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Morphogenesis in *Caenorhabditis elegans*

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In wild-type *Caenorhabditis elegans,* the gonad is a complex epithelial tube that consists of long arms composed predominantly of germline tissue as well as somatic structures specialized for particular reproductive functions. In *gon-1* mutants, the adult gonad is severely disorganized with essentially no arm extension and no recognizable somatic structure. The developmental defects in *gon-1* mutants are limited to the gonad; other cells, tissues, and organs appear to develop normally. Previous work defined the regulatory "leader" cells as crucial for extension of the gonadal arms (J. E. Kimble and J. G. White, 1981, *Dev. Biol.* 81, 208–219). In *gon-1* mutants, the leader cells are specified correctly, but they fail to migrate and gonadal arms are not generated. In addition, *gon-1* is required for morphogenesis of the gonadal somatic structures. This second role appears to be independent of that required for leader migration. Parallel studies have shown that *gon-1* encodes a secreted metalloprotease (R. Blelloch and J. Kimble, 1999, *Nature* 399, 586–590). We discuss how a metalloprotease may control two aspects of gonadal morphogenesis. © 1999 Academic Press

Key Words: gon-1; **organ shape; gonadogenesis; *C. elegans;* morphogenesis.**

INTRODUCTION

Caenorhabditis elegans gonadogenesis involves two morphogenetic processes that shape many organs. The first is extension of a tissue bud to form an elongated tube with a proximal to distal axis (Fig. 1). Examples during vertebrate development include extension of the limb (Johnson and Tabin, 1997; Martin, 1998), ureter (Vainio and Muller, 1997), and lung branches (Hogan, 1999). In the *C. elegans* gonad, long "arms" develop by elongation of buds originating from a gonadal primordium. An emerging theme in bud extension is the presence of specialized regulatory cells at the bud tip that govern elongation. In the *C. elegans* gonad, each gonadal arm possesses a single "leader cell" that serves this regulatory role (Kimble and White, 1981). Indeed, at the current time, the *C. elegans* gonadal leader cells are among the best defined cells that regulate bud elongation, and therefore they serve as a paradigm for investigating this common process of morphogenesis.

A second morphogenetic process during organogenesis is the formation of complex, differentiated epithelial tubes. Vertebrate examples include the kidney tubules (Vainio and Muller, 1997) and the heart tube (Fishman and Olson, 1997). Formation of these complex epithelial tubes involves the initial condensation of mesenchymal cells, followed by epithelialization, lumen formation, and differentiation into modular units. Similarly, during *C. elegans* gonadogenesis, cells coalesce to form a compact larval structure called the somatic gonadal primordium (Fig. 1). Following formation of the somatic gonadal primordium, cell division and differentiation are accompanied by epithelialization and lumen formation to form a complex tube composed of distinct modular units: the uterus, spermathecae, and sheaths in hermaphrodites and the seminal vesicle and vas deferens in males (Fig. 1) (Kimble and Hirsh, 1979). Therefore, the

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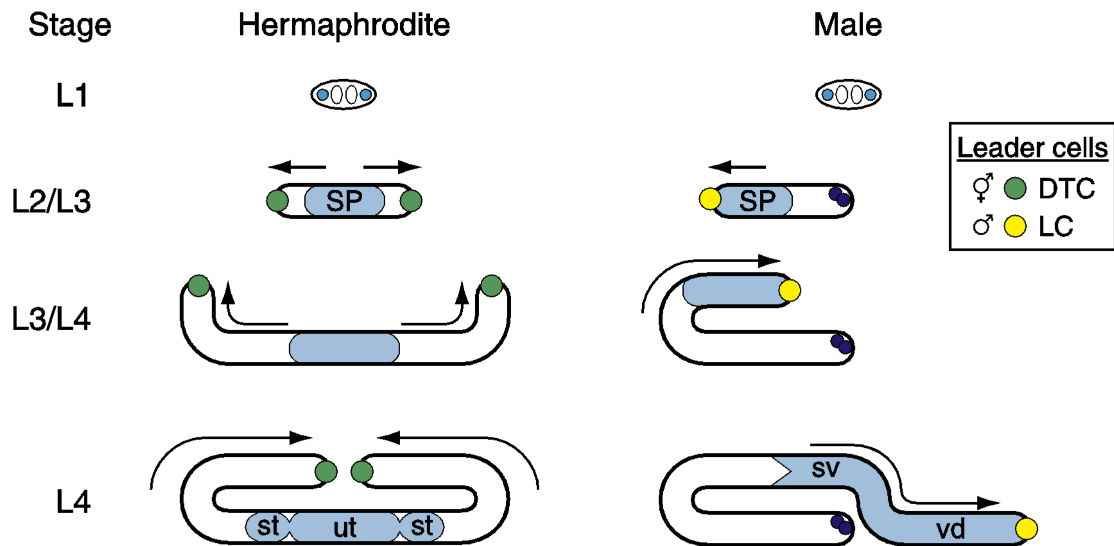

FIG. 1. Morphogenesis in *C. elegans* gonads. Key stages of gonadogenesis in hermaphrodites (left) and males (right); see Schedl (1997) for a more detailed review. Somatic cells and tissues are color-coded (see below); germline is white. The L1 gonadal primordium has two somatic blast cells (Z1 and Z4, aqua) and two germline precursor cells (Z2 and Z3). Gonadal extension requires leader cells to migrate and guide the growing gonadal arms; arrows show the direction of leader cell migration. In hermaphrodites, the distal tip cells (DTC) lead gonadal arms into a final U shape; in males, the linker cell (LC) leads extension into a final J shape. Male DTCs, shown in dark blue, do not lead arm extension. Formation of the somatic gonadal primordium (SP) requires the coalescence of somatic gonadal blast cells. The hermaphrodite SP forms in the center of the gonad, while the male SP forms at the proximal end. The SP cells then divide and differentiate into adult somatic structures: uterus (ut), spermatheca (st), and sheath (not shown) in hermaphrodites and seminal vesicle (sv) and vas deferens (vd) in males.

generation of the *C. elegans* gonadal structures can serve as a simple system for investigating this second common process of morphogenesis.

Previous studies have identified several genes that influence gonadal morphogenesis. One group of such genes includes *unc-5, unc-6,* and *unc-40,* which control the direction of leader cell migration (Hedgecock *et al.,* 1990). Normally, leader cells migrate in one direction along the ventral surface, then move dorsally, and finally move in the opposite direction along the dorsal surface to generate a U-shaped gonadal arm. In the absence of *unc-5, unc-6,* or *unc-40,* the leader cells fail to turn dorsally. Another gene, *ced-5,* causes the leader cell to make extra turns or stop prematurely (Wu and Horvitz, 1998). Therefore, in these mutants, the leader cells migrate, but do not navigate correctly, which results in a failure of the gonadal arms to acquire their normal U shape. In addition to these genes, others are required for specification of cell fates and thereby influence morphogenesis (*lin-12,* Greenwald *et al.,* 1983, Newman *et al.,* 1995; *lin-17,* Sternberg and Horvitz, 1988; *lag-2,* Lambie and Kimble, 1991; *ceh-18,* Greenstein *et al.,* 1994, Rose *et al.,* 1997; *lin-26,* den Boer *et al.,* 1998).

In this article, we report the genetic identification of *gon-1* and show that *gon-1* is a key regulator of gonadal morphogenesis. We find that *gon-1* is specific for morphogenesis of the gonad and is not required for morphogenesis of other structures. Furthermore, we demonstrate that *gon-1* has at least two roles in governing gonadal shape. First, *gon-1* is essential for leader cell migration and, therefore, for gonadal arm extension. Second, *gon-1* is crucial later in gonad development for morphogenesis of adult somatic gonadal structures. In a parallel study, we found that *gon-1* encodes a secreted metalloprotease; we also found that organ shape can be manipulated by controlling the site of *gon-1* expression (Blelloch and Kimble, 1999). In this paper, we discuss how the *gon-1* metalloprotease normally controls the shape of the *C. elegans* gonad and how similar activities may control organ shape more generally.

MATERIALS AND METHODS

Nematode Strains

All strains were derived from the wild-type Bristol strain N2 and were maintained as described (Brenner, 1974). Growth was at 20°C unless otherwise indicated. The following mutations and chromosomes are described in Hodgkin *et al.* (1988) or cited references. LGIV: *gon-1(q18, q517, q518, e1254, e2547, e2551)* (this paper), *unc-24(e138), daf-15(m81), elt-1(zu180)* (Page *et al.*, 1997), *unc-43(e408), dpy-20(e1282), eDf19, mDf7.* Chromosomes: *nT1[unc(n754dm) let]* is a balancer chromosome that is lethal as a homozygote and uncoordinated (Unc) as a heterozygote. *jcIs1* is an integrated GFP reporter (kindly provided by J. Simske) that serves as a recessive lethal balancer for *gon-1.*

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TABLE 1
Percentage of Gonadal Arms Extended in *gon-1(x)* Homozygotes and *gon-1(q518)* Hemizygotes

	15°C		20°C		25°C	
Allele	%AE[a]	*n*	%AE[a]	*n*	%AE[a]	*n*
q518	0	20	0	71	0	70
e2547	0	34	5	44	13	46
q18	nd	nd	8	38	nd	nd
e1254	0	36	26	50	16	43
q517	11	36	23	44	35	52
e2551	31	32	85	27	77	74
q518/eDf19[b]	nd	nd	0	34	nd	nd

Note. nd, not done.
[a] % Arms extended, an arm was scored as extended if, in L4 larvae, the DTC had migrated and reflexed.
[b] *q518/eDf19* was produced by crossing *gon-1(q518) dpy-20/unc-24 dpy-20* hermaphrodites with *eDf19*/+ males and examining all non-Dpy steriles from the cross.

Isolation of gon-1 Alleles

L4 hermaphrodites were mutagenized with 40 mM ethyl methanesulfonate and F1 progeny picked to individual plates and allowed to produce self-progeny at 25°C. From plates on which one-fourth of the F2 progeny possessed a morphologically abnormal gonad, fertile siblings were picked to propagate the Gon (for gonadogenesis defective) mutation in a heterozygous state. The *gon-1* mutants were outcrossed to wild-type N2 animals at least twice. The *gon-1*(*q518*) allele was successively recombined with flanking markers, *dpy-20* first and then *unc-24,* to remove extraneous linked mutations.

Allelic Series of gon-1 Mutations

To compare allelic strengths, we examined the penetrance of arm extension defects in homozygotes for each allele. In *gon-1(q518)* homozygotes, no arm extension was observed at 15, 20, or 25°C. However, in homozygotes for the other *gon-1* alleles, some arms extended at least partially (Table 1). By this measure, the *gon-1* alleles can be placed in an allelic series: *q518* > *e2547* ≈ *q18* ≈ *e1254* > *q517* > *e2551.* Interestingly, the weaker *gon-1* alleles have a more severe defect at lower temperature (Table 1).

Genetic Mapping

Each *gon-1* mutation maps to chromosome IV between *unc-24* and *dpy-20.* Five alleles, *e1254, e2547, q18, q517,* and *q518,* fail to complement the sixth allele, *e2551,* and, therefore, the mutations define a single gene. Three-factor mapping places *gon-1*(*e2551*) 0.08 map units to the right of *elt-1* and 0.12 map units to the left of *unc-43* at position 4.44. Specifically, among Unc-43 non-Elt-1 recombinants isolated from *gon-1/elt-1 unc-43* mothers, 8/13 carried the *gon-1* mutation.

Brood Analysis

From all progeny of two *gon-1(e2551)/unc-24* hermaphrodites, one-fourth (181/690) were Gon, one-fourth were Unc, and the rest were wild type. Brood sizes were comparable to wild type, and no other phenotype was observed. Similarly, heterozygotes for all other *gon-1* alleles segregate about 1/4 Gon with no other phenotypes, although complete broods were not scored. Furthermore, from *gon-1(x)/nT1* heterozygotes, where *x* represents each of the six *gon-1* alleles and *nT1* is *nT1[unc(n754dm) let],* a balancer chromosome with a dominant Unc phenotype, no *gon-1/nT1* Unc heterozygotes were Gon, and all non-Unc homozygotes were Gon. Therefore, all six *gon-1* alleles are fully recessive and 100% penetrant.

Tissue Markers

To score the distal tip cell (DTC), we used two transgenic markers: *qIs19* (*lag-2::GFP V) and evIn54(unc-5::lacZ)* (kindly provided by J. Culotti). To score the hermaphrodite gonadal sheath cells, we used *plim-7::GFP (Hall et al.,* 1999).

Cell Lineaging

Cell lineages were followed as described (Sulston and Horvitz, 1977). Each result described is based on the lineage of at least four animals.

Laser Microsurgery

Laser ablations were done as described (Bargmann and Avery, 1995) using a Micropoint Ablation Laser System. For all laser microsurgeries, at least four animals were analyzed. *gon-1(q518)* homozygotes were identified as nonfluorescent progeny from *gon-1(q518)/jcIs1* mothers. All four gonadal progenitor cells, Z1–Z4, were ablated to remove the entire gonad; DTCs were removed shortly after they were born in L1 larvae. Lineaged animals were mounted as described (Sulston and Horvitz, 1977) approximately 8 h after ablation.

Electron Microscopy

Two each of wild-type and *gon-1(q518)* L3 hermaphrodites were prepared for electron microscopy as described (Bargmann *et al.,* 1993) with modifications. *En bloc* staining and dehydration steps were done in a Pelco 3440 Microwave Oven. Micrographs were obtained at 60 kV with a Philips EM120 microscope.

RESULTS

Identification of the gon-1 Locus

Six *gon-1* alleles were isolated in screens for sterile mutants; all six alleles map to chromosome IV, all are recessive, and all are fully penetrant for sterility (see Materials and Methods). The adult gonads of *gon-1* mutants are severely malformed in both hermaphrodites and males. Normally, the gonad is a tubular structure with specialized regions (Figs. 1 and 2A). In contrast, in *gon-1* mutants, the adult gonadal tissues exist as a disorganized

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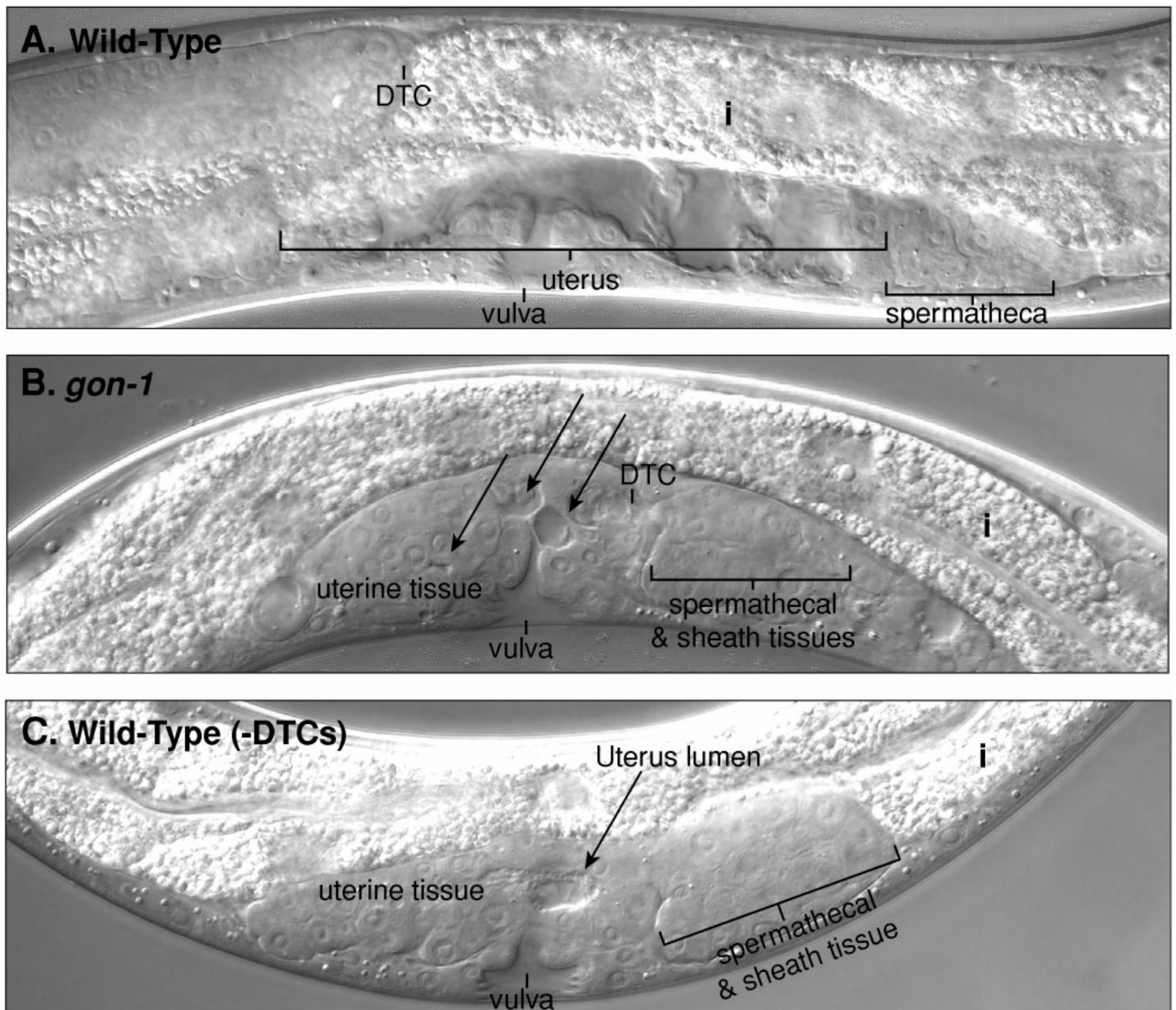

FIG. 2. *gon-1* gonads are severely malformed. Nomarski differential interference contrast (DIC) images. Tissues are defined by their cellular morphology. In each micrograph, the granular intestine (i) and vulva are indicated. (A) Wild type. Uterus consists of thin epithelial cells surrounding a large lumen and forms at center of the gonad. The posterior spermatheca is visible in this plane of focus. The anterior DTC is seen at the distal end of the extended anterior gonadal arm, just dorsal to the anterior end of the uterus. (B) *gon-1(q518)* hermaphrodite. Uterine tissue is present, but fails to form a coherent structure. Fragments of uterine lumen are dispersed throughout the uterine tissue (arrows). A mass of spermathecal and sheath tissues bulge from the posterior. The posterior DTC is marked. (C) Wild-type hermaphrodite that had its DTCs removed by laser ablation (−DTCs). Like *gon-1,* uterine tissue is present, but malformed. However, compared to *gon-1,* a more extensive and coherent uterine lumen has formed (arrow), portions of which are out of the focal plane. A disorganized mass of spermathecal and sheath tissue has bulged from the posterior.

mass with little or no tubular morphology (Fig. 2B). Specifically, neither arms nor somatic gonadal structures (e.g., uterus, spermatheca) are observed. *gon-1(q518)* is the strongest loss-of-function allele (see Materials and Methods). Because the mutant phenotype for *gon-1(q518)* homozygotes is similar to that of *gon-1(q518)* hemizygotes (Table 1) and because *gon-1(q518)* bears a nonsense mutation predicted to remove the bulk of the GON-1 protein (Blelloch and Kimble, 1999), this allele is likely to be a molecular null. Therefore, *gon-1(q518)* was used for

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analyzing the roles of *gon-1* in gonadal morphogenesis and will be referred to as *gon-1(0).*

Leader Cells Are Present and Specified Normally in gon-1 Mutants

The lack of gonadal arms in *gon-1(0)* mutants suggested that the leader cells, which normally govern arm extension (see Introduction), may be defective. To assess whether leader cells were generated during development, we first examined the gonadal cell lineages in *gon-1(0)* mutants during the first two larval stages. Normally, the somatic gonadal progenitor cells, Z1 and Z4, give rise to two leader cells, Z1.aa and Z4.pp, in hermaphrodites, and one leader cell, Z1.pa or Z4.aa, in males (Kimble and Hirsh, 1979). In hermaphrodites, these leader cells are called distal tip cells, and in males, they are called linker cells (LC). The hermaphrodite distal tip cell is both a leader cell and a regulator of germline proliferation (Kimble and White, 1981). In *gon-1(0)* hermaphrodites and males, we found that the timing and pattern of cell divisions of Z1 and Z4 and their descendants were the same as in wild type during L1 and L2 (data not shown). In particular, Z1.aa and Z4.pp in hermaphrodites and Z1.pa/Z4.aa in males were born at the correct time and place.

To ask whether the presumptive hermaphrodite leader cells, Z1.aa and Z4.pp, had adopted the leader fate, we examined expression of a molecular marker for that fate. The *unc-5* gene encodes a netrin receptor and is essential for dorsal migration of leader cells (Leung-Hagesteijn *et al.,* 1992). Using a reporter transgene, *unc-5::lacZ* (J. Culotti, personal communication), we found that *unc-5* expression was the same in wild-type and *gon-1(0)* animals: *unc-5* was not expressed during early larval stages (Figs. 3A and 3B), but was activated in late L3 (Figs. 3C and 3D) when the DTCs normally turn dorsally during wild-type gonadogenesis.

Since the hermaphrodite leader cells, Z1.aa and Z4.pp, also control germline proliferation, we next asked if they were correctly specified for that regulatory function. To this end, we examined expression of the *lag-2* gene, which encodes the DTC signal for germline proliferation (Henderson *et al.,* 1994). Using a *lag-2::GFP* reporter transgene, we found that GFP expression was similar in wild-type (Figs. 4A and 4B) and *gon-1* gonads (Figs. 4C and 4D). Furthermore, we ablated Z1.aa and Z4.pp in *gon-1(0)* mutants and found that germline proliferation was arrested (data not shown). Therefore, the hermaphrodite DTCs, Z1.aa and Z4.pp, appear to be specified correctly both as leader cells and as regulators of germline proliferation.

Leader Cells Do Not Migrate in gon-1(0) Mutants

Since the leader cells appeared to be specified correctly in *gon-1* mutants (see above), we next examined their ability to migrate and lead arm extension. Normally, the hermaphrodite leader cells (distal tip cells) migrate away from the center of the gonad along the anterior–posterior axis, then reflex dorsally and migrate back (Fig. 1). To compare leader cell migration in wild-type and *gon-1(0)* mutants, we followed their movements (Fig. 2) throughout gonadal development. We also measured gonadal length at various stages of development (Table 2). At the mid-L1 stage, just prior to division of the leader cell progenitors, Z1 and Z4, the length of the gonad from anterior to posterior end was 19 μm in both wild type and *gon-1(0)* mutants. Following division of Z1 and Z4 in late L1, a small difference in gonadal length was discerned: 25 μm in wild type vs 22 μm in *gon-1* mutants. However, in older larvae with differentiated leader cells, the length differences were dramatic (Table 2; Figs. 5A and 5B for L3, Figs. 5C and 5D for L4). In *gon-1(0)* hermaphrodites, the distal tip cells had moved little from their birth position and little to no gonad extension had occurred.

A similar defect is observed in males. Normally, the male leader cell (linker cell) migrates anteriorly, then reflexes and migrates to the posterior end of the worm. However, in *gon-1(0)* males, the linker cell failed to migrate, and little to no extension occurred (Table 2; Figs. 5E and 5F). We conclude that *gon-1* is required for leader cell migration and hence gonadal arm extension.

The Hermaphrodite DTCs Have an Abnormal Morphology in gon-1 Mutants

As we observed leader cells during gonadogenesis, we noticed that they assumed an unusual morphology. To explore this further, we examined hermaphrodite DTCs using fluorescence and thin-section electron microscopy (EM). Using *lag-2::GFP,* which reveals the extent of the hermaphrodite DTC cytoplasm, we found that the wild-type and *gon-1(0)* DTCs had dramatically different morphologies. In wild type, the DTC was crescent-shaped with processes extending around the germline (Figs. 4A and 4B), while in *gon-1* mutants, it was round and enlarged (Figs. 4C and 4D). Furthermore, the position of the nucleus within the DTC was variable in *gon-1* mutants, whereas in wild type, it was located at the leading edge of the migrating cell (data not shown). By EM, we confirmed the difference in morphology between wild-type and *gon-1* leader cells and also discovered a difference in subcellular organization (Fig. 6). Whereas wild-type leader cells extend processes along the germline (Fig. 6A), *gon-1(0)* leader cells do not possess such processes (Figs. 6B and 6C). Furthermore, the plasma membrane is abnormally invaginated in *gon-1(0)* L3 leader cells (Fig. 6B), and these membranes accumulate within the cytoplasm of older *gon-1(0)* mutants (Fig. 6C).

Differentiation of the Major Somatic Gonadal Tissues in gon-1 Mutants

The lack of gonadal arms is not the only defect in *gon-1* mutants. In addition, no gonadal structures (e.g., uterus in hermaphrodites, vas deferens in males) can be discerned

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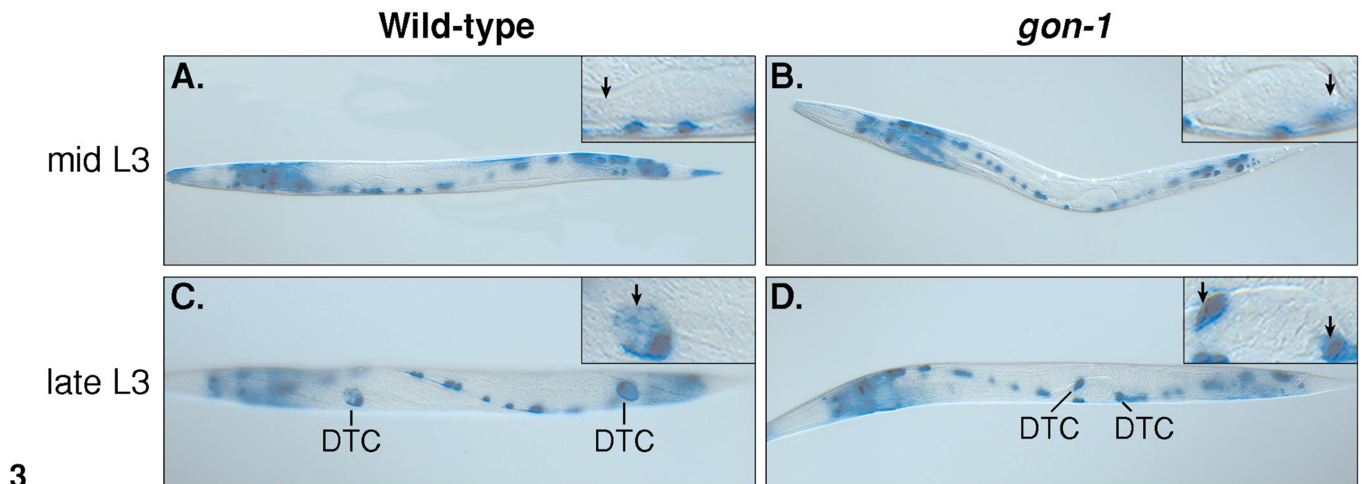

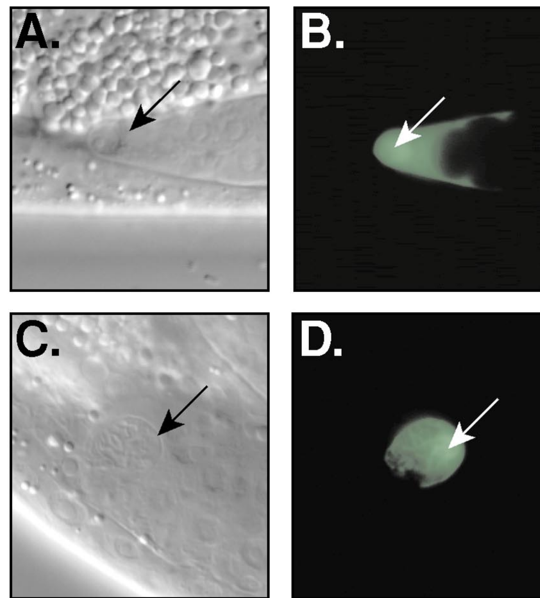

FIG. 3. *unc-5* is expressed in *gon-1(q518)* DTCs at late L3 stage. β-Gal staining of wild-type (A, C) and *gon-1* (B, D) animals carrying *unc-5* 5′∷*lacZ*(NLS) transgene. Insets show a threefold magnification of anterior distal ends for wild type (A, C) and the entire gonad for *gon-1* (B, D) with arrows identifying DTCs. *unc-5* is also expressed in neurons of the ventral nerve cord, head, and tail. In wild-type animals, *unc-5* is not expressed in distal tip cells of mid-L3 larvae (A), but is expressed as the DTCs turn dorsally in late L3 (C). In *gon-1* mutants, *unc-5* expression mimics that of wild type: it is not expressed in DTCs of mid-L3 larvae (B), but is expressed in DTCs of late L3 larvae (D), despite the lack of DTC migration in these animals.

FIG. 4. *gon-1(q518)* hermaphrodites express the DTC marker, *lag-2*∷*GFP.* GFP expressed by a *lag-2*∷*GFP* transgene fills the DTC cytoplasm and nucleus. (A, C) DIC images; arrows identify DTC nucleus. (B, D) *lag-2*∷*GFP* fluorescence. (A, B) Wild-type hermaphrodite DTCs extend processes back along the germline. (C, D) *gon-1* DTCs have no processes and are rounder and larger than wild type.

(Fig. 2B, data not shown). One problem might have been a failure to differentiate gonadal tissues. However, we were able to identify the major somatic gonadal cell types in late L4 *gon-1(0)* mutants (Fig. 7, data not shown). To see somatic gonadal sheath cells, we used *lim-7*∷*GFP,* which expresses the green fluorescent protein in hermaphrodite sheath cells (Hall *et al.,* 1999). In wild type, fluorescence from *lim-7*∷*GFP* encircled the germ cells (Figs. 7A and 7B), while in *gon-1* mutants, only irregularly shaped patches were observed (Figs. 7C and 7D). Similarly, MH27 antibody, which stains spermathecal cells intensely (den Boer *et al.,* 1998), was present in disorganized patches in *gon-1* mutants (data not shown). Finally, cells with a typically uterine morphology were present, but no normal uterine structure was

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TABLE 2
Gonadal Lengths of Wild-Type and *gon-1(q518)* Gonads

	Wild type		*gon-1*	
Sex/stage of development	Length (μm)	*n*	Length (μm)	*n*
Hermaphrodite/mL1[a]	19 ± 2	7	19 ± 2	10
Hermaphrodite/lL1[a]	25 ± 1	7	22 ± 2	6
Hermaphrodite/eL3[b]	124 ± 7	12	27 ± 2	8
Hermaphrodite/mL4[b]	677 ± 24	8	44 ± 3	6
Male/L2 lethargus[c]	98 ± 11	9	25 ± 3	5

[a] Prior to birth of DTCs, length was measured from anterior to posterior ends of gonad. mL1, mid-L1. 1L1, late L1.
[b] After birth of DTCs, length was measured from anterior DTC to posterior DTC along the proximal–distal axes of the gonadal arms. eL3, early L3. mL4, midL4.
[c] In males, length was measured from LC to distal tip.

found in *gon-1* mutants. Therefore, while the gonadal tissues of *gon-1(0)* mutants do not form normal structures, they do appear to differentiate correctly.

gon-1 Function Is Not Limited to Leader Cell Migration

One simple explanation for the gross morphogenetic defects of mature *gon-1* gonads might have been that all aspects of gonadal morphogenesis are disrupted as a consequence of the defect in leader cell migration. Indeed, by killing the distal tip cells in wild-type animals, we could reproduce the *gon-1* mutant phenotype: arms did not extend and gonadal structures were grossly malformed (Fig. 2C). However, closer inspection suggested that *gon-1* has a role in gonad morphogenesis that is independent of leader cells.

To focus on the role of *gon-1* in somatic gonadal development, we removed the germ line (−GL) of *gon-1(0)* hermaphrodites. As a control, we removed both leader cells (−DTCs) and germline from wild-type hermaphrodites. Both *gon-1*(−GL) and control animals failed to extend gonadal arms, but did form an essentially normal somatic gonadal primordium, a structure formed midway through gonadal development (Fig. 8). However, comparison of later stage gonads revealed striking differences between *gon-1*(−GL) and wild-type (−DTC,−GL) hermaphrodites. In *gon-1*(−GL) hermaphrodites, no coherent uterus formed (Fig. 9A). Furthermore, the *gon-1*(−GL) gonad was small, and most gonadal tissue had extruded from the gonad proper. In contrast, the gonad had enlarged and an apparently normal uterus formed in the wild-type (−DTC,−GL) hermaphrodites (Fig. 9B). Therefore, *gon-1* is required not only for arm extension, but also for the more general enlargement of the gonad and morphogenesis of the uterus.

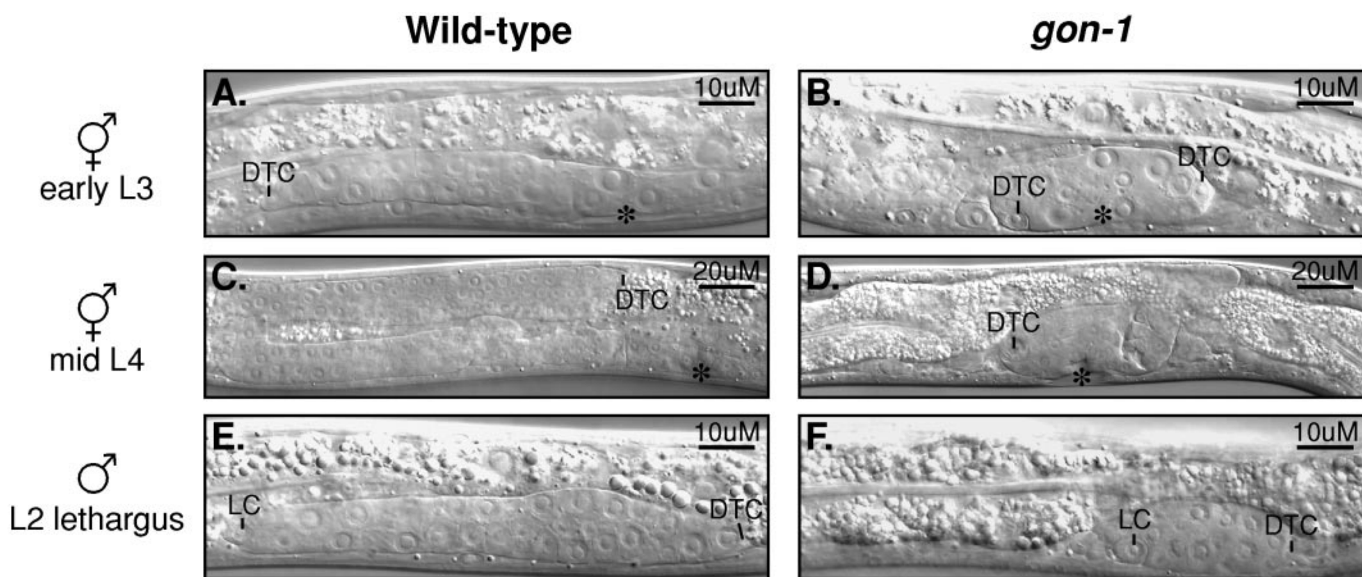

FIG. 5. *gon-1* activity is required for leader cell migration and arm extension. DIC images. Anterior, left; dorsal, up. Asterisk identifies center of hermaphrodite gonads (AC in L3, vulva in L4) (A, C, E) Wild type, (B, E, F) *gon-1.* (A) Wild-type hermaphrodite, early L3. Anterior gonadal arm. Note length from DTC to anchor cell (*). (B) *gon-1* hermaphrodite, early L3. Entire gonad is shown, with DTCs marking the anterior and posterior limits. (C) Wild-type hermaphrodite, mid-L4. Anterior gonadal arm has reflexed and grown posteriorly into a U shape. (D) *gon-1* hermaphrodite, mid-L4. Entire gonad is shown, with anterior DTC indicated. Compare the distances the gonadal arms have extended from early L3 to mid-L4 in wild-type (A, C) and *gon-1* (B, D) animals. (E) Wild-type male, L2 lethargus. Note length of gonadal arm from the LC at the proximal end to the DTCs at the distal end. (F) *gon-1* male, L2 lethargus. The gonadal arm has not extended, so that the distance between the LC and the DTCs is much shorter than in wild type.

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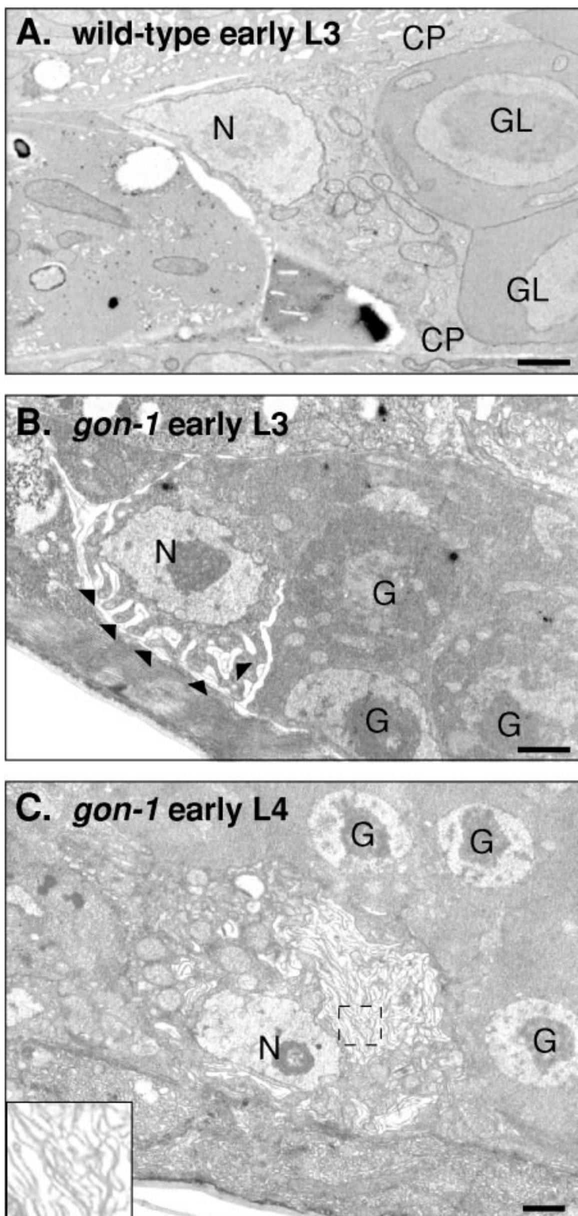

FIG. 6. *gon-1(q518)* hermaphrodite DTCs have an aberrant morphology. Electron micrographs of wild-type and *gon-1* DTCs. Center of the gonad is to the right. Scale bar, 1 μm. (A) Wild-type DTC, early L3. Note the distinct morphology of the DTC. Its nucleus (N) is at the leading edge and cellular processes (CP) extend back toward the center of gonad along the outside edge of the germline (GL). (B) *gon-1* DTC, early L3. Compare to wild-type morphology. There is no leading edge and cell processes are not found. Instead, invaginations of plasma membrane are prominent. G, unidentified gonadal cells; arrowheads, infoldings of plasma membrane. (C) *gon-1* DTC, mid-L4. The cell is filled with membranes, presumably the accumulation of invaginating plasma membranes. Inset, 3× magnification of boxed region showing membranous inclusions.

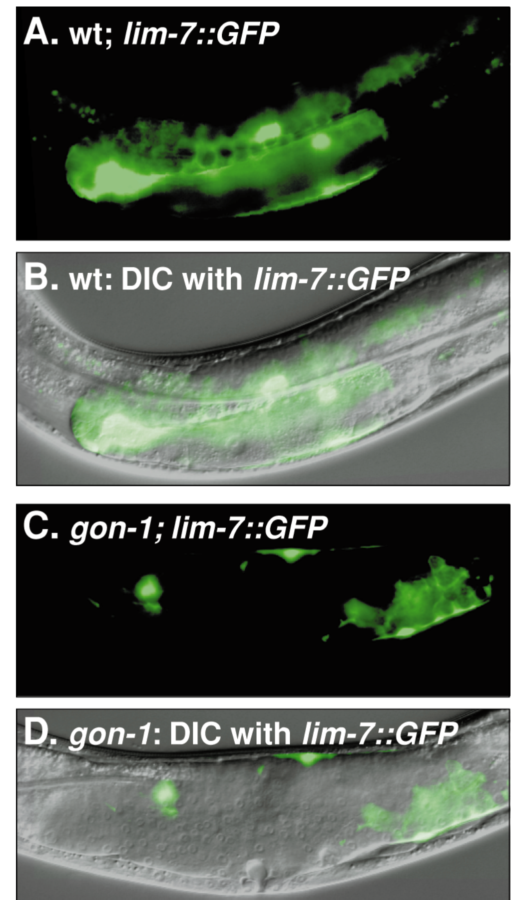

FIG. 7. Sheath differentiation in wild-type and *gon-1(q518)* adult hermaphrodites. (A, C) Fluorescence images showing *lim-7::GFP* expression as a marker of sheath differentiation. (B, D) DIC images with *lim-7::GFP* fluorescence overlay. (A, B) Wild type. *lim-7::GFP* is expressed in a sheet that surrounds the germline and extends along the gonad arm. (C, D) *gon-1(q518). lim-7::GFP* is expressed in irregular patches within the disorganized gonadal mass.

gon-1 Function Is Specific for Gonadal Morphogenesis

Finally, we asked whether *gon-1* functions in the development of nongonadal tissues. We assayed the embryonic viability, the overall shape of the animal, the coordination of its movements, the mating behavior in males, the male tail, the growth rate, and the entry and exit into dauer stage of the life cycle: all were essentially normal in *gon-1(0)* mutants (data not shown). The normal movement and shape of *gon-1(0)* mutants suggest that *gon-1* is not required generally for cell migration. For example, failure in migration of the CAN neuron causes the tail to wither (Forrester *et al.,* 1998), and defects in axon migration lead to an Unc phenotype (Hedgecock *et al.,* 1990). Furthermore, we followed the M sex

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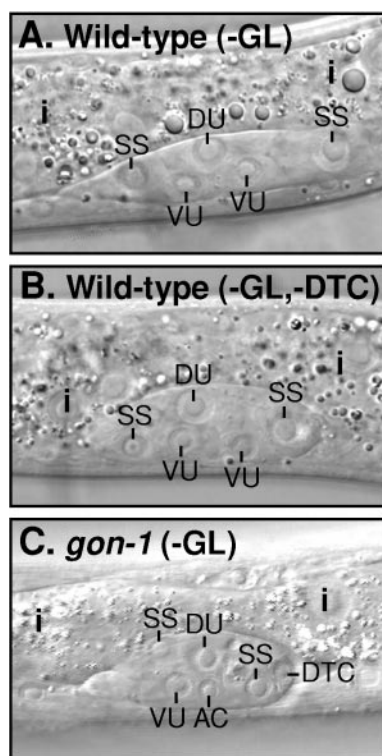

FIG. 8. Formation of the hermaphrodite somatic gonadal primordium (SP) after ablation of germline precursor cells Z2 and Z3; DIC microscopy. The SP cells include three ventral uterine precursors (VU), two dorsal uterine precursors (DU), four sheath/spermathecal precursors (SS), and an anchor cell (AC). Each image shows a single plane of focus representing only half of the SP for each worm. i, intestine. (A) Wild type (−GL). SP cells have adopted their stereotypical arrangement. The uterine precursor cells form a triangle in the center with a sheath/spermathecal cell on either side. The arrangement of SP cells in both (B) wild type (−GL, −DTCs) and (C) *gon-1*(−GL) is similar to that of the wild-type control.

myoblast and the Q neuroblasts migrations (Antebi *et al.,* 1997) in at least five *gon-1(0)* mutants, and both were normal. We conclude that *gon-1* does not affect cell migrations generally. We also examined the nongonadal tissues of *gon-1* mutants whose gonads had been removed, because often the gonad defect results in explosion of adult hermaphrodites and thereby prevents examination of their nongonadal tissues. Although gonadless *gon-1* adults had no gross defects, we observed a reproducible vacuolization in the body wall with differential interference contrast microscopy, which was not seen in similarly treated wild-type animals (data not shown). However, it must be emphasized that this defect has no apparent developmental consequences. The vacuoles were small and did not affect adult movement, behavior, or gross morphology.

DISCUSSION

gon-1 Governs Morphogenesis of the *C. elegans* Gonad

In this paper, we show that the *gon-1* gene governs two distinct events that are essential for shaping the *C. elegans* gonad. First, *gon-1* is required for leader cell migration and extension of the gonadal germline arms. Second, *gon-1* is required for expansion and morphogenesis of the adult somatic gonadal structures. The specification and differentiation of gonadal cells, in contrast, appears to be normal, and nongonadal tissues develop normally in *gon-1* mutants.

gon-1 Is Required for Leader Cell Migration

Previous studies showed that the hermaphrodite DTCs possess two regulatory functions: one to lead gonad arm extension and the other to promote germline proliferation (Kimble and White, 1981). The identification of the DTC signal for germline proliferation (Henderson *et al.,* 1994; Tax *et al.,* 1994), the germline receptor for that signal (Austin and Kimble, 1987; Yochem and Greenwald, 1989; Austin and Kimble, 1989; Crittenden *et al.,* 1994), and the downstream transcription factor (Christensen *et al.,* 1996) revealed that a Notch-like signaling pathway underlies DTC regulation of germline proliferation. However, this signaling pathway does not affect leader function: in mutants lacking the pathway, the leader cells follow their normal course to generate a U-shaped arm. The present work defines a gene with the reciprocal phenotype: *gon-1* is required for leader cell migration, but not for germline proliferation. Other genes important for leader function control *where* the leader cell will go (*unc-5, unc-6,* and *unc-40;* Hedgecock, 1990). In contrast, *gon-1* is the only known gene that is required for leader cell migration itself and, therefore, likely defines a new pathway.

gon-1 Affects Migration of the Leader Cell Specifically

The *gon-1* gene is essential for leader cell migration, but not for other cell migrations. If *gon-1* were involved in some basic mechanism of cell migration, we would expect pleiotropic effects. For example, mutations in members of the rho/rac pathway influence multiple cell migrations in nematodes and have defects in many tissues (Zipkin *et al.,* 1997; Steven *et al.,* 1998). Similarly, if *gon-1* were essential for interpreting or providing general positional cues, other migrations would also likely be affected. For example, the *unc-5*/*unc-6*/*unc-40* pathway guides the dorsal/ventral migration of both leader cells and nerve axons, which results in a gonadal defect as well as an uncoordinated phenotype (Hedgecock *et al.,* 1990). Therefore, *gon-1* is not required for cell migration generally, but instead is required specifically for leader cell migration.

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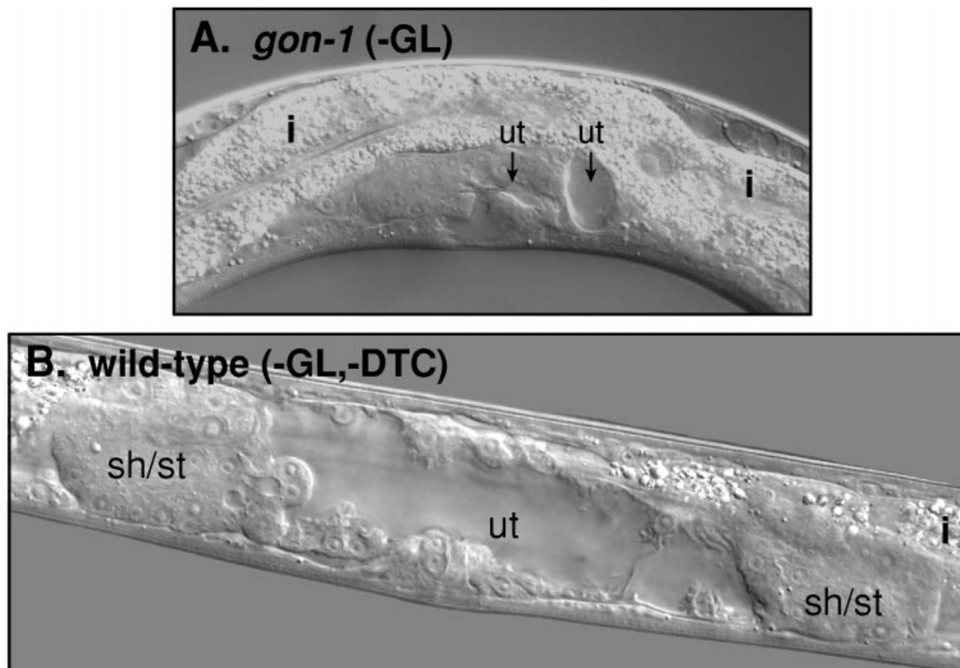

FIG. 9. Hermaphrodite somatic gonadal structures at mid-L4. Both animals have had their germline precursor cells Z2 and Z3 removed by laser ablation during L1(−GL); DIC microscopy. ut, uterine lumen; sh/st, sheath and spermathecal tissue; i, intestine. (A) *gon-1*(−GL). Morphogenesis of uterus is defective. Only fragments of uterine lumen form. Gonad is small with tissue extruding out of gonad proper (out of plane of focus). (B) Wild type (−GL,−DTC). Uterus with large lumen forms (ut).

gon-1 Is Required for Generation of a Normal Mature Uterus

In addition to its role in leader cell migration, *gon-1* also functions in the generation of the adult gonadal somatic structures. Analysis of this later defect is complicated by the earlier defect in arm extension: in both *gon-1* mutants and wild-type hermaphrodites, the failure to form gonadal arms results in a defect in formation of the somatic gonadal primordium (R. Blelloch and J. Kimble, unpublished data). To circumvent this secondary consequence of the early *gon-1* defect, we removed germline cells to permit formation of the somatic gonadal primordium and hence to allow analysis of the somatic gonadal structures. We found that *gon-1* mutants cannot form a normal mature uterus: the uterus is smaller than normal and does not enlarge during organ maturation as it does in wild type. This defect is likely to be independent of the role of *gon-1* in controlling leader cell migration since removal of the leader cells in wild-type controls did not cause a similar defect.

In a parallel study, we reported that expression of *gon-1* in the leader cells rescues leader cell migration and gonad arm extension and can even rescue a *gon-1(0)* mutant to fertility (Blelloch and Kimble, 1999). However, the distal gonadal arms of these animals are unusually slim, the central gonadal structures are often formed abnormally, and fecundity is low (R. Blelloch and J. Kimble, unpublished data). The failure to fully rescue the *gon-1(0)* phenotype by leader cell expression is consistent with a role for *gon-1* in development of the somatic structures that is independent of leader cell migration.

How Does GON-1 Control Gonadal Morphogenesis?

The *gon-1* gene encodes a secreted metalloprotease with multiple thrombospondin type 1 repeats (Blelloch and Kimble, 1999). A major question is how this secreted metalloprotease controls gonadal morphogenesis. Although we do not yet know the answer to this question, we suggest two possible mechanisms. The first is that GON-1 plays a permissive role and influences organ shape by degrading components of the extracellular matrix. Such an activity could explain both the migration and the organ expansion defects. As the leader cell migrates, its surrounding basement membrane must be remodeled to remove the barrier for migration. Similarly, as an organ grows, its surrounding basement membrane must be remodeled to permit expansion and morphogenesis. Therefore, an activity localized at the tip of the extending gonadal arms might act to permit rapid leader cell migration, and a more diffuse activity might support general expansion of the gonad. Consistent with this idea, *gon-1* is expressed in both leader cells and muscle (Blelloch and Kimble, 1999). Furthermore, expres-

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sion of *gon-1* in leader cells promotes gonadal extension, while expression in body wall muscle promotes gonadal expansion along all axes (Blelloch and Kimble, 1999). This hypothesis of a permissive role for *gon-1* is the simplest explanation for its effects on morphogenesis. However, an alternative possibility is that GON-1 plays an instructive role and controls organ shape by cleaving regulators of leader cell migration and somatic gonad formation. A precedent for this model is the Tolloid metalloprotease, which cleaves SOG/Chordin protein and thereby releases a TGF-β-like signal to regulate ventral–dorsal patterning in the embryo (Marqués *et al.,* 1997; Piccolo *et al.,* 1997). Although no such regulator for gonadal morphogenesis has been identified, the existence of such a molecule cannot be excluded.

Genes That Control Organ Shape

gon-1 is one of a growing class of genes that are required for organ shape. Other examples include the *unc-5/unc-6/unc-40* pathway in *C. elegans,* a global guidance system that affects formation of the U shape of the gonadal arm (Hedgecock *et al.,* 1990); the *breathless* pathway in development of the *Drosophila* trachea (Krasnow, 1997); and α8 integrin in murine kidney development (Müller *et al.,* 1997). As far as we know, the *gon-1* phenotype provides the first genetic evidence for a metalloprotease required for organ shape. The identification of other genes in the *gon-1* pathway will provide further insight into how metalloproteases influence this fundamental process of animal development.

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