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# The *lin-11* LIM domain transcription factor is necessary for morphogenesis of *C. elegans* uterine cells

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## SUMMARY

**The *Caenorhabditis elegans* hermaphrodite egg-laying system comprises several tissues, including the uterus and vulva. *lin-11* encodes a LIM domain transcription factor needed for certain vulval precursor cells to divide asymmetrically. Based on *lin-11* expression studies and the *lin-11* mutant phenotype, we find that *lin-11* is also required for *C. elegans* uterine morphogenesis. Specifically, *lin-11* is expressed in the ventral uterine intermediate precursor π cells and their progeny (the utse and uv1 cells), which connect the uterus to the vulva. Like π cell induction, the uterine *lin-11* expression responds to the uterine anchor cell and the *lin-12*-encoded receptor. In wild type animals, the utse, which forms the planar process at the uterine-vulval interface, fuses with the anchor cell. We found that, in *lin-11* mutants, utse differentiation was abnormal, the utse failed to fuse with the anchor cell and a functional uterine-vulval connection was not made. These findings indicate that *lin-11* is essential for uterine-vulval morphogenesis.**

Key words: *Caenorhabditis elegans*, *lin-11*, Uterus, Hermaphrodite

## INTRODUCTION

A central issue in developmental biology is how discrete cells and tissues are assembled into complex structures. The egg-laying apparatus of the *Caenorhabditis elegans* hermaphrodite undergoes concerted morphogenesis to form a functional organ from tissues of diverse origins (Fig. 1A; discussed in Li and Chalfie, 1990; Thomas et al., 1990). Eggs are stored in the mesodermal uterus and released through the epithelial vulva by the contraction of uterine and vulval-specific muscles, which are innervated by the hermaphrodite-specific neurons (HSNs) and the ventral type C (VC) neurons (Hirsh et al., 1976; Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; White et al., 1986).

Formation of a functional vulval passageway requires both that the vulva be induced to form from the ventral epithelium and that, once formed, the vulva connects properly to the uterus. A signal from the uterine anchor cell (AC) mediated by a well-characterized signal transduction pathway (reviewed in Kornfeld, 1997) induces the three most proximal vulval precursor cells (VPCs) to adopt vulval fates. These VPCs, which can have the 1° or 2° vulval fate, are arranged in the pattern 2°-1°-2° by a graded signal from the AC (Sternberg and Horvitz, 1986; Katz et al., 1995) and signaling among VPCs (Sternberg, 1988; Koga and Oshima, 1995; Simske and Kim, 1995). The 22 descendants of these VPCs undergo cell fusions and morphogenesis to produce the seven rings of the mature vulva (Sharma-Kishore et al., 1999). The dorsalmost ring, vulF, is produced by a subset of 1° cell descendants.

The AC induces not only the vulva but also the uterine π cells, the progeny of which organize the connection between these two tissues (Fig. 1B; reviewed in Newman and Sternberg, 1996). Eight π cell daughters fuse to make utse, an H-shaped cell consisting of two lateral extensions that attach the uterus to the lateral epidermis (seam) on each side of the animal, connected by a thin planar process that forms the interface between the uterus and the vulva. The remaining four π cell daughters become uv1 cells, which form adherens junctions with the utse and with vulF, physically connecting the uterus and vulva (Fig. 1C; Newman et al., 1996; Sharma-Kishore et al., 1999). The utse ultimately fuses with the AC. The dual role of the AC in inducing π and vulval cells results in alignment of the specialization of the uterus and epidermis necessary for the connection. However, the AC blocks the developing passageway. As the AC differentiates and fuses with the utse, the bulk of the AC moves away from the developing vulval opening.

*lin-12* is a founding member of the *lin-12*/Notch/*glp-1* family of receptors (Greenwald et al., 1983; Yochem et al., 1988; Gridley, 1995). *lin-12* has a multifaceted role in uterine-

vulval development, mediating three distinct cell-fate decisions. Specifically, *lin-12* functions (1) in the early AC versus VU decision that establishes the identity of the AC (Kimble, 1981; Greenwald et al., 1983; Seydoux and Greenwald, 1989), (2) in lateral signaling between 1° and 2° VPCs (Sternberg 1988; Sternberg and Horvitz, 1989), and (3) in the AC-to-π cell signal (Newman et al., 1995).

*lin-11* is a founding member of the LIM domain family (Ferguson and Horvitz, 1985; Ferguson et al., 1987; Freyd et al., 1990) and encodes a predicted transcription factor with two copies of a cysteine-rich LIM domain upstream of a homeodomain. Some LIM proteins function in development, including in cell-fate specification (for example, Appel et al., 1995) or differentiation (for example, the CRP family of "LIM-only" proteins; Arber et al., 1994) or organogenesis (Sheng et al., 1996). *lin-11* is expressed in the vulva and the VC neurons (Freyd, 1991; Struhl et al., 1993; Hobert et al., 1998) and thus might help to coordinate morphogenesis of the egg-laying system. Here we report that a *lin-11-lacZ* reporter construct is also expressed in the uterine cells that connect to the vulva, specifically in the π cells and their daughters. As in the vulva, in the uterus *lin-11* expression responds to *lin-12* activity, and the *lin-11* mutant phenotype places *lin-11* developmentally downstream of the *lin-12*-mediated cell-fate decision. In *lin-11* mutants, formation of a functional uterine-vulval connection does not occur. The AC fails to undergo the characteristic cell shape changes or migration and remains as a bloated cell blocking the passageway necessary for egg laying.

## MATERIALS AND METHODS

### Strains

Strains used were cultured on OP50 bacteria using standard methods (Brenner, 1974). Wild type is N2. The *lin-12(0)* alleles used were *n676 n909* and *n137 n720* (Greenwald et al., 1983). The *lin-12(d)* allele used was *n137* (Ferguson and Horvitz, 1985). The *lin-11* mutant allele used was *n389* except where otherwise stated. MT5788 was used to characterize the *lin-11-lacZ* expression pattern during wild-type development. For analysis of *cdh-3::*GFP expression, we used a strain containing the pJP#38 reporter construct in a *lin-11(n389)* mutant background and strain NL1008 containing pJP#38 in a *lin-11(+)* background (Pettitt et al., 1996).

### Nomenclature

The ventral uterus is produced by three VU cells that undergo two rounds of division to produce intermediate precursor cells that can be of fate π or ρ. The π cells, which are located proximally to the vulva, undergo one round of cell division and produce utse and uv1 cells, whereas the ρ cells, which are distal, undergo two rounds and generate cells that have neither the utse nor the uv1 fate (see Newman et al., 1995, 1996 for a complete description of π and ρ fates).

The mature vulval cells are the progeny of P(5-7).p granddaughters (Sulston and Horvitz, 1977). These include vulF (generated by the inner P6.p granddaughters) and vulE (produced by the outer P6.p granddaughters; Sharma-Kishore et al., 1999). vulF is the most dorsal cell type in the mature vulva and forms adherens junctions with the uterine uv1 cells (Sharma-Kishore et al., 1999; Newman et al., 1996).

### Cell lineage analysis

Standard procedures were used to observe animals using Nomarski optics (Sulston and Horvitz, 1977). Cell ablations were done with a laser microbeam (Sulston and White, 1980; Avery and Horvitz, 1987).

We examined the uterine lineages and morphogenesis in MT633, a strain of genotype *lin-11(n389); him-5(e1467). n389* is a putative molecular null allele (Freyd et al., 1990). The anatomy of the uterus in *lin-11*(*n389*) mutant animals at the intermediate precursor cell stage is similar to that in the wild type, as are the axis and asymmetry of the intermediate precursor cell divisions. For cell-lineage analysis, animals were observed continuously beginning with either the third or fourth round of VU cell division and for up to 5 hours into the L4 stage. To characterize uterine morphogenesis, either the left or the right side of the animal was observed from the beginning of the L4 stage through L4 lethargus. The position of the AC was followed throughout the L4 stage in six *lin-11(n389)* mutant animals (four animals in which uterine morphogenesis was followed and two additional animals).

All π cell daughters express the *lin-11-lacZ* reporter construct; these cells become either utse or uv1. As described in Results, utse nuclei migrate to the positions of the ut2 cells in wild type but often fail to do so in *lin-11* mutant animals. Since the uv1 cell nuclei remain proximal to the vulva even in wild-type animals, we could not easily assay whether uv1 fates were correctly specified in *lin-11* mutants.

### Electron microscopy

Sample fixation was performed as described (Bargmann et al., 1993). Serial transverse sections were obtained from the vulval region of two animals in L4 lethargus. Initially, we examined sections at intervals of 500-750 nm. In both animals, a large membrane-bound unfused cell clearly identifiable as the AC was observed. For one animal, all cells and cell boundaries were traced out in alternate sections spanning the entire region of the AC (~3 μm).

### Analysis of *lin-11-lacZ* expression

The *nIs2* transgene consists of 10 kb of genomic DNA containing *lin-11* fused to *lacZ* in a vector containing a nuclear localization sequence (Freyd, 1991). Using the MT5788 strain, which contains the *nIs2* transgene, we observed an expression pattern similar to that previously described (Freyd, 1991). To quantitate uterine *lin-11-lacZ* staining, we used animals that had been staged to the π daughter stage by Nomarski optics and transferred worm(s) from individual slides to glass slides with depressions. Fixation and X-gal staining were then done as described (Fire et al., 1990), except that acetone fixation was sometimes omitted as doing so improved the morphology of the animals. In addition to the L3 and early L4 stage staining described in the Results, later uterine and/or vulval staining was also observed. However, individual cell identifications following morphogenesis proved difficult in later-stage fixed animals and we have not pursued these observations further. To examine *lin-11-lacZ* staining in *lin-12* mutant animals, *nIs2* was crossed into strains containing these mutations, and staining was scored in animals homozygous for the mutation and for *nIs2*.

## RESULTS

### A *lin-11-lacZ* reporter construct is expressed in the π cells and their progeny

The *lin-11* gene encodes a predicted LIM domain transcription factor and possesses a homeodomain plus two cysteine-rich metal binding LIM domains (Freyd et al., 1990; Li et al., 1991). *lin-11* loss-of-function mutants fail to exhibit the asymmetric lineage characteristic of vulval 2° cells and generate an LLLL lineage rather than the wild-type LLTN or NTLL lineages (Ferguson et al., 1987). A *lin-11-lacZ* reporter construct is expressed in the portion of the lineage that requires *lin-11* (Fig. 2A; Freyd, 1991; Struhl et al., 1993).

While using the *lin-11-lacZ* reporter construct as a marker for 2° VPC fates, we noticed that it was also expressed in the

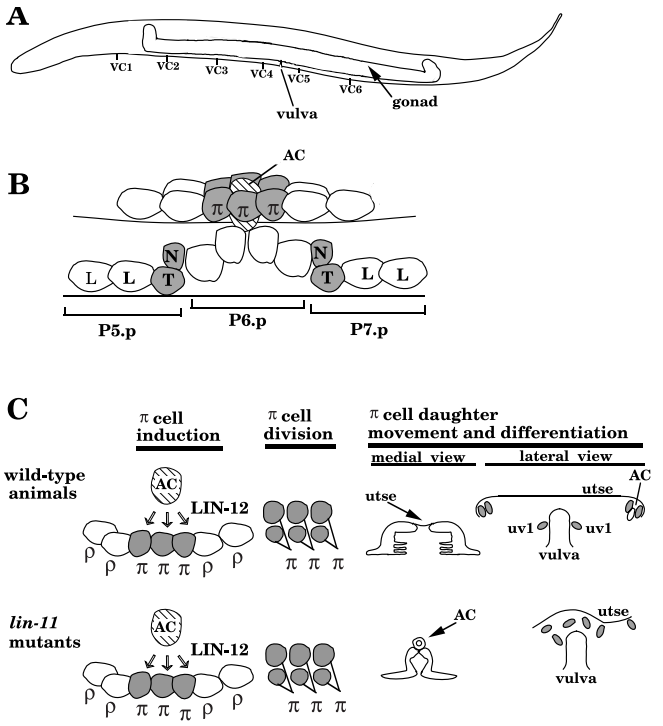


**Fig. 1.** The role of *lin-11* in the egg-laying system. Lateral view (except as noted), anterior is to the left. (A) Components of the egg-laying system described in this paper, including the vulva, gonad and VC neurons (lines indicate the positions of neuronal cell bodies along the ventral cord). Late L3 stage. (B) Enlarged view of the cells of the developing uterine-vulval connection. Ventral uterine cells are at the intermediate precursor cell stage. VPCs have divided twice and their granddaughters have begun to invaginate. *lin-11*-expressing cells are shaded (data from this study; also, Freyd, 1991; Struhl et al., 1993; Hobert et al., 1998). (C) Schematic representation of the development of the uterine-vulval connection in wild-type and in *lin-11* mutant animals. π cells and their progeny are shaded. Induction: ventral uterine cells during the late L3 stage. π cell division: L3 lethargus. Movement and differentiation, medial view: vulva and uterine-vulval connection during the mid-L4 stage. In wild-type animals, the thin laminar process of the utse is visible; in *lin-11* mutants, the vulval lips do not open out, and a bloated AC blocks the connection. Movement and differentiation; lateral view: π progeny nuclei and the line representing the attachment of the utse to the seam during the L4 stage. In the wild type, two cells (on each side) called uv1 remain mononucleate and proximal to the vulva, while four cells per side fuse to form utse, their nuclei subsequently migrating. The utse also fuses with the AC, its nucleus migrating to the left or right. The utse is a multinucleate H-shaped cell comprised of two lateral processes that contain its nuclei and a thin medial process just dorsal to the vulva. There is variability in how many utse nuclei migrate anteriorly and how many migrate posteriorly (Newman et al., 1996); in the conformation shown, the nuclei of two π cell daughters have migrated to the anterior and two have moved posteriorly, as has the AC. In *lin-11* mutant animals, the majority of π progeny nuclei remain proximal to the vulva, and the distinction between utse and uv1 cells is unclear; thus, the six π cell daughters are simply indicated by shaded ovals, while the unfused AC is visible in the medial plane of focus. In some *lin-11* mutant animals, the AC becomes as lateral as the π cell daughters (see Fig. 4). There is variability in the total number of π progeny nuclei migrating (see text).

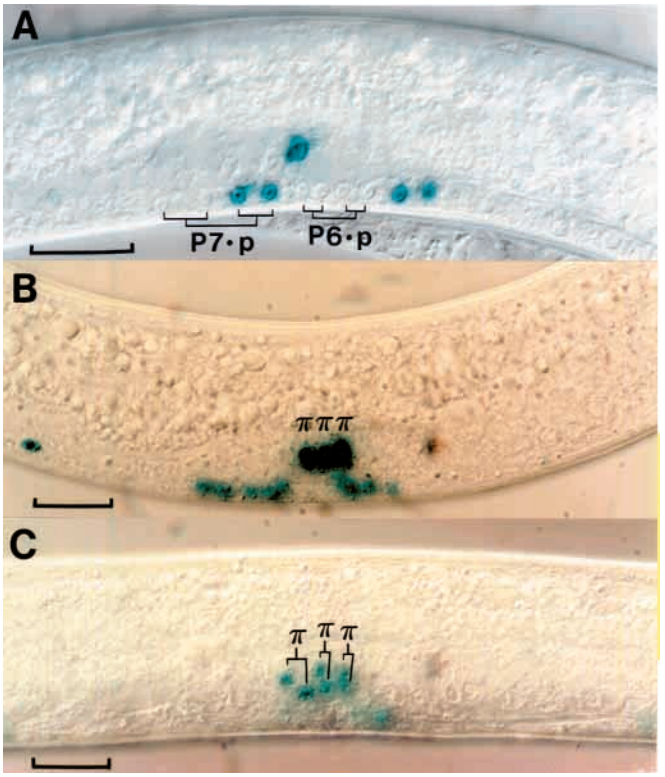


**Fig. 2.** *lin-11-lacZ* staining in the hermaphrodite. (A) X-gal histochemical staining in 2° cell granddaughters during the L3 stage, right lateral view. The four granddaughters of the 2° cell P7.p are indicated; the T and N cells stain, while the LL cells do not. The four granddaughters of P6.p do not stain. To the anterior, the T and N granddaughters of P5.p are also stained. (B) Staining of the π cells during the L3 stage, right lateral view. (C) Staining of π cell progeny during L3 lethargus. Right lateral view. Scale bar, 20 μm.

ventral uterus. The 32 nuclei of the ventral uterus are produced by the three VU cells (Kimble and Hirsh, 1979). The granddaughters of the VU cells are the VU intermediate precursors cells, which can have one of two fates, π or ρ. The π cell fate is induced from among adjacent intermediate precursor cells in response to a signal from the AC (Newman et al., 1995) such that, during the late L3 stage, there are three π cells on each side of the animal in the center of the uterus.

To investigate further the uterine *lin-11-lacZ* expression, we examined staining in animals with ventral uterine cells identified, based on Nomarski optics, to be either at the VU intermediate precursor cell stage or one round of cell division later (i.e. to contain either π cells or their daughters). We found that the *lin-11-lacZ* reporter construct is expressed in the π cells; this expression is first apparent shortly before the π cell division (Fig. 2B). During L3 lethargus, the π cells divide along a dorsoventral axis, producing two rows of three cells each of π progeny on each side of the animal (Kimble and Hirsh, 1979). Of 16 appropriately staged animals, all had staining in some or all of the π progeny (Fig. 2C). The average number of staining cells was 3.3 per side and 3/16 animals had all six π progeny per side stained (we scored only the side that was closest to the cover slip, and only those animals that had a strongly staining pharynx as a control for

fixation). Therefore, *lin-11* is expressed both in the π cells and in their daughters.

### Uterine *lin-11-lacZ* expression is dependent on the *lin-12* gene and a signal from the AC

The restriction of uterine *lin-11* expression to the π cells and their progeny could be a consequence of their cell fate, position, or both. To determine whether *lin-11* expression was dependent on a signal from the AC, we destroyed the AC with a laser microbeam during L2 lethargus or the early L3 stage and scored *lin-11-lacZ* expression during the early L4 stage. We found that 12/13 animals clearly had no uterine *lacZ* staining, indicating that *lin-11* expression was indeed AC-dependent. (In the remaining animal, a single unidentified gonadal cell was stained; however, it was too far anterior to be a π cell and possibly too far anterior to be uterine.)

The π fate also requires the *lin-12* gene: in *lin-12* loss-of-function mutants [*lin-12(0)*], no π fates are specified, whereas extra π cell fates are specified in *lin-12* gain-of-function [*lin-12(d)*] animals (Newman et al., 1995). *lin-12(d)* mutants exhibited excess *lin-11-lacZ*-expressing uterine cells, indicating that *lin-11* expression can be induced in response to activation of the receptor LIN-12. *lin-12(0)* mutant animals exhibited decreased π cell *lin-11-lacZ* expression. Specifically, in *lin-12(n137 n720*) homozygotes, 5/9 animals exhibited no π progeny staining, 1/9 had weak staining, and 3/9 animals exhibited strong to moderate staining (where 'weak' and 'strong to moderate' refer to brightness of staining). In animals homozygous for another *lin-12* reduction-of-function allele, *n676 n909*, 9/18 animals had no π progeny staining, 6/18 had weak staining and 3/18 had moderate to strong staining. Therefore, the extent of *lin-11* expression is specified by *lin-12* activity. The residual staining in putative *lin-12(0)* mutants might occur because the alleles may not be complete nulls, another protein such as GLP-1 can provide some function, or the fusion construct may not perfectly reproduce the endogenous situation.

### *lin-11* is required to make the thin laminar process that forms the L4 uterine-vulval connection

*lin-11* mutants are egg-laying defective (Ferguson and Horvitz, 1985). However, this defect cannot be solely attributable to the aberrant 2° lineage, since all 17 *lin-11* animals in which P(3-5, 7-8).p were ablated with a laser microbeam in the L1 or early L2 stage (so that each animal contained a 1°, but no 2°, vulval cells) were egg-laying defective. By contrast, 16/19 wild-type animals in which P(3-5, 7-8).p were ablated by laser were egg-laying competent.

The *lin-11* expression in the π cells raised the hypothesis that a defect in the π cells was the basis of the egg-laying defect, since π cells are required for egg laying (Newman et al., 1995). A subset of the π progeny make the utse (Newman et al., 1995, 1996), a multinucleate cell that forms the laminar process dorsal to the vulva; replacement of thick uterine tissue with this thin planar process is necessary for egg laying. We examined 27 *lin-11* mutant animals during the L4 stage using Nomarski optics and found that none had a thin process dorsal to the vulva at the appropriate stage. We also observed that, in *lin-11* animals, the dorsalmost cells on either side of the vulva (vulF) did not separate from one another during the mid-L4 stage; rather, the vulva formed a structure that was closed on top (Fig. 1C). We have hypothesized that, in wild-type animals, the differentiated utse provides a planar surface along which the vulval lips can separate (Newman et al., 1996). The *lin-11* mutant phenotype is consistent with this hypothesis and with the focus of the *lin-11* requirement for egg laying being uterine.

To determine whether the phenotype that we observed was specific to the *lin-11(n389)* allele, we used two additional *lin-11* alleles, *sy251* and *ty6*. We found that 11/11 *lin-11(sy251)* and 5/5 *lin-11(ty6)* animals examined during the L4 stage had

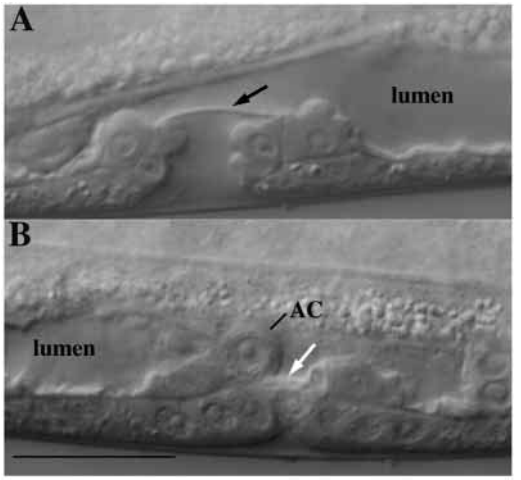


**Fig. 3.** Nomarski photomicrographs of the developing uterine-vulval connection. (A) Wild-type animal in which all VPCs but P6.p have been ablated. A thin laminar process (black arrow) is dorsal to the vulva, as in intact hermaphrodites. (B) *lin-11(n389)* mutant animal with all VPCs but P6.p ablated. No thin process is formed (white arrow), and a large bloated AC is evident. lumen, uterine lumen. Scale bar, 20 μm.

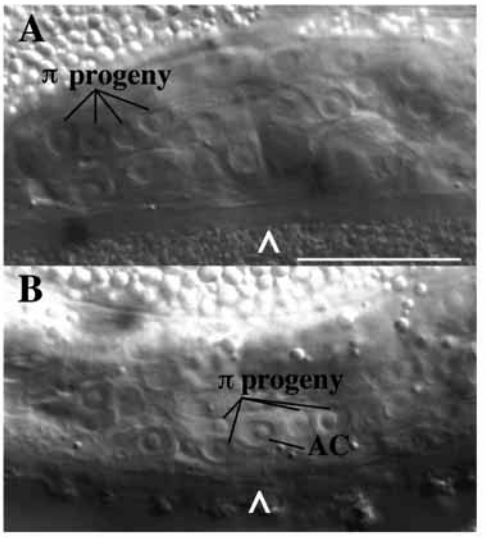


**Fig. 4.** π cell progeny are affected by a *lin-11* mutation. Nomarski photomicrographs. The white arrowhead indicates the position of the vulva. (A) N2, right lateral view. Four π progeny are migrating to the posterior. (B) *lin-11(n389)*, right lateral view. An animal in which none of the π progeny has migrated from the vulva by the late L4 stage. Four of these progeny as well as the AC are visible in a lateral plane. Scale bar, 20 μm.

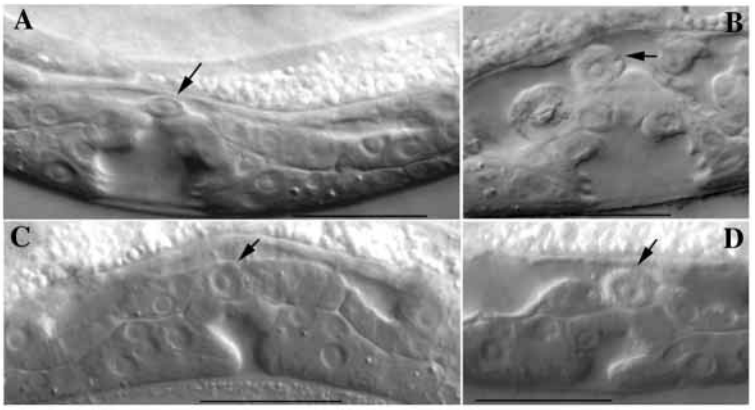


**Fig. 5.** The AC is abnormal in *lin-11* mutants. Nomarski photomicrographs. (A) N2. During the L4 stage, the AC nucleus appears elongate and surrounded by less cytoplasm than earlier in development. (B) An N2 animal in which the three VU precursor cells have been ablated. The AC appears bloated. (C) *lin-11(n389)*, left lateral view. During the mid-L4 stage, the AC (which retains its cytoplasmic mass) remains dorsal to the vulva. (D) Same animal as in C, 2 hours later. The AC remains bloated and distinct from other tissue. Arrow, AC. Scale bar, 20 μm.

uterine anatomies that were indistinguishable from those of *lin-11(n389)* animals. We also examined the anatomies of animals bearing the *lin-11(n566)* allele, in which hermaphrodites have greater mating efficiency than those of genotype *lin-11(n389)* (Ferguson and Horvitz, 1985). We found that only 2/10 appropriately staged *lin-11(n566)* animals had vulval lips that failed to separate, while 8/10 had less severe defects in formation of the uterine-vulval connection.

Since the 2° cell lineage defect leads to a *lin-11* mutant animal with an abnormal vulva, it was possible that a vulval defect could be the cause of the aberrant uterine-vulval connection. To test this hypothesis, we examined the connection in wild-type and *lin-11* mutant animals in which P6.p had been isolated by ablating P(3-5, 7-8).p during the L1 or early L2 stage. We found that, in all 13 wild-type animals with an isolated P6.p cell, the thin process formed normally above the vulva, whereas none of 13 *lin-11* animals with an isolated P6.p exhibited this structure (Fig. 3). Thus, the *lin-11* connection defect, like the egg-laying defect, cannot be attributed to abnormal 2° cells.

### *lin-11* mutant animals have defects in migrations of π progeny nuclei

To test directly whether *lin-11* is required for the π cell fate, we followed the ventral uterine lineages in seven *lin-11* mutant animals. The patterns of cell division were wild type in 5/7 animals. In the other two animals, most cell divisions were wild type but either 2/12 or 4/12 π progeny underwent a fourth round of division, indicative of adopting a ρ fate. Thus, there is no significant defect in the axis of cell division or the number of progeny produced. We therefore looked later in development.

During the L4 stage, four of the six π cell progeny on each side of the animal migrate from the center of the uterus distally in the process of forming the utse (Newman et al., 1996). They migrate to the position of the uterine epithelial ut2 toroids (containing the ventral uterine nuclei VT3a to the anterior and VT10p to the posterior). A thin line evident by Nomarski optics forms with these nuclei at its vertexes at the position where the utse attaches to the lateral epidermis (seam). By contrast, all five *lin-11* mutant animals observed throughout morphogenesis had only from one to three progeny that reached even the uv3 cells (see Newman et al., 1996; Figs 5C,D, 6F) that are more proximal than the ut2 toroids (Table 1; Fig. 4). Furthermore, the shape of the attachment line was generally abnormal. We conclude that *lin-11* is required for proper differentiation of the utse.

**Table 1. Migrations of π cell progeny and AC nuclei in wild-type and *lin-11* animals**

| Genotype | No. of π progeny nuclei migrating past uv3 | AC nucleus migration |
|---|---|---|
| *lin-11(+)* | 4 (16/16 animals)* | Anterior (2/10 animals)<br>Posterior (8/10 animals) |
| *lin-11(n389)* | 3 | none |
| | 2 | none |
| | 2 | none |
| | 2 | none |
| | 1 | nd |

*Data from Newman et al. (1996)

For *lin-11(n389)*, each line represents an animal whose lineage was followed.

### *lin-11* mutants are defective in movement and cell shape changes of the AC

The behavior of the AC in *lin-11* mutants also differs from that in the wild type. During wild-type uterine morphogenesis, the AC fuses with the utse during the L4 stage, and its nucleus migrates with the π progeny nuclei, finally reaching ut2 with them (Fig. 1C; Newman et al., 1996). The appearance of the AC (as observed using Nomarski optics) changes subsequent to fusion, and the nucleus is no longer surrounded by a cytoplasmic mass that clearly belongs to the AC (Fig. 5A). When the π cells are destroyed with a laser microbeam and the AC has no utse with which to fuse, the AC nucleus fails to move from its position dorsal to the vulva and becomes large and bloated (Newman et al., 1996; see Fig. 5B). Furthermore, in a *lin-12(0)* mutant in which π fates are not specified and which also has three or four ACs (*n676 n909*), we observed three or four bloated ACs dorsal to the vulva in each of nine L4 animals examined.

In *lin-11* mutant animals, the behavior of the AC was similar to that of animals lacking π cells, i.e. the AC failed to migrate and became larger and more bloated (Figs 1C, 5C,D). Subsequently, the AC moved to the left or right side of the animal (sometimes becoming as lateral as the π cell progeny), but often remained distinct from other tissue as an isolated cell. In 6/6 animals in which the AC was observed throughout morphogenesis, it failed to migrate to either the anterior or posterior.

Electron micrographs of *lin-11* mutant animals in L4 lethargus confirmed that the AC remains as an unfused cell (Fig. 6). In both animals examined, a cell identifiable as the AC based on its position, shape and ventrally placed nucleus

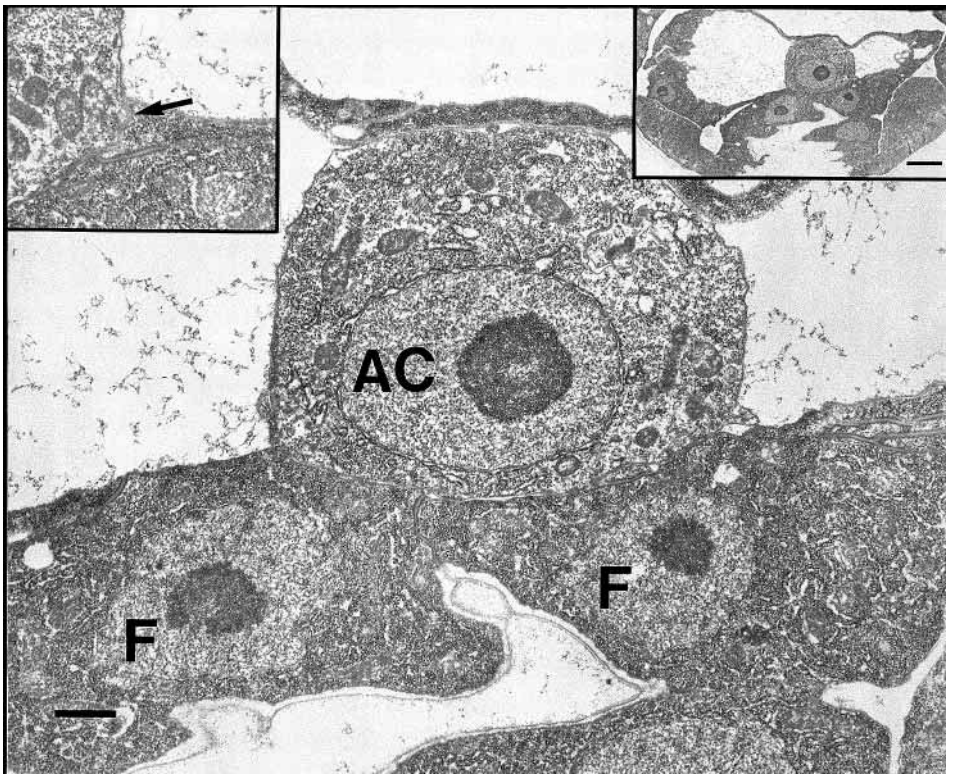


**Fig. 6**. The AC remains unfused in *lin-11(n389)* mutants. Electron micrograph, transverse section. L4 lethargus. The AC, which is surrounded by a double membrane, is in close contact with the vulval vulF cells (F). Right inset shows the entire uterus and vulva from the same section. The uterine lumen is open, while the cells surrounding it have become thin during differentiation. By contrast, the unfused AC occludes the center of the lumen. Left inset: the process of a uterine cell is separated from the AC by a membrane (arrow), indicating that the AC has not fused with the adjacent uterine cells as it would have in the wild type. Scale bar, 500 nm; right inset scale bar, 2 μm.

was observed at a time when in the wild type the AC would have fused with the utse. A double membrane surrounds the entire AC. The AC is in close contact with the vulval vulF cells. By contrast, in wild-type animals at this developmental stage, vulF contacts the uv1 cells, which connect to the utse (Newman et al., 1996).

### Uterine expression of the cadherin-related *cdh-3* gene is altered in *lin-11* mutants

The *cdh-3* gene is a member of the cadherin superfamily, which is dynamically expressed in vulval and uterine cells during their morphogenesis (Pettitt et al., 1996). In the uterus, *cdh-3* is expressed in the AC beginning in the L3 stage and in the utse during the L4 stage. A *cdh-3*::GFP reporter construct serves as a useful marker for uterine development.

To test whether *cdh-3* expression in the utse was dependent on *lin-11* function, we compared expression of a *cdh-3*::GFP reporter construct in animals that were genotypically *lin-11(n389)* or *lin-11(+)* during L4 lethargus, when uterine morphogenesis is complete. Nine of nine *lin-11(+)* animals scored had *cdh-3*::GFP expression in the utse (Fig. 7A), consistent with the observations of Pettit et al. (1996). By contrast, none of 17 *lin-11(n389)* animals exhibited this expression pattern. Instead, three of 17 *lin-11* mutant animals had no (or questionable) uterine expression, while four animals had uterine expression in a single cell identifiable by position and shape as an AC that had failed to fuse with the utse (Fig. 7C,D). The remaining ten *lin-11* animals scored had proximal uterine expression that was irregular in shape and often weaker than the utse expression observed in wild-type animals (Fig. 7B). This pattern may reflect abnormal morphology of the utse in *lin-11* mutants, reduced expression of uterine *cdh-3*, or both. In either case, these data support the conclusion that the *lin-11* gene is required for correct differentiation of the utse.

The expression pattern of *cdh-3* as well as its regulation by *lin-11* suggests a role for *cdh-3* in uterine-vulval development. However, animals in which the *cdh-3* gene is deleted [*cdh-3(pk87)* mutant animals] are not defective in egg laying (Pettitt et al., 1996), perhaps due to genetic redundancy. Furthermore, we found that 23/23 L4 stage *cdh-3(pk87)* animals showed wild-type-like uterine cell positions and uterine-vulval connections. Also, the *cdh-3(pk87)* mutation does not enhance the partial loss-of-function *lin-11(n566)* allele.

## DISCUSSION

### *lin-11* is expressed in three components of the developing egg-laying system

*lin-11* is expressed in a subset of vulval 2° cell progeny (Freyd, 1991; Struhl et al., 1993), in the VC neurons (Freyd, 1991; Hobert et al., 1998), and in the π cells and their progeny (this study), all of which are components of the *C. elegans* egg-laying system (Fig. 1).

The egg-laying system comprises specialized epithelial cells, muscles and neurons of diverse lineal origins and cell types. Mechanisms must exist to ensure its coordinate assembly and function. *lin-11* encodes a transcription factor expressed in three of the component cell types, all of which physically interact with the vulval 1° cell or its progeny. It is possible that *lin-11* regulates some common, but as yet unknown, aspect of the development of these cells.

### Expression of *lin-11* in response to *lin-12* activity

Activation of LIN-12 results in a variety of responses depending on the cell (Greenwald et al., 1983; Newman et al., 1995), raising the issue of how cell-specific responses to a general signal are programmed. We show here that *lin-11* is activated by *lin-12* in both the vulval 2° cells and the uterine π cells. However, *lin-11* does not appear to be a general executor of *lin-12*-mediated responses. According to our studies of the *lin-11-lacZ* fusion gene, *lin-11* is not expressed in the VU cell early, or in the SMs or G2 cell, all of which

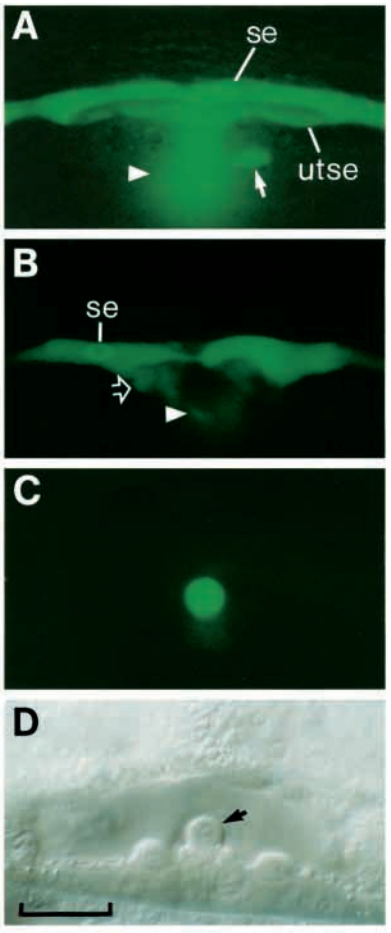


**Fig. 7**. Uterine *cdh-3*::GFP expression in wild-type and *lin-11(n389)* mutant animals during L4 lethargus. Anterior is to the left. (A) Wild-type, left lateral view. *cdh-3*::GFP expression in the seam (se) and the lateral side of the utse that attaches to it. HSN (white arrow) and vulval (arrowhead) expression from other focal planes is also visible. (B) *lin-11(n389)*, left lateral view, showing *cdh-3*::GFP expression in the seam (se) and uterus. In the uterus, *cdh-3*::GFP (open arrow) is expressed weakly and in an irregular pattern. Some vulval expression (arrowhead) from another focal plane is also evident. (C) *lin-11(n389)*, medial view, showing *cdh-3*::GFP expression in the AC. (D) Same animal as C, Nomarski photomicrograph. The AC expressing *cdh-3*::GFP is indicated by the black arrow. Scale bar, 20 μm.

require *lin-12* function (Greenwald et al., 1983). Also, there is no evidence for a role of *lin-12* in VC neuron specification. LIN-11 may be a component of a partially specific response to LIN-12. The ability of a cell to activate LIN-11 in response to LIN-12 might be one of the factors that contributes to the cell specifity of such response.

### The role of *lin-11* in uterine-vulval development

Proper uterine-vulval development depends on induction and differentiation in both tissues. Vulval epithelium is induced in response to a signal from the AC; the vulval 1° and 2° cell fates are then patterned and expressed. In the uterus, the AC induces the π cells, the progeny of which (the utse and uv1 cells) connect to the vulva; as the utse differentiates, it extends a thin laminar process dorsal to the vulva and fuses with the AC.

A requirement for *lin-11* in vulval development (specifically, in execution of the asymmetric 2° VPC lineage) has been previously demonstrated by cell-lineage analysis (Ferguson et al., 1987) and confirmed by *lin-11-lacZ* expression analysis (Freyd, 1991; Struhl et al., 1993). In this study, we used the same techniques to uncover a later role for *lin-11* in the morphogenesis of uterine cells during their connection to the vulva. We observed *lin-11-lacZ* expression in the uterine π cells and their progeny, which connect to the vulva. We found that *lin-11* mutants did not have significant defects in π fate specification per se or in the asymmetric cell division that generates dorsal cells that are larger than their ventral sisters. Thus, in general, the cells divided along the correct axis to produce two progeny as in the wild type, although occasionally extra divisions occurred, perhaps reflecting a partial transformation in cell fates. By contrast, differentiation of π progeny was more severely affected.

The following data support a role for *lin-11* in utse differentiation. First, *lin-11* mutants have (1) a completely penetrant egg-laying defect that cannot be explained by the *lin-11* vulval lineage defect or the VC neuron defect (the VC neurons are not needed for egg laying; Garriga et al., 1993; cf. Waggoner et al., 1998), and (2) defects in migration of π progeny and the AC and in differentiation of the thin laminar process of the utse. Second, a *lin-11-lacZ* gene fusion is expressed in the π cells and their daughters. The simplest explanation is that *lin-11* is required for differentiation of the utse, including its fusion with the AC. In wild-type animals, the utse is formed by fusion of a subset of π progeny, which then fuse with the AC (Newman et al., 1996). Subsequently, the utse extends cytoplasm longitudinally and attaches to the lateral epidermis (seam). The nuclei then migrate from their initial proximal position to the distal ends of the utse, perhaps being squeezed out from the center as the cell attaches to the seam. We found that, in *lin-11* mutants, the defect in AC migration was more severe than that of the π progeny nuclei (see Results). However, the observed defect in migration of the AC can be attributed to a failure of this cell to fuse with the utse since EM analysis revealed a clear membrane between the AC and other cells. We hypothesize that, in *lin-11* mutants, the utse is completely non-functional (resulting in a 100% penetrant egg-laying defect) and partially undifferentiated, so that fusion of the utse with the AC does not occur, but π progeny nuclear migration (and the requisite extension of cytoplasm) sometimes does.

Why does the unfused AC remain attached to the surrounding tissue? In some egg-laying-defective mutants in which the AC fails to fuse with the utse but instead continues to reside at the uterine-vulval interface, the AC later detaches from this tissue and can be seen floating free in the uterine lumen (A. P. N. and N. Cinar, unpublished observations). By contrast, animals in which the π cells were ablated with a laser microbeam and mutants that fail to specify the π cell fate have an AC that appears firmly embedded in the surrounding tissue (Newman et al., 1996; A. P. N., unpublished). Perhaps the AC is retained within the confines of a non-functional or partially functional utse but detaches when the utse has wild-type function and it is the AC that is unable to fuse.

Like *lin-11*, the *cog-2* transcription factor is expressed in the π cells and their daughters and is required for formation of a proper uterine-vulval connection (Hanna-Rose and Han, 1999). Uterine *lin-11* expression does not depend on *cog-2* function (Hanna-Rose and Han, 1999). Also, we found that 10/10 animals examined during the early L4 stage showed normal *cog-2*::GFP reporter construct expression in the π cell lineage in *lin-11(n389)* mutant animals (A. P. N., data not shown). Thus, while both the *lin-11*- and *cog-2*-encoded transcription factors are expressed in the π cell lineage, neither appears necessary for the expression of the other.

### Conclusion

The *lin-11* gene, which encodes a LIM domain transcription factor (Freyd et al., 1990), was first defined by four mutants with fully penetrant egg-laying defects, three of which

consistently failed to form normal vulvae (Ferguson and Horvitz, 1985). It is now clear that the *lin-11* mutant phenotype is caused by two distinct requirements for *lin-11* in uterine-vulval development. First, *lin-11* is required for expression of the 2° vulval lineage (Ferguson et al., 1987). In *lin-11* mutants, the TN portion of the lineage is absent and all 2° cell descendants adhere to the ventral cuticle. (By contrast, in wild-type animals, the TN cell generates descendants that detach from the ventral cuticle, positioning them closer to the uterus.) However, this defect is not responsible for the inability of *lin-11* mutant animals to lay eggs. The *lin-11* gene is also required later in development for uterine morphogenesis (this study). Absence of *lin-11* function results in animals in which the uterine AC does not fuse with the uterine utse and a functional uterine-vulval connection is not made. The dual role of *lin-11* in vulval lineage expression and uterine morphogenesis leads to a vulva with correctly positioned cells that is properly connected to the uterus, forming a functional passageway through which eggs can be laid.

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