Spatial and Temporal Patterns of *lin-12* Expression During *C. elegans* Hermaphrodite Development

Hilary A. Wilkinson*,[1] and Iva Greenwald*,†

**Department of Biochemistry and Molecular Biophysics and †Howard Hughes Medical Institute, Columbia University, College of Physicians and Surgeons, New York, New York 10032*

Manuscript received April 11, 1995
Accepted for publication June 22, 1995

ABSTRACT

The *lin-12* gene encodes a receptor that mediates certain cell-cell interactions during *Caenorhabditis elegans* development. We have examined the expression of a *lin-12::lacZ* reporter gene in individual cells during the development of *C. elegans* hermaphrodites. *lin-12::lacZ* is expressed in a discrete spatial and temporal pattern during development and the *lin-12::lacZ* reporter gene will provide a useful marker for other studies, particularly of somatic gonadal and vulval development. In general, the cells that express *lin-12::lacZ* correspond to cells whose fates are known to be altered in *lin-12* mutants implying that restriction of *lin-12* expression may be an important regulatory mechanism; the exceptions to this statement may reveal the cellular defects that underlie aspects of the *lin-12* phenotype that have not been previously explained. For decisions that are not naturally variable, *lin-12::lacZ* expression does not appear to change before or upon commitment to a cell fate implying that in these cases posttranscriptional regulation of *lin-12* activity may control cell fate specification.

DURING animal development, cell-cell interactions play important roles in causing initially equivalent cells to adopt different fates. Receptors such as *lin-12*, a member of the "*lin-12/Notch* family", mediate such interactions (reviewed in GREENWALD and RUBIN 1992). Genetic and anatomic studies of *lin-12* mutants that have either elevated or reduced levels of *lin-12* activity revealed that *lin-12* controls certain binary decisions during *Caenorhabditis elegans* development (GREENWALD *et al.* 1983). The phenotype of *lin-12* null [*lin-12(0)*] mutants revealed that several different cell fate decisions are mediated by *lin-12*: five cell fate transformations are highly penetrant in *lin-12(0)* mutant hermaphrodites (GREENWALD *et al.* 1983; NEWMAN *et al.* 1995). Additional cell fate decisions are mediated either by *lin-12* or a related gene *glp-1*, and are altered with low penetrance in *lin-12(0)* mutants (LAMBIE and KIMBLE 1991). Mutations that activate *lin-12* [*lin-12(d)*] can cause cell fate transformations that are the opposite of those caused by *lin-12(0)* mutations (GREENWALD *et al.* 1983; GREENWALD and SEYDOUX 1990; STRUHL *et al.* 1993; NEWMAN *et al.* 1995).

The cells affected by mutations in *lin-12* can generally be grouped by shared developmental potential. In most cases, equivalent developmental potential of cells that invariably have different fates is revealed by laser ablation experiments: cells whose fates are altered after ablation of their neighbors are inferred to require cell-cell interactions for their correct specification (for discussion, see SULSTON and WHITE 1980; SULSTON 1988). In a few cases, equivalent developmental potential is suggested by natural variability in the cell lineage and can be confirmed by laser ablation experiments (SULSTON and HORVITZ 1977; KIMBLE and HIRSH 1979; SULSTON *et al.* 1980, 1983; KIMBLE 1981).

Because *lin-12* encodes a receptor, its activity may be controlled at one or more levels. (1) *lin-12* might be ubiquitously expressed, but LIN-12 activity controlled posttranscriptionally. Several different possible posttranscriptional regulatory mechanisms have been observed for other receptors. The availability of ligand or components of the signal transduction machinery may regulate the activity of a receptor: for example, the Torso receptor tyrosine kinase is uniformly distributed in the Drosophila early embryo, but the activity of its ligand is spatially localized, leading to Torso activation only at the termini of the embryo (CASANOVA and STRUHL 1993). The availability of receptor protein itself may regulate its activity: for example, *glp-1* maternal mRNA is found in all of the first eight early blastomeres of *C. elegans*, but is translated only in the four blastomeres that require GLP-1 activity for their correct specification (EVANS *et al.* 1994). (2) *lin-12* might be expressed in a larger set of cells that encompasses the cells affected by mutations in *lin-12*. In this case, the set of cells may be related lineally, temporally or spatially, and localized LIN-12 activation may be achieved by one of the posttranscriptional mechanisms as described above. (3) *lin-12* might be expressed only in cells that need to assess LIN-12 activity for their correct specification, *i.e.*, in the cells that are affected by mutations in *lin-12*.

Corresponding author: Iva Greenwald, Department of Biochemistry, Columbia University, Hammer Health Sciences Center, Room 720A, 701 West 168th St., New York, NY 10032.

[1] *Present address:* Department of Cellular Biochemistry and Physiology, Merck Research Laboratories, Rahway, NJ 07065.

In a previous study, we examined the expression of a *lin-12::lacZ* reporter gene during the specification of two cells, Z1.ppp and Z4.aaa, of the hermaphrodite gonad (WILKINSON *et al.* 1994). Unlike other cells affected by mutations in *lin-12* during hermaphrodite development, Z1.ppp and Z4.aaa have naturally variable fates, with each cell having the potential to become an anchor cell (AC) or a ventral uterine precursor cell (VU) (KIMBLE and HIRSH 1979). However, in a given hermaphrodite, interactions between Z1.ppp and Z4.aaa cause only one to become the AC while the other becomes a VU (KIMBLE 1981; SEYDOUX and GREENWALD 1989). In *lin-12(0)* animals, both cells become ACs; in *lin-12(d)* animals, both cells become VUs. The *lin-12::lacZ* reporter gene is initially expressed in both Z1.ppp and Z4.aaa, but before commitment, *lin-12* expression is seen only in the presumptive VU (WILKINSON *et al.* 1994).

In this study, we have explored the relationship between *lin-12* expression and its activity by examining the expression of a *lin-12::lacZ* reporter gene in cells throughout wild-type hermaphrodite development. We have found that *lin-12::lacZ* is expressed in the groups of equivalent cells that are affected by mutations in *lin-12*. Moreover, we have found that *lin-12::lacZ* expression appears to be uniform and constant in cells with invariant fates, in contrast to what we observed for the naturally variable pair, Z1.ppp and Z4.aaa (WILKINSON *et al.* 1994).

MATERIALS AND METHODS

General methods and strains: Methods for handling and culturing *C. elegans* have been described by BRENNER (1974). The wild-type parent for all strains used was *C. elegans* var. Bristol strain N2 (BRENNER 1974). The LG*I* mutations used were as follows: *smg-1(r861)* and *unc-54(r293)* (HODGKIN *et al.* 1989); and *unc-13(e51)* (BRENNER 1974). The LG*III* mutations used were as follows: *dpy-17(e164)* and *unc-32(e189)* (BRENNER 1974). The LG*X* mutation used was *unc-84(e1410ts)*. Strain GS956, of genotype *smg-1 unc-54; arIs11*, was used for this study (see below).

arIs11: The generation of GS956, the canonical *lin-12::lacZ* reporter gene strain containing *arIs11*, is also described in WILKINSON *et al.* (1994). *arIs11* is an integrated array composed of the plasmids pRF4 [*rol-6 (su1006)*] (MELLO *et al.* 1991) and pBGSLE (a *lin-12::lacZ* chimeric gene) (WILKINSON *et al.* 1994). pBGSLE contains all sequences required to rescue a *lin-12(0)* mutant (FITZGERALD *et al.* 1993). A modified *lacZ* gene (FIRE *et al.* 1990) was inserted into the *Bam*HI site at position 6 in frame to the *lin-12* ATG. The *lacZ* gene encodes a β-galactosidase protein that contains a nuclear localization signal and includes its own stop codon but no polyadenylation signal. The mRNA encoded by the *lin-12::lacZ* gene is therefore predicted to be unstable due to the presence of a large 3′ untranslated region containing most of the *lin-12* coding sequence. For this reason, β-galactosidase activity is only detectable in the presence of a *smg-1* mutation, which stabilizes mRNAs with long 3′ untranslated regions (PULAK and ANDERSON 1993).

We have taken a number of precautions to help ensure that expression of *arIs11* accurately reflects expression of the endogenous *lin-12* gene (data not shown). First, we established five independent transgenic lines carrying extrachromosomal arrays of this reporter gene and created seven independent attached lines deriving from two different extrachromosomal arrays. All of the integrated and extrachromosomal arrays displayed similar expression patterns, although some lines displayed some variability in the intensity or penetrance of staining. Second, we analyzed an integrated array to circumvent variability due to mosaicism (FIRE 1986; HERMAN 1995). Third, the marker plasmid used, pRF4, has been used as a coinjection marker with the *lin-12(+)* rescuing clone and these arrays are able to rescue all of the defects associated with loss of *lin-12* activity (FITZGERALD *et al.* 1993). Thus, it appears that this marker does not interfere with *lin-12* expression in the cells in which it is required. Fourth, the expression pattern of the *lin-12::lacZ* is altered in different *lin-12* mutants consistent with the cell fate transformations in these mutants (WILKINSON *et al.* 1994; this work). Fifth, the *lacZ* gene used encodes a modified β-galactosidase protein that appears to be unstable and does not appreciably perdure (WAY and CHALFIE 1989; FIRE *et al.* 1990; WILKINSON *et al.* 1994).

We also note that the use of *smg-1* is not likely to have any effect on the pattern of transcription, because *smg-1* appears to act exclusively by stabilizing unstable mRNAs (PULAK and ANDERSON 1993). The stabilization of *lin-12::lacZ* mRNA in *smg-1* mutants does not necessarily lead to perdurance of the reporter gene mRNA, since we have observed an apparently rapid change in expression pattern within a cell and nonstaining daughters of staining mothers (WILKINSON *et al.* 1994; this paper).

arIs11 was mapped between *dpy-17* and *unc-32 III* at 20° in the following manner: *unc-13; arIs11* hermaphrodites were mated to *dpy-17 unc-32/++* males. Cross progeny of the genotype *unc-13/+; arIs11/dpy-17 unc-32* were picked and their progeny scored for the number of Dpy non-Unc-32 recombinant animals that had also picked up *arIs11*; 6/16 Dpy non-Unc-32 animals segregated Roller progeny. These data indicate that the array is ~1.5 map units (m.u.) to the left of the *lin-12* locus.

β-galactosidase activity assay and identification of cells: *smg-1 unc-54; arIs11* animals were grown at 25°. Individual animals of a particular age were identified under Nomarski differential interference microscopy and then tested for β-galactosidase activity using an acetone fixation protocol described by FIRE (1993). Pictures of the staining pattern were taken at ×1000 with a flash using TMAX400 film (Kodak).

Individual staining nuclei were identified by three criteria: the age of the animal as determined by Nomarski examination before fixation; the size and shape of the nuclei that stained; and the position of the staining nuclei in the animal relative to other nuclei, as defined by SULSTON (1976), SULSTON and HORVITZ (1977), and SULSTON *et al.* (1983). Counterstaining with 4,6-diamidino-2 phenylindole (DAPI) allowed visualization of all nuclei in the animal by fluorescence microscopy (ELLIS and HORVITZ 1986).

We were particularly interested in the staining pattern observed in the ventral hypodermal cells that give rise to the vulva and examined this pattern in more detail. VPCs: The *unc-84* gene is required for the correct nuclear migration of P1-P12, the cells that generate P1.p-P12.p in the ventral hypodermis (SULSTON and HORVITZ 1977; FIXSEN 1985). In *unc-84* mutants the nuclei of the P cells do not migrate to the ventral cord and instead die during the L1 larval stage. Animals of the genotype *smg-1 unc-54; arIs11; unc-84* were examined in the L2 larval stage for the staining pattern observed in the ventral cord. In most animals, all P nuclei failed to migrate, and no β-galactosidase staining was observed. In some animals, one or two P nuclei migrated. If P3-P8 mi-

grated, β-galactosidase staining was observed in the ventral cord. If P1, P2, or P9-P12 migrated, no staining was observed. VPC descendants: A monoclonal antibody that recognizes desmosomal junctions of cells in the ventral hypodermis (MH27) (FRANCIS and WATERSTON 1985; PRIESS and HIRSH 1986) was used to examine the staining pattern observed in the "N" and "T" cell descendants of P5.p and P7.p. This antibody stains the outline of the grandaughters of P5.p, P6.p and P7.p soon after they are born (KENYON 1986). We used the MH27 antibody in conjunction with a rabbit anti-β-galactosidase antibody (Cappel Laboratories) for immunofluorescent analysis (using a protocol described by FINNEY and RUVKUN 1990) in the late L3 stage. This examination confirmed that β-galactosidase protein was not detected in the nuclei of the central four cells that are the T descendants of P6.p and the protein was detected in the nuclei of the N and T descendants of P5.p and P7.p.

RESULTS

In *C. elegans*, the best available method for the study of gene expression in larvae is the analysis of transgenic strains carrying gene fusions with reporter genes such as *lacZ*, which can be assayed for β-galactosidase activity (FIRE *et al.* 1990). As described previously, we have constructed a *lin-12::lacZ* reporter gene that contains all genomic sequences necessary for rescue of a *lin-12(0)* mutant (WILKINSON *et al.* 1994). Here we report a detailed description of the expression pattern of *arIs11*, an integrated array containing the *lin-12::lacZ* reporter gene, during larval development. We have taken a number of precautions to help ensure that expression of the reporter gene accurately reflects expression of the endogenous gene, as described in MATERIALS AND METHODS.

lin-12::lacZ expression in somatic gonadal development

Background: HIRSH *et al.* (1976) and KIMBLE and HIRSH (1979) described the anatomy and development of the hermaphrodite gonad in *C. elegans* (Figure 1). At hatching, the hermaphrodite gonad consists of two somatic progenitors, Z1 and Z4, and two germline progenitors, Z2 and Z3. Gonadal development may be divided in two phases, based on the two periods of somatic proliferation. The first phase of gonadal development begins with a period of somatic proliferation during the L1 stage, when Z1 and Z4 each produce six descendants. During the L2 stage, no further cell divisions occur in the somatic gonad, although there is germline proliferation. In the late L2 stage, the gonad primordium forms: the 10 proximal somatic gonadal cells rearrange themselves and displace the germ nuclei into the gonadal arms, and one of these cells, the presumptive AC, moves to occupy a central position.

The second phase of gonadal development begins in the L3 stage and continues into the L4 stage. During this time the somatic gonadal cells in the primordium (with the exception of the anchor cell and the distal tip cells) all divide. The progeny of these divisions differentiate to give rise to the uterus, anterior and posterior sheaths and the anterior and posterior spermathecae. During this time the gonad arms continue to elongate and form a reflexed tube. The proximal germ nuclei begin to differentiate into sperm. The distal germ nuclei remain mitotic. After the L4 molt, oocytes begin to form and are fertilized as they pass through the spermatheca to the uterus.

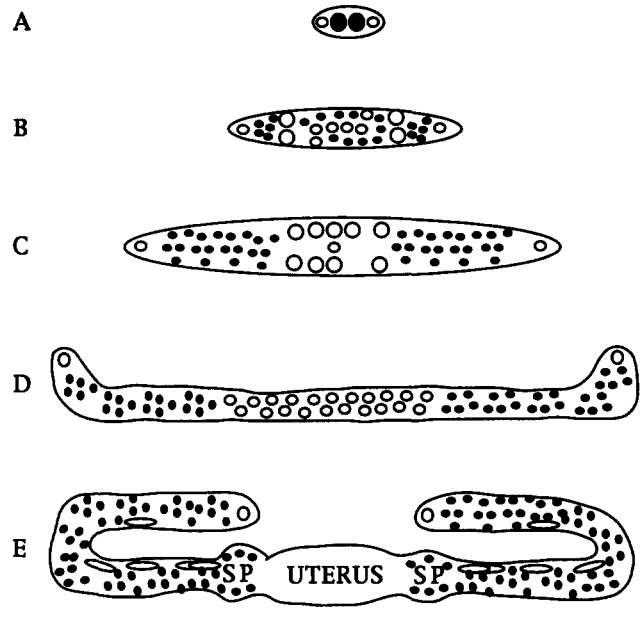

FIGURE 1.—Overview of gonadal development. Schematic diagram of the first (A–C) and second (D and E) phase of gonadal development (adapted from HODGKIN 1988). ○, somatically derived nuclei; ●, germline nuclei. (A) Dorsal view of the gonad during the early L1 stage. There are four cells that give rise to the gonad. (B) Dorsal view of the gonad during the L1 molt. The two somatic progenitors generate 12 progeny. The germ nuclei begin to divide. (C) Dorsal view of the gonad during the L2 molt. The germ nuclei proliferate extensively during the L2 stage and the somatic nuclei rearrange their position to form the gonad primordium. (D) Lateral view of the gonad during the L3 stage. The two gonad arms extend and the somatic nuclei proliferate to generate the cells that will form the structures of the gonad. The germ nuclei continue to proliferate. (E) Lateral view of the late L4 gonad. The two arms of the gonad reflex and continue to grow out. The somatic nuclei generate the spermatheca (SP), uterus, and the sheath cells that surround the gonad arms. The germ nuclei continue to proliferate and those in the spermatheca differentiate into sperm. After the L4 molt, the germ nuclei in the proximal gonad arms will begin to differentiate into oocytes.

lin-12 activity is required for at least two cell fate decisions in somatic gonad development. In the early L2 stage, Z1.ppa, Z1.ppp, Z4.aap, and Z4.aaa have equivalent developmental potential: each of them has the ability to become an AC or a VU (KIMBLE 1981; SEYDOUX and GREENWALD 1989; SEYDOUX *et al.* 1990). Z1.ppa and Z4.aap always become VUs in wild type (KIMBLE and HIRSH 1979). *lin-12* activity plays a role in the specification of these cells: in certain *lin-12(0)* mutants, Z1.ppa and Z4.aap sometimes become ACs (GREENWALD *et al.*

1983). Moreover, in wild-type hermaphrodites, Z1.ppa and Z4.aap have the potential to become ACs early in the L2 stage; in a *lin-12(0)* mutant, this potential is extended until late in the L2 stage (SEYDOUX *et al.* 1990). The fates of Z1.ppp and Z4.aaa are naturally variable. Each cell has an equal chance of becoming the AC or a VU. In any given hermaphrodite, only one of these cells will become the AC, while the other becomes a VU (KIMBLE and HIRSH 1979). Interactions between Z1.ppp and Z4.aaa specify their fates (KIMBLE 1981; SEYDOUX and GREENWALD 1989). These interactions are mediated by *lin-12*: in *lin-12(0)* mutants, both Z1.ppp and Z4.aaa become ACs, while in *lin-12(d)* mutants, which have elevated levels of *lin-12* activity, both Z1.ppp and Z4.aaa become VUs (GREENWALD *et al.* 1983). The three VUs undergo slightly different lineages (KIMBLE and HIRSH 1979) (see Figure 3). A. NEWMAN, J. G. WHITE and P. W. STERNBERG (1995) have shown that the daughters of the VUs are initially equivalent, but that a signal from the AC appears to induce these cells to generate a certain number of π cells, which undergo a characteristic lineage. They have also shown that this interaction depends on *lin-12* activity: in a *lin-12(0)* mutant, the VU daughters do not divide and no π cells are generated, whereas in a *lin-12(d)* mutant, excess π cells are generated.

***lin-12::lacZ* is expressed in all three VUs:** *lin-12::lacZ* expression in the early phase of the Z1 and Z4 lineages is summarized in Figure 2. Expression begins in Z1.pp and Z4.aa, and is seen in Z1.ppp, Z4.aaa, Z1.ppa and Z4.aap. All four cells continue to express the reporter gene until the middle of the L2 stage. Some additional staining is seen elsewhere before primordium formation at low frequency (see Figure 2 legend). After the primordium has formed, *lin-12::lacZ* expression is detected only in the three VUs: Z1.ppa and Z4.aap, and either Z1.ppp or Z4.aaa. During the early L3 stage, *lin-12::lacZ* staining disappears in the VUs; staining reappears in their daughters just after division (see below). We have described elsewhere how the *lin-12::lacZ* expression pattern in Z1.ppp or Z4.aaa changes before primordium formation and its relationship to the decision of these cells between the AC and VU fates (WILKINSON *et al.* 1994).

***lin-12::lacZ* is expressed in the VU daughters:** Many different cells express the *lin-12::lacZ* reporter gene during the second phase of somatic gonadal development. There are six cells that express the reporter gene in the somatic gonad in the mid-L3 stage (Figure 3). This staining corresponds to the daughters of the VUs, that is VU1(l/r), VU2(a/p) and VU3(a/p) [the actual lineage of the cells depends on whether Z1.ppp or Z4.aaa has become an AC: if Z1.ppp adopts the AC fate, then Z4.aap adopts the VU1 fate, Z1.ppa adopts the VU2 fate, and Z4.aaa adopts the VU3 fate; or if Z4.aaa adopts the AC fate, then Z1.ppa adopts the VU1 fate, Z4.aap adopts the VU2 fate, and Z1.ppp adopts the VU3 fate (KIMBLE and HIRSH 1979)]. As noted above, there appears to be a time during the early L3 stage when the VUs no longer express the *lin-12::lacZ* reporter gene, so that the staining that is observed in their daughters appears to represent "new" expression.

The *lin-12::lacZ* reporter is also expressed in all twelve of the granddaughters of the VUs (Figure 3). We have been unable to determine whether the reporter construct is expressed in the great-granddaughters because it is expressed in a cluster of up to 28 cells in the somatic gonad, making it difficult to distinguish individual nuclei.

Significance of the staining pattern observed in the VUs and their daughters: During the first phase of gonadal development, *lin-12::lacZ* is expressed in Z1.ppa, Z1.ppp, Z4.aap, and Z4.aaa, the cells whose fates are altered in *lin-12* mutants. For Z1.ppp and Z4.aaa, *lin-12::lacZ* expression is maintained in the presumptive VU and not in the presumptive AC (WILKINSON *et al.* 1994). For Z1.ppa and Z4.aap, which both become VUs, *lin-12::lacZ* staining appears to be constant throughout the L2 stage. Thus, for cells that become VUs, *lin-12::lacZ* is expressed throughout the L2 stage. A difference between the two pairs of cells, Z1.ppp/Z4.aaa and Z1.ppa/Z4.aap, emerges from an examination of the staining pattern in a *lin-12(0)* mutant. In a *lin-12(0)* mutant staining is observed throughout the L2 stage in Z1.ppa and Z4.aap, but staining is observed in Z1.ppp and Z4.aaa only prior to primordium formation (WILKINSON *et al.* 1994). This observation is consistent with the interpretation that *lin-12* activity is not required *per se* for *lin-12* expression to be maintained in Z1.ppa and Z4.aap.

In a *lin-12(+)* background there is no temporal change in the staining pattern observed in the VU daughters or VU granddaughters that express the *lin-12::lacZ* reporter gene. Ablation studies have indicated that an interaction between the AC and the VU daughters is required for the correct number of π cells to be generated (NEWMAN *et al.* 1995). It appears unlikely that *lin-12* activity is regulated by restricting its expression only to the π cells themselves or to the VU daughters that generate them. β-galctosidase activity is also detected in all six of the VU daughters in both *lin-12(0)* and *lin-12(d)* mutants; this is consistent with the interpretation that *lin-12* activity is not required *per se* to generate the pattern observed (data not shown).

***lin-12::lacZ* is expressed in sheath cells Nos. 1–3:** There is a total of 20 sheath cells, five pairs in each arm, that arise from four different lineages (KIMBLE and HIRSH 1979) (Figure 4). Sheath cells No. 1 are born during the mid-L3 stage. The rest (sheath cells Nos. 2–5) are born in the early L4 stage. Note that the sheath cells move as pairs that are designated by a number that relates their position on the gonad arm; however, each member of a pair arises from a different lineage (for example, there are two sheath cells No. 1 in the right arm of the gonad: one is a descendant of

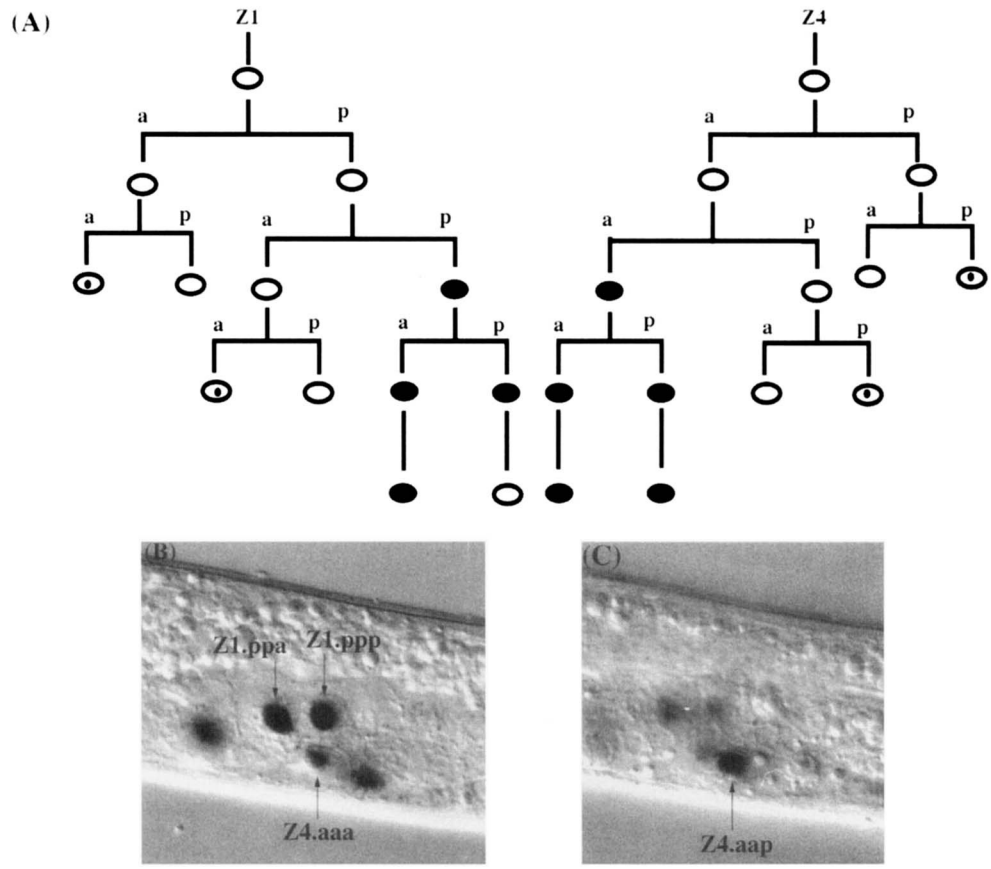

FIGURE 2.—*lin-12::lacZ* expression in the first phase of somatic gonadal development. (A) Schematic diagram of the Z1 and Z4 lineages during the first phase of somatic gonad development (adapted from KIMBLE and HIRSH 1979). ●, cells that express the reporter gene; ⊙, staining is only sometimes observed in Z1.aa and Z4.pp (the distal tip cells) or in Z1.paa or Z4.app. None of these staining patterns is found in a high percentage of animals and the reason for this variability is unknown. (B and C) Nomarski photomicrographs of the somatic gonad of an L2 larva that has been fixed and stained for β-galactosidase activity as described in MATERIALS AND METHODS. Anterior is to the left. Lateral view of an L2 animal. B and C show different planes of focus. Due to the Roller phenotype, all *lin-12::lacZ* expressing cells in the somatic gonad are visible from this angle. All four cells that have the potential to adopt the VU fate, Z1.ppa, Z1.ppp, Z4.aaa and Z4.aap, express the *lin-12::lacZ* reporter gene at this stage. Note that the VPCs also express the *lin-12* transgene at this time and these cells appear as dark spots or patches in the photographs.

Z1.paa and the other is a descendant of Z1.ap; and there are two sheath cells No. 1 in the left arm of the gonad: one is a descendant of Z4.app and the other is a descendant of Z4.pa). Soon after they are born, the morphology of these cells changes. They flatten out and elongate and either move or are carried out along the arms of the gonad and eventually wrap around the gonad.

During the L3 stage, β-galactosidase activity is detected in two sheath cells in each gonad arm: sheath cells No. 1 (Z1.paaa, Z1.apa, Z4.pap, and Z4.appp) (Figure 4). During the early L4 stage, eight more sheath cells express the transgene: sheath cells No. 2 (Z1.paapaaa, Z1.appaaa, Z4.paappp, and Z4.appappp) and sheath cells No. 3 (Z1.paapaap, Z1.appaap, Z4.paappa, and Z4.appappa). The staining in the sheath cells is very reproducible in the early L4 stage, however, it is less reproducible late in the L4 stage and in the young adult stage. Staining is almost always observed in sheath cells No. 1 in the L3 and L4 stages as well as in the young adult; however, only a subset of animals consistently express the reporter gene in sheath cells No. 2 once the nuclei have begun to migrate far out along the arm. We have never been able to detect staining in sheath cells No. 3 once the nuclei have begun to migrate far out along the arm. One important observation to note is that the staining is observed in 12 sheath cells during the late L3/early L4 stage soon after they are born (that is, in all the lineages in which they arise). However, as the cells move out the gonad arm, staining is only detectable in one member of pair No. 1 and one member of pair No. 2 in each arm. It is unknown which member of the pair stains or whether it is always the same member of the pair that continues to express the *lin-12::lacZ* gene.

***lin-12::lacZ* is expressed in a subset of spermathecal cells:** There is a subset of cells in the DU (dorsal uterine) lineage that contribute to the structure of the spermatheca (KIMBLE and HIRSH 1979) (Figure 5). These cells arise from four progenitors: two contribute to the

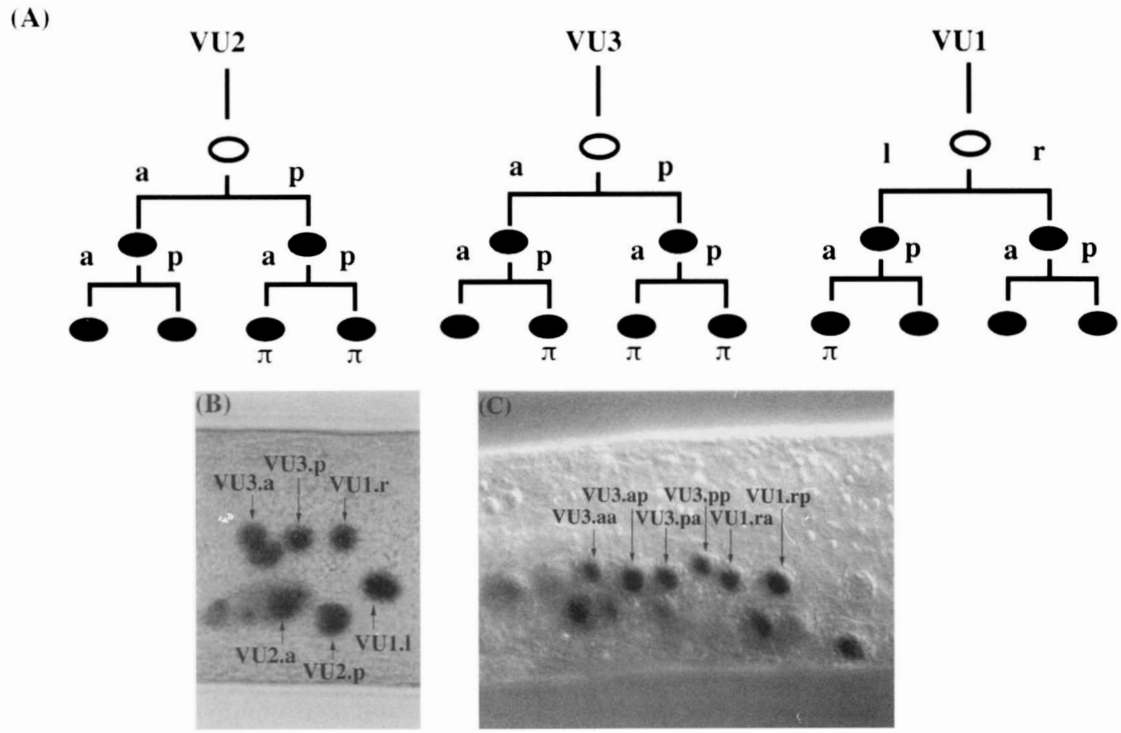

FIGURE 3.—*lin-12::lacZ* expression in the VU descendants. (A) Schematic diagram of the partial lineage of the VU descendants during the L3 stage (adapted from KIMBLE and HIRSH 1979). ●, cells that express the reporter gene. π indicates the cells that adopt the π cell fate (see RESULTS). (B) Nomarski photomicrograph of the somatic gonad of an L3 larva that has been fixed and stained for β-galactosidase activity as described in MATERIALS AND METHODS. Anterior is to the left. Dorsal view of the mid-L3 larval stage. All six VU daughters are visible in this plane. Dark smudges indicate other cells that are staining at this time such as the VPC descendants. (C) Nomarski photomicrograph of the somatic gonad of an L3 larva that has been fixed and stained for β-galactosidase activity as described in MATERIALS AND METHODS. Lateral view of the late L3 larval stage. Only the VU descendants on the left side of the animal are visible in this plane of focus. Dark smudges indicate other cells that are staining at this time such as the vulval precursor cell descendants and other somatic gonadal cells.

anterior spermatheca and two contribute to the posterior spermatheca. Each progenitor gives rise to four cells that form the part of the spermatheca that is adjacent to the uterus. One cell from each progenitor contributes to the core of the spermathecal-uterine junction, a highly specialized structure formed by the fusion of two cells.

The expression of the reporter gene in the spermathecal cells is most easily detected during the L4 larval stage (Figure 5). There are up to eight spermathecal cells [Z1.papaa(a/p)(d/v), Z4.apaaa(a/p)(d/v), Z1.pappp-(a/p)(d/v), and Z4.apapp(a/p)(d/v)] in each arm that express the reporter gene; the position and number is consistent with a group of cells in lineage "B" (KIMBLE and HIRSH 1979) that are present at the spermathecal uterine junction. The progenitors of these cells [Z1.papaa(a/p), Z4.apaaa(a/p), Z1.pappp(a/p), and Z4.apapp(a/p)] also appear to express the reporter gene. There is some variablity in the number of staining cells detected and this description may include only a subset of the total number of spermathecal cells that express the reporter construct.

Significance of the staining pattern observed in the sheath cells and the spermatheca: During the second phase of gonadal development, *lin-12::lacZ* expression in the spermatheca and sheath cells was not predicted from the *lin-12* mutant phenotypes. However, *lin-12(0)* and *lin-12(d)* mutants both have fertility defects: *lin-12(0)* mutants are generally sterile, and several *lin-12(d)* mutants have reduced brood sizes (GREENWALD *et al.* 1983). The cellular basis for the sterility is not known, but the *lin-12::lacZ* expression pattern suggests that spermatheca and/or sheath defects may underlie the fertility defects. Recently, J. MCCARTER and T. SCHEDL (personal communication) have demonstrated that the sheath and spermathecal cells are involved in at least three processes in the developing germline: proliferation of germ nuclei, germline sex determination, and oocyte maintenance of meiotic arrest. The germline anatomy of *lin-12* mutants has not been well characterized and may display abnormalities in one or more of these processes. *lin-12(0)* and *lin-12(d)* mutants appear to have abnormal spermathecal-uterine junctions (NEWMAN *et al.* 1995; J. G. WHITE, personal communication). Moreover, it is possible that *lin-12* and *glp-1* are functionally redundant for cell fate determination in the sheath, spermatheca and vulval lineages (see LAMBIE and KIMBLE 1991; FITZGERALD *et al.* 1993), so that the phenotype of *lin-12* (or *glp-1*) single mutants would not necessarily reveal the defects; because the *lin-12(0)*

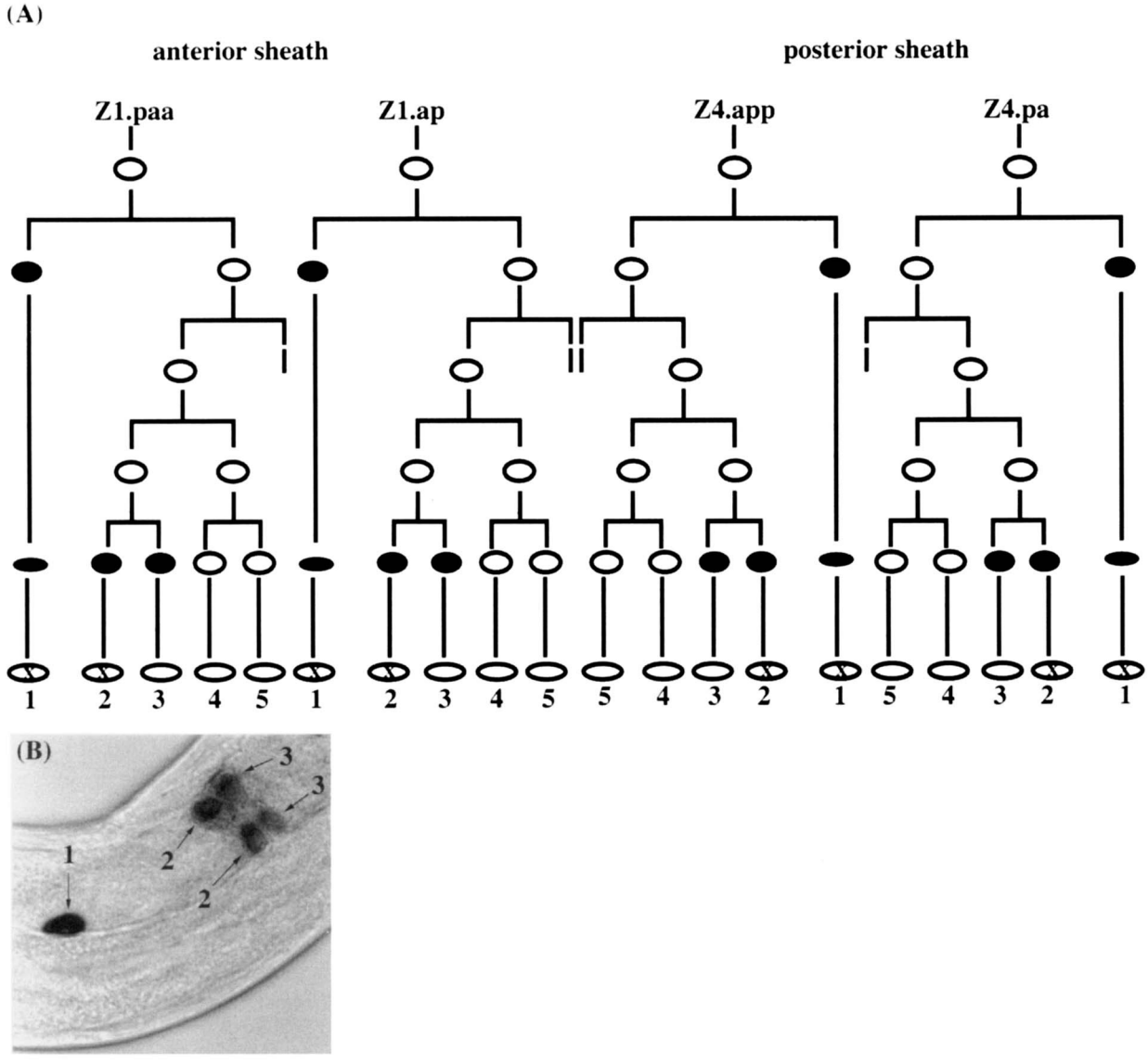

FIGURE 4.—*lin-12::lacZ* expression in sheath cells. (A) Schematic diagram of the lineages of the sheath cells (adapted from KIMBLE and HIRSH 1979). ●, cells that express the reporter gene. ⊗, expression that is observed only in one of the two pairs of cells indicated (*i.e.*, one of the No. 1 sheath cells and one of the No. 2 sheath cells in each sheath). It is unknown in which No. 1 or No. 2 sheath cell expression of the reporter gene is maintained. Note that the No. 3 sheath cells express the reporter gene during the early L4 stage but do not express the reporter gene in the late L4 stage. (B) Bright field photomicrograph of an L4 larva that has been fixed and stained for β-galactosidase activity as described in MATERIALS AND METHODS. Anterior is to the left. Lateral view. Note that only one of the two No. 1 sheath cells has visible staining. Due to the Roller phenotype, both Nos. 2 and both Nos. 3 sheath cells are visible.

glp-1(-) mutant dies in the L1 stage (LAMBIE and KIMBLE 1991), functional redundancy for later developmental events would not have been seen.

Given the observation that *lin-12::lacZ* staining persists in only one of the pair of sheath cells, it is intriguing to speculate that these cells are not equivalent as was previously thought (KIMBLE and HIRSH 1979). It may be that there are subtle differences in the functions of individual sheath cells that are not obvious based on morphogenetic or ablation criteria. We have observed the same sheath cell staining pattern in a *lin-12(d)* mutant; however, in a *lin-12(0)* mutant, staining persists in both members of pairs Nos. 1 and 2 (data not shown). These observations are similar to those described for Z1.ppp and Z4.aaa in the sense that the staining pattern observed in these two cells is altered in *lin-12* mutants. This may indicate that the sheath cells have a naturally variable cell fate decision that is mediated by *lin-12* and that the level of *lin-12* activity affects the pattern of *lin-12* expression.

lin-12::lacZ expression in vulval development

Background: We will consider vulval development as comprising two phases: one phase includes specification of the vulval precursor cells (VPCs) and the other in-

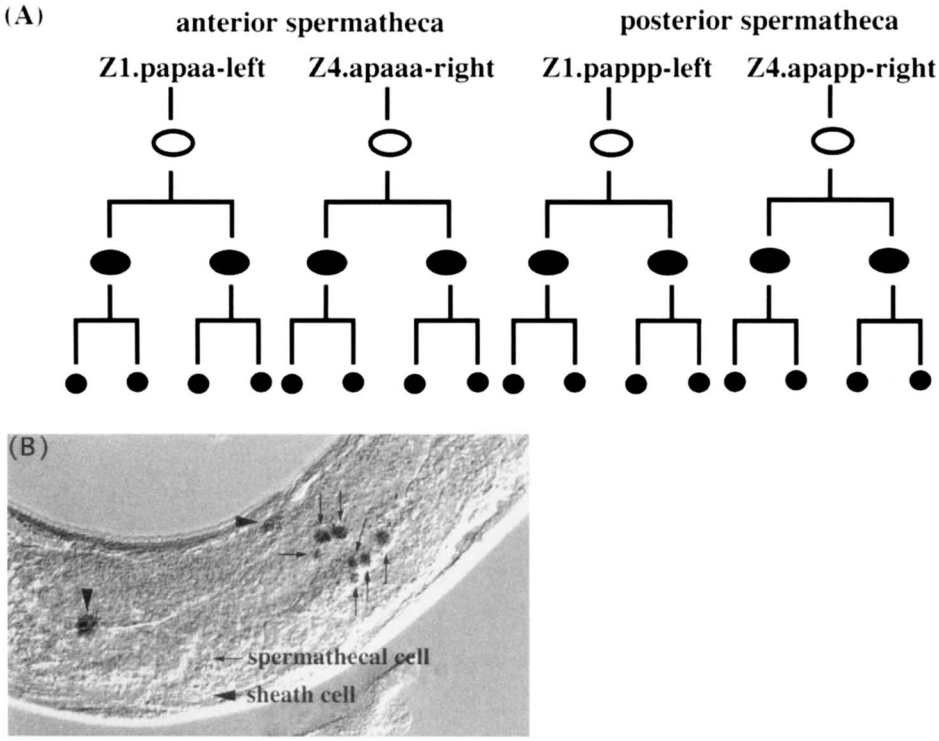

FIGURE 5.—*lin-12::lacZ* expression in the spermathecal cells. (A) Schematic diagram of the lineages of the cells thought to stain in the spermatheca (adapted from KIMBLE and HIRSH 1979). ●, cells that express the reporter gene. (B) Nomarski photomicrograph of young adult that has been fixed and stained for β-galactosidase activity as described in MATERIALS AND METHODS. Anterior is to the left. Lateral view. Due to the Roller phenotype, cells on both the left and right sides of the gonad are visible. Arrows indicate spermathecal cells and arrowheads indicate sheath cells.

cludes execution of vulval cell fates and morphogenesis. In wild-type hermaphrodites, the vulval cells are descended from three of six VPCs, consecutively numbered P3.p-P8.p (SULSTON and HORVITZ 1977). Each VPC has the potential to adopt one of three fates, termed "1°," "2°" and "3°" (SULSTON and WHITE 1980; STERNBERG and HORVITZ 1986). Normally, P6.p adopts the 1° fate, P5.p and P7.p adopt the 2° fate, and P3.p, P4.p and P8.p adopt the 3° fate (SULSTON and HORVITZ 1977; SULSTON and WHITE 1980; STERNBERG and HORVITZ 1986). The 3° fate is to produce daughters that fuse with the hypodermal syncytium, *hyp7*. The 1° and 2° fates are to generate the vulval cells that form the opening through which the eggs are laid. The 1° and 2° fates can be distinguished from each other by the plane of the terminal division of the lineage and by the adhesive properties of the penultimate and ultimate cells (STERNBERG and HORVITZ 1986) (see Figures 6 and 8).

There are at least three signaling systems involved in VPC specification (HORVITZ and STERNBERG 1991), but the decision to adopt the 2° fate is controlled by *lin-12* activity: in *lin-12(0)* hermaphrodites, none of the VPCs express the 2° fate, while when *lin-12* is activated, all VPCs express the 2° fate (GREENWALD *et al.* 1983; STRUHL *et al.* 1993). *lin-12* is thought to function as the receptor for a lateral signal among the VPCs (STERNBERG 1988; STERNBERG and HORVITZ 1989).

In wild-type hermaphrodites the structure of the vulva is formed by a series of morphogenetic movements and cell fusions that take place during the L3-L4 stage (SULSTON and HORVITZ 1977; J. WHITE, personal communication). The phase comprising the execution of vulval cell fates and morphogenesis of the vulval cells during the L3 and L4 stages has not been as well characterized as the earlier phase of VPC specification. By phenotypic analysis and/or genetic epistasis, several genes have been placed downstream of events involved in VPC specification, and hence appear to be involved in execution and morphogenesis (FERGUSON *et al.* 1987; FREYD 1991; SEYDOUX *et al.* 1993; T. HERMAN and H. R. HORVITZ, personal communciation; D. LEVITAN and I. GREENWALD, unpublished observations). These studies did not provide direct evidence that *lin-12* is involved in these processes. Temperature shift studies of *lin-12* hypomorphs have demonstrated a requirement for *lin-12* activity for proper egg-laying during the L4 stage when these events occur, however, the identity of the cells that require *lin-12* activity at this time has not been determined (SUNDARAM and GREENWALD 1993).

***lin-12::lacZ* is expressed in all six VPCs and their daughters:** During the L2 and early L3 stages, *lin-12::lacZ* is expressed in all six VPCs (Figure 7). All 12 daughters of the VPCs express the reporter gene. Eventually (within 2–3 hr after fusion with the hypodermal syncytium), the P3.p, P4.p and P8.p daughters no longer express the reporter gene (Figure 7).

Significance of the staining pattern observed in the VPCs: We have observed expression of the *lin-12::lacZ* reporter gene in the VPCs and their daughters. Temperature-shift experiments indicated that *lin-12* function in the specification of the VPCs occurs in the VPCs themselves, and not in their daughters (GREENWALD *et al.* 1983). Moreover, laser ablation studies demonstrated that the VPCs become committed at some point

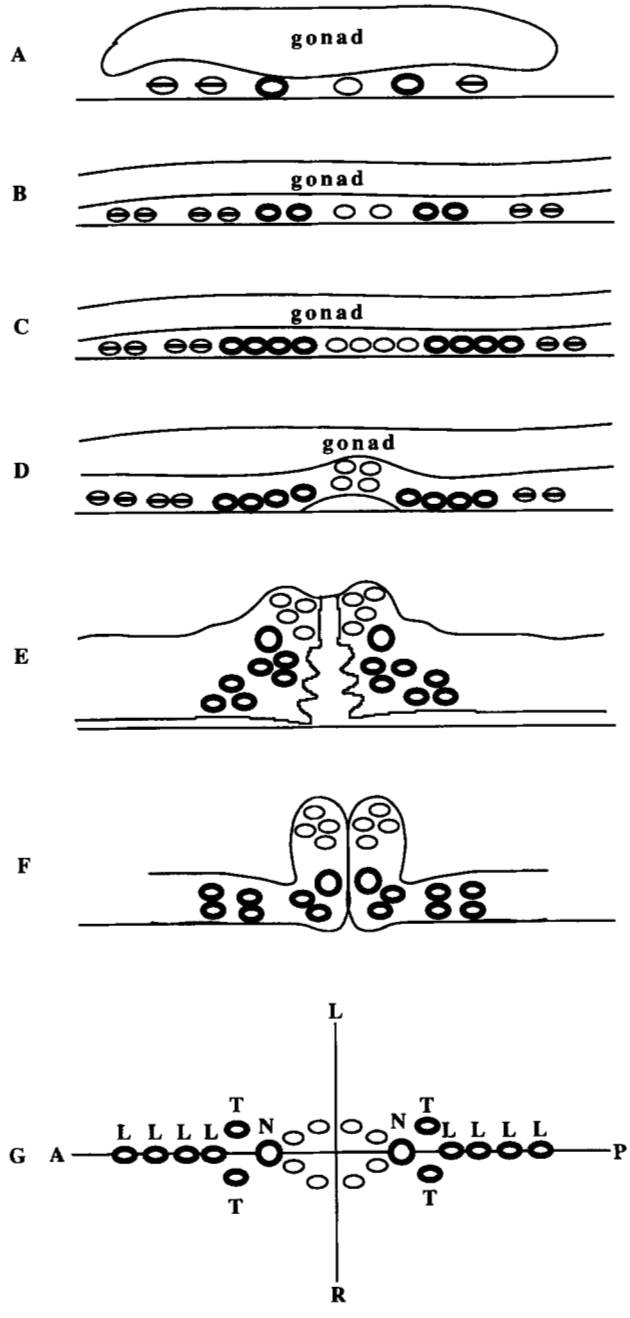

FIGURE 6.—Overview of vulval development. Schematic diagram of vulval devlopment from the L3 stage to the adult (adapted from SULSTON and HORVITZ 1977; STERNBERG and HORVITZ 1986). Circles indicate P6.p and its descendants. Heavily outlined circles indicate P5.p and P7.p and their descendants. Circles with a horizontal line indicate P3.p, P4.p, and P8.p and their descendants. (A) Left lateral view of the position of P3.p-P8.p in the L3 stage. (B) Left lateral view of the position of the descendants of P3.p-P8.p after the first round of divisions in the L3 stage. (C) Left lateral view of the position of the descendants of P3.p-P8.p after the second round of divisions in the L3 stage. (D) Left lateral view of the position of the descendants of P3.p-P8.p as they begin to invaginate. (E) Left lateral view of the position of the descendants of P5.p-P7.p as they invaginate farther. Note that the P6.p descendants have undergone a third round of divisions. Only three of the descendants of P5.p and three of the descendants of P7.p have undergone a third round of divisions. (F) Left lateral view of the position of the descendants of P5.p-P7.p as they evert to form the lips and opening of the vulva in the late L4 stage. (G) Ventral view of the adult vulva showing the positions of the descendants of P5.p-P7.p. A, anterior; P, posterior; L, left; R, right.

before they divide (SULSTON and WHITE 1980; STERNBERG and HORVITZ, 1986). For example, if P6.p is ablated, P5.p or P7.p sometimes adopts the 1° fate, but if P6.pa and P6.pp are ablated, the P5.p and P7.p descendants do not change their fates. Thus, we might have seen initial *lin-12::lacZ* expression in the VPCs, followed by expression only in P5.p and P7.p and followed by an absence of expression in the VPC daughters. In contrast, we found that *lin-12::lacZ* appears to be expressed continuously in the VPCs and their daughters, suggesting that transcriptional regulation is not the mechanism by which *lin-12* activity is restricted to P5.p and P7.p. Instead, expression or activity of the ligand or other members of the signal transduction pathway may be localized to P6.p or to P5.p and P7.p.

***lin-12::lacZ* is expressed in N and T descendants of P5.p and P7.p:** In the VPC granddaughters, the staining pattern is modified in the different VPC lineages (Figure 8). β-galactosidase activity is weakly detectable in two of the granddaughters of P5.p and P7.p, the L cells, but is undetectable in their progeny. *lin-12::lacZ* expression is not detectable in any of the four granddaughters of P6.p, the T cells. *lin-12::lacZ* expression is always detectable in the other two granddaughters of P5.p and P7.p, the N and T cells. The lack of staining in the T descendants of P6.p, which adopts the 1° fate, and the presence of staining in the T descendants of P5.p and P7.p, which adopt the 2° fate, is an indication that the two groups of T cells are not the same. The N cells do not divide and β-galactosidase activity remains detectable in both N cells throughout vulval morphogenesis in the L4 stage and often can be detected in young adults. The reproducibility of detecting expression of the *lin-12::lacZ* reporter gene in the daughters of the T cells descended from P5.p and P7.p is more variable (*i.e.*, only 10–20% of the L4 stage larvae that had staining in N had staining in the daughters of T), but staining can be detected in the T daughters in the L4 stage and in the young adult as well. In *lin-12(d)* mutants the reproducibility of detecting expression of the *lin-12::lacZ* in the daughters of the T cells descended from P5.p and P7.p is greatly enhanced. β-galactosidase activity is detected in all L4 and young adults in both the N cells and the daughters of the T cells.

Significance of the staining pattern observed in the VPC granddaughters: The involvement of *lin-12* in the choice of VPCs to adopt the 2° fate may have obscured the recognition of the role of *lin-12* in descendants of the VPCs. However, analysis of *lin-12* partial loss-of-function mutants has indicated that *lin-12* activity has a role in the development of the egg-laying system that

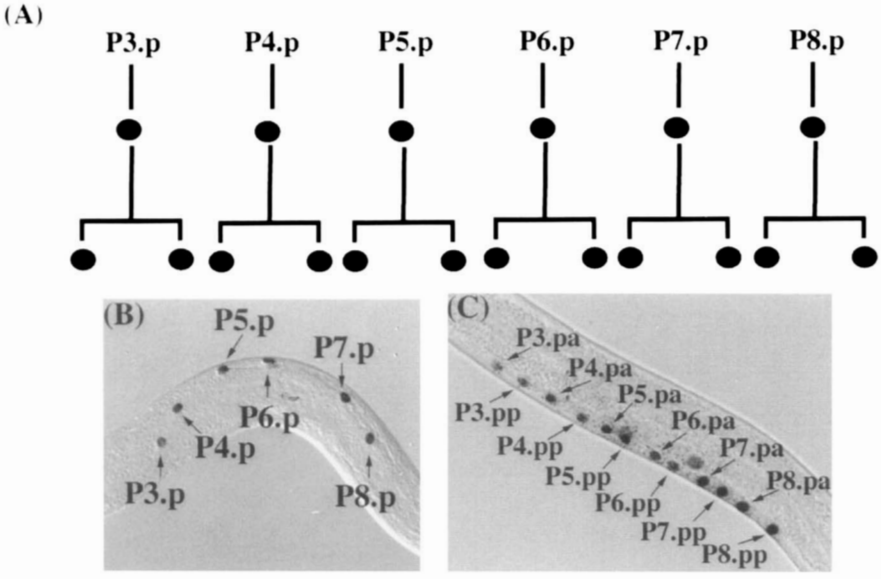

FIGURE 7.—*lin-12::lacZ* expression in the VPCs and their daughters. (A) Schematic diagram of the partial lineages of the VPCs (adapted from SULSTON and HORVITZ 1977). ●, cells that express the reporter gene. Both photographs are a lateral view, and anterior is to the left. (B) Nomarski photomicrograph of an L2 animal that has been fixed and stained for β-galactosidase activity as described in MATERIALS AND METHODS. Due to the Roller phenotype, cells on the ventral side appear on the diagonal. (C) Nomarski photomicrograph of an L3 animal that has been fixed and stained for β-galactosidase activity as described in MATERIALS AND METHODS. The animal was fixed just after all the VPCs had divided. Dark smudges indicate other cells that are staining at this time in the somatic gonad.

is independent of its involvement in VPC specification or the sex mesoblast/body wall muscle precursor cell specification (SUNDARAM and GREENWALD 1993). This role may reflect a requirement for *lin-12* activity in the VPC descendants, specifically in the N and T cells of P5.p and P7.p, where the reporter gene is expressed during the L4 larval stage. It is interesting to note that in a *lin-12(d)* mutant, which has up to six 2° lineages (due to the transformation of the VPCs), expression of the reporter gene is more consistently detected in the N cells and the daughters of the T cells of each pseudovulva than it is in the N cells and the daughters of the T cells in a *lin-12(+)* background (data not shown). This observation may suggest that *lin-12* activity positively autoregulates *lin-12* expression during a cell fate decision in these cells.

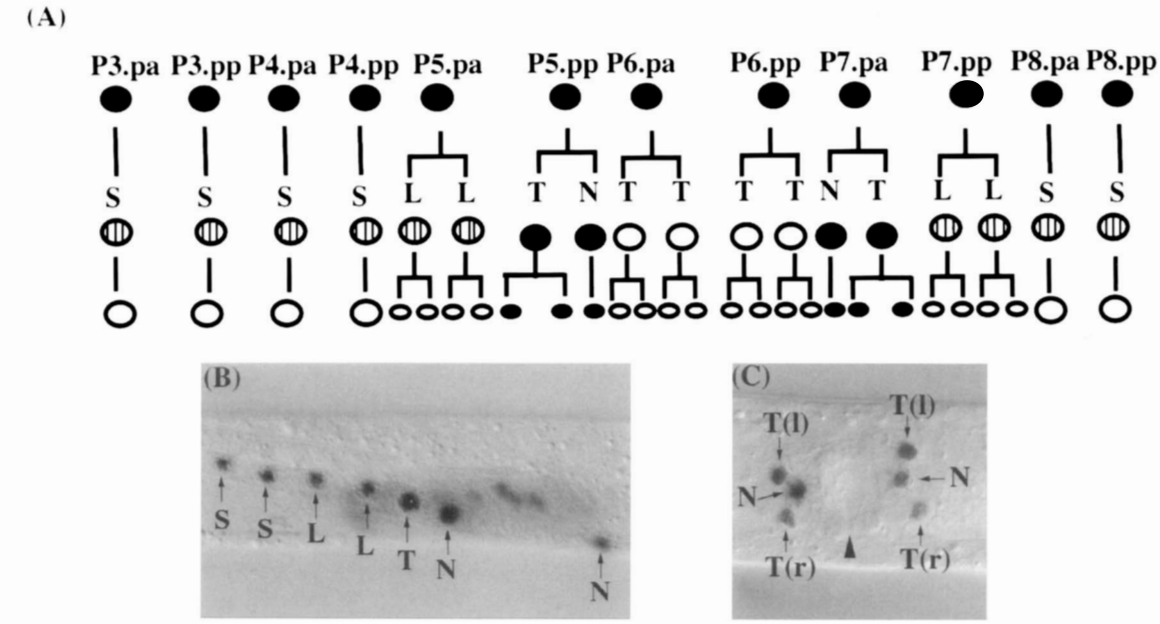

FIGURE 8.—*lin-12::lacZ* expression in the descendants of P5.p and P7.p. (A) Schematic diagram of the lineages of the descendants of the VPCs (adapted from STERNBERG and HORVITZ 1986). ●, cells that express the reporter gene; ▨, cells in which expression of the reporter gene is reduced. Both photomicrographs show a ventral view, and anterior is to the left. (B) Nomarski photomicrograph of an L3 animal that has been fixed and stained for β-galactosidase activity as described in MATERIALS AND METHODS. The animal was fixed just before invagination of the vulval cells. Due to the Roller phenotype, cells on the ventral side appear on the diagonal. Note that expression of the reporter gene is reduced in the S and L cells, but is still intense in the N and T cells; the posterior N cell is blurred because it is in a different plane of focus. No expression is detected in the descendants of P6.p, which are located in this plane of focus between the N cells. (C) Nomarski photomicrograph of a young adult animal that has been fixed and stained for β-galactosidase activity as described in MATERIALS AND METHODS. Arrowhead indicates the vulva.

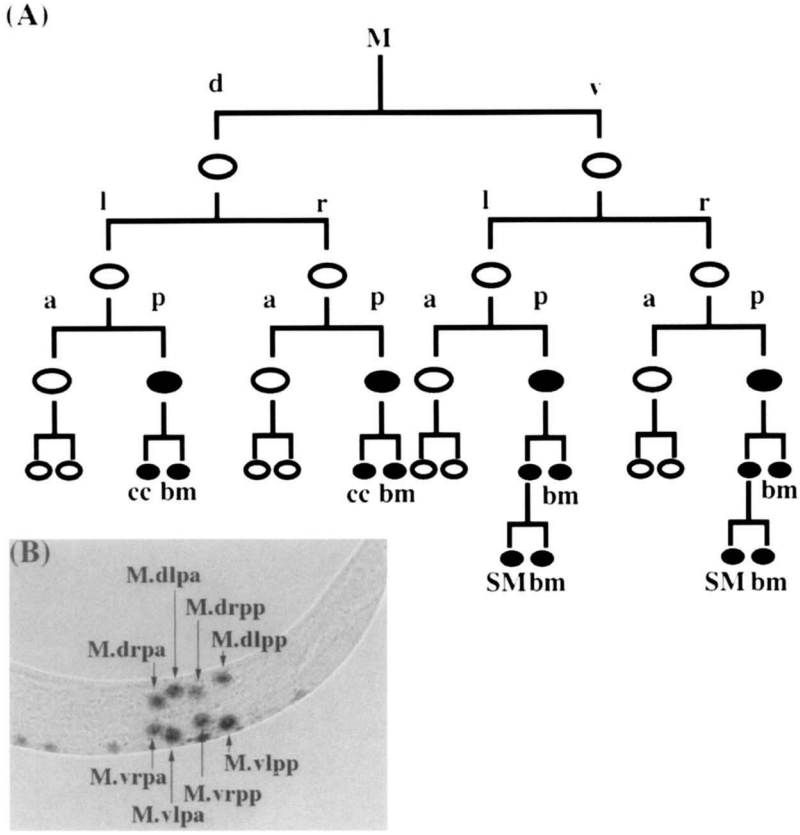

FIGURE 9.—*lin-12::lacZ* expression in the M lineage. (A) Schematic diagram of the M lineage (adapted from SULSTON and HORVITZ 1977). ●, cells that express the reporter gene. (B) Bright field photomicrograph of an L1 animal that has been fixed and stained for β-galactosidase activity as described in MATERIALS AND METHODS. The animal was fixed prior to division of the SM/bm precursor cell and the *lin-12::lacZ* expressing M cell descendants are indicated. Anterior is to the left. Right lateral view. Other cells that stain appear to be neurons located in the ventral cord.

lin-12 expression in the M lineage

Background: The M mesoblast begins to divide during the late L1 stage. The M lineage eventually produces two coelomocytes (cc), 14 body muscle cells (bm), and two sex myoblasts (SM) (SULSTON and HORVITZ 1977) (see Figure 9). During the L2 stage the sex myoblasts migrate anteriorly to a position near the middle of the developing gonad and in the L3 stage they divide to produce muscles required for egg laying.

lin-12 controls specification of the body muscle/sex myoblast precursor cells, M.vlpa and M.vrpa (GREENWALD *et al.* 1983). In wild-type animals, M.v(l/r)pa each divide to produce a body muscle cell and a sex myoblast. In *lin-12(0)* mutants, these cells are transformed into their dorsal equivalents and adopt coelomocyte fates, whereas in *lin-12(d)* mutants, M.dlpa and M.drpa are transformed into their ventral equivalents.

***lin-12::lacZ* is expressed in a subset of both dorsal and ventral cells of the M lineage:** We have examined the expression pattern of the *lin-12::lacZ* reporter gene in the M lineage. The parents of the SM/bm precursor cells, M.vlp and M.vrp, and their dorsal equivalents, M.dlp and M.drp, all express the *lin-12::lacZ* reporter gene (Figure 9). After division of the parent cells, the SM/bm precursor cells and their dorsal equivalents also express the reporter gene as do the sisters of these cells. The SM/bm precursor cells divide soon after they are born and expression of the reporter gene is detected in both the SM and bm cells on the left and right sides of the animal. It is interesting to note that staining often persists in these cells on the ventral side even after it is no longer detectable in the cells on the dorsal side of the animal.

Significance of the staining pattern observed in the M lineage: Laser ablation experiments have not revealed any cell-cell interactions between the dorsal and ventral equivalent cells in the M lineage (J. THOMAS, personal communication; C. KENYON, personal communication). It is thought that M.vlpa and M.vrpa are induced by neighboring ventral cells, and that there may be no source of inducer dorsally (SEYDOUX 1991). The staining pattern is consistent with this prediction because the cells on the dorsal side of the animal also express the *lin-12::lacZ* reporter gene; the simplest model to explain why only the ventral cells execute the SM/bm precursor cell fate is that their extracellular environment is different from that of the cells on the dorsal side of the animal. The relatively longer persistence of β-galactosidase activity on the ventral side compared to the dorsal side may indicate that *lin-12* activation promotes *lin-12* expression in the M lineage.

lin-12::lacZ expression in other cells

The expression in the Z1 and Z4 lineages, VPC lineages, and M lineage accounts for all of the staining observed from the late L1 stage on. *lin-12::lacZ* expression is also seen in the embryo and early L1 stage. *lin-12* is known to be required in a number of other cell

fate decisions that occur during this time, such as the G2/W decision, the G1/excretory duct cell decision, the excretory cell/neuroblast decision and in the specification of the intestinal valve cells (GREENWALD *et al.* 1983; LAMBIE and KIMBLE 1991; BOWERMAN *et al.* 1992). The role of *lin-12*, and the cell-cell interactions involved, are less well understood for these decisions. We did not examine these decisions in detail, but have made some general observations.

The *lin-12::lacZ* reporter construct is expressed in a discrete subset of cells during embryogenesis. We did not identify the cells that stain; however, staining was only observed in pairs or groups of cells from the 28-cell stage to about the 400-cell stage. We did note two cells that express the reporter gene in the >300-cell embryo. Based on the age of the embryos and the central positioning of the staining cells, these two cells may be the intestinal valve cells that are known to require cell-cell interactions for proper fate specification (BOWERMAN *et al.* 1992).

The *lin-12::lacZ* reporter gene is expressed in a discrete subset of cells in the ventral cord of L1 larvae. There are different patterns of staining nuclei in the ventral nerve cord, although we do not know the identity of the individual neurons. There are also at least three small nuclei that stain in the head region that may be G2, W, the excretory duct cell, G1 or the neuroblast that is the equipotent equivalent of the excretory cell. We observed staining in the excretory cell. We did not observe staining in the tail region consistent with the location of other cells that are known to require *lin-12* activity such as the cell that forms the rectum, the anal depressor muscle cell, the rectal sphincter cell, or the L1 intestinal cells F, U, K and K′ (LAMBIE and KIMBLE 1991); however, we did observe staining of cells in this region in a *lin-12(n137)* background [*n137* is a *lin-12(d)* mutant]. We postulate that the inability to detect this staining in a wild-type background is due either to a very short window of detectable expression and/or to a very low level of expression, which is increased when LIN-12 is activated (WILKINSON *et al.* 1994).

DISCUSSION

We have examined the expression of a *lin-12::lacZ* reporter gene that contains all sequences required for rescue of a *lin-12(0)* mutant. A detailed discussion of the expression pattern is presented in RESULTS. In this section, we discuss more general points.

The *lin-12::lacZ* reporter gene: Studies of the development of the *C. elegans* gonad and vulva have provided important general paradigms of cell fate decisions (reviewed in HORVITZ and STERNBERG 1991; GREENWALD and RUBIN 1992). The expression of *arIs11*, the *lin-12::lacZ* transgene, described in this study, will be a useful marker for specific cell types during gonadal and vulval development. During gonadal development, *arIs11* will be particularly useful to mark Z1.ppa, Z1.ppp, Z4.aaa and Z4.aap during the L2 larval stage, the VU daughters and granddaughters during the L3 larval stage, and sheath cells No. 1 and a subset of the spermathecal cells during the L4 larval stage. During vulval development, *arIs11* will be useful in identifying the VPCs and will join *nIs2* [*lin-11::lacZ*] (FREYD 1991; G. FREYD and H. R. HORVITZ, personal communication) as a marker for the adoption of the 2° fate by a VPC. *arIs11* will also be a useful marker for the early dorsal and ventral descendants of the M lineage.

During the course of this work, we have tried many other reporter constructs. These constructs included reporter genes containing the *lin-12* 5′ region fused to *lacZ*, and derivatives in which one or more introns were added. These reporter constructs displayed very limited or no expression, even when coinjected with the *lin-12(+)* rescuing construct (WILKINSON 1994; H. A. WILKINSON and I. GREENWALD, unpublished observations). Our experience with *lin-12* and other genes in our laboratory suggests that the design of the pBGSLE plasmid used to generate *arIs11* may be a general solution to the problem of obtaining an accurate reflection of gene expression by *lacZ* reporter genes for genes with complicated regulation.

Pattern of *lin-12* expression: We have found that *lin-12::lacZ* is expressed in a discrete spatial and temporal pattern during development, implying that regulation of *lin-12* expression is one way that LIN-12 activity is regulated. It is possible that *lin-12* expression is restricted to prevent undesirable effects of inappropriate LIN-12 activation in other cells. Alternatively, *lin-12* expression may be restricted as a consequence of the evolution of the *lin-12* gene: it has been proposed that *lin-12* arose from an ancestral *glp-1* gene that was duplicated and placed under the control of different regulatory elements (YOCHEM and GREENWALD 1989; FITZGERALD *et al.* 1993).

Most of the cells that express *lin-12::lacZ* correspond to cells that are known to be affected in *lin-12* mutants (GREENWALD *et al.* 1983; SEYDOUX *et al.* 1990; LAMBIE and KIMBLE 1991; BOWERMAN *et al.* 1992; NEWMAN *et al.* 1995). There are two exceptions to this statement: later expression in the gonadal and vulval lineages, and expression in parents of cells known to require *lin-12* activity. However, later expression in the gonadal and vulval lineages may account for previously unexplained fertility and egg-laying defects associated with mutations in *lin-12* (see RESULTS), and parental expression may suggest that "maternal" product provided to the daughter cells may help prepare them to receive intercellular signals when they are born. Alternatively, *lin-12* expression in these cells may not be functionally significant or the defects associated with the absence of *lin-12* activity may not be apparent because of possible functional redundancy with *glp-1*.

Commitment and *lin-12* expression: During the deci-

sion of Z1.ppp and Z4.aaa between the AC and VU fates, there is a change in the expression of *lin-12::lacZ* in Z1.ppp or Z4.aaa before commitment (WILKINSON *et al.* 1994). Z1.ppp and Z4.aaa have naturally variable fates, which are specified as a result of *lin-12*-mediated interactions between the two cells (KIMBLE and HIRSH 1979; KIMBLE 1981; SEYDOUX and GREENWALD 1989). One of the two cells becomes the AC and the other becomes a VU (KIMBLE and HIRSH 1979). When Z1.ppp and Z4.aaa are in the process of choosing which will become the AC and which will become the VU, *lin-12::lacZ* expression is positively autoregulated in the presumptive VU and negatively regulated in the presumptive AC (WILKINSON *et al.* 1994).

Unlike Z1.ppp and Z4.aaa, the other cells affected by mutations in *lin-12* in hermaphrodites have invariant fates that can be predicted by their ancestry. For these cells, regulation of *lin-12* expression does not seem to be an important component of the process of making a cell fate decision, because *lin-12::lacZ* expression appears to be constant. Precise details of the cell-cell interactions and timing of commitment are known for the VPCs. A VPC may receive up to three different signalling inputs: there is an inductive signal emanating from the AC, a lateral signal thought to originate in the VPCs and received by LIN-12, and an inhibitory signal thought to emanate from the hypodermal syncytium (reviewed in HORVITZ and STERNBERG 1991). The VPCs become committed before they divide in the mid-L3 stage (GREENWALD *et al.* 1983; STERNBERG and HORVITZ 1986). We have observed that *lin-12::lacZ* is expressed in the VPCs from the L2 stage on, and when they divide, *lin-12::lacZ* is expressed in their daughters. Thus, there is no apparent change in *lin-12::lacZ* expression at the time of commitment. We expect that ligand availability or activity may lead to LIN-12 activation in P5.p and P7.p, the VPCs that adopt the 2° fate. Indeed, there is reason to think that the inductive signal produced by the AC may control the activity of the ligand for *lin-12*, because a reduction in the activity of the inductive signalling pathway can lead to a reduction in the efficacy of lateral signalling (TUCK and GREENWALD 1995).

We thank JIM MCCARTER and TIM SCHEDL for help in identifying the sheath cells and spermathecal cells that express the *lin-12::lacZ* reporter gene; DIANE LEVITAN for help in identifying the N and T cell staining in the L3 larval stage; MIKE BECK for help in identifying and characterizing lines carrying integrated arrays of the *lin-12::lacZ* reporter gene; ANDY FIRE and GARY STRUHL for advice on obtaining expression; and BARTH GRANT, DIANE LEVITAN, CHEN-WEI WEN, and JANE HUBBARD for critical reading of this manuscript. H.A.W. was a graduate student *in absentia* in the Department of Molecular Biology at Princeton University. This work was supported by National Institute of General Medical Sciences grant GM-37602. I.G. is an Associate Investigator of the Howard Hughes Medical Institute.

LITERATURE CITED

BOWERMAN, B., F. E. TAX, J. H. THOMAS and J. R. PRIESS, 1992 Cell interactions involved in development of the bilaterally symmetrical intestinal valve cells during embryogenesis in *Caenorhabditis elegans*. Development **116:** 1113–1122.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

CASANOVA, J., and G. STRUHL, 1993 The torso receptor localizes as well as transduces the spatial signal specifying terminal body pattern in *Drosophila*. Nature **362:** 152–155.

ELLIS, H. M., and H. R. HORVITZ, 1986 Genetic control of programmed cell death in the nematode Caenorhabditis elegans. Cell **44:** 817–829.

EVANS, T. C., S. L. CRITTENDEN, V. KODOYIANNI and J. KIMBLE, 1994 Translational control of maternal *glp-1* mRNA establishes an asymmetry in the *C. elegans* embryo. Cell **77:** 183–194.

FERGUSON, E. L., P. W. STERNBERG and H. R. HORVITZ, 1987 A genetic pathway for the specification of the vulval lineages of *Caenorhabditis elegans*. Nature **326:** 259–267.

FINNEY, M., and G. RUVKUN, 1990 The *unc-86* gene product couples cell lineage and cell identity in *C. elegans*. Cell **63:** 895–905.

FIRE, A., 1993 Histochemical techniques for locating *Escherichia coli* β-galactosidase activity in transgenic organisms. Gene Anal. Tech. Appl. **9:** 151–158.

FIRE, A., S. W. HARRISON and D. DIXON, 1990 A modular set of *lacZ* fusion vectors for studying gene expression in *Caenorhabditis elegans*. Gene **93:** 189–198.

FITZGERALD, K., H. A. WILKINSON and I. GREENWALD, 1993 *glp-1* can substitute for *lin-12* in specifying cell fate decisions in *Caenorhabditis elegans*. Development **119:** 1019–1027.

FIXSEN, W. D., 1985 The genetic control of hypodermal lineages during nematode development. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, MA.

FRANCIS, R., and R. WATERSTON, 1985 Muscle organization in *Caenorhabditis elegans*: localization of proteins implicated in thin filament attachment and I-band organization. J. Cell Biol. **101:** 1532–1549.

FREYD, G., 1991 Molecular analysis of the *Caenorhabditis elegans* cell lineage gene *lin-11*. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, MA.

GREENWALD, I., and G. M. RUBIN, 1992 Making a difference: the role of cell-cell interactions in establishing separate identities for equivalent cells. Cell **68:** 271–281.

GREENWALD, I., and G. SEYDOUX, 1990 Analysis of gain-of-function mutations of the *lin-12* gene of *Caenorhabditis elegans*. Nature **346:** 197–199.

GREENWALD, I. S., P. W. STERNBERG and H. R. HORVITZ, 1983 The *lin-12* locus specifies cell fates in *Caenorhabditis elegans*. Cell **34:** 435–444.

HERMAN, R. K., 1995 Mosaic analysis. Methods Cell Biol (in press).

HIRSH, D., D. OPPENHEIM and M. KLASS, 1976 Development of the reproductive system of *Caenorhabditis elegans*. Dev. Biol. **49:** 200–219.

HODGKIN, J., 1988 Sexual dimorphism and sexual determination, pp. 243–280 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

HODGKIN, J., A. PAPP, R. PULAK, V. AMBROS and P. ANDERSON, 1989 A new kind of informational suppression in the nematode *Caenorhabditis elegans*. Genetics **123:** 301–313.

HORVITZ, H. R., and P. W. STERNBERG, 1991 Multiple intercellular signalling systems control the development of the *Caenorhabditis elegans* vulva. Nature **351:** 535–541.

KENYON, C., 1986 A gene involved in the development of the posterior body region of *C. elegans*. Cell **46:** 477–487.

KIMBLE, J., 1981 Alterations in cell lineage following laser ablation of cells in the somatic gonad of *Caenorhabditis elegans*. Dev. Biol. **87:** 286–300.

KIMBLE, J., and D. HIRSH, 1979 Postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev. Biol. **81:** 208–221.

LAMBIE, E., and J. KIMBLE, 1991 Two homologous genes, *lin-12* and *glp-1*, have overlapping functions. Development **112:** 231–240.

MELLO, C. C., J. M. KRAMER, D. STINCHCOMB and V. AMBROS, 1991 Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J. **10:** 3959–3970.

NEWMAN, A., J. G. WHITE and P. W. STERNBERG, 1995 The *Caenorhab-*

ditis elegans lin-12 gene mediates induction of ventral uterine specialization by the anchor cell. Development **121:** 263–271.

PRIESS, J. R., and D. HIRSH, 1986 *Caenorhabditis elegans* morphogenesis: the role of the cytoskeleton in elongation of the embryo. Dev. Biol. **117:** 156–173.

PULAK, R., and R. P. ANDERSON, 1993 mRNA surveillance by the *Caenorhabditis elegans smg* genes. Genes Dev. **7:** 1885–1897.

SEYDOUX, G., 1991 Analysis of the function of *lin-12* in cell fate decisions in the gonad of *C. elegans*. Ph.D. Thesis, Princeton University, Princeton, NJ.

SEYDOUX, G., and I. GREENWALD, 1989 Cell autonomy of *lin-12* function in a cell fate decision in *C. elegans*. Cell **57:** 1237–1245.

SEYDOUX, G., T. SCHEDL and I. GREENWALD, 1990 Cell-cell interactions prevent a potential inductive interaction between soma and germline in *C. elegans*. Cell **61:** 939–51

SEYDOUX, G., C. SAVAGE and I. GREENWALD, 1993 Isolation and characterization of mutations causing abnormal eversion of the vulva in *Caenorhabditis elegans*. Dev. Biol. **157:** 423–436.

STERNBERG, P. W., 1988 Lateral inhibition during vulval induction in *Caenorhabditis elegans*. Nature **335:** 551–554.

STERNBERG, P. W., and H. R. HORVITZ, 1986 Pattern formation during vulval development in *Caenorhabditis elegans*. Cell **44:** 761–772.

STERNBERG, P. W., and H. R. HORVITZ, 1989 The combined action of two intercellular signalling pathways specifies three cell fates during vulval induction in *C. elegans*. Cell **58:** 679–693.

STRUHL, G., K. FITZGERALD and I. GREENWALD, 1993 Intrinsic activity of the *lin-12* and *Notch* intracellular domains *in vivo*. Cell **74:** 331–345.

SULSTON, J. E., 1976 Post-embryonic development in the ventral cord of *Caenorhabditis elegans*. Philos. Tans. R. Soc. Lond. B Biol. Sci. **275:** 287–297.

SULSTON, J. E., 1988 Cell lineage pp.123–156 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SULSTON, J. E., and H. R. HORVITZ, 1977 Postembryonic cell lineages of the nematode *Caenorhabditis elegans*. Dev. Biol. **56:** 110–156.

SULSTON, J. E., and J. G. WHITE, 1980 Regulation and cell autonomy during post-embryonic development of *Caenorhabditis elegans*. Dev. Biol. **78:** 577–597.

SULSTON, J. E., D. G. ALBERTSON and J. N. THOMSON, 1980 The *Caenorhabditis elegans* male: post-embryonic development of nongonadal structures. Dev. Biol. **78:** 542–576.

SULSTON, J. E., E. SCHIERENBERG, J. G. WHITE and J. N. THOMSON, 1983 The embryonic cell lineage of the nematode *C. elegans*. Dev. Biol. **100:** 64–119.

SUNDARAM, ,M. and I. GREENWALD, 1993 Genetic and phenotypic analysis of hypomorphic *lin-12* mutants in *Caenorhabditis elegans*. Genetics **135:** 755–763.

TUCK, S., and I. GREENWALD, 1995 *lin-25*, a gene required for vulval induction in *Caenorhabditis elegans*. Genes Dev. **9:** 341–357.

WAY, J. C., and M. CHALFIE, 1989 The *mec-3* gene of *Caenorhabditis elegans* requires its own product for maintained expression and is expressed in neuronal cell types. Genes Dev. **3:** 1823–33.

WILKINSON, H. A., 1994 Regulation and expression of the *lin-12* gene in *Caenorhabditis elegans*. Ph.D. Thesis, Princeton University, Princeton, NJ.

WILKINSON, H. A., K. F. FITZGERALD and I. GREENWALD, 1994 Reciprocal changes in expression of *lin-12* (receptor) and *lag-2* (ligand) prior to commitment in a *C. elegans* cell fate decision. Cell **79:** 1187–1198.

YOCHEM, J., and I. GREENWALD, 1989 *glp-1* and *lin-12*, genes implicated in distinct cell-cell interactions in *Caenorhabditis elegans*, encode similar transmembrane proteins. Cell **58:** 553–563.

Communicating editor: R. K. HERMAN