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# On the Control of Germ Cell Development in *Caenorhabditis elegans*

J. E. KIMBLE AND J. G. WHITE

*MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, England*

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After hatching, the germ line progenitor cells in *C. elegans* begin to divide mitotically; later, some of the germ line cells enter meiosis and differentiate into gametes. In the adult, mitotic germ cells, or stem cells, are found at one end (the distal end) and meiotic cells occupy the rest of the elongate gonad. Removal of two somatic gonadal cells, the distal tip cells, by laser microsurgery has a dramatic effect on germ cell development. In either sex, this operation leads to the arrest of mitosis and the initiation of meiosis in germ cells. The function of the distal tip cell in the intact animal appears to be the inhibition of meiosis (or stimulation of mitosis) in nearby germ cells. During development, this permits growth and, in the adult, it maintains the germ line stem cell population. A change in the position of the distal tip cell in the gonad at an early point in development is correlated with a change in the axial polarity of the germ line tissue. This suggests that the localization of the distal tip cell's inhibitory activity at the distal end of the gonad establishes the axial polarity of the germ line tissue in the intact animal.

## INTRODUCTION

Many fundamental problems of germ cell development are not understood. What controls the onset of meiosis during development? How is the germ line stem cell population maintained in adults? And why do germ cells differentiate in one region of the gonad rather than another? In this paper, we report experiments performed in the small nematode *Caenorhabditis elegans* that bear on these questions. *C. elegans* is particularly advantageous for these studies, because of the simplicity of its gonadal anatomy (Hirsh *et al.*, 1976; Klass *et al.*, 1976) and the accessibility of its gonadal development to experimental intervention.

Nematode development is generally considered to proceed by invariant cell lineages (e.g., Boveri, 1899; Wilson, 1925). Recently, descriptions of the cell lineages of *C. elegans* have confirmed this principle of invariance for embryonic development (Deppe *et al.*, 1978) and for postembryonic development of the somatic tissues (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979). Laser microsurgery, in which individual cells are destroyed with no apparent damage to their neighbors, has been used to study the influence of cell-cell interaction on cell fate in the postembryonic lineages of *C. elegans* (Sulston and Horvitz, 1977; Kimble *et al.*, 1979; Sulston and White, 1980). These experiments show that, in a limited number of cases, cell-cell interaction plays a significant role in the development of the somatic cells.

In contrast to the development of somatic tissues in *C. elegans*, the germ cells follow a division pattern that is variable from animal to animal (Kimble and Hirsh, 1979). In this paper we use the technique of laser ablation to elucidate interactions between somatic cells and germ cells in *C. elegans*. Two somatic cells in the gonad are shown to play a crucial role in the control of proliferation of germ cells, in their entry into meiosis, and in the establishment and maintenance of their spatial organization.

## MATERIALS AND METHODS

The maintenance of *C. elegans*, var. Bristol, has been 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 anesthetized 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× on a Zeiss Universal microscope equipped with Nomarski optics, and was centered at a point previously aligned with an auxiliary He/Ne gas laser. Then, pulses from a 250-mJ coumarin 2 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 had recovered, or if neighboring cells appeared damaged, the animal was discarded. If the ablation had been successful (Figs. 3, 11), the effect of the ablation was followed by observation of the living animal with



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Normarski optics at the appropriate times after the operation.

*Feulgen staining.* Feulgen staining of individual worms was achieved by placing single animals on a collagen-coated slide in a small drop of 10% ovalbumen. Excess ovalbumen was recovered until only a meniscus of the solution remained around the worm. The slide was then put into Carnoy's fixative (Pearse, 1968), and, after an overnight fixation, was transferred to 70% ethanol for 1-14 days. Staining was performed as described by Sulston and Horvitz (1977). The stained specimens were examined using bright-field illumination through a green filter.

*Determination of developmental age at which pachytene figures are first seen.* Animals were fixed at defined stages of development and were Feulgen stained to examine the chromosomal morphology of germ cell nuclei. The appearance of pachytene nuclei was used as an unambiguous sign of entry into meiosis. Before fixation, the animals were observed with Nomarski optics for landmark developmental events. According to time measurements made by Sulston and Horvitz (1977) and Sulston (personal communication), these events provide standards for the developmental age of the animal in hours (20°C) as follows: 25 hr, newly molted L3 (⚥ and ♂); 29 hr, first division of P5.p, P6.p, and P7.p (⚥) or P10.p and P11.p (♂); 33-34 hr, L3 lethargus (⚥ and ♂); 35 hr, newly molted L4 (⚥ and ♂); 36-37 hr, vulva invaginating (⚥); 37-39 hr, tail shrinking (♂); 38-40 hr, vulval orifice expanding (⚥); 40 hr, vulval "teeth" formed (⚥); 41-43 hr, vulval orifice open (⚥); 44-45 hr, L4 lethargus (⚥ and ♂). Most of the animals scored in these studies were maintained at 20°C. However, some of the experimental animals were placed at 15 or 10°C overnight to slow their development. Therefore, similar temperature shifts were performed on unoperated animals. We found that the first appearance of pachytene nuclei coincided with the same landmarks under all temperature conditions tested so we have used these landmarks as a biological time scale.

## RESULTS

### *Background Information*

Figure 1 summarizes the postembryonic development and the adult anatomy of hermaphrodite and male gonads with special emphasis on the development of germ cells.[1] The following brief account is based on Hirsh *et al.* (1976), Klass *et al.* (1976), and Kimble and Hirsh (1979) unless noted otherwise.

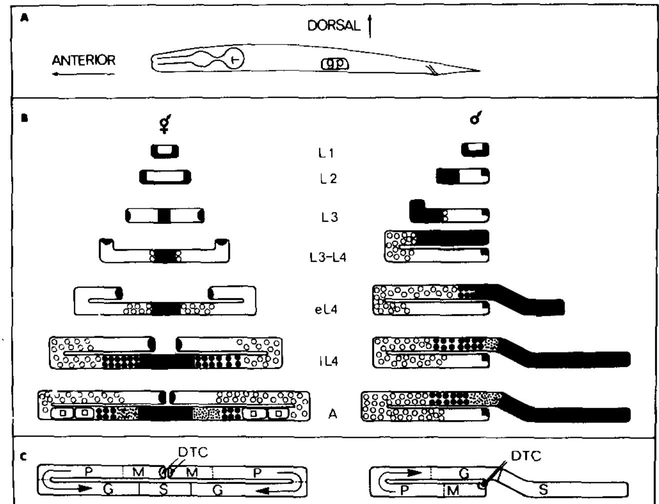


FIG. 1. Gonadogenesis and adult anatomy in hermaphrodites (left) and males (right). (A) The midventral position of the gonadal primordium (gp) is the same in both sexes. The anterior-posterior and dorsal-ventral coordinates indicated here are used for all the diagrams in the paper. (B) Morphology of the gonad and distribution of cell types within the gonad at consecutive stages of postembryonic development. L1, L2, and L3, first, second, and third larval stages; eL4 and lL4, early and late L4; A, adult. Somatic tissue is black; germ line tissue is clear. Mitotic regions of germ line tissue are left blank. ○, Pachytene nuclei; ●, primary spermatocyte nuclei; ⁘, sperm; □, oocyte nuclei. (C) Spatial organization of adult gonad. The mitotic (M), pachytene (P), and gamete-forming (G) regions are demarcated by dashed lines. S, somatic tissue; DTC, distal tip cells. Arrows indicate the polarity of maturation of the germ line tissue, and point proximally.

*Adult anatomy (Fig. 1C).* The hermaphrodite gonad consists of two equivalent reflexed tubes and the male gonad consists of a single reflexed tube. Each tube displays a distal-proximal axis defined by the maturation of germ cells. At the distal or immature end, the germ cells are mitotic and serve as stem cells. More proximally, the nuclei enter meiosis and remain in pachytene. At the proximal or mature end, gamete formation occurs. Within this region, an ordered progression of maturation, both in stages of meiotic prophase and gametogenesis, is arranged from distal to proximal. In males, this gradient culminates proximally in the meiotic divisions which generate mature sperm. In hermaphrodites, first, the most proximal gametes of each tube become sperm, and then, more distal gametes become oocytes as they move into the gamete-forming region.

The bulk of the somatic tissue of the gonad is found proximal to the germ line tissue. In males, the germ cells are encapsulated by a basal lamina, but are not ensheathed by somatic cells (except most proximally). In hermaphrodites, the germ cells are encapsulated by

[1] The term *germ cells* is used here in a general sense to refer to descendants of the two germ line progenitor cells, Z2 and Z3. In fact, most of the germ line tissue is syncytial (Hirsh *et al.*, 1976). However, each germ line nucleus occupies its own membrane-bound alcove of cytoplasm which is located at the edge of a common anuclear cytoplasm. Each germ line nucleus and its cytoplasm is called a germ "cell" in this paper.

a basal lamina and they are ensheathed almost completely by a single layer of sheath cells. At the distal end of each tube, one (hermaphrodites) or two (males) somatic cells are found. These cells are the *distal tip cells.*

*Postembryonic development (Fig. 1B).* The worm hatches with a four-celled gonadal primordium consisting of two somatic progenitor cells (Z1 and Z4) and two germ line progenitor cells (Z2 and Z3) in both sexes. In the hermaphrodite, growth of this primordium occurs in two directions to generate a symmetrical structure consisting of two reflexed tubes (Fig. 1B, left column). In the male, growth occurs in one direction to generate a single reflexed tube (Fig. 1B, right column). In hermaphrodites, the two elongating tips become the distal (or immature) ends of the adult half gonads, whereas in males, the elongating tip becomes the proximal (or mature) end. Thus, the distal-proximal axes of the hermaphrodite and male gonads are opposite to each other with respect to the elongation of the developing gonads.

The number of germ cells increases during larval growth from 2 to about 1000 in hermaphrodites and to about 500 in males (Kimble, unpublished observations). This increase follows a simple exponential curve (Delavault, 1959). During the first and second larval stages (L1 and L2) the germ cells are arranged evenly throughout the developing gonad in both sexes. This is also true of males in later larval stages. In hermaphrodites, however, the germ cells become physically separated around the time of the L2-L3 molt by a rearrangement of somatic gonadal cells. This establishes two separate populations of germ cells—one occupying the anterior- and one the posterior-half gonad.

*The distal tip cells.* In both hermaphrodites and males, two distal tip cells arise in homologous positions of the lineage of Z1 and Z4 (Fig. 2). These cells occupy a position at the distal end of the gonad throughout gonadogenesis. In hermaphrodites, the anterior or posterior distal tip cell (Z1.aa and Z4.pp, respectively, Fig. 2A) each precedes the elongating tip of its respective half of the gonad. In males, both distal tip cells (Z1.a and Z4.p, Fig. 2B) reside at the stationary end of the gonad. The elongating tip is led progressively away from the distal end by a different somatic cell, the *linker cell* (Z1.paa or Z4.aaa, Fig. 2B).

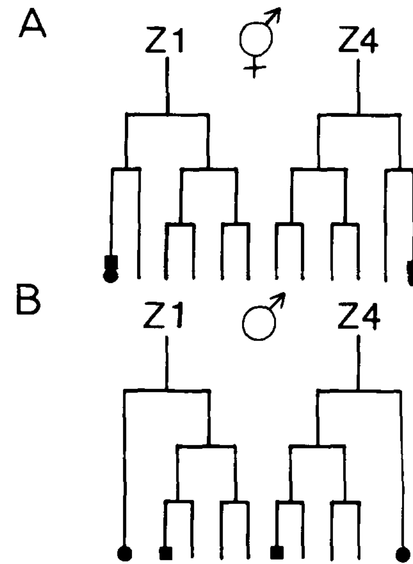


FIG. 2. Ancestry of distal tip cells (●) and cells with leader potential (■) in hermaphrodites (above) and males (below). Each vertical line represents a cell, and each horizontal line represents a cell division. The left-hand branch indicates the anterior and the right-hand branch the posterior daughtor at each division. Daughters are named by adding "a" if it is the anterior, or "p" if the posterior daughter, to the name of the mother cell. Thus, the anterior daughter of Z1 is Z1.a, and the posterior daughter of Z1.a is Z1.ap. In hermaphrodites, both distal tip cells possess leader function. In males, this function is allocated to either Z1.paa or Z4.aaa.

## *Effects of Laser Ablation of the Distal Tip Cells on Germ Cell Development*

Laser ablation of the distal tip cells in either sex (Fig. 3) leads to an arrest of mitosis and an initiation of meiosis in all germ cells. In males, both distal tip cells must be destroyed to obtain this effect. In hermaphrodites, if one distal tip cell is ablated, only germ cells in the half gonad of the killed distal tip cell are affected. Ablation of the immediate precursors of the distal tip cells in hermaphrodites (Z1.a and Z4.p, Fig. 2) mimics the effect of killing the distal tip cells. However, if both somatic gonadal progenitor cells are killed in either sex, the germ cells do not divide mitotically and do not enter meiosis. Indeed, if the precursors to Z1 and Z4 are killed in the egg, the two germ cells die during L1.

The major consequences of killing the distal tip cells in hermaphrodites and males are shown schematically in Fig. 4, and are described below.

*Effect on mitotic-meiotic state of germ cells.* In intact animals, some germ cells always remain in mitosis. However, the ablation of both distal tip cells at any time during gonadogenesis in either sex leads to the entry of all descendants of Z2 and Z3 into meiosis. Within 24 hr of the ablation, only pachytene nuclei are observed in regions of the gonad that normally harbor only nonmeiotic nuclei (Fig. 5).

*Effect on proliferation of germ cells.* In unoperated animals the proliferation of germ cells begins shortly after hatching, and the number of germ cells increases exponentially during larval development. Ablation of the distal tip cells during L1, L2, or L3 results in a decrease in the number of germ cells present in the young adult animal by an order of magnitude compared

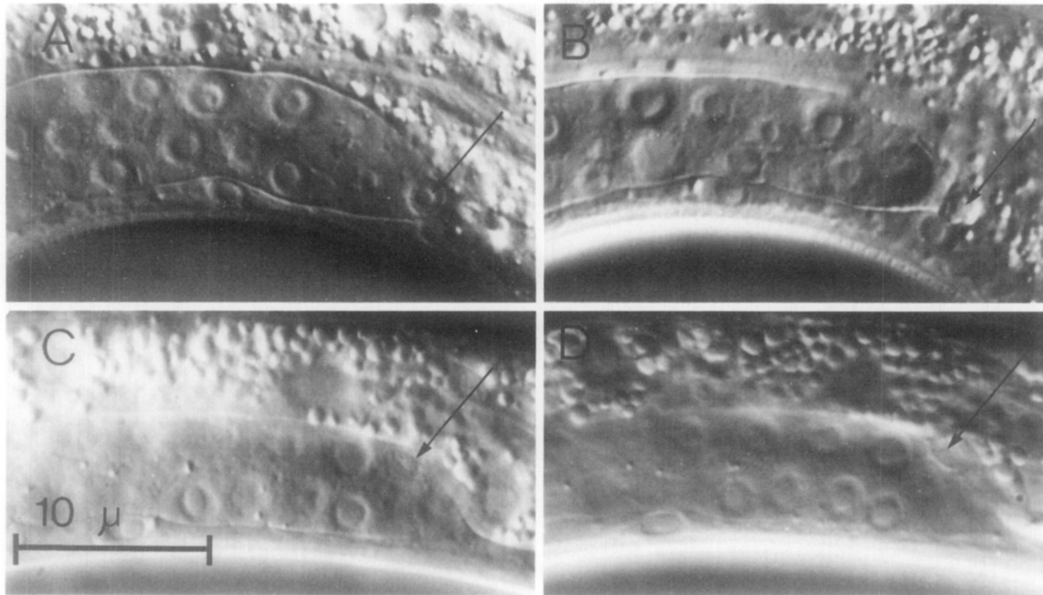


FIG. 3. Nomarski micrographs of distal tip cells (arrows). A posterior distal tip cell is shown in an L3 hermaphrodite before (A) and after (B) ablation. A posterior distal tip cell is shown in an L3 male before (C) and after (D) ablation.

to controls in both hermaphrodites (Fig. 6) and in males (data not shown).

After the distal tip cells are killed, the germ cells continue dividing for a certain period. If the operation is performed as soon as possible during L1 (ablation of Z1.a and Z4.p soon after their birth), germ cells typically undergo two to four rounds of division in hermaphrodites (fig. 7) and two to three rounds of division in males (data not shown). If it is performed during L2 or L3, one round of division is typical. Rounds of division were calculated from the number of germ cells at the time of the operation and the number of germ cells after divisions were finished. Continuous observation

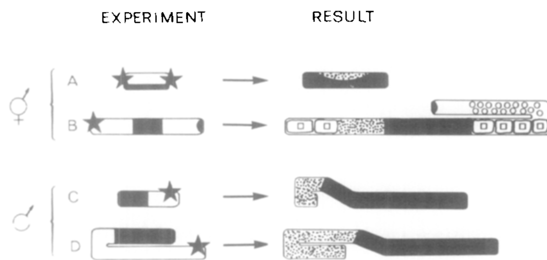


FIG. 4. Effects of laser ablation (depicted by stars) of distal tip cells on germ cell development. Representation of different cell types is the same as in Fig. 1B. In hermaphrodites, if both distal tip cells are ablated (A) development of both anterior- and posterior-half gonads is blocked. If one is ablated (B), the development of that half is blocked. If ablation is early (A), few germ cells are made, and they all become sperm. If ablation is later (B), both sperm and oocytes are made in their normal relative positions. In males, if both distal tip cells are ablated earlier (C) or later (D), all germ cells become sperm. The normal shape of the gonad is maintained. The anterior–posterior and dorsal-ventral coordinates are the same as shown in Fig. 1.

of the rounds of division in a few animals showed that the majority of germ cells divided a few times rather than that a few cells divided many times to account for the observed increase in germ cell number.

*Effect on gamete differentiation.* In the male, all germ cells give rise to sperm after ablation of the distal tip cells (Fig. 8). Although proximal cells enter spermatogenesis and make mature sperm before distal cells, spermatogenesis occurs in all regions of the germ line tissue.

In the hermaphrodite, germ cells give rise to either sperm only or to both sperm and oocytes, depending on when the distal tip cells are killed. If the operation is performed during L1, L2, or early in L3, all germ cells differentiate as sperm (Fig. 9A). (No germ cell nuclei, other than sperm, were visible after completion of spermatogenesis, and no cell death was observed during postoperative development. Also, examination of Feulgen-stained preparations of these animals revealed no nonsperm germ cell nuclei in the young adult.) If the operation is performed later, the normal complement of sperm is made proximally (about 150/half gonad from about 37 spermatocytes), and oocytes are made by the germ cells remaining distally (Figs. 9B, 10). However, oocyte differentiation does not occur in the distal region of the gonad. Instead, cells in the distal arm gradually enter the normal region of oocyte differentiation and become oocytes. In contrast to spermatogenesis in males, then, oogenesis in hermaphrodites is confined to a particular region of the gonad.

*Effect on shape of the gonad.* In hermaphrodites, elimination of a distal tip cell blocks elongation of the

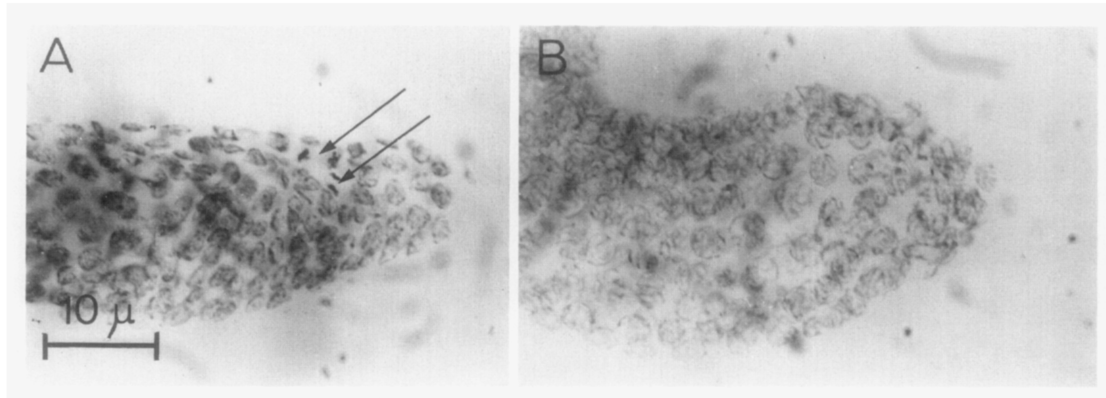


FIG. 5. Chromosome morphology of nuclei before and after distal tip cell ablation observed in Feulgen-stained preparations of dissected gonads. (A) Nuclei in the distal end of an unoperated hermaphrodite gonad are not meiotic. Most are in mitotic interphase and two are dividing (arrows). (B) Nuclei in the distal end of a hermaphrodite gonad about 24 hr after killing its distal tip cell are all in pachytene. No mitotic figures are seen.

half gonad associated with the killed cell at whatever point has been reached at the time of the operation (Figs. 4A and B, 9A and B). In contrast, distal tip cell ablation at any time in males does not stop formation of the reflexed shape of the gonad (Figs. 4C and D, 8). In males, elimination of the linker cell (which precedes the elongating gonad in males as the distal tip cells do in hermaphrodites) stops generation of the reflexed gonad. However, linker cell ablation does not affect the proliferative capacity or the differentiation of the germ cells.

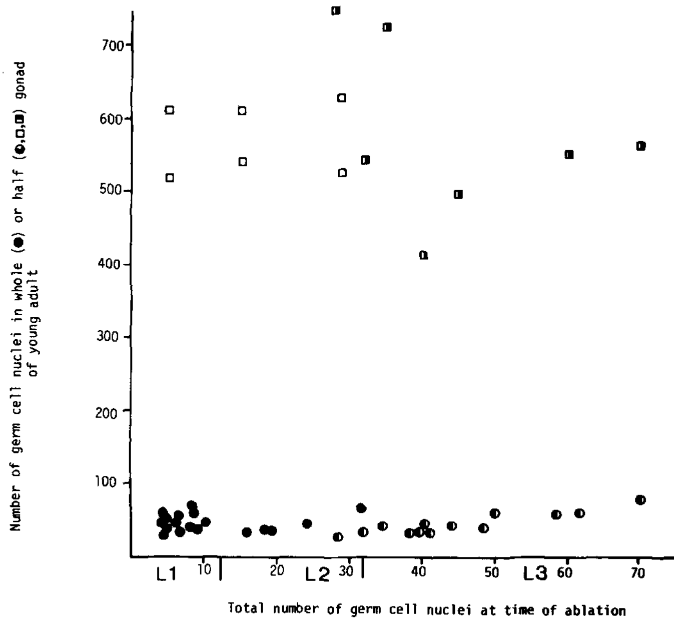


FIG. 6. Effect of laser ablation of hermaphrodite distal tip cells on number of germ cell nuclei produced. The number of germ cells present at time of ablation (abscissa) was counted using Nomarski optics. The number of germ cells present in the young adult (ordinate) was determined by counting nuclei in individually Feulgen-stained animals fixed within 5 hr after their final molt. The latter number was obtained by adding one-fourth of the number of mature sperm to the number of meiotic cells that had not undergone meiotic divisions. (In *C. elegans*, each primary spermatocyte makes four sperm). Experiments, ●, both distal tip cells ablated and total number of germ cells scored; ◐, one distal tip cell ablated and number of germ cells in experimental half gonad scored. Controls: ◨, one distal tip cell ablated and number of germ cells in the nonexperimental half scored; □, several nongonadal cells ablated. The correlation of larval stages with number of germ cells is approximate.

*Controls.* Cells in the region adjacent to the distal tip cells were killed with the laser to study the possibility that secondary damage might cause the effects attributed to the distal tip cell ablation:

(1) All progeny of Z1 and Z4, except the distal tip cells, were deleted by killing cells during L1 as early as possible in both hermaphrodites and males. Although the only somatic cells in these gonads were the two distal tip cells, the proliferation of germ cells continued, and the organization of the germ cells was essentially normal in these gonads (if the distal tip cells assumed their normal positions).

(2) Three nongonadal cells or nuclei (a ventral hypodermal cell, a lateral hypodermal nucleus, and a muscle cell), all positioned as close as possible to a distal tip cell, were destroyed in single animals. These ablations were performed in two hermaphrodites and two males at each of three stages of gonadogenesis (L1, L2, and L3). All produced gonads of normal size and organization. Three of the hermaphrodites were fixed as young adults and the number of germ cell nuclei was counted (Fig. 6).

(3) One or more germ cells located in the distal region of the gonad were killed during L1, L2, or L3. The remaining germ cells continued to divide as normal, and the regional organization of the gonad was not affected.

### *Time of Onset of Meiosis during Development*

Results in the previous section demonstrate that cells that normally are mitotic enter meiosis after ablation

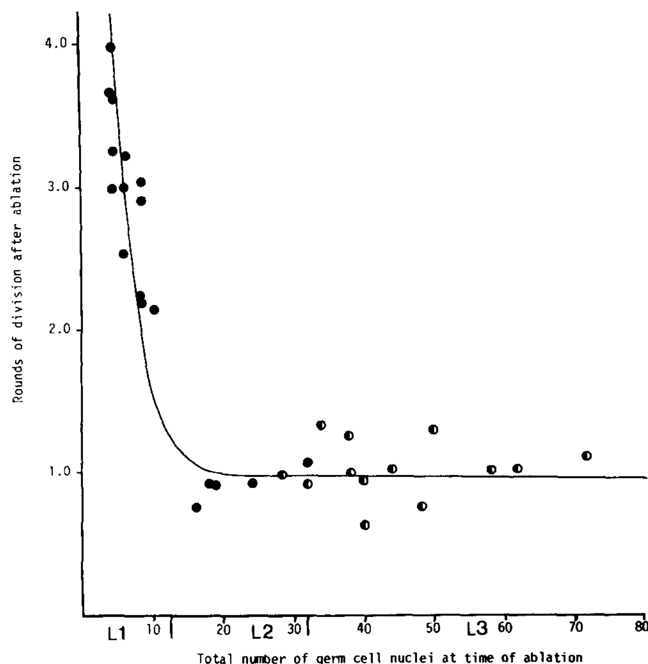


FIG. 7. Effect of laser ablation of distal tip cells on number of mitotic divisions following ablation. The number of germ cells present at time of ablation (abscissa) was counted using Nomarski optics. The number of rounds of division of germ cells after ablation was calculated by solving for $n$ in the following equation: $2^n = y/x$, where $y$ is the final number of germ cells made and $x$ is the number of germ cells made at the time of ablation. The shape and position of the curve were estimated by eye. ●, Both distal tip cells ablated; ◐, one distal tip cell ablated.

of the distal tip cells. It seemed plausible, therefore, that the onset of meiosis during development might be controlled by the release of germ cells from the influence of the distal tip cells. To test this hypothesis, the time at which meiosis begins during development was investigated in unoperated animals and in two experimental situations. In these studies, the appearance of pachytene nuclei was used to indicate entry into meiosis. Therefore, the time at which the germ cells leave the mitotic cell cycle to enter meiosis must be some hours before the times given in Table 1.

*Intact animals.* Pachytene figures are first seen at L3-L4 lethargus (33-34 hr, 20°C) in unoperated hermaphrodites (Table 1A) and in the middle of L3 (29-32 hr, 20°C) in unoperated males (Table 1B).

*Attempt to induce meiosis earlier than normal.* Since distal tip cells seem to prevent the onset of meiosis in adjacent germ cells, we thought that if the distal tip cells were killed early in development, the onset of meiosis might occur precociously. Therefore, Z1.a and Z4.p were killed shortly after their birth (about 24 hr, before the first appearance of pachytene nuclei in intact animals). However, no significant change in the time of first appearance of pachytene figures in hermaphrodites (Table 1C) or males (data not shown) was observed after such ablation. This finding is consistent with the observation mentioned earlier that mitoses continue for several rounds after early ablation of the distal tip cells (Fig. 7).

*Attempts to delay the onset of meiosis.* The first cells that enter meiosis in the intact animal are the ones furthest away from the distal tip cells. We thought that the onset of meiosis might be retarded if the distance between the most proximal germ cells and the distal tip cells was decreased. If one of the germ line progenitor cells (Z2 or Z3) is ablated in the newly hatched worm, the developing gonad possesses significantly fewer germ cells than normal throughout gonadogen-

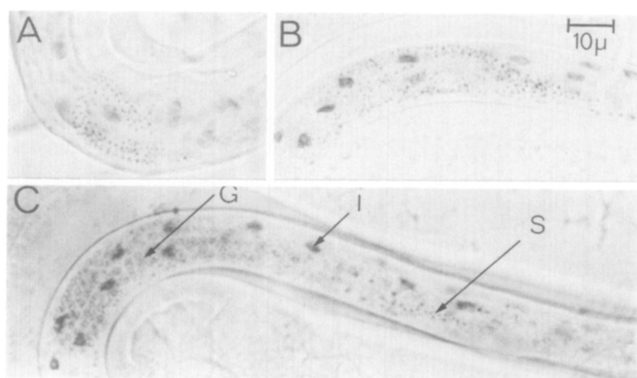


FIG. 8. Gamete differentiation in male gonads after distal tip cell ablation as seen in Feulgen-stained preparations of late L4 animals. (A) Gonad contains a small number of sperm (small dots of Feulgen-positive material) after ablation in L1. (B) Gonad contains a larger number of sperm after ablation in L3. (C) Unoperated animal. Sperm are made by all germ cells in (A) and (B), and are found only in a proximal position in (C). S, sperm; I, intestinal nucleus; (G), germ cells not differentiated into gametes.

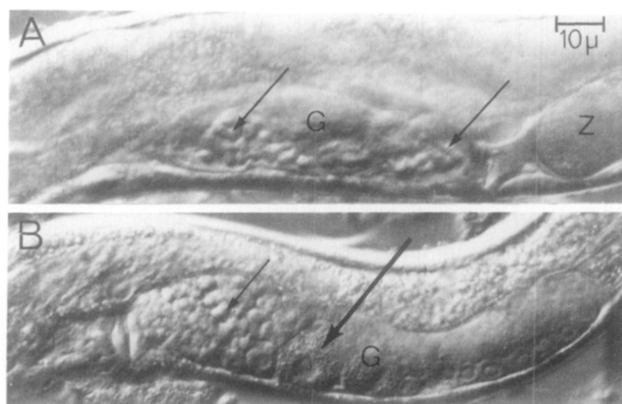


FIG. 9. Gamete differentiation in hermaphrodite gonads after ablation of a single distal tip cell as seen with Nomarski in young adult animals. (A) Half gonad (G) contains sperm only (arrows) after ablation of one distal tip cell during L2-L3 lethargus. A zygote (Z) is seen in the spermatheca of the unoperated half-gonad. (B) Half gonad (G) contains sperm (small arrow) and oocytes (large arrow) after ablation during L3.

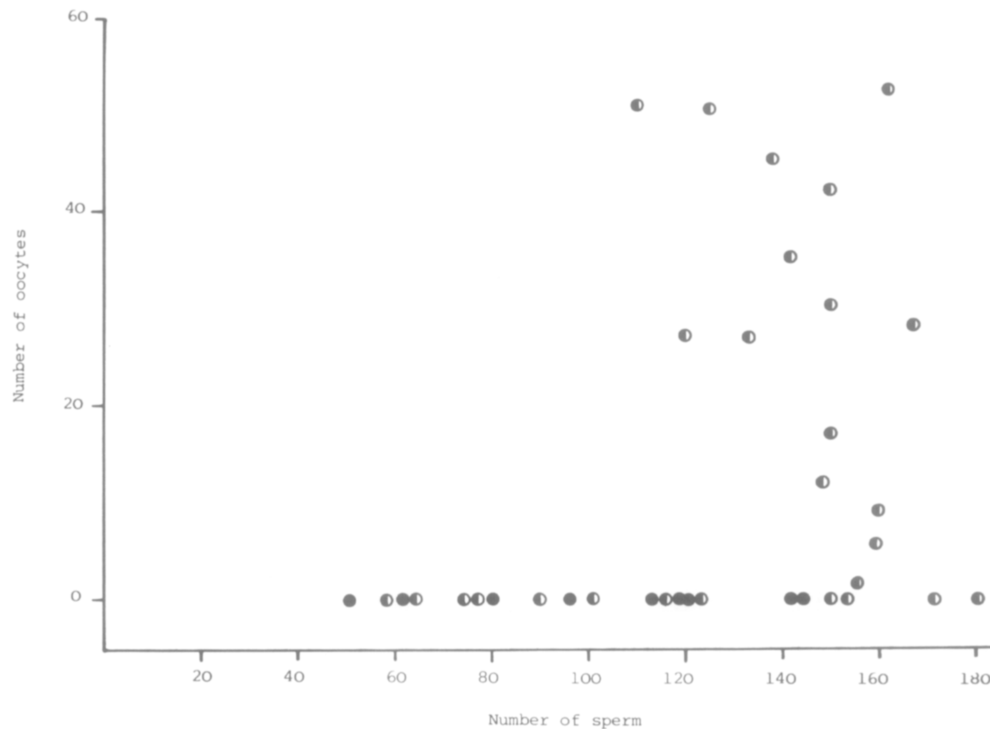


FIG. 10. Number of sperm and oocytes made in the half gonads of individual ablation experiments. In the intact animal, each half gonad makes about 140–160 sperm. In distal tip cell-ablated animals, each half gonad makes the normal complement of sperm before any oocytes are produced. ●, both distal tip cells ablated and half the number of sperm scored. No oocytes were made in these gonads because the operation was performed at a relatively early time of development. ◐, one distal tip cell ablated and the number of sperm and oocytes made in the experimental half gonad scored.

esis, and consequently is smaller than normal (Fig. 11). Z3 was therefore ablated in a number of hermaphrodites. This operation caused a delay in the appearance of pachytene figures of about 5 hr (Table 1D). The first appearance of mature sperm was also delayed by about 5 hr in such animals (data not shown). Similar results were obtained after ablation of Z2 in hermaphrodites (data not shown). Both oocytes and sperm were produced in gonads after ablation of either Z2 or Z3.

Two controls for the Z3 ablation experiment show that the observed delay in meiosis is not due to damage to the germ cells. First, ablation of Z3 was followed by ablation of both distal tip cells later in L1 and fixation in the newly molted L4 (35 hr, 20°C) in three animals. All three animals exhibited pachytene figures after Feulgen staining, and thus, did not show the delay typical of animals in which only Z3 was killed. Second, in one animal, all the descendants of Z2 were squeezed by chance into the anterior half of the gonad so that the normal size of the half gonad was maintained despite killing Z3. This animal was also fixed at 35 hr and Feulgen stained. Again, pachytene figures were present. Thus, the delay does not occur if the distal tip cells are not present (first control) or if the size of the gonad is corrected by redistribution of all descendants of Z2 into one-half (second control).

### *Control of Axial Polarity by Distal Tip Cells*

The normal regional organization of germ cells is lost after ablation of distal tip cells; all germ cells enter meiosis and eventually differentiate. It seemed plausible, therefore, that the organization of the germ cells is established by the distal tip cells. This hypothesis was tested by studying the effect of a change in distal tip cell position on germ cell organization.

The ability to manipulate distal tip cell position depends on a natural positional change of distal tip cells in males (Fig. 12). During male gonadogenesis, the anterior distal tip cell arises at the anterior edge of the gonad, and then moves posteriorly to join the posterior distal tip cell. When the sisters of the distal tip cells (Z1.p and Z4.a, Fig. 2B) are ablated, a change in distal tip cell position often results. This change may occur because the anterior migration of Z1.p and Z4.a normally displaces the anterior distal tip cell from the anterior tip, or because the direction of germ cell growth is no longer controlled by the linker cell (which is produced by Z1.p or Z4.a).

Successful ablation of Z1.p and Z4.a was obtained in six animals (Fig. 12). Camera lucida drawings of the gonads in these six (Figs. 12A–F) show that the axial polarity of the germ line tissue is directly related to the

TABLE 1
DEVELOPMENTAL AGE OF FIRST APPEARANCE OF PACHYTENE NUCLEI

| Experiment | Age at fixation[a] (hr, 20°C) | Total number of animals scored | Number of animals with pachytene nuclei[b] | First appearance of pachytene nuclei (hr, 20°C) |
|---|---|---|---|---|
| A. Intact | 29–32 | 6 | 0 | |
| hermaphrodite | 33–34 | 7 | 4 | 33–34 |
| | 35–37 | 6 | 6 | |
| | 38–40 | 4 | 4 | |
| B. Intact male | 26–28 | 5 | 0 | |
| | 29–32 | 12 | 10 | 29–32 |
| | 33–35 | 8 | 8 | |
| | 36–38 | 6 | 6 | |
| C. Both distal tip cells | 25–28 | 3 | 0 | |
| killed in L1 | 29–32 | 5 | 0 | 33–34 |
| hermaphrodite | 33–34 | 5 | 5 | |
| | 35–37 | 5 | 5 | |
| D. Z3 killed | 35–37 | 4 | 0 | |
| in L1 | 38–39 | 5 | 1 | 38–39 |
| hermaphrodite | 40–43 | 7 | 7 | |
| | 44–45 | 5 | 5 | |

[a] Age at fixation was determined by scoring each animal with Nomarski for standard stages as described under Materials and Methods.
[b] Animals were scored positively for pachytene nuclei if one or more pachytene nuclei were present in one or both half gonads.

position of the distal tip cell during development. The position of the distal tip cells was visible until L3 in all animals, but was obscured in L4 and adult stages by the increase in number of germ cells. In two animals (Figs. 12A and B), both distal tip cells became located, as normal, at the posterior edge of the germ cell mass and no change in polarity was observed. In two animals (Figs. 12C and D), both distal tip cells became located

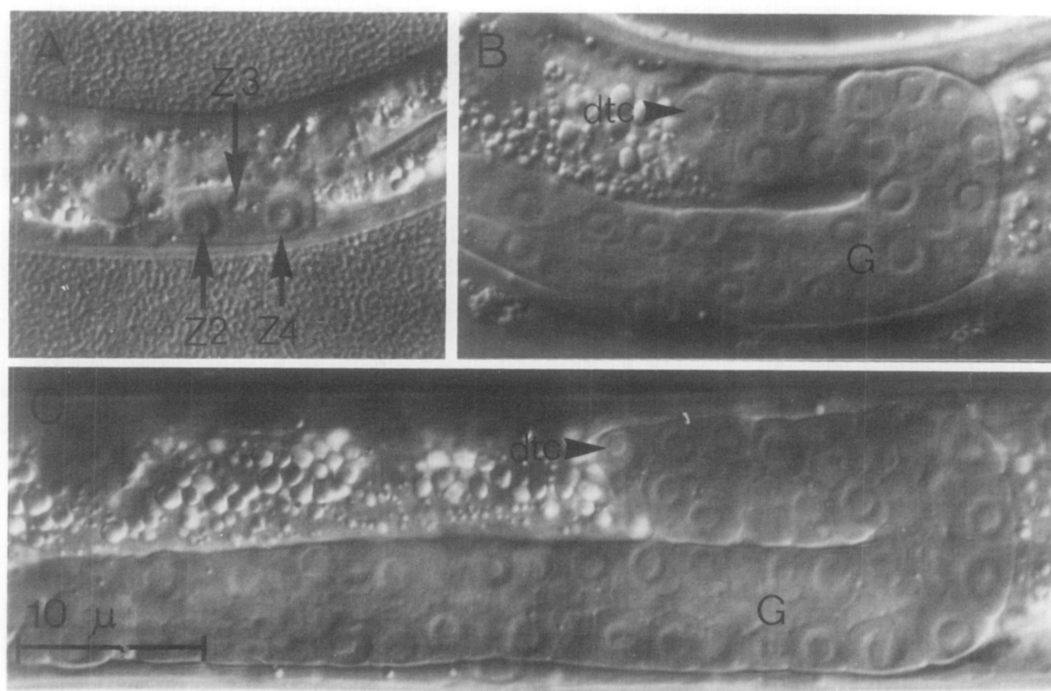


FIG. 11. Nomarski micrographs showing (A) ablation of Z3 in the gonadal primordium, (B) size of a half gonad (G) in a young L4 worm after ablation of Z3, and (C) size of a half gonad (G) in an unoperated animal of the same age as (B). All pictures are at the same magnification. dtc, distal tip cell.

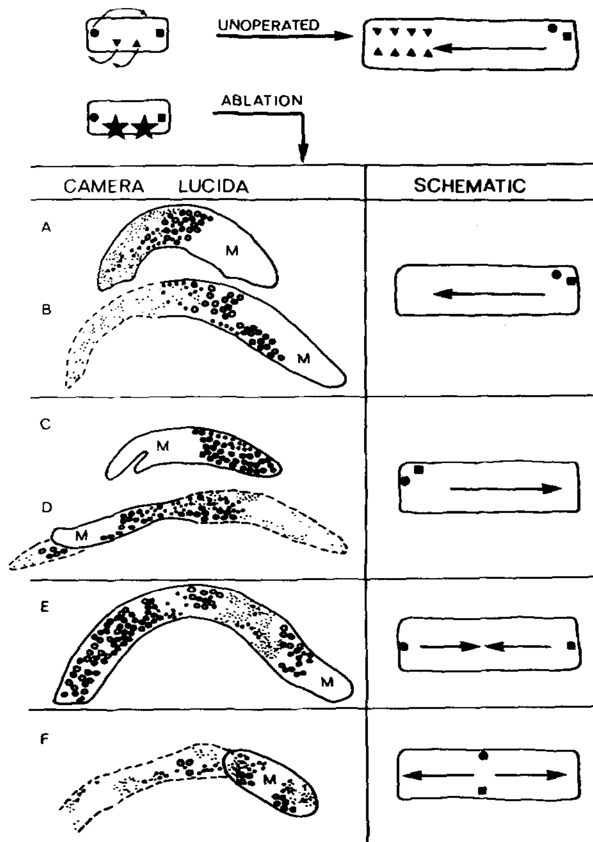


FIG. 12. Change in distal tip cell position corresponds to a change in polarity in the gonad. The anterior-posterior and dorsal-ventral coordinates are the same as shown in Fig. 1. In the unoperated male gonad (top diagram) somatic gonadal cells, Z1.a, ●, Z1.p, ▼; Z4.a, ▲; and Z4.p, ■ undergo rearrangement so that Z1.a and Z4.p, the distal tip cells, become located posteriorly. Z1.p and Z4.a divide further at the anterior end, and the axial polarity from distal to proximal is oriented away from the distal tip cells, in the simplified, unreflexed gonad diagrammed at top right. If Z1.p and Z4.a are ablated (stars), the positions assumed by Z1.a and Z4.p can vary. Camera lucida drawings of adult gonads resulting from such ablation experiments are shown on the left side, and the positions of the distal tip cells (as observed in L3 gonads) and the basic polarity of the adult gonad are diagrammed on the right side. M, Mitotic zone; O, meiotic nuclei; ●, primary spermatocytes; ⁘, sperm. Solid lines surround the gonadal cells where they form a coherent structure; broken lines surround germ cells that appear in Nomarski to have broken out of the normal confines of the gonad. Not all nuclei have been traced in the camera lucida drawings; instead, representative nuclei have been traced where overlapping nuclei are seen in many focal planes. See text for further explanation.

at the anterior edge of the germ cell mass, and the polarity of the germ line tissue was reversed. In one of these animals, the two distal tip cells separated from each other resulting in a forked anterior end with one distal tip cell located at the distal tip of each projection (Fig. 12C). In one animal (Fig. 12E), the anterior and posterior distal tip cells remained at their respective anterior and posterior ends, and a bipolar gonad was formed with mature sperm found between the two ends. No mitotic cells were observed at the anterior end of this gonad which suggests that the anterior distal tip cell was displaced posteriorly during L4 when it could no longer be seen. Finally, in one animal (Fig. 12F), both distal tip cells were displaced from the ends toward the middle of the germ line mass. In this case, a bipolar gonad antiparallel to that seen in Fig. 12E was the result; mature sperm were seen at either end of the gonad. In these experiments no gonads were observed that were inconsistent with the idea that the distal tip cells control the axial polarity of the germ line tissue. In unsuccessful animals, either the cells ablated were not killed, or the entire gonad was destroyed.

## DISCUSSION

In this paper, we focus on the control of germ cell development by two somatic cells, the distal tip cells, in *Caenorhabditis elegans*. We have used laser microsurgery to study the effect on the germ line cells of elimination of the distal tip cells or of altering the position of the distal tip cells. Our results bear on a number of questions concerning the control of postembryonic germ cell development, which will be discussed individually.

### *Control of Postembryonic Germ Cell Differentiation*

Kimble and Hirsh (1979) showed that the pattern of divisions followed by germ line cells after hatching is variable from individual to individual both in timing and orientation of divisions. Despite this variability, it might have been possible that individual germ cells become committed to a particular fate, i.e., stem cell, sperm, or oocyte, during early development and that all the progeny of the committed precursors assume their fate according to ancestry. The pattern of germ cell differentiation observed after distal tip cell ablation argues against this model. In unoperated hermaphrodites and males, germ cells are segregated into a distal mitotic or stem cell group and a proximal meiotic group. However, after ablation of the distal tip cells, the stem cells enter meiosis indicating that the stem cell population of germ cells is fundamentally equivalent to the meiotic population. In males, where all germ cells subsequently become sperm, it is clear that all the germ cells are equivalent.

In intact hermaphrodites, a further segregation of germ cells into sperm and oocytes takes place. However, after ablation of the distal tip cells in L1, L2, or early in L3, all germ cells differentiate as sperm. An oocyte precursor has therefore not been set aside up to this time of development. Yet, experiments with a temper-

ature-sensitive mutant, *tra-2 (b202)*, indicate that the potential to produce oocytes or not in the hermaphrodite gonad is determined during L1 (Klass *et al.*, 1976). These data argue that the decision taken in L1 to make oocytes does not involve the segregation of an oocyte precursor. Our findings, however, do not address the possibility that a sperm precursor might be set aside early in development. Ablation of the distal tip cell in a mutant that does not make sperm (*isx-1*, Nelson *et al.*, 1978) shows that all germ cells can enter the oocyte pathway (Kimble, unpublished results). An analogous result has not been obtained in wild type as yet, however, so the possibility of a committed sperm precursor remains.

Oocytes are made only if more germ cells are produced after ablation of the distal tip cells than are required to make the normal complement of sperm. (The number made in unoperated animals has a fairly wide range, but usually is 140–160/half gonad). Thus, the number of sperm made is kept as close as possible to normal—whether that means that all germ line cells become sperm as seen after killing the distal tip cells in L1 or L2, or that a small fraction of the germ cells become sperm as seen in the unoperated animal. The mechanism by which the number of spermatocytes is determined is not understood. Preliminary experiments (Kimble, unpublished results) have shown that the normal number of sperm are made even after a delay of 5–10 hr in the onset of meiosis and spermatogenesis (the delay being caused by ablation of Z3 as shown in Table 1D). The control of sperm number therefore seems to be independent of the time of sperm maturation.

*Control over Gonadal Shape*

During gonadogenesis, the gonad grows first in one direction, and then it reflexes and grows in the antiparallel direction. In both hermaphrodites and males, a single undividing somatic cell precedes the elongating gonad. In hermaphrodites, this single cell is the distal tip cell, and its ablation blocks both the directed growth of the gonad and germ cell divisions. In males, this single cell is the linker cell, and its ablation blocks directed growth, but germ cell divisions continue. Ablation of the distal tip cells in the male stops germ cell divisions, but does not alter the course of elongation. These experiments identify two separate functions necessary for normal gonadogenesis. A leader function is responsible for directed elongation, and a distal tip cell-specific function is necessary for proliferation of germ line cells. The two functions both reside in the distal tip cells in hermaphrodites, but they are allocated to the linker cell and the distal tip cells, respectively, in males.

*Control over Entry into Meiosis*

The laser ablation experiments reported here identify cells in the somatic gonad that are critical to the entry of germ cells into meiosis. First, the somatic gonadal progenitor cells, Z1 and Z4, are necessary for the initiation of postembryonic germ cell development; germ cells neither divide further mitotically nor enter meiosis when Z1 and Z4 are killed. Second, the distal tip cells are required to keep germ cells in mitosis postembryonically; germ cells enter meiosis only if located at some distance from the distal tip cell or if the distal tip cell is killed. The bulk of the somatic gonad can be eliminated after one division of Z1 and Z4 in males by ablation of two daughters of Z1 and Z4, Z1.p and Z4.a. This operation leaves only the two distal tip cells, Z1.a and Z4.p, and germ cells in the gonad; yet the germ cells continue dividing, enter meiosis, and make sperm. Since the distal tip cells prevent entry into meiosis and the rest of the somatic gonad seems to be irrelevant to the decision to enter meiosis, the onset of meiosis during L3 is probably controlled by the germ cells autonomously or by cells outside the gonad. Z1 and Z4 may act simply to reinitiate mitoses after a period of embryonic quiescence.

The nature of the distal tip cell inhibition of meiosis is not understood. It appears that the onset of meiosis per se mediates the arrest in germ cell mitotic divisions after distal tip cell ablation. If the distal tip cells are killed in L1, the germ cells nonetheless continue dividing until the time at which meiosis begins in the unoperated animal. If the distal tip cells are mitogenic, the simplest model would predict that germ cells would stop mitosis at a fixed time after distal tip cell ablation and that they would then either enter meiosis somewhat earlier than normal or remain suspended in mitotic interphase until meiosis normally begins—depending on the nature of the signal to initiate meiosis. The consistency with which the germ cells continue mitosis until the correct time for onset of meiosis argues against this simple model. Furthermore, it appears that the attainment of a critical number of mass of germ cells does not trigger the onset of meiosis since considerable variability is seen in the number of cells made after distal tip cell ablation (meiosis can begin when as few as 20 or as many as 64 cells have been produced after early ablation of the distal tip cells).

The cellular and molecular natures of distal tip cell control are also a matter of speculation. Since the distal tip cells seem to influence considerably more cells than they are in contact with, it is likely that this influence is mediated by a diffusable factor. The ultrastructure of the male distal tip cell (which has no known function other than preventing meiosis) reveals no internal membrane or junction specialization (Kimble, 1978).

*Control over Spatial Organization of Germ Cells*

The adult germ line tissue exhibits a polarity with mitotic nuclei found at the distal end and meiotic nuclei found at the proximal end. In addition, a gradient of maturation is found among the meiotic nuclei with the most mature stages of meiosis and gametogenesis located most proximally. In hermaphrodites, sperm are made by the most proximal cells and oocytes are made by cells originally located more distally that move into the gamete-forming region after the sperm are made. This organization of germ cells is lost after distal tip cell ablation—all mitotic nuclei become meiotic, and all meiotic nuclei mature into gametes. A remnant of the polarity persists in some hermaphrodite experiments, in that, if the distal tip cells are ablated after enough germ cells have been produced for the normal complement of sperm, oocytes are made in the appropriate relative position to sperm.

The hypothesis that the position of the distal tip cell might be responsible for establishing the polarity of the germ line tissue was tested by altering the position of distal tip cells very early in gonadogenesis in males. Such experiments indicate that the primary signal for establishing the polarity emanates from the distal tip cell. Since the germ line polarity is defined in terms of the mitotic/meiotic state of the nuclei, and since the distal tip cells have been shown to influence this state, it seems probable that the control of polarity by distal tip cells results from positioning the meiotic inhibitory signal at the distal end of the gonad. How the position of the distal tip cells is controlled is not understood.

The first morphological sign of the polarity of the germ line tissue is the entry of the most proximal cells into meiosis during L3. This suggests that the distal tip cells act over a distance, and that it is the release from distal tip cell influence that allows the cells furthest from the distal tip cells to enter meiosis first. The delay in meiosis observed in gonads made smaller by killing Z3 (Fig. 11; Table 1D) strongly supports this hypothesis. The onset of meiosis is no longer retarded if the distal tip cells are killed in addition to Z3, or if the normal size of the gonad is restored by the chance sequestering of all Z2 descendants into a single half gonad. Thus, the observed delay in meiosis probably results from an extension of the time of distal tip cell influence over the germ cells located proximally. This argues that the distal tip cells act over a distance.

Figure 13 presents a simple model to explain how the gradient of maturation of the germ line tissue in *C. elegans* might be established. The distal tip cells provide a localized inhibitory activity which acts over a distance, and growth causes the germ cells to escape this inhibitory influence sequentially. Thus, as the number of germ cells increases, cells become positioned outside the distal tip cell influence at progressively later times, and therefore initiate meiosis and gametogenesis at progressively later times. Consequently, a spatial gradient of maturation is established.

*Comparison with Other Organisms*

The distal tip cell control of germ cells in *C. elegans* suggests the possibility of a universal mechanism by which somatic—germ line interactions control germ cell proliferation during development and maintenance of a stem cell population for the germ line in the adult. At present, this is a matter of speculation. Tarkowski (1969) has suggested that the somatic gonad of mammals inhibits the onset of meiosis based on grafting experiments, and Byskov (1974) has identified an activity in somatic tissue of mammals that initiates meiosis in nonmeiotic germ cells, but no unifying principle has emerged.

The gonadal anatomy of many invertebrates is similar to that of *C. elegans* (e.g., Beklemishev, 1969). The shared features include an elongate shape and a polarity with a stem cell population at one end and a gradient of maturation in the meiotic region at the other end. In insects, one or a few nondividing somatic cells are located at the immature end of both ovaries and testes (King, 1970; Roosen-Runge, 1977). Early workers (Zick, 1911; Buder, 1917) postulated on morphological grounds that one such cell might inhibit the neighboring germ cells from entering meiosis. The similarity in the organization of these gonads may reflect a similarity in

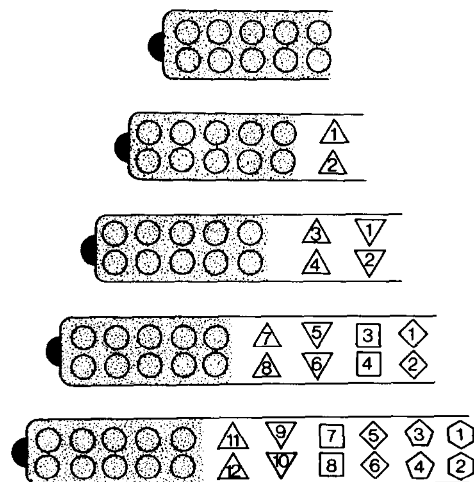

FIG. 13. Generalized model for establishing a gradient of maturation based on the distal tip cell control over meiosis. The control element (solid half circle) is localized on one end of a growing tube, and the controlling activity acts over a distance (stippled area). Due to proliferation of the target cells, some escape the controlling influence and begin to differentiate. Numbers identify individual cells; shapes represent stages of maturation, from least mature (△) to most mature (○).

the mechanism by which that organization is established. If so, it may be possible to identify somatic cells in these gonads that are analogous in function to the distal tip cells of *C. elegans*.

The gonads of a few nematodes are not organized like those of *C. elegans* (Chitwood and Chitwood, 1950). Instead, mitotic germ cells are found along the entire length of these "hologonic" gonads with the germinal zone located either to one side or all around the circumference of the gonad. One possible explanation of such a drastic departure from the more typical "telegonic" gonad seen in *C. elegans* is that the distal tip cell-like function has been assigned in the hologonic gonads to part or all of the sheath cells that encapsulate the germ line tissue along its length.

## CONCLUSIONS

The evidence presented in this paper supports the following main conclusions concerning the control of germ cell development in *C. elegans*:

(1) Two somatic gonadal cells, the *distal tip cells*, are responsible for the continued proliferation of germ cells and the local inhibition of germ cells from entry into meiosis in both sexes during postembryonic development and in the adult. The germ line stem cell population in the adult is therefore set aside, not by a lineage mechanism, but by local somatic-germ cell interactions.

(2) The distal tip cells are also responsible for establishing the axial polarity of the germ line tissue. The control of distal tip cells over polarity is probably due to the localization of its influence over the mitotic/meiotic state of the germ cells.

(3) No oocyte precursor is set aside by early L3 suggesting that ancestry does not play a role in the decision to make sperm or oocytes.

(4) The gross shape of the gonad is controlled by the distal tip cells in hermaphrodites and by the linker cell in males. These somatic gonadal cells precede the elongating tip during gonadogenesis, and serve a "leader" function which is distinct from the distal tip cell-specific function that is the main focus of this paper.

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## REFERENCES

BEKLEMISHEV, W. N. (1969). "Principles of Comparative Anatomy of Invertebrates," Vol. 2. Oliver & Boyd, Edinburgh.

BOVERI, T. (1899). "Die Entwicklung von *Ascaris megalocephala* mit besonderer Rucksicht auf die Kernverhaltnisse." Fischer, Jena.

BRENNER, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.

BUDER, J. E. (1917). Die Spermatogenese von *Deilephele euphorbiae*. L. *Arch. Zellforsch.* **14**, 26-78.

BYSKOV, A. G. (1974). Does the rete ovarii act as a trigger for the onset of meiosis? *Nature (London)* **252**, 396-397.

CHITWOOD, B. B., and CHITWOOD, M. B. (1950). "Introduction to Nematology." Univ. Park Press, Baltimore.

DELAVAULT, R. (1959). Developpement, croissance, et fonctionnement des glandes genitales chez les nematodes libres. *Arch. Zool. Exp. Gen.* **97**, 109-208.

DEPPE, U., SCHIERENBERG, E., COLE, T., KRIEG, C., SCHMITT, D., YODER, B., and VON EHRENSTEIN, G. (1978). Cell lineages of the embryo of the nematode *Caenorhabditis elegans*. *Proc. Nat. Acad. Sci. USA* **75**, 376-380.

HIRSH, D., OPPENHEIM, D., and KLASS, M. (1976). Development of the reproductive system of *Caenorhabditis elegans*. *Develop. Biol.* **49**, 200-219.

KIMBLE, J. E. (1978). "The Post-Embryonic Cell Lineages of the Hermaphrodite and Male Gonads in *Caenorhabditis elegans*," Ph.D. Thesis. University of Colorado, Boulder.

KIMBLE, J. E., and HIRSH, D. (1979). The post-embryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. *Develop. Biol.* **70**, 396-417.

KIMBLE, J. E., SULSTON, J. E., and WHITE, J. G. (1979). Regulative development in the post-embryonic lineages of *Caenorhabditis elegans*. *In* "Stem Cells, Cell Lineages, and Cell Determination" (N. LeDouarin and A. Monroy, eds.) Elsevier-North Holland, New York.

KING, R. (1970). "Ovarian Development in *Drosophila melanogaster*." Academic Press, New York.

KLASS, M., WOLF, N., and HIRSH, D. (1976). Development of the male reproductive system and sexual transformation in the nematode *Caenorhabditis elegans*. *Develop. Biol.* **52**, 1-18.

NELSON, G. A., LEW, K. K., and WARD, S. (1978). Intersex, a temperature sensitive mutant of the nematode, *Caenorhabditis elegans*. *Develop. Biol.* **66**, 386-409.

PEARSE, A. G. E. (1968). "Histochemistry, Theoretical and Applied," Vol. I, p. 603. Churchill, London.

ROOSEN-RUNGE E. C. (1977). "The Process of Spermatogenesis in Animals." University Press, Cambridge.

SULSTON, J. E., and HORVITZ, H. R. (1977). Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Develop. Biol.* **56**, 110-156.

SULSTON, J. E., and WHITE, J. G. (1980). Regulation and cell autonomy during post-embryonic development of *Caenorhabditis elegans*. *Develop. Biol.*, **78**, 577-598.

TARKOWSKI, A. K. (1969). Are genetic factors controlling sexual differentiation of somatic and germinal tissues of a mammalian gonad stable or labile? *In* "Environmental Influences on Genetic Expression" (N. Kretchmar and D. N. Walcher, eds.), Fogarty Int. Center Proc. No. 2, pp. 49-60. U.S. Gov. Print. Off.

WILSON, E. B. (1925). "The Cell in Development and Heredity," 3rd ed. Macmillan, New York.

ZICK, K. (1911). Die post-embryonale Entwicklung des mannlichen Geschlectsapparat der Schmetterlinge. *Z. Wiss. Zool.* **98**, 430-477.