea.

NID-1-Dependent and -Independent Pathways Downstream of MIG-17. Because the *nid-1(cg119)* null mutant has essentially WT U-shaped gonads, it appears that MIG-17 and NID-1 have redundant functions in DTC migration. However, the fact that NID-1 localization is reduced in *mig-17* mutants and overexpression of NID-1 can markedly rescue the DTC migration defects suggests that MIG-17 also acts through NID-1. Thus, it is likely that both NID-1-dependent and -independent pathways are downstream of MIG-17 and that these two pathways are redundant. Although *fbl-1* function in the WT background was essential for normal NID-1 localization,

overexpression of FBL-1C in the *mig-17* background did not enhance NID-1 localization. Therefore, MIG-17 activity is probably required not only for recruiting FBL-1C (9), but also for activating FBL-1C to recruit NID-1. Although it is possible that FBL-1C or LET-2 is the substrate of MIG-17, so far we have failed to detect such interactions. We speculate that the cleavage of an unknown substrate by MIG-17 activates FBL-1C by, for example, altering the conformation of FBL-1C, which then recruits NID-1 and causes the remodeling of the gonadal BM that is required for directional DTC migration (Fig. 5G).

We observed that accumulation of NID-1 at the BM increased in the suppressor *fbl-1* and *let-2* mutants in the absence of MIG-17. The specific amino acid substitutions in mutant FBL-1C or LET-2 might mimic MIG-17 function to increase the affinity of the BM for NID-1. Because the suppression by *fbl-1* is *nid-1*-dependent, it is reasonable that mutant FBL-1 gained the function of NID-1 localization. However, enhancement of NID-1 localization in *let-2* mutants is not expected because of the *nid-1*-independent suppression by *let-2* mutants. Because abnormal DTC migration in *mig-17* mutants can be substantially suppressed by NID-1 overexpression, it seems that the recovery of NID-1 gonadal BM accumulation in suppressor mutants is functionally important for suppression of DTC migration defects. It might be possible that both NID-1-dependent and -independent MIG-17 pathways are activated in suppressor *let-2* mutants and that the latter pathway is still intact even in the *nid-1; let-2* double mutants. The reduced localization of NID-1 at the gonadal BM in *let-2(b246)* mutants supports the idea that WT LET-2 is also required for efficient accumulation of NID-1. Furthermore, we showed that gonadal FBL-1C-Venus localization was not affected in *let-2(b246)* mutants grown at nonpermissive temperatures (see Fig. S7 F and G), indicating that LET-2 recruits NID-1 independently of FBL-1C. Although it is not required for localization of LET-2, MIG-17 may regulate the function of WT LET-2 in the BM to induce NID-1-dependent and -independent pathways for DTC migration control (Fig. 5G). For example, LET-2 may contain a cryptic guidance cue that is exposed by MIG-17-dependent proteolysis or by suppressor *let-2* mutations. Cryptic fragments of type IV collagen having angiogenic and anti-angiogenic activities have been described in mammals (22–24).

Possible Functions of Nidogens in Cell Migration. Recently, regulated expression of two α -integrins, INA-1 and PAT-2, was shown to be important for turning and cessation of movement of DTCs: INA-1 is required for continuous migration, whereas PAT-2 is required for pathfinding during migration (25). Because integrins are major receptors for ECM proteins, the meandering migration of DTCs in *mig-17* mutants may be due to misrecognition of the BM by the integrin receptors. In mammals, integrins expressed in neutrophils bind the Arg-Gly-Asp (RGD) sequence in the rod domain of nidogen and activate chemotaxis (26). Integrins containing the $\beta 1$ subunit have been shown to be required for nidogen-dependent migration of Schwann cells (27). Therefore, nidogen plays an important role in the interaction between integrins and the BM.

In DTC pathfinding, dorsal migration is guided by the membrane-bound receptors UNC-5 and UNC-40, which respond to the UNC-6/netrin gradient (28). Because we previously showed that *mig-17(k174)* enhances a weak allele of *unc-6* (7), the DTCs in *mig-17* mutants may not be sufficiently sensitive to the UNC-6 signal. Interestingly, NID-1 has been reported to interact with UNC-40 in the guidance of commissural axons in *C. elegans*; NID-1 appears to negatively regulate UNC-40 when the dorsally migrating axon of the SDQR neuron reorients anteriorly (29). Reduction of NID-1 in the gonadal BM may also reduce the guidance function of UNC-40 expressed in DTCs.

Interactions Among Fibulin-1, Nidogen and Type IV Collagen May Be Evolutionarily Conserved. Type IV collagen is a major component of the BM and is associated with other ECM molecules such as nidogen, laminin, and heparan sulfate proteoglycans (30–32). Mammalian fibulin-1 binds to nidogen-1, laminin-1, and some proteoglycans (15, 17, 33). Mouse fibulin-1 also interacts weakly with type IV collagen (34). Therefore, LET-2 and FBL-1 may bind directly or indirectly through other ECM molecules to form a supramolecular network in the BM to support proper gonad morphogenesis. Our findings that both mutant FBL-1C and LET-2 can enhance NID-1 BM accumulation and that the suppression of DTC migration defects of *mig-17* by mutant FBL-1C depends on NID-1 implies functional interactions among these four molecules.

There are two nidogen genes in mammals (35). *nidogen-1* and *nidogen-2* double-KO mice and *fibulin-1* KO mice exhibit similar delayed embryonic lung development with thickened parenchymal septa and improperly expanded saculi (35, 36). These results suggest that fibulin-1 and nidogens also functionally interact in mammals.

Finally, our finding that in *C. elegans* MIG-17 interacts with fibulin-1, nidogen, and type IV collagen, all major components of the BM, suggests the possibility that a similar functional interaction between ADAMTS and these ECM molecules may also occur in mammals during organogenesis.

Materials and Methods

Strains and Genetic Analysis. Culture, handling and ethylmethane sulfonate mutagenesis of *C. elegans* were performed using standard methods (37). Worms were grown at 20 °C unless otherwise noted. The following mutations and genetic balancers were used in this work: *ced-1(cg120)*, *emb-9(g23cg46)*, *epi-1(gm57)*, *tbl-1(k201, k206 and tk45)*, *let-2(b246, g25, k193 and k196)*, *mig-17(k174)*, *nid-1(cg118 and cg119)*, *unc-7(e139)*, *unc-42(e270)*, *unc-52(e1421)*, *unc-119(e2498)*, *nT1[qIs51]* and *hT2[qIs48]*.

Microscopy. DTC migration was analyzed as described (38). The strains with *let-2* suppressor mutations often showed somewhat shorter phase III migra-

tion than WT animals. This phenotype was not scored as a defect here. The localization of LET-2, FBL-1C, and NID-1 was analyzed using a confocal laser scanning microscope (LSM5, Zeiss) equipped with a C-apochromat 63× (water immersion; NA 1.2) lens and controlled by PASCAL version 3.2 SP2 software.

Constructs and Germline Transformation. Construction of the *nid-1::HA* and *let-2* plasmids and methods for germline transformation are described in the *SI Materials and Methods*.

Preparation of Antisera and Ab Staining. The *nid-1* cDNA clone yk1531C10 was obtained from Yuji Kohara at the National Institute of Genetics, Mishima, Japan. Part of the G3 domain (residues 1281–1414 for NID-1A) (17) of NID-1 tagged with histidines was cloned into pET-28a(+) (Novagen). The tagged protein was isolated from *E. coli*. Rabbit antiserum against NID-1 was purified on a column fixed with the histidine-tagged NID-1. L3 to L4 animals were fixed with 4% paraformaldehyde in PBS for 12 h on ice. Frozen sections were prepared as described (39). After blocking the sections with 0.1% (wt/vol) Triton X-100 and 3% BSA in PBS, they were immunostained by incubation with anti-LET-2 (NW68, 1:200) (14), mouse anti-GFP IgG (3E6, 1:200; Molecular Probes), mouse monoclonal anti-DAF-21(HSP90) (608F, 1:10) (40), anti-NID-1 (5 μg/ml) or rat anti-HA IgG (3F10, 2 μg/ml; Roche) for 12 h at 4 °C followed by incubation with the appropriate Alexa Fluor-conjugated secondary Ab (1:500; Molecular Probes) for 1 h at room temperature and then stained with DAPI (2 μg/ml; Wako) for 10 min at room temperature. Anti-NID-1 was preabsorbed with the acetone powder of *nid-1(cg119)* null mutants.

Western Blotting. Mixed stage worm extracts (20 μg) were immunoblotted with rabbit anti-NID-1 (2 μg/ml) or mouse monoclonal anti-α-tubulin (12G10, 1:1,000, J. Frankel and M. Nelson, provided by Developmental Studies Hybridoma Bank at the University of Iowa).

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