

The *Caenorhabditis elegans* *lin-12* gene mediates induction of ventral uterine specialization by the anchor cell

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SUMMARY

The anchor cell (AC) of the *Caenorhabditis elegans* gonad has a critical role in the development of a functional egg-laying system, which is accomplished through cell-cell interactions. Lateral inhibitory *lin-12*-mediated signaling among two bipotential cells causes one to adopt the ventral uterine precursor (VU) cell fate while the other becomes the AC. The AC then induces formation of vulval tissue. We find that the AC also induces a particular ventral uterine intermediate precursor fate (π) by a mechanism that is genetically and temporally distinct from vulval

induction. This process requires *lin-12*, but unlike previously described *lin-12*-mediated decisions, signaling is unidirectional, is between dissimilar cells and does not involve lateral inhibition. The π fates are necessary for egg laying and appear to produce a distinct specialized cell type. Thus, patterning of the ventral uterus by the AC is crucial to the development of a functional egg-laying system.

Key words: *Caenorhabditis*, *lin-12*, anchor cell, uterine development, induction

INTRODUCTION

The development of complex organs involves the specification of individual cell fates and the integration of differentiated cells into a single functioning unit. The capacity of *C. elegans* hermaphrodites to lay eggs is dependent on a number of different cell types of diverse lineal origins. These include the epithelial cells of the uterus and of the vulva, through which eggs are laid, the uterine and vulval muscle cells and the neurons that innervate them (Hirsh et al., 1976; Kimble and Hirsh, 1979; Sulston and Horvitz, 1977; White et al., 1986). Studies of the specification of many of these cells have revealed signaling events between different cell types (Desai et al., 1988; Garriga et al., 1993; Horvitz and Sternberg, 1991; Kimble, 1981; Li and Chalfie, 1990; Thomas et al., 1990). Thus, the *C. elegans* egg-laying apparatus allows an analysis of the orchestration of numerous cell types into a functional organ.

The developing hermaphrodite somatic gonad occupies a central role in organizing the egg-laying system. In mutants in which the gonad is anterior to its normal position in the animal, a functional egg-laying system can be assembled, albeit at an ectopic location (Thomas et al., 1990). The developing gonad produces signals that induce production of vulval tissue and refine the migrations of the precursors that generate the egg-laying muscles (Kimble, 1981; Thomas et al., 1990). The vulva in turn helps to position correctly incoming axons and muscle attachments (Garriga et al., 1993; Li and Chalfie, 1990; Thomas et al., 1990).

The AC is a cell of the somatic gonad with a unique role in

forming the connection between uterus and vulva that permits eggs to be laid. It both participates physically in this structure and induces other cells that cooperate in its formation (see below). During *C. elegans* development, lateral inhibitory signaling among two developmentally equivalent bipotential cells causes one to adopt the ventral uterine precursor (VU) cell fate while the other becomes an anchor cell (AC; the AC versus VU decision; (Kimble, 1981; Seydoux and Greenwald, 1989). This specification is mediated by LIN-12, a member of a family of presumptive receptors for intercellular signals which includes *C. elegans* GLP-1, *Drosophila* Notch and the vertebrate proteins Xotch, Motch and Tan1 (Artavanis et al., 1991; Austin and Kimble, 1987, 1989; Coffman et al., 1990; Ellisen et al., 1991; Franco del Amo et al., 1992; Greenwald et al., 1983; Priess et al., 1987; Weinmaster et al., 1992; Yochem and Greenwald, 1989). Both LIN-12 and Notch have been shown to mediate numerous lateral inhibitory signaling events. Elegant mosaic analysis (Seydoux and Greenwald, 1989) and related studies have made the AC versus VU decision a paradigm for the study of the role of LIN-12/Notch in lateral inhibition. In contrast, GLP-1 appears to function in induction (reviewed in Lambie and Kimble, 1991a).

Once the AC fate is determined, the AC induces three of six vulval precursor cells (VPCs) to generate vulval tissue (Kimble, 1981; Sternberg and Horvitz, 1986). The genes that mediate this induction include the *lin-3* signal (a member of the EGF family of growth factors; Hill and Sternberg, 1992), the receptor tyrosine kinase *let-23*, (Aroian et al., 1990) and downstream genes whose role in signaling appears conserved in a wide variety of organisms (Sternberg, 1993).

The *C. elegans* hermaphrodite ventral uterus is composed of a complex array of cells whose pattern is symmetric with respect to the AC (Kimble and Hirsh, 1979). In this paper, we show that differentiated ventral uterine cells are produced by intermediate precursors that can have one of two fates. We find that the AC is required to specify one of these fates in nearby cells and that this event is *lin-12* dependent. However, AC-mediated signaling of ventral uterine cells is unidirectional and inductive, in contrast to previously characterized *lin-12*-dependent processes that involve lateral inhibition. This induction occurs later than AC induction of the vulva and is also genetically distinct.

MATERIALS AND METHODS

Strains

Strains were cultured using standard techniques (Brenner, 1974). Genotypes are as follows. Wild-type strain is N2 (Brenner, 1974). The *lin-12* alleles used were *n137* (Greenwald et al., 1983), *n676n909* (Greenwald et al., 1983) and *n137n720*. *n676n909* was maintained in MT2375, a strain of genotype + *dpy-19(e1259) lin-12(n137)/unc-32(e189) + lin-12(n676n909)*; *him-5(e1467)*. *n137n720* was maintained in + *dpy-19(e1259) lin-12(n137)/unc-32(e189) + lin-12(n137n720)* (Sternberg and Horvitz, 1989). The *lin-3* alleles used were *n378*, *1059* and *n1058*. *n378/n1059* was maintained in PS1031, a strain of genotype + *let-312(s1234) lin-3(n378) + unc-22(s7)/unc-24(e138) + lin-3(n1059) dpy-20(e1282) +* (J. Liu, unpublished). *n1058* was maintained in the balanced line *n1058/DnT1* (Ferguson and Horvitz, 1985). PS1238 contains multiple copies of a transgene encoding the *lin-3* EGF domain under control of a heat shock promoter (genotype, *unc-31(e169); syEx23[pRH51 C14G10 pMOB]*; R. J. Hill and P. W. Sternberg, unpublished results). The *let-23* alleles used were *sy97* and *sy10*, which was maintained in the balanced line *let-23(sy10) unc-4(e120)/mnC1[dpy10(e128)unc-52(e444)]* (Aroian and Sternberg, 1991). The *sur-1* allele used was *kul* and the *lin-45* allele, *sy96*.

Cell lineage analysis and laser ablations

Cell lineages were observed using Nomarski optics as described (Sulston and Horvitz, 1977). For mutant strains, full or partial lineages were observed for 8 to 43 animals per strain. Only those animals for which all final divisions were observed are represented in the tables. In general, these animals were observed continuously beginning with the third round of VU cell division, although observation was sometimes begun with the first, second, or fourth round of division. Animals were followed through the end of the fourth round and generally for 1-4 hours (and for up to 7 hours) after the end of this round to ensure that no further divisions occurred. Divisions of the dorsal uterine lineage were followed in most animals, providing a means of correlating the timing of rounds of uterine cell division with the wild-type situation. Ablations were performed using a laser microbeam as described (Avery and Horvitz, 1987; Sulston and White, 1980).

Interpretation of cell lineage data

π and ρ intermediate precursors differ from one another in axis of division, number of progeny produced and morphology of their progeny. Specifically, the π cells undergo an asymmetric cell division along a predominantly dorsal-ventral axis to produce a small lower daughter and a larger upper one. Since the altered geometry of the uterus in mutants and ablated animals often results in a general skewing of division axes, we have used only the number of progeny produced as our assay for cell fate. In general, cell fate transformations caused a change in both morphology and number of progeny.

However, in some $\pi \rightarrow \rho$ cell fate transformations, the morphology of the progeny remained π -like, perhaps reflecting only a partial transformation of cell fate. This occurred most often in the progeny of Z1.ppp and Z4.aaa and may reflect a cell-intrinsic component to specification. The distal ρ cell at each end generates progeny that contribute to the spermathecal valve as well as to the uterus. We have not distinguished between these and the other ρ cells.

In calculating the percent of presumptive π and ρ cells induced to π for mutant animals, all animals were considered to have 50% presumptive π cells and 50% presumptive ρ cells, as in the wild type.

Percent vulval induction was calculated as described (Han et al., 1990).

Heat shock

Animals in L2 lethargus were subject to heat shock by floating sealed plates in a 33°C water bath for 90-120 minutes.

EM reconstruction

Serial section reconstructions were undertaken as described (White et al., 1986).

RESULTS

Ventral uterine development

Early events in the development of the hermaphrodite gonad lead to the generation of twelve somatic precursor cells, their dispersal by proliferation of the germ line and the subsequent movement of ten of these cells into the center of the gonad to form the somatic primordium (Kimble and Hirsh, 1979). As the somatic primordium is forming at the end of the L2 stage, lateral inhibitory signaling among two developmentally equivalent bipotential cells (Z1.ppp and Z4.aaa) results in either cell adopting the AC fate and the other becoming a VU cell (the AC versus VU decision; Kimble, 1981; Seydoux and Greenwald, 1989). The three VU cells whose divisions produce the 32 nuclei of the hermaphrodite ventral uterus consist of the sisters of both Z1.ppp or Z4.aaa plus one of these cells (Kimble and Hirsh, 1979). Each VU cell generates four intermediate precursors during the L3 stage. The results of Kimble and Hirsh (1979) and of this study indicate that these intermediate precursors can have one of two fates, which differ in the morphology of their progeny and the number of descendants generated (Figs 1A, 2). We have designated these fates π and ρ ; π cells produce two descendants, whereas ρ cells produce four. π cells divide along a dorsal-ventral axis, while ρ divisions are predominantly anterior-posterior.

In wild-type animals, two conformations of the somatic primordium are equally probable; the outcome of the AC versus VU decision determines which is adopted (Kimble and Hirsh, 1979). The conformation of the somatic primordium in turn determines which of three possible VU lineages (VU1, VU2 or VU3) a VU cell will generate. VU1, VU2 and VU3 produce one, two or three π cells, respectively. For both conformations of the somatic primordium, the total number of π cells produced, as well as the pattern of π and ρ cells, is identical (Fig. 1A).

If the AC is ablated when the somatic primordium is forming, it is replaced by the alternative precursor (Kimble, 1981). In addition, ablation of single VU cells sometimes causes changes in the lineages of the remaining VU cells. One interpretation is that each VU cell has a ground state which is altered by cell-cell interactions between VU cells. If all the cells of the somatic

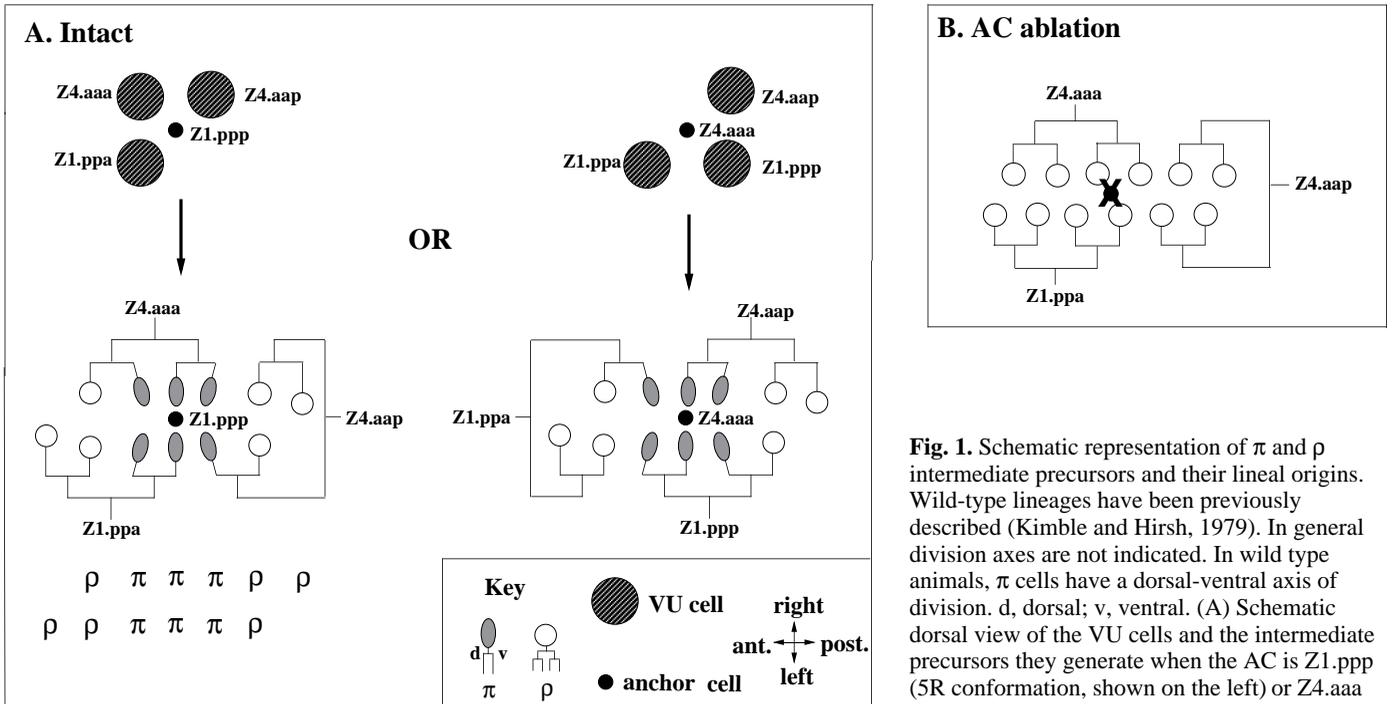


Fig. 1. Schematic representation of π and ρ intermediate precursors and their lineal origins. Wild-type lineages have been previously described (Kimble and Hirsh, 1979). In general division axes are not indicated. In wild type animals, π cells have a dorsal-ventral axis of division. d, dorsal; v, ventral. (A) Schematic dorsal view of the VU cells and the intermediate precursors they generate when the AC is Z1.ppa (5R conformation, shown on the left) or Z4.aaa (5L conformation, shown on the right) (Kimble

and Hirsh, 1979). Beneath the former diagram is a representation of the pattern of ρ and π cells in the notation used in the tables. (B) After ablation of the AC in L2 lethargus or early L3, all intermediate precursors become ρ . Only a 5R conformation is shown.

primordium except one VU cell are ablated, that VU would be expected to adopt its ground state. When we did this experiment in two animals, the remaining VU followed a lineage not observed in wild-type animals, generating four ρ cells and no π cells. This suggested to us that cell fate decisions might be taking place at the level of the intermediate precursors and led to the following line of experiments.

The AC induces the π fate

While cell boundaries cannot be well visualized using Nomarski optics, it is clear that all π cells are close to, and most likely touching, the AC. To test whether the AC has a role in uterine patterning, we ablated the AC at a time when it could no longer be replaced by the alternative precursor and followed the ventral uterine lineages. When this was done before the VU cells had divided (1-cell stage) or when they had recently divided (early 2-cell stage), no π fates were specified and only ρ fates were observed (Table 1A; Figs 1B, 2D). The π to ρ transformation was not observed when the AC was ablated after the intermediate precursors had formed (4-cell stage), but sometimes occurred following ablations done when cells were in the process of dividing to produce the intermediate precursors (2 \rightarrow 4-cell stage; Table 1A). While it is possible that induction is occurring at the time of this division, given the perdurance of debris following ablations, it seems likely that cell fate determination is occurring in the intermediate precursors, rather than in their parents. We propose that the AC induces nearby intermediate precursors to adopt the π fate (Fig. 3).

Since the AC induces production of vulval tissue (Kimble, 1981; Sternberg and Horvitz, 1986), the absence of π cells in AC-ablated animals could be an indirect consequence of the absence of vulval tissue. However, ablation of the six vulval precursor cells (VPCs) that produce vulval tissue does not

abolish the π fate (Table 1B). Thus, the VPCs are not required for induction of the π fate.

Vulval induction occurs at the time when VPCs are at the 1-cell stage, at approximately 27 hours after hatching (Kimble, 1981; Sternberg and Horvitz, 1986). Ablation of the AC after that time has no effect on vulval induction. We found that some animals in which the AC was ablated too late to have any effect on vulval induction nonetheless had a complete π to ρ transformation (Table 1A). Therefore, induction of π and vulval fates by the AC are temporally distinct. We estimate that induction of π fates occurs at about 31 hours after hatching, or approximately 4 hours after vulval induction.

Induction of the vulva by the AC requires proteins that function in a conserved signal transduction pathway (Sternberg, 1993). These include the ligand LIN-3, a member of the EGF family of growth factors (Hill and Sternberg, 1992), the LET-23 receptor tyrosine kinase (Aroian et al., 1990; Ferguson and Horvitz, 1985), LIN-45 raf (Han et al., 1993), and the MAP kinase MPK-1/SUR-1 (Lackner et al., 1994; Wu and Han, 1994). These proteins seem not to be required for specification of the π fate since the ventral uterine lineages are essentially wild type in strains defective in *lin-3*, *let-23*, *lin-45* or *sur-1*, or that overexpress *lin-3* (Table 2A). With the caveat that we did not analyze complete loss-of-function alleles (see legend to Table 2), the AC induces π and vulval fates using distinct sets of signaling proteins.

The geometry of the uterus is altered in strains with abnormal vulval development and the AC can become slightly mispositioned with respect to the ventral uterine cells. This mispositioning sometimes results in presumptive π cells (presumptive based on their lineage history) becoming distal from the AC and adopting the ρ fate. Since this was observed in VPC-ablated animals (1/42 presumptive π cells), *lin-3* and *let-*

Table 1. AC ablation leads to an absence of π fates

Number of animals	Stage of VUs at ablation*	Lineage	Presumptive π cells induced to π (%) [†]	Vulval induction
(A) AC ablations				
	Intact [‡]	$\rho\pi\pi\rho\rho$ $\rho\rho\pi\pi\rho$	100%	100%
6	1-cell stage	$\rho\rho\rho\rho\rho$ $\rho\rho\rho\rho\rho$	0% [§]	0%/partial
5	mixed 1- & 2-cell stage and early 2-cell stage [¶]	$\rho\rho\rho\rho\rho$ $\rho\rho\rho\rho\rho$	0%	66%/100%/induced
12 [◊]	{ late 2-cell stage 2→4-cell stage early 4-cell stage	variable	37%	Induced
			74%	
			98%	
3	mid 4-cell stage	$\rho\pi\pi\rho\rho$ $\rho\rho\pi\pi\rho$	100%	100%
(B) VPC ablations				
7	1-cell stage or earlier	$\rho\pi\pi\rho\rho$ $\rho\rho\pi\pi\rho$	98% [#]	

For each lineage designation, the upper line depicts intermediate precursors on the right side of the animal and the lower line depicts those on the left.

(A) All AC ablations were done during L3, except for one animal in which the AC was ablated during L2 lethargus (when the VUs were at the 1-cell stage). AC ablations were not done earlier than L2 lethargus because prior to this time, replacement regulation results in the alternative precursor's becoming an AC (Kimble, 1981).

*1-cell stage designates the VU cells; 2-cell stage: daughters of the VU cells; 4-cell stage: granddaughters of the VU cells, which are the intermediate precursors ρ and π .

[†]In the AC and VPC ablations, presumptive ρ cells always became ρ .

[‡]Data from Kimble and Hirsh, 1979.

[§]Presumptive π cells from Z1.ppp or Z4.aaa sometimes divided later than those from Z1.ppa and Z4.aaa.

[¶]Three animals were from the mixed 1- and 2-cell stage; two animals were from the early 2-cell stage.

^{||}One animal had 66% vulval induction, two animals had 100% induction and two animals were scored as 'induced.' Induced indicates that induction appeared complete, but was not scored at an informative stage.

[◊]Of these animals, one was at the late 2-cell stage. For the remaining 11 animals, at least one cell per animal was in metaphase, anaphase, or telophase of the division producing intermediate precursors (2→4-cell stage). Other cells were either at the late 2-cell or early 4-cell stage. All presumptive π and ρ cells were scored. For 70/72 intermediate precursors scored, both progeny behaved similarly. In two cases, one daughter divided and the other did not.

(B) VPC ablations were performed either during L1 or L1 lethargus ($n=4$) or during L2 lethargus ($n=3$) when VPCs were at the 1-cell stage and VUs were at the 1-cell stage or before the divisions which produce the VU cells had occurred.

[#]One animal had a slightly mispositioned AC resulting in a presumptive π becoming ρ .

23 vulvaless strains (1/24 presumptive π cells in each case) and strains overexpressing *lin-3* (2/24 presumptive π cells), we do not believe that it reflects anything other than altered geometry. Since a slight change in the AC position can result in the cell fate transformation of an individual intermediate precursor and π nuclei are proximal to the AC, it seems likely that specification of the π fate is dependent on direct contact with the AC. In these experiments, presumptive π cells sometimes adopted a ρ fate, but we did not observe hybrid π/ρ lineages. In fact, in the vast majority of animals, intermediate precursor lineages were transformed as a unit (Tables 1-2), suggesting that fate specification is occurring at this level. However, since we did

see occasional hybrid lineages (see footnotes to Tables 1, 2), the progeny of intermediate precursors may be subject to some later regulation as well.

π fate specification requires *lin-12*

The AC versus VU decision is mediated by *lin-12* (Greenwald et al., 1983; Seydoux and Greenwald, 1989). In *lin-12* loss-of-function mutants [*lin-12(0)*], one to three of the VU cells are transformed into ACs. We found that in *lin-12(0)* animals that had either one or two VU cells, no π fates were specified (Table 2B; Fig. 4A). If *lin-12* were not required for the π fate, the presence of extra ACs (and presumably extra signal) might be expected to increase production of π cells. Furthermore, the ACs are clearly functional in *lin-12(0)* mutants as vulval induction does occur. Thus, the absence of π cells in *lin-12(0)* cannot be an indirect consequence of the earlier defect in the AC versus VU decision. We conclude that *lin-12* is required for induction of the π fate by the AC.

Gain-of-function alleles of *lin-12* [*lin-12(d)*] lead to the opposite transformation from the loss-of-function phenotype. In the ventral uterus, this results in specification of four VU cells and no AC (Greenwald et al., 1983). When we determined the ventral uterine lineages of *lin-12(d)* mutants, we found that they generate predominantly π cells, but exhibit some extra cell divisions (i.e. ρ fates) at the distal ends of the uterus (Table 2B, Fig. 4B). These extra divisions may be caused by additional intercellular signaling, possibly a signal that normally specifies the distal ends of the uterus. To determine the phenotype when all potential signaling cells had been eliminated, we ablated all gonadal cells other than the VU cells and followed the ventral uterine lineages. We found that all intermediate precursors were π cells, the opposite transformation from *lin-12(0)* mutants with respect to the π fate (Table 2B, Fig. 4C). Thus, *lin-12* activity is both necessary and sufficient to specify π fates. In *lin-12(d)* animals, the π fate is specified despite the absence of an AC, suggesting that *lin-12* functions in the intermediate precursors. This hypothesis is consistent with mosaic analysis of *lin-12* showing that it is required in the signaled cell in the AC versus VU decision (Seydoux and Greenwald, 1989).

In wild-type animals, the distal ρ cell at each end generates progeny that contribute to the spermathecal valves as well as to the uterus (Kimble and Hirsh, 1979). If this difference in progeny cell types is specified at the level of the intermediate precursor cells, then these distal cells differ in fate from their more AC-proximal ρ neighbors; however, this is not evident by differences in number of rounds of division, as both produce four progeny (The different ρ cells have different axes of division, but since these axes are readily changed when the uterine geometry is altered, it is not clear that they represent distinct fates). However, in a *lin-12(d)* background where most cells are π , signaling of distal ventral uterine intermediate precursor cells may allow them to override the action of *lin-12(d)* and to adopt a fate that is readily distinguished by cell lineage from that of their neighbors. Unablated *lin-12(d)* animals have four VU cells and therefore 16 intermediate precursors. The configuration of these intermediate precursors is such that there is an 'overhang' of cells at each end not seen in the wild-type (Fig. 4B). Since it is the cells at these ends that exhibit some ρ fates, this may be caused by signaling from other gonadal cells, i.e., those of the germ line or the dorsal uterine and/or sheath-spermathecal lineages. However, while

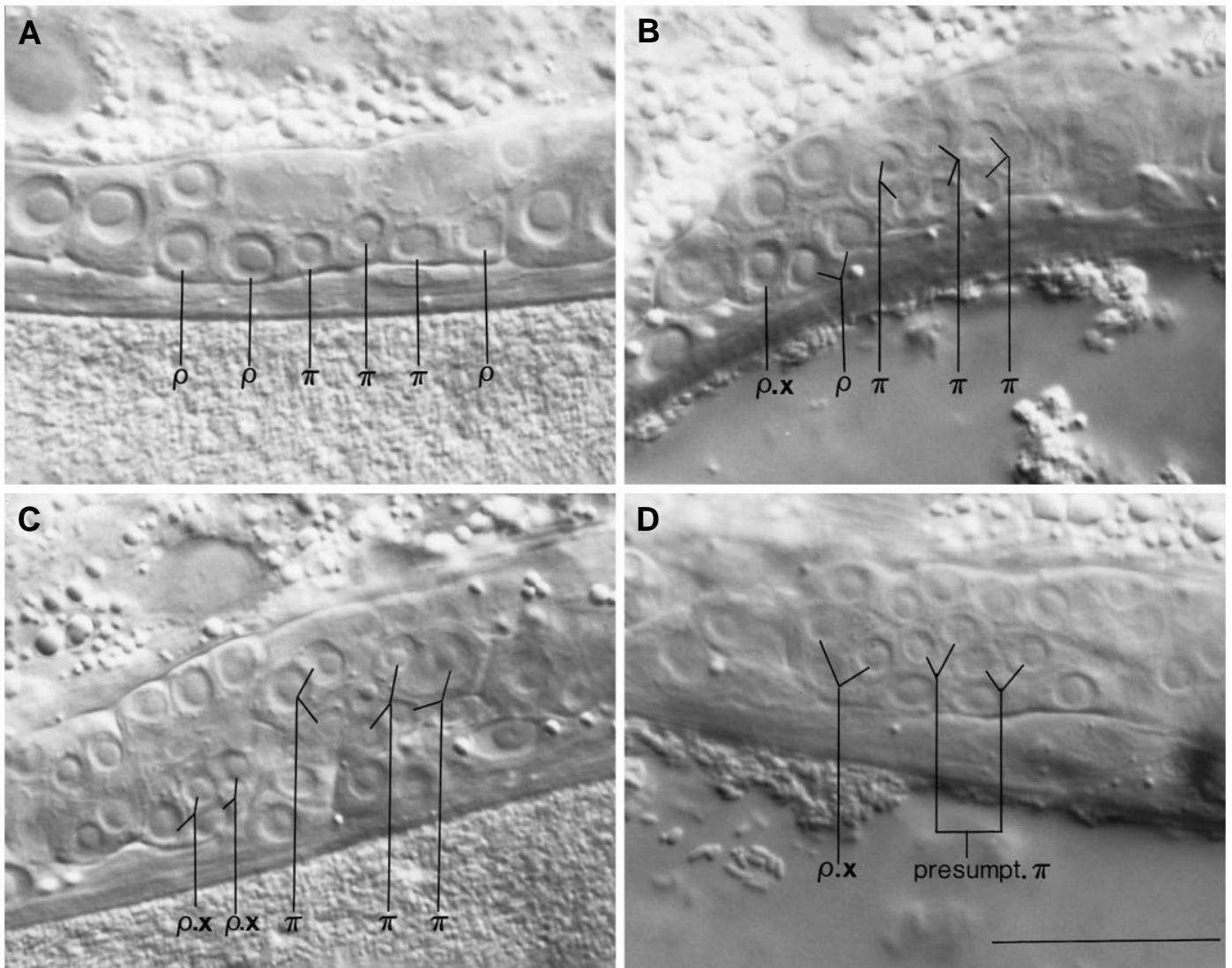


Fig. 2. Nomarski photomicrographs of the ventral uterine lineage in intact (A-C) and AC-ablated (D) animals. (A) L3 stage. VU cells have divided twice to produce π and ρ intermediate precursors. (B) L3 lethargus. π and ρ cells have each divided once. (C) Early L4 stage. ρ progeny have divided; π progeny have not. (D) Early L4 stage. In the AC-ablated animal, presumptive π cells adopt a ρ fate and each produce four progeny. Right (A) and left (B-C) lateral views. Scale bar, 20 μm .

isolation of the four VU cells abolished ρ fates at the ends, we could not find any one type of blast cell whose ablation consistently gave this result (unpublished data). In the VU isolation experiment, the steric configuration of intermediate precursors is altered such that there is no longer an overhang of cells at the ends (Fig. 4C). Thus, cells at the uterine ends of unablated *lin-12(d)* animals might be specified by a mechanism that distinguishes whether a cell's neighbors are 'self' or 'non-self.' Other explanations, including redundancy of signaling cells, could also account for our inability to find a single source of signal that specifies the distal uterine ends.

***lin-12* mutations lead to altered identity of final differentiated ventral uterine progeny**

Examination of differentiated ventral uterine progeny by electron microscopy (EM) in *lin-12* mutants revealed alterations in identity which paralleled those deduced by lineage

analysis. During the L4 stage, in wild-type animals, eight ventral uterine progeny fuse together and also fuse with the AC to make a large multinucleate cell, the uterine seam cell (utse), which attaches to the lateral epithelial (seam) cells (J. White and E. Southgate, unpublished observations). In a *lin-12(d)* mutant, a large multinucleate cell was observed that formed a specific attachment to the lateral epithelial (seam) cells (Fig. 5A). This feature and the characteristic conspicuous appearance of the endoplasmic reticulum identifies this as corresponding to the utse cell of the wild-type. Serial section reconstructions showed that the *lin-12(d)* utse had 33 nuclei at the expense of other uterine cell types. In a reconstructed *lin-12(0)* animal, none of the cells in the L4 uterus had the appearance of a utse cell and no lateral attachments to the seam cells were seen (Fig. 5B).

The final uterine divisions take place during early L4; following this, morphogenesis leads to a formation of a uterine lumen

Table 2. Ventral uterine lineages in mutant animals

Number of animals	Strain	Lineage	Presumptive π cells induced to π (%)	Presumptive ρ cells induced to π (%)
(A) Mutants defective in vulval induction				
10	<i>lin-3</i> reduced function#	$\rho\pi\pi\rho\rho$ $\rho\rho\pi\pi\rho$	98%*	0%
4	<i>lin-3</i> overexpressed (transgene)	$\rho\pi\pi\rho\rho$ $\rho\rho\pi\pi\rho$	92%*	4%†
11	<i>let-23</i> reduced function‡	$\rho\pi\pi\rho\rho$ $\rho\rho\pi\pi\rho$	98%*	0%
4	<i>lin-45</i> reduced function Δ	$\rho\pi\pi\rho\rho$ $\rho\rho\pi\pi\rho$	100%	0%
3	<i>sur-1</i> reduced function ∇	$\rho\pi\pi\rho\rho$ $\rho\rho\pi\pi\rho$	100%	0%
(B) <i>lin-12</i> mutants \diamond				
5	<i>lin-12(0)</i> , 1 VU \blacklozenge	$\rho\rho\rho\rho$	0%	0%
3	<i>lin-12(0)</i> , 2 VUs \blacksquare	$\rho\rho\rho\rho$ $\rho\rho\rho\rho$	0%	0%
5	<i>lin-12(d)</i>	(ρ or π) $\pi\pi\pi\pi\pi\pi$ $\pi\pi\pi\pi\pi\pi$ (ρ or π)‡	100%	79%
3	<i>lin-12(d)</i> , 4 VU cell isolation \S	$\pi\pi\pi\pi\pi\pi$ $\pi\pi\pi\pi\pi\pi$	100%	100%

For each lineage designation, the upper line depicts intermediate precursors on the right side of the animal and the lower line depicts those on the left.
 #Two strains were used, neither of which are null for *lin-3* activity, as this results in larval lethality (Ferguson and Horvitz, 1985). Four animals were *lin-3(n378)/lin-3(n1059)* heterozygotes, which have the most severe vulvaless defect. Six animals were *lin-3(n1058)* homozygotes, which are sterile.

*Strains with 92-98% of presumptive π cells induced to π occasionally had a slightly mispositioned AC resulting in a presumptive π cells becoming ρ .
 †In this strain, 1/24 presumptive ρ cells produced only two progeny and therefore was scored as π . However, the timing and axes of division were ρ -like.

‡Two strains were used, neither of which is null for *let-23* activity, as this results in larval lethality (Ferguson and Horvitz, 1985). Four animals were *let-23(sy97)* homozygotes, which have a highly penetrant vulvaless defect. Seven animals were *let-23(sy10)* homozygotes, which are sterile and vulvaless.

Δ *lin-45(sy96)* is a severe hypomorph (Han et al., 1993).

∇ *sur-1(ku1)* is likely non-null (Wu and Han, 1994).

\diamond Genetic analysis indicated that the *lin-12(0)* and *lin-12(d)* alleles are null and gain-of-function, respectively (Greenwald et al., 1983).

\blacklozenge The alleles used were *n676n909* ($n=4$) and *n137n720* ($n=1$).

\blacksquare *n137n720*.

‡(ρ or π): these intermediate precursors, which are distal to the position at which the anchor cell normally sits, became ρ (7/10), π (2/10) or ρ/π hybrid (1/10). In one animal, an additional distal cell became ρ . In addition to the five animals shown above, six animals for which precise lineages were not determined were found to contain predominantly π precursors.

\S *lin-12(d)* has four VU cells and therefore 16 intermediate precursors. The four VU cells were isolated by either two or three rounds of ablation. The cells ablated were Z2, Z3, Z1.a, Z4.p, Z1.pa and Z4.ap, so that the only gonadal cells remaining were Z1.pp and Z4.aa.

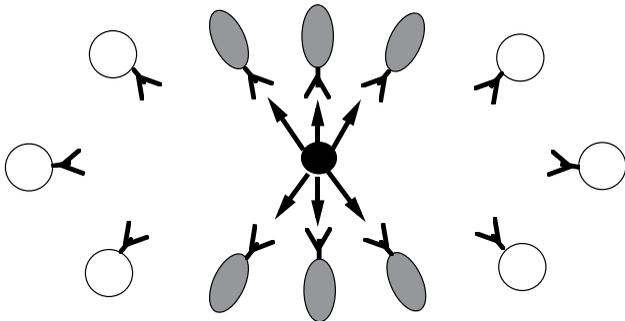


Fig. 3. Model for induction of the π fate by the AC in the most proximal intermediate precursors. Black circle, AC; grey oval, π cell; white circle, ρ cell. In the simplest interpretation of our results, the receptor for the inductive signal is *lin-12*.

and other features of the mature reproductive system (Kimble and Hirsh, 1979). Because of these complex cellular movements, it has been difficult to determine the precise lineal ori-

gins of the terminal differentiated progeny. Our correlations between the cell fate transformations of intermediate precursors, based on Nomarski criteria, and final differentiated progeny, as judged by EM reconstruction, in *lin-12* mutants has provided a clue to the origins of one differentiated uterine cell, the utse. Specifically, its absence in a *lin-12(0)* mutant, which has no π cells, and its over-representation in a *lin-12(d)* mutant, which has excess π cells, strongly suggests that the majority of π progeny form the utse. Since the ultimate fate of the AC is to fuse with the utse (J. White and E. Southgate, unpublished observations), it would appear that the AC both induces the π cells and fuses with their progeny. The observation that there are twelve π progeny and only eight ventral uterine utse nuclei strongly suggests that there are subsequent cell fate decisions which further delimit the roles of subsets of π progeny.

π cells are required for generation of a functional egg-laying system

Since π cells appear to produce a differentiated cell type not made by ρ cells, we were curious whether both sets of intermediate precursors generated cell types with essential roles in

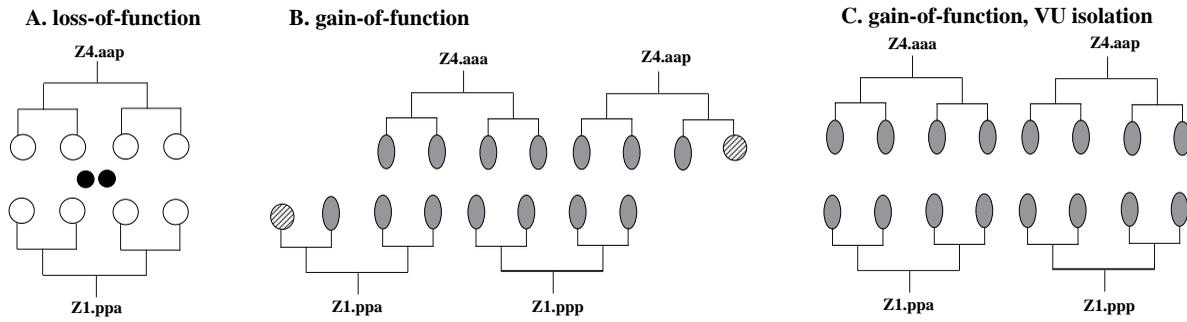


Fig. 4. Intermediate precursor fates in *lin-12* mutants. Symbols as in Fig. 1. Hatched circle represents a cell that became π or ρ . (A) In *lin-12* loss-of-function mutants, all intermediate precursors become ρ . In the example shown, there were two ACs and two VU cells; in *lin-12* loss-of-function mutants with three ACs and one VU cell, the intermediate precursors are also all ρ . (B) In *lin-12* gain-of-function mutants, most of the intermediate precursors are π . The most distal sometimes become ρ (see Table 2B for frequency). (C) In a *lin-12* gain-of-function mutant in which all gonadal cells other than the four VU cells were ablated, all intermediate precursors are π .

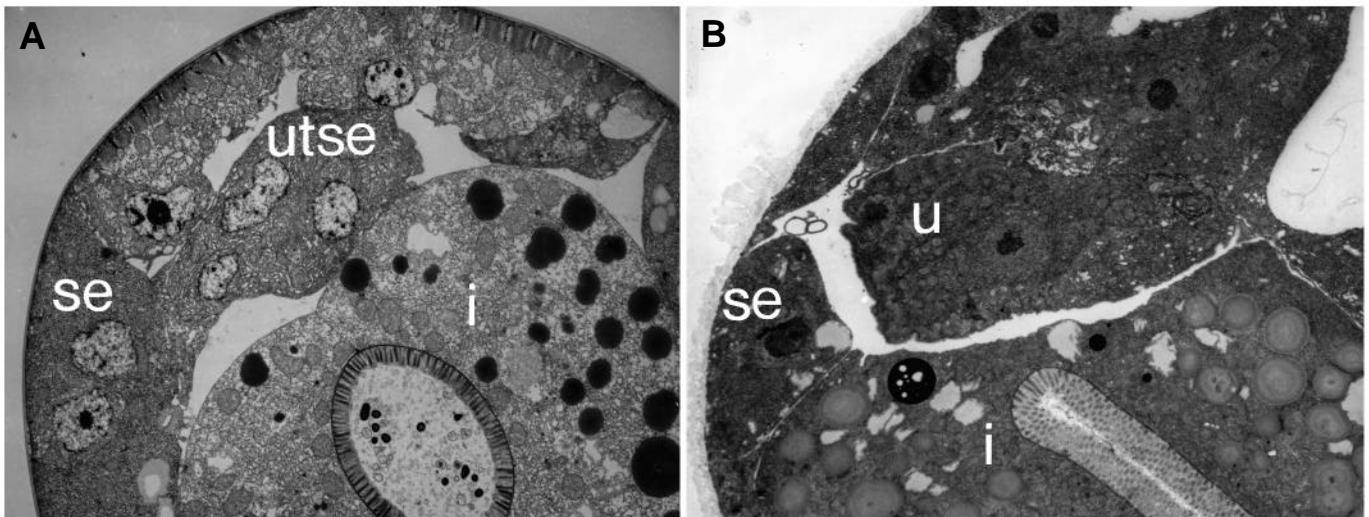


Fig. 5. Representative electron micrographs from serially sectioned and reconstructed animals depicting the differentiated uterine structures in (A) a *lin-12(d)(n137)* and (B) a *lin-12(0)(n137n720)* animal. The multinucleate utse cell can be seen making its characteristic attachment to a seam cell in A, whereas no utse cell is seen in B. u, uterine cells; utse, uterine seam cell; se, seam cell; i, intestine. *lin-12(0)* animals give poor results in the electron microscope, which may correspond to their sickly appearance under the light microscope (Greenwald et al., 1983). Magnification: 1820.

egg laying. We therefore ablated either all of the π cells or all of the ρ cells and examined the phenotype of the resulting animals. We found that 20/20 hermaphrodites with all π cells ablated were incapable of laying eggs and their progeny hatched internally. In contrast, only 5/10 ρ cell-ablated animals were egg-laying defective. Therefore, ρ cells may produce progeny that are redundant with those produced by other lineages, such as those of the dorsal uterus. In contrast, the finding that π cells are essential for egg laying may reflect the fact that this cell type represents a modification of the basal uterine program – induced as part of the AC’s coordinated signaling program that establishes a specialized uterine/vulval structure required for eggs to be laid.

DISCUSSION

Cell fate specification in the ventral uterine lineages

Cell-cell interactions play an important role in specifying

ventral uterine fates. The four cells that will become the AC and three VU cells interact relatively early in *C. elegans* post-embryonic development when they join the somatic primordium (Kimble and Hirsh, 1979). Our results indicate that a second stage of cell fate specification occurs at a later time and determines whether a ventral uterine intermediate precursor cell will have a π or ρ fate. The early AC versus VU decision and the resultant positioning of the AC in the center of the gonad results in an asymmetric arrangement of cells in the somatic primordium such that there are two VU cells on one side of the animal and one on the other (Fig. 1A). The axes of the subsequent divisions restore the symmetry, generating six intermediate precursors on each side of the animal. There exist specialized cell types in the ventral uterus of the mature animal (J. White and E. Southgate, unpublished observations). Our results demonstrate that the central AC is critical to make the central uterus distinct from the distal portions by inducing those intermediate precursors closest to it to become π .

Our data also permit a reinterpretation of the cell lineage regulation seen following single VU cell ablations at or shortly after somatic primordium formation (Kimble, 1981). Following ablation of a single VU cell in the somatic primordium, steric constraints cause the remaining VU cells to move so that the set of intermediate precursors adjacent to the anchor cell are generated by different VU cells than in the intact animal. Thus, the observation that the remaining VU cells generate altered lineages may be an indirect result of the altered position of the intermediate precursors with respect to the AC, and therefore of specification of the π fate in an altered group of intermediate precursors.

A $\pi \rightarrow \rho$ cell fate transformation can be produced physically (by AC ablation during L3) or genetically (by a loss-of-function mutation in *lin-12*). AC ablation causes this transformation if performed before or during the division that produces intermediate precursors, suggesting that cell fate determination is occurring in these cells and not their parents. Furthermore, in general, intermediate precursors are transformed as a unit, suggesting that fate specification is occurring in these cells and not their progeny.

The role of *lin-12* in π fate specification

lin-12 is required for induction of the π fate by the AC. The simplest interpretation of our results is that *lin-12* is the receptor for the AC signal that specifies π fates. While other possibilities exist, none involve lateral inhibition. For instance, a responding cell might need both an inductive signal and a 'lateral excitatory' signal from similarly responding cells to become π , in a phenomenon similar to the community effect (Gurdon, 1988), with *lin-12* acting in the latter process. In either case, *lin-12* activity is required to specify the induced fate and not to prevent two equipotential cells from adopting the same state as in its previously documented functions. As seen in other *lin-12*-dependent cell fate decisions, a *lin-12* gain-of-function mutation causes the opposite transformation (excess π cells at the expense of ρ cells) from a loss-of-function mutation, indicating that *lin-12* activity state specifies the cell fate.

Use of sequential binary decisions to generate complex cellular patterns

The family of proteins that includes Notch, LIN-12 and GLP-1 appears, in some cases at least, to mediate signals between adjacent cells (Mello et al., 1994; Seydoux and Greenwald, 1989; Sternberg, 1988; Sternberg and Horvitz, 1989; this study), and has been shown to control binary decisions of cell fate (reviewed in Greenspan, 1990; Greenwald and Rubin, 1992). Binary decisions must be utilized several times in succession if they are to generate complex patterns of cells. Notch mediates sequential rounds of lateral inhibition in the *Drosophila* external sensory organs (Posakony, 1994), while *glp-1* mediates two stages of inductive signaling in the *C. elegans* embryo (Mello et al., 1994).

A third way in which one receptor for binary decisions can be used twice in succession is in a switch from lateral inhibition to inductive signaling. The AC versus VU decision begins with two equivalent cells which appear to use the same ligand and receptor (*lin-12*) to signal one another mutually. The outcome of this process is that one cell (the AC) becomes the signaling cell and the other (the VU cell) becomes the

receiving cell, with the apparatus for receiving signal in the AC and for sending it in the VU cell presumably down-regulated. The simplest interpretation of our results is that two generations later, this asymmetry is maintained and used in an inductive interaction, so that the signaling cell (the AC) utilizes the same ligand to induce the grandprogeny of the receiving (VU) cell, which may still be expressing the *lin-12* receptor or may re-express it. These two signaling events occur approximately 8 hours apart (Kimble, 1981; this study). *apx-1* and *lag-2* are two *C. elegans* genes with sequence similarity to the Notch ligands Delta and Serrate that function in the *glp-1* and *lin-12* signaling pathways (Fortini and Artavanis-Tsakonas, 1993; Lambie and Kimble, 1991b; Mello et al., 1994; Tax et al., 1994). Loss-of-function mutations in *lag-2* result in early larval lethality, but certain hypomorphic alleles allow occasional animals to survive past this point (Lambie and Kimble, 1991b). Such survivors sometimes have two anchor cells, suggesting that *lag-2* may be the ligand for the AC versus VU decision. However, the ventral uterine lineages that we observed in survivors of genotype *lag-2(q393)* failed to demonstrate a convincing defect in π fate specification (our unpublished results), leaving the identity of the ligand for π fate induction an open question.

The intracellular apparatus used downstream of a receptor when two bipotential cells interact to adopt different fates stochastically, and that used to mediate the coordinated response of a group of cells to an inductive signal, might be expected to differ in at least some feedback response loops. Our results indicate that *lin-12* acts in one modality in the VU cell and in another in its grandprogeny. The observation that π cell fates are required for egg laying will enable us to screen for genes that are specific to one of these modalities.

The AC has multiple interrelated functions in egg-laying system organogenesis

Our data add a further dimension to the multifaceted role of the AC during generation of the egg-laying system. Lateral inhibitory *lin-12*-mediated signaling first determines which of two equipotential ventral uterine cells will become the AC. Subsequently, the AC induces vulval formation via *lin-3* and then π fate specification using *lin-12*. The AC is required for correct vulval morphogenesis as well (Kimble, 1981). Finally, when the AC has completed all the above functions, it fuses with the ventral uterine cells of the utse, a cell which forms part of the specific connection between the mature uterus and vulva (J. White and E. Southgate, unpublished observations). Thus the AC both induces the VPCs and ventral uterine intermediate precursor cells whose progeny are critical to forming a passage from the uterus to the outside and continues to interact with these progeny in a highly specific fashion. Further study of how the AC's roles in induction and morphogenesis are coordinated will provide fundamental insight into the relationship between cell fate specification and higher order organization of tissues and organs during development.

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