



Ablating the fixed lineage conjecture: Commentary on Kimble 1981

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In 1981 Judith Kimble published what became a classic paper in developmental biology (Kimble, 1981). The history of nematode cell lineage dating back to Boveri and zur Strassen in the 19th century indicated that nematodes had a fixed cell lineage. This invariance was confirmed in a modern study by Sulston and Horvitz (1977). Kimble's PhD work confirmed this except for two cases of indeterminate development (Kimble and Hirsh, 1979). As a postdoc, Kimble followed up using a laser microbeam system developed by John White (Sulston and White, 1980a, 1980b) to selectively remove cells. With this system, if you can see the cell with Nomarski DIC optics, you can kill it. This paper laid the groundwork for analysis of the AC-VU decision, hence for the genetic analysis of LIN-12/Notch signaling (Greenwald, 2012), and for vulval induction, hence for the genetic analysis of the EGFR-RAS pathway (Sternberg, 2006).

The AC-VU Decision. Kimble and Hirsh (1979) had observed indeterminacy in the formation of the gonadal anchor cell (AC). Specifically, either of the two somatic gonad precursors (Z1 and Z4) could generate the single anchor cell. If Z1.ppp became the anchor cell, then Z4.aaa was one of three Ventral Uterine precursor (VU) cells; while conversely, if Z4.aaa became the anchor cell, then Z1.ppp was a VU cell. In this paper, Kimble showed that a single precursor gave rise to an anchor cell, or that isolated Z1.ppp became an anchor cell. This observation indicated that intercellular communication specified the AC and VU fates, and suggested that the anchor cell inhibits the presumptive VU cells from also becoming an anchor cell. Subsequent studies suggest that there is reciprocal signaling between the two cells (Seydoux and Greenwald, 1989).

Vulval induction. Kimble showed that in the absence of the gonad and more specifically of the anchor cell, the three epidermal precursor that form the vulva instead undergo one round of mitosis and their progeny differentiate as epidermis. The demonstration in this paper that the anchor cell is necessary and also sufficient to induce the vulva paved the way for interpretation of Vulvaless mutants (Sulston and Horvitz, 1981; Ferguson et al., 1987) as defective in this signaling process. In particular, Vulvaless mutants have the same phenotype as does ablation of the anchor cell, suggesting that they might have defects in either an inductive signal from the anchor cell to the underlying epidermal vulval precursor cells or in the response of those epidermal

precursor to such a signal. This was indeed the case (e.g., Hill and Sternberg, 1992).

The paper also delves into what at the time was intriguing but confusing polarity reversal in the ventral uterine lineages. Ironically, these were interpreted as fixed lineages at the time and for some time thereafter, but turned out to be based on another cell interaction, namely induction of the VU granddaughters (uterine pi cells) by the anchor cell using the same ligand and receptor (Newman et al., 1995). The three types of VU lineages differ by another induction by the anchor cell! This bit of history highlights the strong bias toward intrinsically determined cell lineages in nematodes.

One type of classic paper is defined by a simple experimental design with clear cut results and deep implications. It took decades to flesh out many of the observations made in this paper such paper. Not all the observations have yet been pursued, for example the invariant parts of the sheath-spermathecal lineages.

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Alterations in Cell Lineage following Laser Ablation of Cells in the Somatic Gonad of *Caenorhabditis elegans*

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The postembryonic cell lineage of the somatic gonad is essentially invariant in *Caenorhabditis elegans* (J. E. Kimble and D. Hirsh, 1979, *Develop. Biol.* **70**, 396-417). The two exceptions to this rule of invariance involve a natural ambiguity in the ancestry of certain cells such that each of two precursor cells assumes one of two alternative fates in a given animal. In this paper, experiments are reported in which laser microsurgery is used to kill individual cells in the developing somatic gonad. Such intervention perturbs the normal environment of the remaining cells; a change observed in the expected behavior of these cells suggests that extrinsic cues may normally play a role in controlling that behavior. Several different lineage alterations have been observed after laser microsurgery in the somatic gonad. These include switches in the *type* of lineage followed by a given precursor cell, reversals in lineage *polarity*, *duplications* of a lineage, and alterations in the *number* of cells produced in the lineage. The only cases in which cells switch from one lineage type to another involve pairs of cells which exhibit natural ambiguity. In most cases, the interactions inferred from these changes seem to occur between neighboring somatic gonadal cells. In one case, induction of the vulva, the interaction occurs between a single somatic gonadal cell, the anchor cell, and the precursors to the vulva in a neighboring tissue, the hypodermis. The roles of intrinsic and extrinsic cues in controlling normally invariant cell lineages are discussed.

INTRODUCTION

In many invertebrates, development normally occurs by invariant cell lineages. A given precursor cell follows a specific pattern of cell divisions and the cells generated assume fates that correspond precisely to their position in the lineage tree. The control of such programs of cell behavior is not understood. Individual lineages might be specified by intrinsic factors, or they might be influenced by reproducible interactions between the cell and its environment.

Classical studies (reviewed by Davidson, 1976), in which embryonic blastomeres were either deleted or isolated, suggested that a cell's fate is determined by its ancestry in animals developing by invariant lineages. Thus, each cell was considered to behave autonomously in the developing organism as a result of instructions received from its parent. However, in the same types of animals, several cases have now been reported in which a cell's fate is influenced by interactions with its neighbors (reviewed by Cather, 1971; Morrill *et al.*, 1973; van den Bigelaar and Guerrier, 1979). These include both regulative interactions, in which a cell alters its fate to compensate for a deleted cell, and inductive interactions, in which a particular structure does not develop after deletion of a neighboring tissue.

Descriptive studies have shown that the small non-parasitic nematode *Caenorhabditis elegans* develops by

essentially invariant lineages (Sulston and Horvitz, 1977; Deppe *et al.*, 1978; Kimble and Hirsh, 1979; Sulston and Schierenberg, personal communication). Cells have been shown to behave autonomously in the early embryonic lineages of *C. elegans* by blastomere isolation experiments (Laufer *et al.*, 1980), and in the postembryonic lineages by initial experiments using laser microsurgery (Sulston and Horvitz, 1977). Recently, however, Sulston and White (1980) have done a more extensive series of laser ablation studies and have discovered examples of both regulation and induction in the postembryonic development of nongonadal lineages.

In the development of both the gonadal and the nongonadal tissues, a limited variability in cell ancestry occurs naturally. For example, in the lineage of the somatic gonad, there are cases of variability in which two cells each assumes one of two alternative fates. Such variability might have been the result of choosing individual animals at random from a pool of two genetically distinct populations. However, Kimble and Hirsh (1979) demonstrated that individuals developing by either pathway give rise to some progeny developing by the parental pathway and other progeny developing by the alternative pathway. The experiments reported here were initiated to distinguish between two further possible explanations of this natural ambiguity. The precursor cells might be committed to a single fate by ancestry during embryogenesis, but assume alternative positions in the hatchling. Or, the precursor cells might

be uncommitted at hatching, and become committed later as a result of interactions experienced after hatching. Results presented in this paper suggest that these cells are not committed to one of the two fates at hatching, and that cell-cell interactions play a critical role in influencing the choice. Further results suggest that cell-cell interactions may have a more general importance in controlling certain aspects of cell lineages that are normally invariant.

MATERIALS AND METHODS

C. elegans var. Bristol was maintained at 20–22°C on agar-filled petri plates seeded with *Escherichia coli* as described by Brenner (1974).

Laser microsurgery. The laser microbeam system and the procedure for killing individual cells in *C. elegans* have been described elsewhere (Sulston and White, 1980). Briefly, selected worms were anaesthetized in 0.5% 1-phenoxy-2-propanol (Koch-Light Laboratories, Ltd) and mounted on an agar pad under a coverslip. The cell of interest was brought into focus at 1250× using a Zeiss Universal microscope equipped with Nomarski differential interference contrast optics, and was centered at a point previously aligned with an auxiliary He/Ne gas laser. Then, pulses from a 250 mJ coumarin dye laser microbeam were directed through the objective to kill the cell. The condition of the target cell and neighboring cells was monitored between pulses. When the nucleus of the target cell appeared to be destroyed, the worm was returned to a petri plate for recovery. After 1–4 hr, it was remounted to validate destruction of the desired cell. If the nucleus of that cell could be seen, or if neighboring cells appeared damaged, the animal was discarded. If the target cell remained only as debris, with no visible sign of a nucleus, the ablation was scored as successful (Fig. 4). The effect of the ablation on the fate of the remaining cells was followed by observation of the cells in the living animal with Nomarski optics either continuously to obtain a lineage, or at intervals as necessary for the particular experiment. In some cases, where timing of the ablation was not critical, a partially recovered cell was pulsed again to kill it.

Explanation of nomenclature and lineage diagrams. The postembryonic life of *C. elegans* includes four larval stages, L1, L2, L3, L4, and adulthood.

The four gonadal precursor cells present at hatching have been named Z1, Z2, Z3, and Z4 from anterior to posterior (Kimble and Hirsh, 1979). Z2 and Z3 produce only germ line cells; Z1 and Z4 produce only somatic cells.

In lineage diagrams (e.g., Fig. 5), each vertical line

represents a cell, and each horizontal line represents a cell division. The left-hand branch indicates the anterior and the right-hand branch the posterior daughter at each division unless marked otherwise—d (dorsal), v (ventral), l (left), or r (right). Daughters are named by adding a letter (e.g., a if it is the anterior, or p if the posterior daughter), to the name of the mother cell. Thus, the anterior daughter of cell X is X.a, and the posterior daughter of X.a is X.ap.

Instead of referring to the descendants of Z1 and Z4 that make up the somatic primordium as Z1.pap, Z4.aap, etc., they have been renamed SP1–SP10 (hermaphrodites) and SP11–SP18 (males) for ease of reading. Furthermore, the lineages followed by these precursors in the somatic primordium are named according to the structures to which they contribute descendants as indicated in Figs. 2 and 3. In most cases these fate names comprise two letters (e.g., ss, sheath-spermathecal lineage; or vd, vas deferens lineage). The lineages of the ventral uterine precursors are an exception; here, a number is added to distinguish similar, but unique lineages. The number added indicates how many pairs of lateral uterine cells are produced by each lineage (see Figs. 5 and 6). An additional complication of these lineages is the necessity to indicate the polarity of the lineage. This is done by placing an arrow above the fate name.

Electron microscopy. Individual animals were cut transversely through both pharynx and tail, and prepared for electron microscopy as described previously (Ward *et al.*, 1975).

RESULTS

The somatic structures of hermaphrodite and male gonads develop from two progenitor cells, Z1 and Z4, which are present in the gonadal primordium at hatching. In this paper, the effects of laser ablation of cells in the Z1 and Z4 lineages on the fates of the remaining somatic cells are described. The effects of laser ablation of somatic gonadal cells on germ cell fate have been described elsewhere (Kimble and White, 1981; Kimble and Sharrock, manuscript in preparation).

For background information the reader is referred to Figs. 1, 2, and 3 and their legends. Figure 1 summarizes the major events in the development of hermaphrodite and male gonads after hatching. Briefly, Z1 and Z4 undergo a period of *early divisions* during L1 and early in L2 to generate 10 (male) or 12 (hermaphrodite) cells. All except two of these descendants become arranged in a *somatic primordium* during L2, and all but one of the cells in the somatic primordium undergo a period of *late divisions* during L3 and L4. These di-

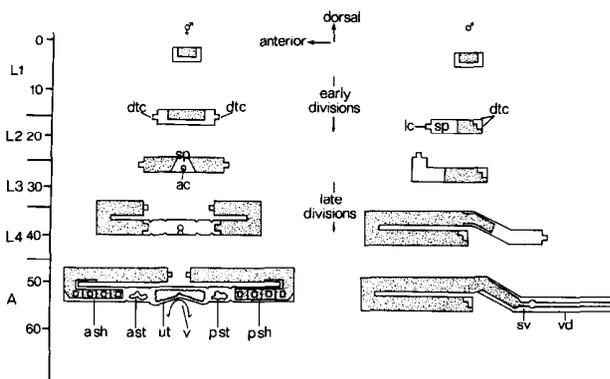


FIG. 1. Postembryonic development of the hermaphrodite (left) and male (right) gonads (based on Hirsh *et al.* (1976) and Kimble and Hirsh (1979)). Coordinates of the animal are indicated at the top. The morphology of the developing gonad is shown at consecutive stages of development from top to bottom corresponding to times after hatching (vertical scale to left, in hours, at 20°C). The major events in the development of the somatic gonadal structures in both sexes are (1) a period of early divisions (upper arrow), (2) organization of most of the early descendants of Z1 and Z4 into a somatic primordium (sp), and (3) a period of late divisions (lower arrow) that generate the constituent cells of the adult somatic structures. The vulva (v) is a hypodermal, rather than a gonadal derivative. L1, L2, L3, L4, first to fourth larval stages; A, adult; dtc, distal tip cell; ac, anchor cell; lc, linker cell; ash, anterior sheath; ast, anterior spermatheca; ut, uterus; pst, posterior spermatheca; psh, posterior sheath; sv, seminal vesicle; vd, vas deferens.

visions of the precursor cells in the somatic primordium generate the somatic cells of the adult gonad. Figures 2 and 3 show, for hermaphrodites and males, respectively, the ancestry and fates of the cells in the Z1 and Z4 lineages discussed in this paper. The nomenclature used here is described under Materials and Methods.

The Results are organized as follows. First, experiments are presented in which regulative changes in cell fate are seen. Second, experiments in which cell fates remain essentially unaltered are briefly described. Third, experiments concerning the induction of the vulva by the gonad are reported. The Discussion includes a brief summary of the results for those readers who would prefer to forego the detailed description given below.

Linker Cell Regulation

In males, a natural variability is seen in the ancestry of the linker cell, the cell that guides the elongating gonad during development and links the vas deferens to the cloaca (Fig. 1). This variability involves two precursors, SP11 and SP15 (Fig. 3) that assume one of two alternative positions in the somatic primordium. One of the two becomes the linker cell and the other becomes a vas deferens precursor cell.

If both SP11 and SP15 are killed before assuming their positions in the somatic primordium, no linker cell is produced (two animals). And, if the linker cell alone is killed, once it has assumed its position in the somatic primordium, no linker cell is made (Table 1). However, if one of the linker cell precursors, or one of its ancestors, is killed before the linker cell is recognizable, a linker cell is made in all cases (Table 1). The ancestry of this linker cell has not been determined directly, because the linker cell is established shortly after the operation while the animal is recovering from the anaesthetic. However, since no linker cell is made

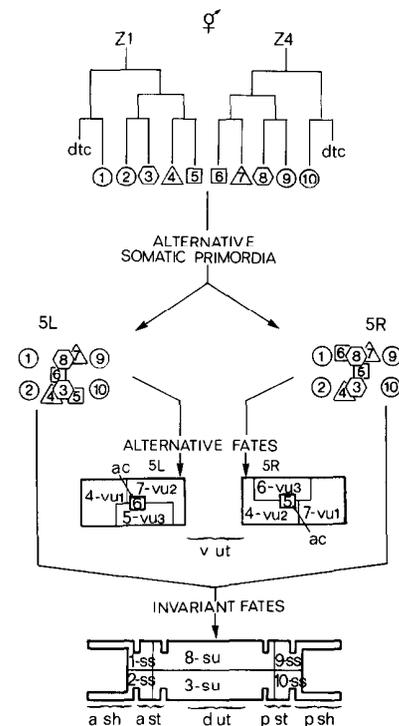


FIG. 2. Ancestry and fates of cells in the hermaphrodite somatic primordium (based on Kimble and Hirsh, 1979). Anterior is to the left for all diagrams. The early divisions of Z1 and Z4 (top) generate 12 cells in hermaphrodites. Ten of these cells, named SP1-SP10 but represented by only the relevant number here, become arranged in one of two alternative somatic primordia (middle, dorsal views). The two somatic primordia are morphologically distinct because of the difference in position of two cells (SP5 and SP6). Cells represented by the same geometric shape are equivalent in developmental potential. The descendant cells of each of the precursors in the somatic primordia assume positions in the adult somatic structures as shown in the bottom diagrams (dorsal views). The diagrams of the ventral uterus and the dorsal uterus are separated in this figure; in the animal, the ventral uterus would lie ventral to the dorsal uterus as a single structure. The ancestry of anterior and posterior sheaths (ash and psh), anterior and posterior spermathecae (ast and pst), and the dorsal part of the uterus, dut, is invariant, and the ancestry of the ventral part of the uterus (vut) and anchor cell (ac) is variable as shown (bottom diagrams, dorsal views).

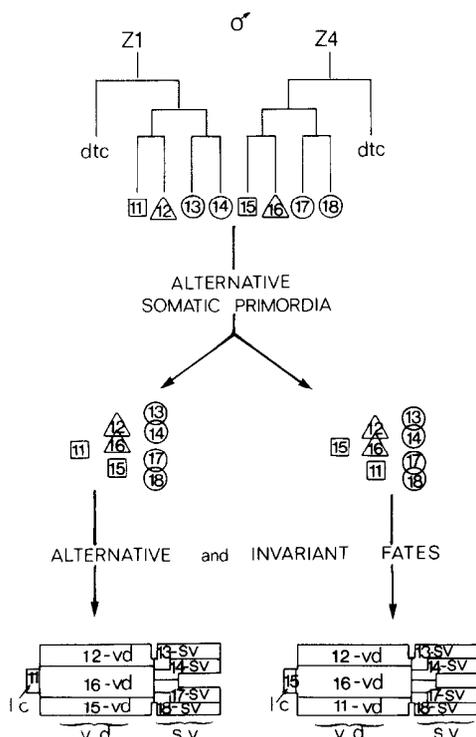


FIG. 3. Ancestry and fates of cells in the male somatic primordium (based on Kimble and Hirsh, 1979). Anterior is to the left for all diagrams. The early divisions of Z1 and Z4 (top) generate 10 cells in males. Eight of these cells, named SP11-SP18 but represented by only the relevant number here, become arranged in one of two alternative somatic primordia (middle, dorsal view). The two somatic primordia are morphologically indistinguishable, though they differ in the positions of two cells (SP11 and SP15). Cells represented by the same geometrical shape share both a similar ancestry and fate. The descendant cells of each of the precursors in the somatic primordia assume positions in the adult somatic structures as shown in the bottom diagrams (dorsal view). The ancestry of the seminal vesicle (sv) is invariant, and the ancestry of the vas deferens (vd) and linker cell (lc) is variable as shown.

when both SP11 and SP15 are killed, it is probable that SP15 becomes the linker cell when SP11 is killed.

Ventral Uterine and Anchor Cell Regulation

In hermaphrodites, a natural variability is seen in the ancestry of the ventral uterus and the anchor cell (Fig. 2). This variability involves two pairs of apparently equivalent cells—the *ventral uterine/anchor cell group* of precursor cells. The four cells assume positions in one of two alternative somatic primordia and each cell in a pair assumes one of two alternative fates. One pair of cells, the *anchor cell pair* (SP5 and SP6), contributes the anchor cell and a ventral uterine precursor. The anchor cell induces the underlying hypodermis to make a vulva (as shown in a later section). The alternative, or redundant, anchor cell precursor follows a unique ventral uterine lineage (designated vu3; Figs. 2,

6). The other pair of cells, the *nonanchor cell pair* (SP4 and SP7), contributes two ventral uterine precursors. Each of these follows one of two unique ventral uterine lineages (designated vu1 and vu2; Figs. 2, 5).

The precise fates of each of these four cells can be predicted from the configuration of the somatic primordium in which the cells reside. The two primordia differ only in the positions of the two cells of the anchor cell pair (Fig. 2). In the 5L configuration, SP6 acquires a central position and becomes the anchor cell, whereas SP5 assumes a lateral position on the left side of the somatic primordium and becomes a uterine precursor. Thus, in 5L primordia, the left side consists of five cells (hence the name) and the right side of four cells. The 5R primordium is related to the 5L primordium by two-fold rotational symmetry.

Three alterations in lineage have been observed after ablations of individual cells among the ventral uterine-anchor cell group of precursor cells. In all cases, a functional uterus is made from the two remaining ventral uterine precursors.

1. *Anchor cell replacement.* If either Z1 or Z4 is ablated, an anchor cell is made (52/53 animals), suggesting that the commitment of one of the two precursors to make an anchor cell is not made at this early point in the lineage. If both anchor cell precursors, SP5 and SP6, are ablated, no anchor cell is made (five animals). If the prospective anchor cell is killed before the somatic primordium is formed, the redundant anchor

TABLE 1
TIMING OF LINKER CELL REGULATION

Cell ablated	Stage of development ^a	Number of animals	Linker cell replacement	
			+	-
Z1	Not formed	7	7	0
Z1.p	Not formed	2	2	0
Z1.pa	Not formed	2	2	0
Z1.paa(SP1)	Forming	4	4	0
Presumptive linker	Just formed	5	0	5
Linker cell	Formed	4	0	4

^a Stage of development refers to the stage of formation of the somatic primordium. Not formed describes the period before the cells of the somatic primordium have been born (e.g., two to six cells found in the somatic gonad); forming means that the two linker cell precursors (SP11 and SP15) have not yet assumed their positions in the somatic primordium; just formed means that one linker cell precursor has assumed its position at the apex of the gonad and that the worm is in L1 lethargus or is a newly molted L2; and formed covers the rest of L2 when the established primordium is simply growing in size. A linker cell is "presumptive" if it can be distinguished morphologically as a linker cell but has not acquired the appearance of a fully mature linker cell.

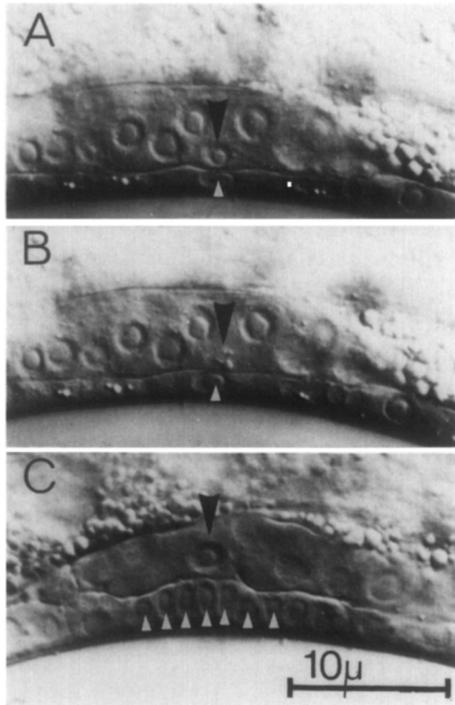


FIG. 4. Anchor cell regulation, Nomarski optics, lateral view. (A) Presumptive anchor cell (black arrowhead) in an unoperated hermaphrodite in L2 lethargus. (B) Debris of same cell as in A (black arrowhead), just after the cell's ablation. (C) Anchor cell (black arrowhead) derived by replacement regulation in an L3 hermaphrodite. White arrowheads indicate hypodermal precursors to the vulva (A and B) or progeny of hypodermal precursors to the vulva after induction (C).

cell can replace it (Fig. 4; Table 2A). This replacement is not seen when the anchor cell is killed later (Table 2A).

2. *Switching of a nonanchor cell precursor to its alternative fate.* Ablation of the presumptive anchor cell leads not only to its replacement by the cell expected to follow the *vu3* fate, but also to a switch in the fate of another ventral uterine precursor. Thus, the sister of the new anchor cell switches from its predicted fate (*vu1*) to its alternative fate (*vu2*) (Figs. 5, 7B). This same lineage switch is also observed if the alternative anchor cell precursor is removed by ablating it during somatic primordium formation (Fig. 7C). However, when the same cell is ablated a few hours later, the lineage switch is no longer observed (Table 2B).

3. *Reversal in polarity of the alternative anchor cell precursor.* The ablation of one of the ventral uterine precursors, if performed before formation of the somatic primordium, leads to a reversal in the polarity of its sister's pattern of cell divisions (Figs. 6A, 7D). This regulation, called *vectorial regulation*, does not alter the number or kinds of cells produced, but alters their distribution in the uterus. If the same operation

is performed soon after the somatic primordium forms, a novel ventral uterine lineage is sometimes observed (Fig. 6B, Table 2C). This new lineage appears to duplicate half of the normal *vu3* lineage. If the operation is performed a few hours after somatic primordium formation, no change in the expected lineage is observed (Table 2C).

A Second Example of Vectorial Regulation

If Z1 or Z4 is killed in an L1 hermaphrodite, a fertile adult animal develops with one instead of two reflexed

TABLE 2
TIMING OF HERMAPHRODITE LINEAGE ALTERATIONS

Cell ablated	Stage of development ^a	Number of animals	Lineage alteration ^b		
			+	n	-
A. Anchor cell	Forming	15	13		2
	Just formed	17	1		16
	Formed	13	0		13
	First division	15	0		16
B. SP5(5L) or SP6(5R)	Forming	10	7		3
	Just formed	5	1		4
	Formed	5	2		3
	First division	3	0		3
C. SP4(5L) or SP7(5R)	Forming	7	5	0	2
	Just formed	3	1	0	2
	Formed	10	2	2	6
	First division	6	0	0	6

^a Stage of development refers to the stage of formation of the somatic primordium. Forming refers to the period (about 2 hr) when the configuration of the primordium can be observed but when the constituent cells have not assumed their final position; just formed refers to the period (about 1 hr) when the cells are in position, but the worm is still in L2 lethargus; formed refers to the period (about 1 hr) when no divisions have occurred and the worm is a newly molted L3; first division means that the first divisions of cells in the primordium are occurring or have occurred; thus, one or both of the spermathecal-uterine precursors have divided, but none of the ventral uterine precursors has divided.

^b Lineage alteration: (A) The animal is scored for presence (+) or absence (-) of the anchor cell in early L4. (B) The animal is scored for having undergone (+) or not (-) the change in lineage in SP4(5L) or SP7(5R), as shown in Fig. 5, after ablation of SP5(5L) or SP6(5R). The fate is observed by continuous observation in some animals, or by checking the number of lateral uterine cells produced in L3 lethargus or early L4 since this is diagnostic of the particular ventral uterine lineage followed. (C) The animal is scored for having undergone (+) or not (-) the reversal in polarity of the ventral uterine precursor SP5(5L) or SP6(5R) as shown in Fig. 6A. The fate is observed by continuous observation of the entire lineage in some animals, or of the last two rounds of divisions in other animals, since they are diagnostic of the polarity of the lineage. In two cases, a novel lineage was observed (n) as shown in Fig. 6B.

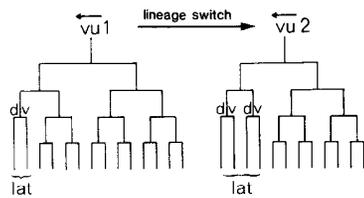


FIG. 5. Lineage switch in the nonanchor cell pair. The cell expected to follow the vu1 lineage (left) follows the vu2 lineage (right) instead. The vu2 lineage makes two more lateral uterine cells (lat) and contributes four fewer cells to the main body of the uterus. The polarity of the lineage, indicated by arrows above fate name, remains unchanged. The contribution to the spermathecal-uterine junction was not followed in these experiments.

gonadal tubes. Such a half-gonad has a single complete spermatheca and sheath, a half uterus, and makes both sperm and oocytes (Fig. 8). Since, in the intact animal, both spermathecae and the uterus consist of descendants of both Z1 and Z4 (Fig. 9A), some change is necessary to make functional structures from Z1 or Z4 alone.

The alteration involves a reversal in the polarity of the lineage followed by one daughter of SP3 (if Z4 is killed) or of SP8 (if Z1 is killed). Consider the ablation of Z4. Normally, SP3 gives rise to two equivalent daughters which follow lineages of opposite polarity to generate descendants occupying mirror symmetrical positions in the anterior and posterior halves of one side of the adult somatic structures (Figs. 9B, 10A). When Z4 is killed, the two daughters of SP3 do not maintain their normal anterior-posterior relationship, but instead the posterior daughter (SP3.p) moves laterally. Both daughters subsequently follow lineages with the same polarity and make descendants in bilaterally symmetric positions (Figs. 9C, 10B). The first division of SP3.p is asymmetric, and the polarity reversal of the SP3.p lineage is first detected by a reversal in orientation of the asymmetry of that first division. Vectorial

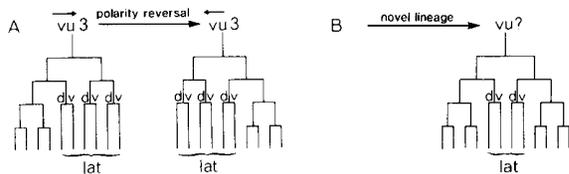


FIG. 6. Two lineage changes observed in SP5 (5L) or SP6 (5R) after ablation of its sister. (A) Vectorial regulation simply reverses the polarity of the vu3 lineage so that the three pairs of lateral uterine cells (lat) are produced in the anterior part of the lineage instead of the posterior part as expected. (B) In two animals, the cell expected to follow the vu3 lineage followed a novel symmetrical lineage instead, producing only four lateral uterine cells instead of six, and producing eight uterine structural cells instead of four. This lineage change appears to duplicate the anterior half of the vu3 lineage.

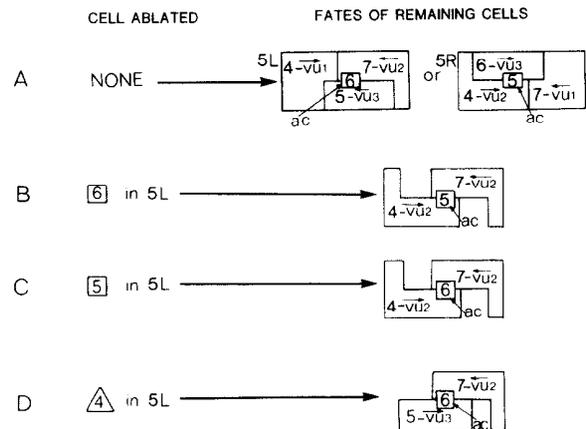


FIG. 7. Summary of regulation in the ventral uterine/anchor cell group of precursors. The ablated cell is shown in the left column; the contribution of each precursor to the ventral uterus is shown in the right column as dorsal views. Fate names: vu1, vu2, and vu3, are three unique ventral uterine lineages (shown in Figs. 5 and 6). The arrow over the fate name indicates the polarity of the lineage, and points to the part of the lineage that produces the lateral uterine cells; the number after vu refers to how many pairs of lateral uterine cells are made by the lineage. (The lateral uterine cells arise by an asymmetric dorsal-ventral division and are morphologically distinct. The larger dorsal cells flank the main body of the uterus in the same focal plane as the sex muscle cells and may serve as a link between the uterine muscles and the uterus; the smaller ventral cells form a collar around the juncture between the vulva and the main body of the uterus.) ac, anchor cell. All experiments are shown as occurring in a 5L primordium for ease of comparison; actual experiments were performed in both 5L and 5R primordia with similar frequency. The changes shown are seen when the ablation is done sufficiently early in the development of the somatic primordium (Table 2). (A) Alternative fates assumed by precursors in the two alternative configurations (5L and 5R) in the unoperated animals. (B) When the presumptive anchor cell is killed, it is replaced by its alternative precursor (SP5) and one member of the nonanchor cell pair (SP4) switches to its alternative fate. (C) When the redundant anchor cell is killed, the same member of the nonanchor cell pair (SP4) switches to its alternative fate. (D) When SP4 is killed, the polarity of its sister (SP5) is reversed (see Fig. 6 for a novel lineage seen in two animals).

regulation, then, does not alter the number or type of cells produced by a lineage, but does change the distribution of the descendant cells in the developing structure (Fig. 9D). This regulation involves an early switch in the polarity of the regulating precursor.

Stability of Cell Fate

A number of experiments have been performed in which no change has been observed in the types of cells (e.g., vas deferens or spermathecal cells) produced by the remaining precursors. In one series of experiments, one of each kind of somatic primordial precursor was ablated, and yet the only fate changes observed were the anchor cell and linker cell replacements already

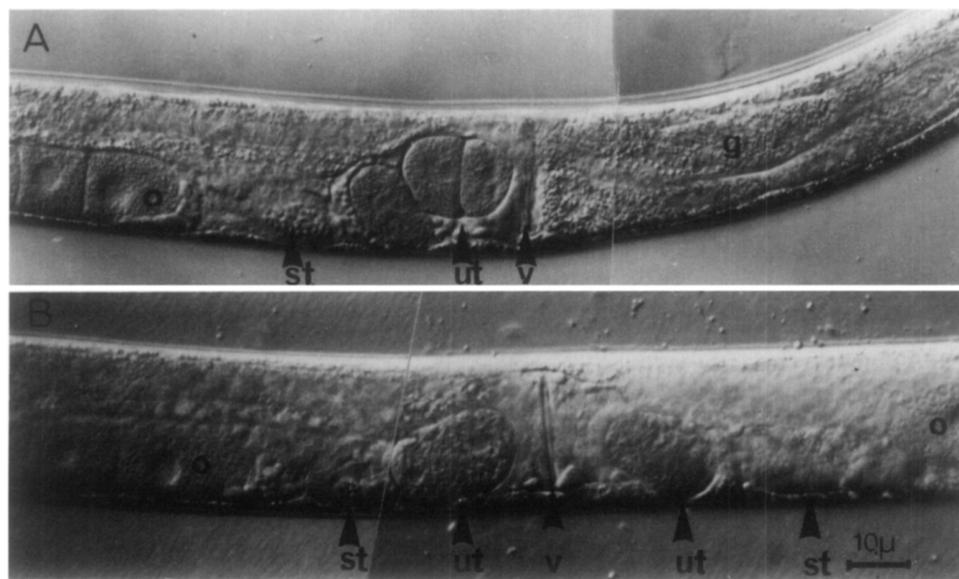


FIG. 8. Formation of an anterior half-gonad after ablation of Z4; Nomarski optics. Adult animals are shown in ventral view; anterior is to the right. (A) Experimental animal. A functional half-gonad is formed anterior to the vulva, whereas the region posterior to the vulva is occupied only by the gut (g). (B) Unoperated animal. Functional half-gonads are seen both anterior and posterior to the vulva. o, Oviduct or proximal arm; st, spermatheca; ut, uterus; v, vulva.

described. These experiments included the ablation during L2 of one sheath-spermathecal precursor (ss, Fig. 2), one spermathecal-uterine precursor (su, Fig. 2), one of each of the various ventral uterine precursors (vu, Fig. 2), one vas deferens precursor (vd, Fig. 3), or one seminal vesicle precursor (sv, Fig. 3). In all cases, the remaining precursor cells gave rise to the expected kind of descendant cells of approximately the correct number (the number was not determined accurately). In addition, these cells became organized into a tubular structure although normally they would form only part of the tube. In another series of experiments *all* of each kind of somatic primordial cell were ablated in indi-

vidual animals by ablation of the cells or their precursors. Again, the remaining precursors produced descendants of the expected cell type in the expected place and of approximately the expected number (the number was not determined accurately). Finally, ablation of the two germ-line progenitor cells (Z2 and Z3) has no apparent effect on the development of the somatic gonad in either sex.

The isolation of a precursor from its normal neighbors provides a more stringent test of the stability of that cell's fate. The sheath-spermathecal precursors, SP1 and SP10, were chosen for such isolation experiments because their normal neighbors could be removed by ablation of fewer cells at an earlier point in the Z1-Z4 lineage than any of the other precursors in either the hermaphrodite or male somatic primordium. In

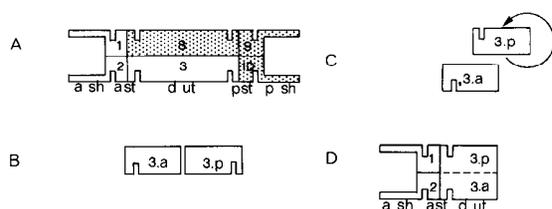


FIG. 9. Vectorial regulation after ablation of Z4. Somatic structures are named as in Figs. 1 and 2. (A) Fates of Z1 (clear) and Z4 (stippled) descendants in the unoperated animal. Removal of Z4 would, without some change, lead to the formation of asymmetric somatic structures. (B) The two daughters of SP3 give rise to equivalent mirror symmetric parts of the uterus and spermatheca in the unoperated animal. (C) After the polarity reversal of SP3.p, the two daughters give rise to equivalent bilaterally symmetric structures. (D) A half-uterus and complete anterior spermatheca are made as a result of this regulation.

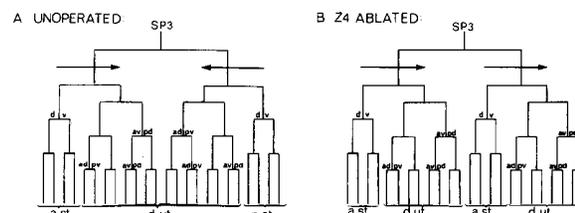


FIG. 10. Lineages followed by SP3 in the unoperated animal (A) and after ablation of Z4 (B). The arrows point to the larger daughter in the asymmetric division of SP3.a and SP3.p. The contribution of the descendant cells to adult structures is indicated below each lineage tree. Somatic structures are named as in Figs. 1 and 2.

three animals, either SP1 or SP10 was isolated by ablation of Z4 (or Z1) initially, and Z1.p (or Z4.a) later (Fig. 11). In addition, SP1 and SP10, which usually are not in contact, were isolated together by ablation of Z1.p and Z4.a (two animals), or they were separated by only the ventral uterine/anchor cell group by ablation of Z1.pa and Z4.ap (two animals).

The lineages followed by SP1 (or Sp10) under these conditions are different from that observed in intact animals, yet they share a number of features with the normal lineage (Fig. 12). The first division is always asymmetric, generating a smaller distal sheath precursor and a larger cell. And, the larger cell always divides symmetrically, giving rise to a proximal sheath precursor and a spermathecal precursor.

The details of the lineages, however, are variable from animal to animal. This variability involves differences in the polarity of divisions and in the number of descendants produced. Polarity reversals occur infrequently. In one animal, where SP1 and SP10 were isolated together, the lineage of both precursors was reversed compared to normal (lineages not shown). In another animal, only the first division and the spermathecal sublineage were reversed (Fig. 12C). In all cases, the reversal in the polarity of a cell division or of an entire lineage was accompanied by a reversal in the orientation of an asymmetric cell division of the switching precursor cell.

Extra divisions are the rule rather than the exception in these isolated cells. An extra division in the distal sheath branch occurred in one animal. Both daughters appeared to be distal sheath cells when examined in the electron microscope, since neither of them exhibited muscle fibers typical of proximal sheath cells. The spermathecal sublineages were variable producing 10, 11, or 12 cells instead of the usual 9. In most cases, the pattern of divisions was asymmetric (Figs. 12B, C, D) and in one of these, a stem cell pattern was followed

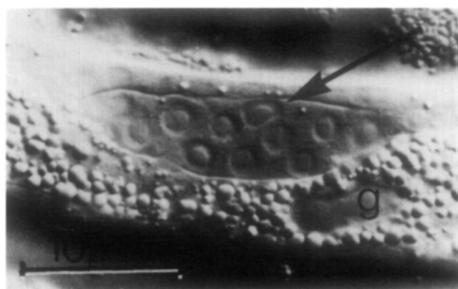


FIG. 11. Isolated sheath-spermathecal precursor; Nomarski optics. Arrow indicates SP1 in a late L2 gonad after ablation of Z4 and Z1.p during L1. The precursor's sister, a distal tip cell, occupies a position at the anterior tip of the developing gonad, and is not seen here. The other nuclei seen in the gonad are germ-line cells. g, Gut nucleus.

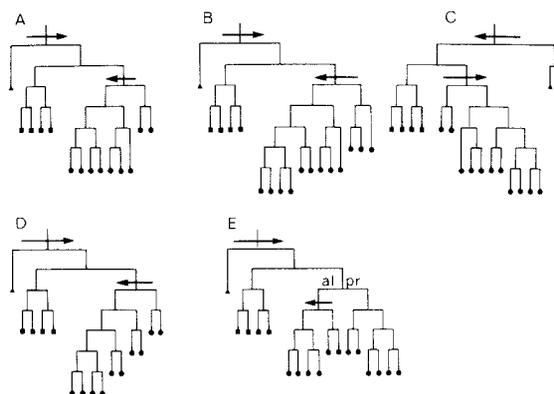


FIG. 12. Lineages of isolated sheath-spermathecal precursors. All lineages have been drawn in the orientation of SP1 for ease of comparison. Arrows point to the larger cell of an asymmetric division. Geometric symbols indicate the fate of each descendant: ●, spermathecal cell; ■, proximal sheath cell; ▲, distal sheath cell. In most cases, these fates were assigned by cell position and appearance using Nomarski optics. In lineages C and E, this assignment was confirmed by electron microscopy (see Fig. 13). (A) Lineage observed in the unoperated animal, or after ablation of the other sheath-spermathecal precursor, SP2 (or SP9) which normally joins SP1 (or Sp10) to make the complete anterior (or posterior) sheath and part spermatheca. (B) Lineage observed in three animals: in one, after ablation of Z1.pa and Z4.ap; in another, after ablation of Z1.p and Z4.a, and in another, after ablation of Z4 and Z1.p. (C). Lineage observed in one animal after ablation of Z4 and Z1.p resulting in complete isolation of the precursor from its normal somatic contacts. (D) Lineage observed in one animal after ablation of Z1.pa and Z4.ap. (E) Lineage observed in one animal after isolation as described in C above.

(Fig. 12D). The sublineage was symmetric in only one animal (Fig. 12E). The descendants of all these various division patterns appeared to be spermathecal cells in the light microscope, and this classification was verified in two animals by examination in the electron microscope (Fig. 13).

SP5, one of the potential anchor cell precursors, was isolated by ablation of Z2, Z3, and Z4 initially, and Z1.a, Z1.pa, and Z1.ppa sequentially thereafter. In the two animals in which this ablation was successful without killing all cells in the gonad, the isolated cell became the anchor cell (Fig. 14A, B).

Induction of the Vulva

In the intact animal, the hypodermal cells underlying the developing gonad divide during L3 and early L4 to make the vulva. Six hypodermal cells (called P3.p-P8.p) undergo one round of division (first-stage divisions) at 29-30 hr, and then, the daughters of three of those cells (P5.p, P6.p, and P7.p) continue dividing (second-stage divisions) with one round at 32 hr and one round at 35 hr to generate a total of 22 vulval cells (Sulston and Horvitz, 1977). Sulston and White (1980) showed that

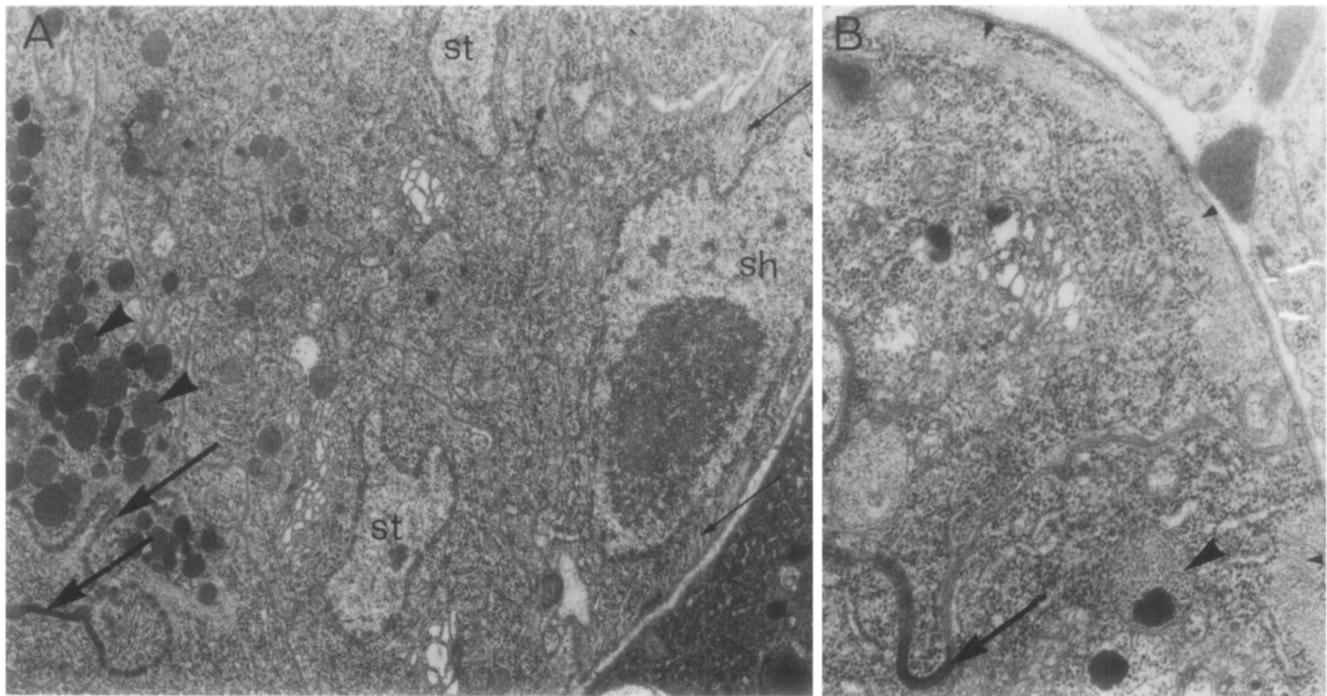


FIG. 13. Electron micrographs of descendants of an isolated sheath-spermathecal precursor (that of lineage shown in Fig. 12C). (A) Cells classified as spermathecal cells by light microscopy exhibit secretory granules (arrowheads) and specialized junctions (large arrows) typical of spermathecal cells in the unoperated animal (Wolf and Hirsh, personal communication). st, Spermathecal cell nucleus. A cell classified as a proximal sheath cell (sh) exhibits muscle filaments (small arrows) typical of such cells in the unoperated animal (Hirsh *et al.*, 1976). Magnification 13,300 \times . (B) Spermathecal cells also exhibit the band of cortical filaments (small arrowheads) typical of those cells in the unoperated animal (Wolf and Hirsh, personal communication). Other symbols are same as in A. Magnification 26,500 \times .

ablation of the gonadal primordium at hatching blocks the second-stage divisions, but not the first-stage divisions. Indeed, ablation of the embryonic precursors to Z1 and Z4 has the same effect (Sulston and Kimble, unpublished results). These results suggest that the gonad induces vulval development. The following experiments show that the anchor cell is both necessary and sufficient for vulval induction.

Ablation of both anchor cell precursors during mid-L2 eliminates vulval development in the same way as ablation of the gonad (five animals), and ablation of the anchor cell at the earliest point possible without replacement regulation (24–26 hr, 20°C) usually has the same effect (Fig. 15A). Ablation of all cells in the gonad (somatic and germ line) *except* one of the potential anchor cell precursors (described in previous section) results in the normal induction of the vulva (Fig. 14B). Although lineages were not followed, the morphology of the vulva was checked at 2-hr intervals in L3 and L4, and each stage looked normal.

Ablation of the anchor cell in the few hours after somatic primordium formation, and before divisions of the vulval precursors, usually leads to partially induced vulvae (Table 3). In some of these, only a few daughters

of the first-stage vulva divisions divide further.¹ This partial induction often generates animals with two “mini-vulvae” (Fig. 15B). Other intermediate vulvae possess the normal 22 cells (presumably by the correct lineage), but they are not organized into normally shaped vulvae (Fig. 15C). If the anchor cell is ablated at or soon after the time of the first divisions of the vulval precursors (29–31 hr), neither the second-stage divisions nor the morphogenesis of the vulva is blocked (Fig. 15D). Instead, the attachment of the vulva to the uterus is not made and the passage necessary for the exit of eggs through the vulva is not formed.

DISCUSSION

Summary

In this paper, the ability of cells to alter their normal fate is explored in the postembryonic lineages of the somatic structures of the gonad in *C. elegans*. A laser

¹ Although the lineages were not followed, the probable identity of the induced cells was determined in eight animals during early L4: (1) P5.pa, (2) P5.pp, (3) P6.pa, P6.pp, (4) P6.pa, P6.pp, (5) P7.pa, P7.pp, (6) P5.pa, P7.pa, P7.pp, (7) P5.pa, P5.pp, P7.pa, P7.pp, and (8) P6.pa, P6.pp, P7.pa, P7.pp.

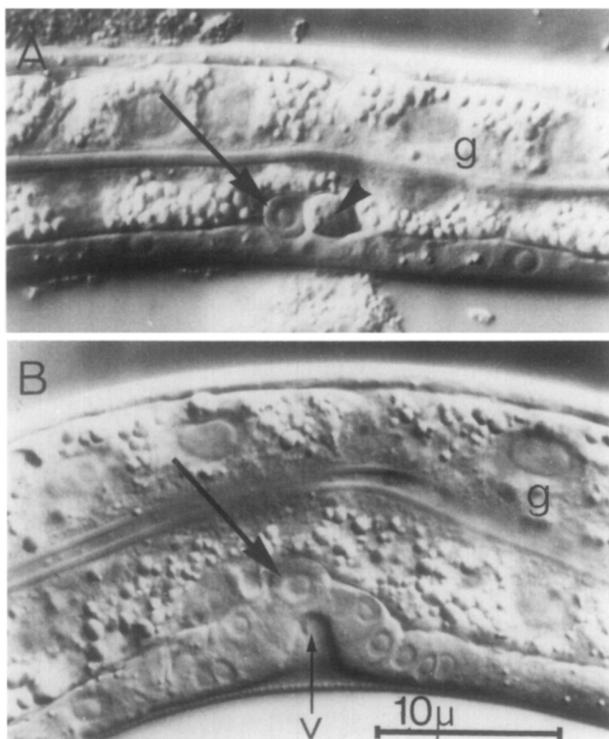


FIG. 14. Isolation of an anchor cell precursor; Nomarski optics. g, Gut. (A) Anchor cell (arrow) is the only remaining cell in the gonad. The debris left by the ablation of six cells (precursors to the rest of the gonad killed in L1 and early L2) is minimal (arrowhead). (B) Isolated anchor cell (large arrow) induces the vulva (small arrow, v).

microbeam is used to kill individual cells, and, thereby, to disturb the environment of the remaining cells. The major results are summarized below.

1. There are three cases in which a precursor cell normally capable of assuming either of two alternative fates can be channelled into one of those fates. Two of these cases are the same as *replacement regulation* observed by Sulston and White (1980) in the nongonadal tissues. In the male, if one of the natural precursors to the linker cell is ablated, the other natural precursor invariably becomes the linker cell. In the hermaphrodite, if one of the anchor cell precursors is ablated, even after it is identifiable as the presumptive anchor cell, the other anchor cell precursor becomes the anchor cell. Both of these cases indicate that the two alternative precursors are equivalent in their potential to become the linker cell or the anchor cell.

In the third case, if either of the anchor cell precursors is removed, both members of a different pair of cells (the nonanchor cell pair) assume the same fate. This change is similar to replacement regulation in that two cells which normally assume different fates are led to follow the same fate in the same animal. However, the lineage change does not involve replacement.

2. Reversals in lineage polarity have been observed

in two different kinds of experiments. In one kind, the reversal appears to compensate for an aberrant geometrical asymmetry in a developing structure introduced by killing a particular cell. This type of regulation has so far only been observed in the gonad and is called *vectorial regulation*. In the other kind, polarity reversals are sometimes seen after the isolation of a precursor from its normal neighbors. Such reversals, seen in both the gonadal tissues and the nongonadal tissues, are

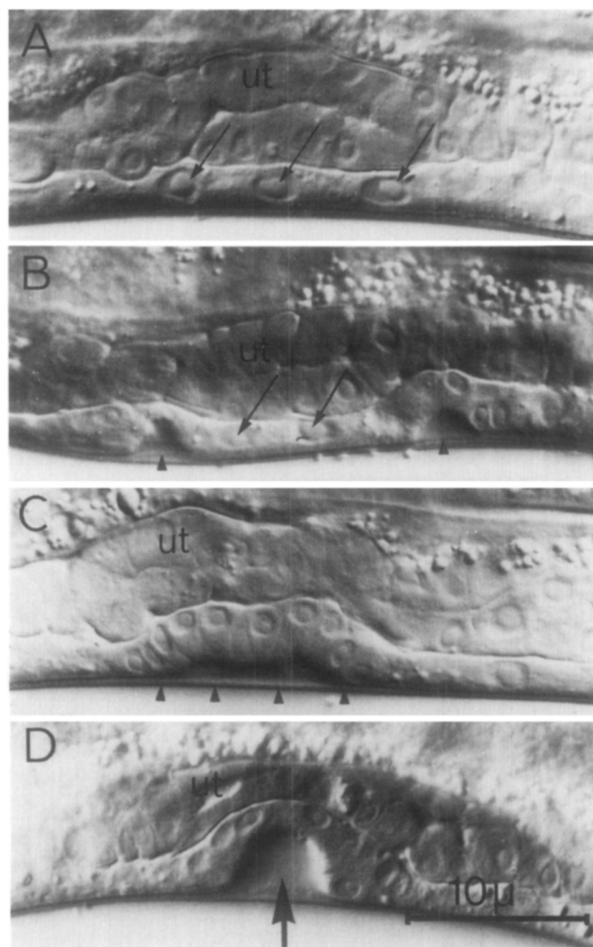


FIG. 15. Morphology of hypodermis in prospective vulval region after ablation of anchor cell; Nomarski optics. All photographs show early L4 animals, lateral view. (A) No induction. Arrows point to descendants of the first-stage division of the normal vulval precursors which, in this case, have not divided further to make a vulva. (B) Partial induction. Each of two invaginations (arrowheads) results from the induction of a single vulval precursor. Arrows point to descendants of another vulval precursor that has not been induced to make vulval cells. (C) Partial induction. A broad invagination (arrowheads) is formed if all vulval precursors are induced, but the descendant vulval cells do not cooperate to form a vulva. (D) Full induction. All vulval cells are produced, and cooperate to make a single invagination (arrow). Later stages of vulva morphogenesis are also normal, except that the uterus and the vulva are not attached. Therefore, no orifice is formed for exit of the eggs. For further explanation see legend to Table 3.

TABLE 3
TIMING OF VULVA INDUCTION

Cell ablated	Stage of development ^a	Number of animals	Vulva induction ^b			
			-	+	++	+++
Anchor cell	24-26	5	4	1	0	0
	27	8	4	4	0	0
	28	13	0	3	9	1
	29-31	13	0	0	4	8

^a The time of the operation was scored in hours after hatching (20°C) by standards obtained by Sulston and Horvitz (1977) and by Kimble and Hirsh (1979): 24-26 hr, late L2 lethargus and early L3 with no divisions in the somatic primordium; 27 hr, first division of the spermathecal-uterine precursors is occurring or has just occurred; 28 hr, first division of the ventral uterine precursors is occurring or has just occurred; 29-31 hr, first round of divisions of vulval precursors, Pn.p, is occurring or has just occurred.

^b The development of the vulva was scored in early L4 as follows: -, no second-stage divisions and no invagination (Fig. 15A); +, a few second-stage divisions and local invagination (Fig. 15B); ++, 22 cells in vulva and broad invagination (Fig. 15C); +++, 22 cells in vulva and cooperative vulval assembly (Fig. 15D).

variable in that they are infrequent and that they do not switch the polarity of the same division in different animals. Moreover, they do not seem to be compensatory. Both types of polarity reversal indicate that cell polarity is fixed by cell-cell interactions.

3. The production of more descendants than usual in a lineage is frequently seen after isolation of its precursor cell from its normal neighbors. Such a proliferative response is seen in the lateral hypodermal precursors after their isolation (Sulston and White, 1980), and in the sheath-spermathecal precursor after its isolation. In both tissues, the additional divisions extend the pattern of divisions normally followed by the precursor.

4. Two examples have been seen in which a lineage that normally has an asymmetric pattern of divisions changes to a lineage with a symmetric pattern of divisions. In both cases (Figs. 6B, 12E), the changes appear to involve a duplication of one-half of the lineage.

5. The timing of cell ablations is critical to the ability of the remaining cells to change their fate. The naturally ambiguous precursors (those that follow one of the two fates) can be channelled into one particular fate only if the appropriate ablation is done around the time that the precursors become rearranged to make the somatic primordium (Tables 1 and 2). This is true in hermaphrodites where divisions of the somatic primordial precursors begin 1-2 hr after the rearrangement and in males where they begin about 10 hr after. This suggests that the fates of the variable precursors are fixed around the time of somatic primordium formation.

6. Isolation of either the anchor cell or a sheath-spermathecal precursor does not change the "basic character" of their respective fates. Thus, the isolated anchor cell still induces the vulva, and the isolated sheath-spermathecal precursor still generates the same types of descendants as usual, (though the number made and the polarity of the divisions can vary). In addition, many precursors have been ablated with no essential change in fate (i.e., the remaining cells give rise to same types of progeny as usual, though the precise lineages were not followed). This suggests that some aspects of a precursor's fate are determined by intrinsic factors.

7. The *anchor cell* is both necessary and sufficient for normal development of the vulva in the hypodermis. Ablation of the anchor cell can block vulval induction, and furthermore, ablation of all the other gonadal cells, leaving an isolated anchor cell, does not block vulval induction. Partially induced vulvae are seen if the anchor cell is killed just before the first divisions of the vulval precursors.

The Anchor Cell and Induction of the Vulva

If the anchor cell is killed just before the precursors of the vulva begin to divide, the vulva is usually only induced partially. The intermediate morphologies observed provide some clues about the nature of vulval induction. Sometimes, only a few precursors undergo the extra divisions typical of the normal vulval lineage. The induced cells are not preferentially located under the anchor cell nor are they preferentially neighbors (unless they are sisters). This suggests that the initial signal for induction is distributed to all 3 vulval precursors instead of to just the one directly beneath the anchor cell. Moreover, it suggests that the initial response of one cell does not activate the induction of its neighbor. In this sense, the initiation of vulval development does not appear to be cooperative.

In some partially induced vulvae, the normal complement of 22 cells is seen, but, although some invagination is seen, the usual morphogenesis of the vulva does not take place. The role of the anchor cell in vulval morphogenesis is not simply one of attachment. If the anchor cell is killed as the vulval precursors begin to divide, there is no gonadal attachment, but vulval morphogenesis proceeds normally. This indicates that the influence of the anchor cell is required before the vulval divisions begin, not only for the initiation of the appropriate lineage, but also for the cooperative assembly of the vulval cells into a normally shaped vulva.

These results cast doubt on the possibility of an integral "vulval program" that is 'switched on' in the vulval precursors by action of the anchor cell at an early point in vulva development. Instead, the anchor cell

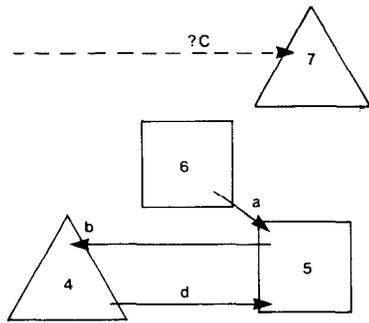


FIG. 16. Proposed cell-cell interactions in the ventral uterine-anchor cell group. The anchor cell pair are squares, and the nonanchor cell pair are triangles; cells are numbered as in Fig. 2. The *proposed* interactions among this group of cells are as follows: The anchor cell (SP6) inhibits its alternative precursor (SP5) from becoming the anchor cell (arrow a). The redundant anchor cell precursor (SP5) influences the fate of its sister (SP4; arrow b). This latter interaction suggests, by argument of symmetry, that the absence of the anchor cell from its alternative position may affect the fate of its sister (SP7; dashed arrow c). Cell insertion experiments, required to test this possibility, cannot yet be done. Finally, the polarity of the redundant anchor cell (SP5) is influenced by the presence of its sister (SP4; arrow d).

seems to function over a period of time to regulate multiple steps of the vulva's morphogenesis. Such action may rely on one signal over a period of time, or multiple signals that control successive steps of vulva development.

Group Behavior in the Gonad

Replacement regulation, in which one cell replaces an ablated cell by assuming its position and fate, is limited to discrete groups of cells in both the gonadal and nongonadal tissues. The constituent cells of such a group are probably equivalent in developmental potential, but assume different fates in the unoperated animal by interactions among the cells in the group. These groups have been called *equivalence groups* (Kimble *et al.*, 1979; Sulston and White, 1980). The equivalence of the cells in one group (the vulval equivalence group in the hermaphrodite ventral hypodermis) is documented by both laser ablation and mutational studies. All six cells in this group can be induced to follow the same vulval lineage by replacement regulation (Sulston and White, 1980) or the same hypodermal lineage by anchor cell ablation. Furthermore, all six cells follow the same vulval lineage in certain mutants with a *Multivulva* phenotype or the same hypodermal lineage in other mutants with a *Vulvaless* phenotype (Sulston and Horvitz, 1981). It seems likely that the cells in other groups, delineated solely by replacement regulation, are also equivalent in a more general sense by analogy with the vulval equivalence group.

Two such equivalence groups have been identified in the somatic gonad: the anchor cell pair in hermaphrodites and the linker cell pair in males. In hermaphrodites, one cell in the anchor cell equivalence group seems to influence, and be influenced by, a cell outside that group (Fig. 16). Thus, unlike other equivalence groups, the anchor cell pair does not seem to be an autonomous unit of development. Instead, the four cells of the anchor and nonanchor cell pairs seem to behave as a group. Interactions among these cells influence their neighbors' fates in a fashion similar to cells in an equivalence group. Since the cluster of interacting cells in the developing uterus transgresses equivalence group boundaries, the equivalence of cells *per se* seems not to be a critical factor in determining the limits of which cells can interact. Alternatively, it is conceivable that these four cells represent an ancestral equivalence group of ventral uterine precursors which has evolved to induce a vulva by two of its four cells.

Regulation of Cell Polarity

A reversal in the polarity of a lineage probably reflects a reversal in the cell polarity of the precursor to that lineage. When the first division of a precursor is asymmetric, the reversal in polarity of its lineage is always foreshadowed by a reversal in polarity of that asymmetric division. This indicates that the switch has taken place in the precursor itself. It is not understood whether the cell's polarity is changed by an internal reorganization of the cell or by the cell simply turning around. Since polarity switches are seen most often when a cell is isolated from its normal cell-cell contacts, it seems likely that, in the unoperated animal, cell polarity is fixed by local cell-cell interactions.

In two cases, (Figs. 6 and 10), a cell reverses its polarity predictably after ablation of the appropriate cell in the gonad. In both cases, this results in a redistribution of the descendants of the regulating precursor and a change in symmetry of the structure made. Regulation of cell polarity also appears to be used during normal development. A comparison of the early lineages of Z1 and Z4 in the two sexes (Kimble and Hirsh, 1979) reveals several reversals in polarity in the male lineage compared to the hermaphrodite lineage. These reversals appear to be critical for generating gonads with different symmetries in the two sexes. Morphogenesis of both gonads depends on a special regulatory function, the *leader function*, that is carried out by cells preceding the developing gonadal arm as it elongates (distal tip cells in hermaphrodites and linker cell in males (Kimble and White, 1981)). Cells with leader function are produced by Z1.a and Z4.p in hermaphrodites, but by Z1.p or Z4.a in males. This suggests a polarity switch with respect to segregation of leader

function between hermaphrodites and males in the first division of Z1 and Z4. Such a switch separates the leader function away from the distal tip cell in males, both in position and in lineage. This separation is required for making the male gonad since the male leader cell occupies a proximal position and leads the developing gonad away from the distal end and the distal tip cells. Furthermore, the polarities of Z1.p and Z4.a in males are both reversed (though in different ways) with respect to their polarities in hermaphrodites (Fig. 17). In this way, Z1.p acquires the same polarity as Z4.a, which also seems essential for making the asymmetric male gonad. In the reversal of both Z1.p and Z4.a, the apparent segregation of developmental potential is uncoupled from the asymmetry of the division suggesting that the two are not intrinsically linked. Such uncoupling has not been observed in polarity reversals observed after laser ablation. A similar reversal in polarity has been observed in the male gonad lineage of *Panagrellus redivivus*, another nematode. In that animal, the polarity of Z1 is reversed in the male compared to the polarity of Z1 in the female. This has led to a similar conclusion concerning the effect of switching the polarity of a cell on the symmetry of the structure made (Sternberg and Horvitz, 1981).

Other polarity reversals observed in lineages during normal development have also been documented. Sulston and Horvitz (1977) attributed reversals in the polarity of lineages of terminal lateral hypodermal and ventral cord precursors to "end effects" possibly mediated by "positional influences." The results reported here suggest that the cellular basis of these "positional influences" might be that the interactions with neighbors experienced by cells at the end of a linear array of precursors are different from cells in the middle.

Another example in which the polarity of cells seems to be regulated during normal development is in the vas deferens of the male. This lineage fits into a stem cell pattern in which, at each division, one daughter divides only once and the other daughter serves as a stem cell. However, the polarity of the backbone of this stem cell pattern of divisions switches in the middle of the lineage (Kimble, 1981). This switch in polarity may be an evolutionary relic reflecting a mechanism of controlling gonadal reflection that is more primitive than reliance on a cell with a special leader function.

Lineage duplications, in which both daughters of a division follow the same lineage, are observed rarely in experiments which otherwise generate polarity reversals. In particular, the cell in the ventral uterus that undergoes vectorial regulation, duplicates half its lineage instead of reversing its polarity in experiments performed near the time that the vectorial regulation can no longer be effected (Fig. 6B; Table 2C). This sug-

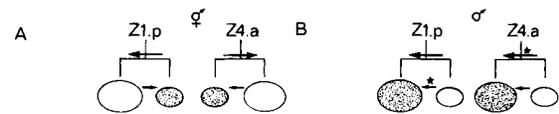


FIG. 17. Alterations in cell polarity during normal development. Z1.p and Z4.a divide asymmetrically in both sexes. The larger arrows point to the larger daughter of the division, and the smaller arrows point to the daughter that produces an anchor cell or linker cell (represented by stippling). The homology between the anchor cell and the linker cell was argued in Kimble and Hirsh (1979). (A) In hermaphrodites, the polarities of Z1.p and Z4.a are opposite and symmetrical. (B) In males, two reversals in polarity occur (arrows indicated by stars). Z4.a reverses the asymmetry of its division, and Z1.p switches the branch in which the linker cell is made.

gests that a duplication in lineage is mediated by the same cellular mechanism as a reversal in cell polarity. In this case, the lineage duplication may result from the fixation of an intermediate state in the cell as it responds to the environment and begins to reverse its polarity (by whatever mechanism).

A second example of lineage duplication was seen in an isolated sheath-spermathecal lineage (Fig. 12E) where polarity reversals have also been seen. The duplication was foreshadowed by a change in the division plane from anterior-posterior to left-right. Such transverse divisions are typical of duplications in the unoperated animal (Sulston and Horvitz, 1977) suggesting that the regulation of the plane of division is used during normal development to duplicate lineages. However, it should be noted that lineage duplications along the anterior-posterior axis also occur in the unoperated animal.

Generation of Complex Lineage Patterns from Simple Instructions

The lineages of several precursors follow simple patterns of cell division in both the nongonadal and the gonadal tissues (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979). For example, lateral hypodermal, vas deferens, and seminal vesicle precursors in the unoperated, wild-type animal follow a stem cell pattern (or parental reiteration in the terminology of Chalfie *et al.* (1981)). It seems plausible that other precursors are also instructed to follow simple patterns of division, but that these instructions can be modified to generate more complex lineage patterns. Kimble and Hirsh (1979) suggested, for example, that the spermathecal precursor in the sheath-spermathecal lineage seemed to embark on a stem cell pattern of division and then diverge from it. This idea of complex patterns deriving from simple patterns is supported by mutations that alter complex lineages to reiterative lineages (Chalfie *et al.*, 1981).

The patterns of division followed by the spermathecal precursor (when the normal neighbors of its grand-

mother, a sheath-spermathecal precursor, are eliminated) also support this idea. All, but one, of the variable patterns share a simple backbone of stem cell divisions (Fig. 18, solid lines). The exception undergoes a left-right division initially, and then each daughter follows this same asymmetrical pattern partially, as if a duplication had occurred. In one animal, the precursor follows a stem cell pattern precisely, and in the rest, extra divisions extend this pattern in a regular way (Fig. 18, dashed lines). Furthermore, in *Panagrellus redivivus*, another nematode, the lineage of the homologous precursor contains this pattern (solid lines, Fig. 18) precisely (Sternberg and Horvitz, 1981). Thus, the spermathecal precursor may have an intrinsic potential to follow a stem cell pattern which becomes modified by cell-cell interactions in the unoperated animal.

Autonomy of Cell Fate

In many deletion experiments in *C. elegans* (Sulston and Horvitz, 1977; Sulston and White, 1980; this paper), the fates of the remaining precursors are not altered by ablation of a neighboring cell. This suggests that the unaltered cells behave autonomously during development. Sulston and White (1980) have discussed various caveats to this conclusion which need not be repeated here.

Isolation of a cell within the animal is a more stringent test of its autonomy. This can be achieved by ablating all of a cell's normal neighbors (or precursors to those neighbors). In both gonadal and nongonadal

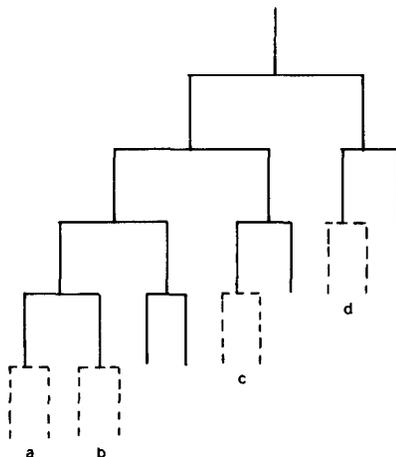


FIG. 18. Backbone of divisions (solid lines) proposed as a simple program of proliferation for the spermathecal precursor (a sublineage of the sheath-spermathecal precursor). The addition of some, or all, of certain divisions (dashed lines) to this backbone generates the lineage observed in the unoperated animal (Fig. 12A) as well as the lineages seen after isolation of the sheath-spermathecal precursor (Figs. 12B-E).

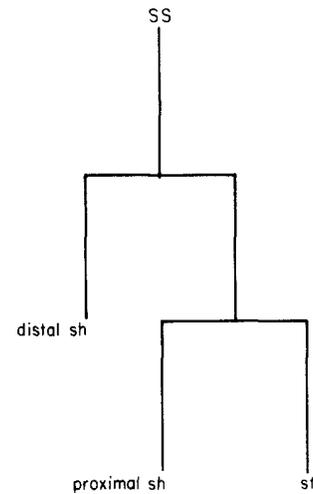


FIG. 19. Generation of distal sheath, proximal sheath, and spermathecal precursors by the sheath-spermathecal lineage. This pattern is invariant in all lineages so far observed in *C. elegans* (see Fig. 12), and has also been observed in *Panagrellus redivivus* (Sternberg and Horvitz, 1981). Since it has not been altered by isolation of the precursor, it may be determined by intrinsic factors in the precursor.

tissues, such isolation experiments do not alter the basic developmental potential of the isolated cell. Isolation of an anchor cell precursor, for example, channels the cell into the anchor cell fate, but does not uncover any new developmental potential for the precursor. Similarly, the isolation of precursors that subsequently divide does not change the types of cells made by the precursor, or the lineal positions in which particular cell types arise. Thus, the sheath-spermathecal precursor generates precursors to the distal sheath, proximal sheath, and spermatheca as normal (Fig. 19). The precursors in *P. redivivus* that are homologous to the sheath-spermathecal precursors of *C. elegans* follow this same division pattern (Sternberg and Horvitz, 1981). The invariance with which these precursors are generated both in isolation experiments and in a nematode of a different taxonomic family suggests that this sublineage may be an intrinsic property of the precursor cell itself.

Conclusions

In conclusion, the complex cell lineages observed in the unoperated animal appear to result from the integration of several types of information. Both the potential to produce descendants of a particular type (e.g., spermatheca or vas deferens cells) and the basic patterns of division followed (e.g., repeated equal or asymmetric divisions) have proven difficult to alter by disturbing a precursor's normal environment. This suggests that these parameters of a cell's fate are controlled by factors intrinsic to the cell.

In a few cases, cells have been observed to switch their fate to replace a deleted neighbor. Such replacements identify groups of interacting cells that are equivalent in developmental potential. This establishes that cell fate can be influenced, within a limited number of choices, by extrinsic information. In addition, the polarity of divisions and the number of cells produced in a given lineage appear to be influenced by controls extrinsic to the cell.

It seems intriguing that the alterations in lineage induced by perturbing the external environment of a cell are essentially identical to the types of lineage differences observed by comparing the lineages of different nematode species (Sternberg and Horvitz, 1981). Such a similarity suggests that those aspects of cell lineage that are influenced by extrinsic cues may be modifiers of a more fundamental and intractable set of instructions that are common to many nematodes.

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