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Commentary

Building a worm: Complete development one cell at a time

From 1977 to 1983 *Developmental Biology* published a remarkable series of papers that together described the first complete cell-by-cell development of a metazoan from zygote to adult, the *C. elegans* cell lineage, as well as ways that that lineage could be modified and interrogated. In fact, *Developmental Biology* became the *de facto* house organ for the field. In honor of the eightieth anniversary of the Society of Developmental Biology, we have collected and reprinted these foundational studies. (For more personal perspectives on the early work, see Horvitz and Sulston, 1990; Chalfie, 2018.)

The first paper (Sulston and Horvitz, 1977) built on the lineaging techniques that John Sulston had developed to examine the cell divisions that produced the postembryonic ventral cord neurons (Sulston, 1976) to describe all the postembryonic cell divisions giving rise to non-gonadal cells of the *C. elegans* hermaphrodite. Soon afterward Kimble and Hirsh (1979) described the somatic, gonadal divisions in both males and hermaphrodites (the germ cells did not follow a fixed pattern of divisions). One year later, Sulston et al. (1980) added the description of the lineages giving rise to the specialized somatic cells of the male. Finally, Sulston et al. (1983) described the embryonic lineages thus completing this remarkable tour de force. Interlaced with these studies were the characterization of the first of many genes that could be mutated to alter the cell lineage (Sulston and Horvitz, 1981) and the demonstration that certain cells could have alternative cell fates if other cells were removed by laser ablation (Sulston and White, 1981; Kimble, 1981; Kimble and White, 1981). These latter experiments defined the concept of equivalence groups in development.

This body of work was aided by several factors. The animals grew quickly, developing from eggs to egg-laying adults in 3.5 days at 20 °C. They were effectively transparent, especially using differential interference contrast (Nomarski) optics which allowed the visualization of all the nuclei and their divisions in living organisms. Moreover, adults only had a limited number of somatic nuclei, 959 in the hermaphrodite and 1031 in the male, making the task challenging but not impossible (nuclei are counted rather than cells because some tissues are syncytial). Most importantly, the pattern of the somatic cell divisions was reproducible (with a few exceptions) from animal to animal.

What made these papers unique and groundbreaking was that they described the entire development of a multicellular organism on a division-by-division basis. Never before had development been described in such minute detail. And this fine detail yielded an astonishing series of observations. The patterns of cell division were quite diverse; no single pattern generated all sets of cells. Some cells were derived clonally, e.g., the intestine, many of the muscle cells, and to an extent the six DD motor neurons. Other cells arose from a set of precursor cells that each divided to yield a cluster of similar cells, as was seen in the generation of the postembryonic motor neurons of the ventral cord. Still other sets of cells arose from a diverse set of precursors, e.g., the six touch receptor neurons). In fact, as Sulston et al. (1983) remarked, the lineage was a living fossil, having accumulated various changes and modifications over time [an idea reinforced by examination of similar lineages in the related nematode *Panagrellus redivivus* by Sternberg and Horvitz (1981, 1982) and other nematodes (Sulston et al., 1983)].

The observations in these lineage papers were more than just a catalogue of cell divisions. These papers also presented a detailed description of programmed cell death and its importance in the development of the animal. In one case, perhaps an example of accumulated development change during the worm's evolution, an embryonic cell divided twice, but at each division one of the daughter cells died. In the end the two divisions yielded a single cell. These papers also described patterns of cell migration, sexual dimorphisms, and developmental modifications (including a muscle cell that reorients its attachments and myofibrillar orientation as the male tail developments).

These papers sum to produce a tour-de-force in developmental biology. The importance of the *C. elegans* cell lineage, however, as Sulston et al. (1983) remarked in their discussion, lay in the use of the lineage information as the "basis for more detailed studies of development." Subsequent research has shown that it has served this purpose extremely well and continues to do so.

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Abnormal Cell Lineages in Mutants of the Nematode *Caenorhabditis elegans*

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The phenotypes of cell lineage mutants of the nematode *Caenorhabditis elegans* are described. The mutants, which define 14 genes, differ in the breadth and nature of their phenotypic defects. Mutants in four genes display very general abnormalities in cell division and may be altered either in cellular maintenance and growth or in the mechanics of cell division and/or DNA replication. Mutants in two genes are specifically defective in the movements and divisions of certain hypodermal nuclei. Mutants in six genes are affected in vulva development in the hermaphrodite and, in some cases, in homologous aspects of sexual maturation in the male; some of these mutants are blocked in vulval cell divisions, whereas others undergo extra divisions to generate multiple ectopic pseudovulvae; these genes appear to specify which of two alternative fates is assumed by a set of six cells involved in vulva development. Mutants in two genes produce reiterations in certain cell lineages, i.e., specific cells generate descendants similar to themselves in both their division patterns and the fates of their progeny; these genes may control primary determinative events that occur during the course of a cell lineage.

I. INTRODUCTION

The postembryonic cell lineages of the small soil nematode *Caenorhabditis elegans* are rigidly determined: invariant patterns of cell division generate a fixed number of progeny cells of strictly specified fates (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston *et al.*, 1980). The constancy of this developmental program and the ease of its study facilitate the examination of the mechanisms involved. For example, specific cells can be ablated with a laser microbeam and the effects of such ablations on other cells established (Sulston and Horvitz, 1977; Kimble *et al.*, 1979; Sulston and White, 1980).

Genetics provides an alternative method of disrupting the normally invariant program for nematode development. Mutants lacking specific cells or groups of cells can be examined to explore the roles these cells play during development. Mutants provide an advantage over laser-ablated animals in that large numbers of mutant individuals, such as might be needed for biochemical analyses, can be readily obtained. Furthermore, it may be possible to utilize mutants to help reveal the underlying genetic program for nematode development.

We have described elsewhere the isolation and genetic characterization of 24 mutants of *C. elegans* that are abnormal in their postembryonic cell lineages (Horvitz and Sulston, 1980). Both mutants blocked in cell divisions and mutants with abnormal cell proliferations were found. These mutants were obtained by identifying strains defective in structures that had been previously shown (Sulston and Horvitz, 1977) to be generated from postembryonic cell divisions. To determine the specificities of the defects of these mutants, we have now studied many features of postembryonic development in both hermaphrodites and males for representative alleles of the 14 genes defined by these cell lineage mutants.

II. MATERIALS AND METHODS

1. Strains

Caenorhabditis elegans var. Bristol strain N2 was obtained from Brenner (1974) and is the wild-type parent of all nematode strains used. We have described elsewhere the isolation and genetic characterization of the cell lineage mutants studied in this work (Horvitz and Sulston, 1980). Other mutants are described by Brenner (1974) or by Hodgkin *et al.* (1979). The mutant genes and alleles utilized are listed below; at least one allele of each gene affecting cell lineages has been examined in detail for anatomical and developmental lesions.

LGI *unc-35(e259), lin-6(e1466), him-1(e879), dpy-5(e61), unc-59(e261,e1005).*

LGII *lin-8(n111), unc-85(e1414), lin-4(e912), dpy-10(e128), lin-5(e1348), lin-7(e1413).*

LGIII *lin-9(n112), unc-86(e1416,e1507).*

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LGIV *lin-1(e1026,e1275,n176), lin-3(e1417).*
LGV *unc-83(e1408), him-5(e1467,e1490), dpy-21(e428).*
LGX *lin-2(e1309,e1453), unc-84(e1410).*

Hermaphrodites studied microscopically were obtained as the self-progeny of homozygous hermaphrodite parents, except for *lin-5* and *lin-6* animals, which are sterile and were obtained from balanced heterozygous hermaphrodite parents, *lin-5/dpy-10* and *lin-6/dpy-5*, respectively. Males were obtained in a number of ways: from matings involving either heterozygous or homozygous mutant males and hermaphrodites; from hermaphrodites of strains carrying a *him* ("high incidence of males") mutation (Hodgkin *et al.*, 1979); and in the case of sex-linked mutations, from crosses of wild-type males with homozygous mutant hermaphrodites. Specifically, the following were used as sources of males: *unc-86(e1416)* hermaphrodites (*unc-86* is Him; Hodgkin *et al.*, 1979), *lin-3* males crossed with *lin-3* hermaphrodites, *unc-83/+* males crossed with *unc-83* hermaphrodites (homozygous *unc-83* progeny were recognized by nuclei in the dorsal cord), *lin-5/dpy-10* males crossed with *lin-5/dpy-10* hermaphrodites, *lin-6/unc-35* males crossed with *lin-6/unc-35* hermaphrodites, *unc-59(e261); him-5(e1490)* hermaphrodites, *unc-85; him-5(e1490) dpy-21* hermaphrodites, (*dpy-21* is not expressed in XO males; Hodgkin and Brenner, 1977), *lin-4; him-5(e1467)* hermaphrodites, *lin-1(e1275); him-5(e1490)* hermaphrodites, *him-1; lin-7* hermaphrodites, *lin-8; lin-9; him-5(e1467)* hermaphrodites, wild-type males crossed with *unc-84(e1410) X* hermaphrodites, and wild-type males crossed with *lin-2(e1453) X* hermaphrodites.

All strains were maintained and crosses performed at 20°C.

2. Microscopy

Most of the characterization of the cellular anatomy and cell lineages of the mutants described in this manuscript involved the observation of living nematodes using Nomarski differential interference contrast optics, as described previously (Sulston and Horvitz, 1977). Fixed specimens stained by the Feulgen procedure (Sulston and Horvitz, 1977), with Hoechst 33258 [Albertson *et al.*, 1978; S. Ward and M. Chalfie, personal communication (see Fig. 1)] or by the technique of formaldehyde-induced fluorescence (Sulston *et al.*, 1975) were also studied.

3. Nomenclature

Cells have been named as defined in Sulston and Horvitz (1977). The blast cells present in the newly hatched larva are as follows: head lateral ectoblasts (H1,H2); body ventrolateral ectoblasts (V1–V6); tail lateral ectoblasts (T); lateral neuroblasts (Q1, Q2); ventral cord precursor cells (P0.a, P1–P12); mesoblast (M); intestinal nuclei (I); ventral ganglion neuroblasts (G1, G2); tail ectoblast (K); and male-specific ectoblasts (B, C, E, F). When a blast cell divides, each daughter is named by adding to the name of its mother cell a single lowercase letter representing its position immediately after division relative to its sister cell; for example, P12.pa is the anterior daughter of the posterior daughter of P12.

This paper conforms to the standardized nomenclature for *C. elegans* genetics (Horvitz *et al.*, 1979).

III. RESULTS

C. elegans cell lineage mutants defining 14 genes have been identified based upon their defects in the ventral nerve cord, vulva, or postdeirids (Horvitz and Sulston, 1980). We have now examined many other aspects of postembryonic development in these mutants, using Nomarski optics to observe individual cells in living nematodes. We have studied primarily the nongonadal cell lineages (Sulston and Horvitz, 1977), but gross gonadal defects also have been noted.

Tables 1 and 2 summarize the anatomical and developmental phenotypes of these mutants. Various classes of cell lineage abnormalities have been observed. Lineages can be missing entirely (because the relevant precursor cell either is absent or fails to divide; in the latter case, DNA replication may or may not continue). Lineages can be proliferative, generating more cells than usual by a series of apparently symmetrical cell divisions. Lineages can be reiterative, with certain progeny cells repeating division patterns displayed by either their parental or grandparental precursors. Lineages can also be abnormal in other ways, aberrant in either patterns of cell division and/or in cell fates. The mutants differ in the specificities of their defects with respect to both the lineages affected and the nature of the lineage abnormalities displayed.

More detailed phenotypic descriptions are presented below in four sections. The first section describes mutants in four genes that have very general effects on cell division. Because of defects in gonadal cell lineages, mutants in two of these genes are sterile and are maintained as balanced heterozygotes. Mutants in the other two genes display abnormalities in many cell lineages, particularly in terminal cell divisions involved in neuronal development; gonadal development is sufficiently normal that these strains are maintained as homozygotes. The second section describes mutants in two

TABLE 1
PHENOTYPES OF CELL LINEAGE MUTANTS: HERMAPHRODITES

Mutants	se	pd	T(neur)	P(neur)	P(vul)	M	G	I	K	Q	Comments
General defects, sterile											
lin-5	en	–	–	en	–	en	en	abn	en	(en)	No postembryonic nuclear divisions
lin-6	abn	abn	abn	abn	–	abn	abn	+	abn	+	No postembryonic DNA synthesis
General defects, fertile											
unc-59	abn	abn	abn	abn	abn	+	abn	+	(+)	abn	Defective cytokinesis
unc-85	(+)	abn	abn	abn	+	+	+	+	+	abn	Defective cytokinesis
Nuclear migration defects											
unc-83	+	+	+	mig	+/–	(+)	+	+	+	+	Embryonic nuclear migration defects
unc-84	+	+	+	mig	+/–	(+)	+	+	+	+	Embryonic nuclear migration defects
Vulvaless											
lin-2	+	+	+	+	abn	+	+	+	+	+	
lin-3	+	+	+	+	abn	+	(+)	+	+	+	
lin-7	+	+	+	+	+/–	+	(+)	+	+	(+)	
Multivulva											
lin-1	+	+	+	+	prolif	(+)	abn	+	+	+	
lin-8; lin-9	+	+	+	+	prolif	+	abn	prolif	+	+	
Reiterations											
unc-86	+	reit	reit	+	+	+	+	+	+	reit	Embryonic abnormalities
lin-4	reit	prolif	reit	+	abn	prolif	(+)	prolif	reit	+	

Note. Effects of mutations on the postembryonic cell lineages. se, lineages of seam cells (hypodermal blast cells H, V, and T); pd, formation of postdeirid cell group (from V5.pa); T(neur), neuronal lineages of T (from neuroblast T.p); P(neur), neuronal lineages of Pn (from neuroblasts Pn.a); P(vul), vulval lineages of Pn (from ventral hypodermal cells Pn.p); M, G, I, K, Q, lineages of these blast cells; rays, formation of sensory rays; VCdiv, divisions of Pn.aap in L3; PAG, divisions of P10.p and P11.p in L3; SM, divisions of sex mesoblasts; B, C, E, F, divisions of these blast cells; +, lineage and differentiation normal; –, lineage absent; abn, some cell division, but lineage or cell fates generally abnormal; en, endomitosis without nuclear or cell division; mig, stochastic failure of nuclei to migrate and divide; prolif, more divisions than in wild type; reit, reiterative lineage, as defined by branching pattern and cell fates. Parentheses indicate that probable exceptions have been observed. In these tables, we have summarized only the principal phenotypes. The entries are based upon spot checks of a number of individuals at various ages, using Nomarski and polarization optics, supplemented by observation of cell lineages when anomalies were detected. Defects seen only once in a given mutant have been ignored for the present.

genes that affect the movements and divisions of certain hypodermal nuclei. The third section desribes mutants defining six genes that are specifically defective in vulva development in the hermaphrodite and, in some cases, in apparently homologous aspects of sexual maturation in the male. The fourth section describes mutants in two genes in which certain cell lineages are altered in a reiterative fashion: specific cells generate descendants similar to themselves in both their division patterns and the fates of their progeny.

1. Cell Division Mutants

lin-5 II. The newly hatched animal is normal. Postembryonic nuclear division and cytokinesis are virtually absent, except in the germ line; occasionally, an early division [i.e., of the ventral cord precursor cells (P) or of the lateral neuroblasts (Q)] has been observed. Some intestinal nuclei divide, but their daughters seldom separate completely. DNA replication continues in the various blast cells, and polyploid nuclei result (Fig. 1c). Albertson *et al.* (1978) have described certain aspects of the phenotype of *lin-5* in detail.

The germ line appears normal until 10–20 cells have been generated. Division then slows and virtually ceases. Many of the germ cells become polyploid, although in rare individuals sperm are produced.

lin-5 animals grow normally. Adult hermaphrodites are healthy but sterile and abnormally thin; males usually die during the late L3 or L4 stage, either of constipation or by bursting at the tail. *lin-5* animals are normal in locomotion during the L1 stage; however, from the middle of the L2 stage onward they can move forward only in a somewhat uncoordinated fashion and cannot move backward at all. This behavioral defect is shared by a number of cell lineage mutants (*lin-6*, *unc-85*, *unc-59*, *unc-83*, *unc-84*) and presumably results from abnormal development of the ventral nervous system. The onset of the uncoordinated phenotype may well reflect the reorganization of the DD motor neurons, which removes all innervation from ventral body muscles in animals lacking those neurons that normally are generated by postembryonic cell divisions (White *et al.*, 1978).

Both the adult and the dauer larva frequently fail to form complete lateral alae, which are longitudinal cuticular ridges normally produced by the lateral seam cells derived from H, V, and T (Sulston and Horvitz,

TABLE 2
PHENOTYPES OF CELL LINEAGE MUTANTS: MALES[a]

Mutants	rays	VCdiv	PAG	SM	B	C	E	F	Other
General defects, sterile									
lin-5	−	−	−	−	(en)	en	en	en	Dies as L3-L4
lin-6	−	−	−	−	abn	abn	abn	abn	Dies as L2-L3
General defects, fertile									
unc-59	+/−	+	(+)	abn	abn	+	abn	abn	
unc-85	+/−	+	(+)	abn	abn	+	abn	abn	
Nuclear migration defects									
unc-83	+	+	+/−	+	+	+	+	+	
unc-84	+	+	+/−	+	+	+	+		
Vulvaless									
lin-2	+	+	+	+	+	+	+	+	
lin-3	+	+	+	+	+	+	+	+	
lin-7	+	+	+	+	+	+	+	+	
Multivulva									
lin-1	+	+	prolif	+	+	+	+	+	
lin-8; lin-9	+	+	prolif	+	+	+	+	+	
Reiterations									
unc-86	+	+	+	+	+	+	+	+	Cephalic companions absent
lin-4	−	(−)	abn	prolif	abn	abn	abn	abn	

[a] See note to Table 1.

1977; Singh and Sulston, 1978); in *lin-5*, the lineages of H, V, and T are blocked, and the resultant polyploid cells eventually fuse with the large hypodermal syncytium. Although normal in L1 larvae, the hermaphrodite-specific neuron cell bodies (hsns; Sulston and Horvitz, 1977) are often displaced in older animals. Similar postembryonic displacement and/or loss of the hsns appears to occur in all of the other cell lineage mutants defective in ventral cord development (*lin-6*, *unc-85*, *unc-59*, *unc-83*, *unc-84*), suggesting that some of the ventral cord neurons generated during the L1 stage may be necessary for hsn survival and/or development.

lin-6 I. The newly hatched hermaphrodite is normal; the left coelomocytes are displaced anteriorly in the newly hatched male. Although DNA synthesis is largely absent, except in the germ line (Fig. 1d), both gonadal and nongonadal somatic blast cells continue to divide (Fig. 2); the nuclei generated become smaller in each generation, and the cells eventually die. Thus, the adult animal contains principally those juvenile cells that do not divide.

A few postembryonic somatic cell progeny can survive: (a) the descendants of Q1 and Q2 are usually normal; (b) the distal tip cells of the gonad are present and presumably functional, since in their absence the germ cells fail to divide (J. Kimble, personal communication); (c) small but morphologically recognizable dorsal coelomocytes that are formed postembryonically can sometimes be found (in wild type, these cells are descendants of the mesoblast, M; Sulston and Horvitz, 1977).

The germ line divides extensively and forms a gonad with reflexed arms of nearly normal size; however, most of the normal structural cells (Hirsh *et al.*, 1976; Klass *et al.*, 1976; Kimble and Hirsh, 1979) are missing. Sperm are generated in the young adult and small oocytes may be formed.

lin-6 adults are sterile and uncoordinated. Both adults and dauer larvae fail to form complete lateral alae (Singh and Sulston, 1978). The hsn cell bodies often appear to be missing in older animals.

unc-59 I. The newly hatched animal is normal. During postembryonic development, various lineages can be blocked. The blocks are variable among individuals, so that different animals can be quite distinct in phenotype. Blocks have been observed in the lineages of H2, Vn, T, Qn, Gn, and Pn in both sexes, and in ray formation, the preanal ganglion, B, E, and F, in the male. The seam cells frequently fail to divide after the L1 stage and tend to fuse with the large hypodermal syncytium; consequently, the formation of lateral alae is erratic. The dopamine characteristic of V5.paaa, a postdeirid neuron, is often absent when animals are examined using the technique of formaldehyde-induced fluorescence (Sulston and Horvitz, 1977). Almost no sensory rays are made in the male. In the hermaphrodite, the mesodermal lineage appears normal; in the male,

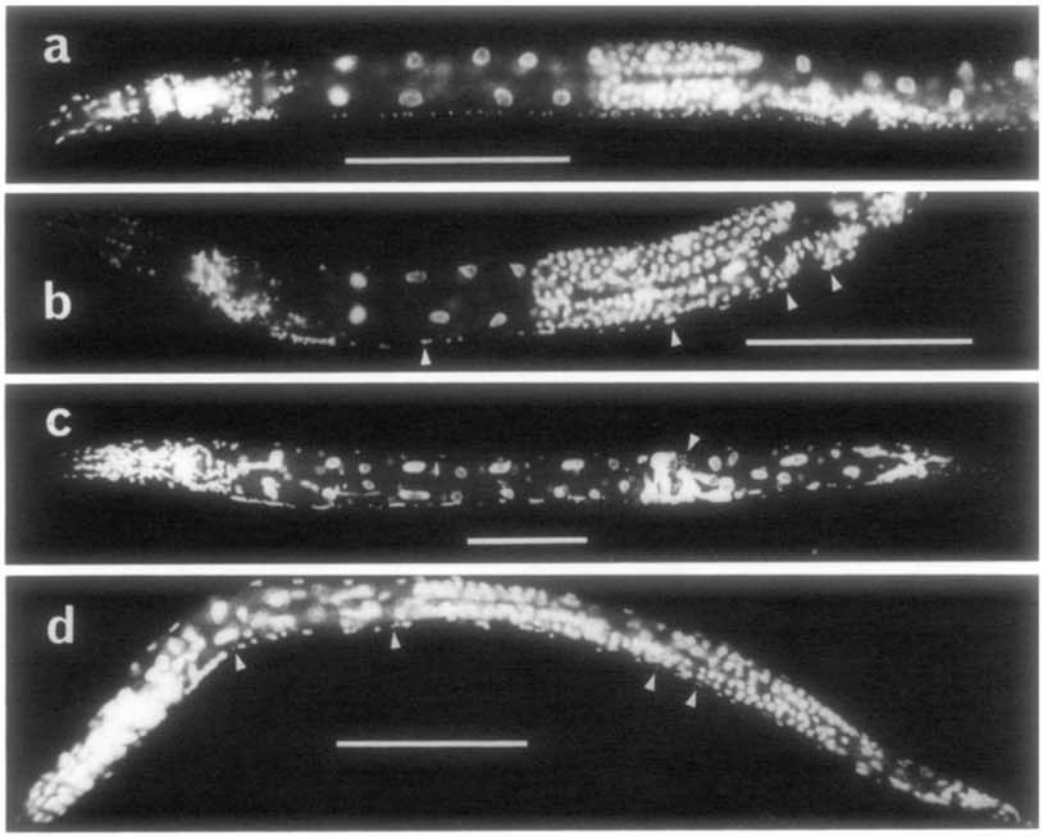

FIG. 1. Nuclear stained whole mounts. Animals mounted in M9 buffer containing 0.5% 1-phenoxy-2-propanol and 5 μg/ml Hoechst 33258 (Albertson *et al.*, 1978; S. Ward and M. Chalfie, personal communication). Photographed after 1–5 hr on Kodak PanX film, using a Zeiss Standard microscope with UG1 exciter and 41 barrier filters. Left lateral views. Bar, 100 μm. (a) Wild type, L4. Anterior ventral cord, along lower edge. (b) *unc-59(e1005)*, L4. Ventral cord has fewer neurons and contains polyploid nuclei (arrow heads). (c) *lin-5(e1348)*, L4. Ventral cord has few neurons and contains elongated polyploid nuclei. The gonad contains large polyploid nuclei and some sperm (arrow head). (d) *lin-6(e1466)*, L3. Ventral cord has few neurons and contains subdiploid nuclei (arrow heads).

the spicule retractor muscles are sometimes not formed and binucleate cells have been observed. The somatic gonad is sometimes defective, one striking character being a binucleate anchor cell (Kimble and Hirsh, 1979) in the hermaphrodite. The vulva is generally formed after a normal cell lineage, yet invariably protrudes in the adult. It is largely terminal cell divisions that are blocked in *unc-59*. Older animals are uncoordinated and often appear to lack the hsn cell bodies.

The nature of the blocks has been studied principally in the ventral cord. Feulgen-stained animals can be seen to contain polyploid nuclei in the cord, indicating that the block occurs subsequent to DNA replication (Fig. 1b). Using Nomarski microscopy, we have found that polyploid nuclei can arise in either of two ways. First, a nucleus may enter anaphase but fail to divide, similar to the blocks seen in *lin-5*. Second, two apparently normal nuclei may fuse at telophase; examples of cell lineage in which such fusions have been seen are shown in Fig. 3. Nuclear fusion may result from a previous failure in cytokinesis, as binucleate cells have been observed in the ventral cord (J. White, personal communication), as well as in the gonad and in muscle (see above). Thus, the primary lesion in *unc-59* could be in cytokinesis. In addition to blocked lineages, rare *unc-59* individuals have supernumerary neuron-like nuclei embedded in the lateral hypodermis.

unc-85 II. This mutant bears a striking resemblance to *unc-59*. Again, the newly hatched animal is normal, and blocks can occur in numerous terminal postembryonic lineages. Blocks have been seen in Vn, T, Qn, and Pn in both sexes and in ray formation, B, E, and F in the male. The male sex muscles are usually defective, and in one individual the hook (formed by preanal ganglion cell P10.papp; Sulston *et al.*, 1980) was missing. In contrast to *unc-59*, most of the seam cells survive and produce lateral alae, the vulva does not protrude and the gonad generally appears more normal. The dopamine characteristic of the postdeirid is often absent. The postdeirid lineage is sometimes blocked, but even an apparently normal postdeirid lineage can fail to produce a dopaminergic cell. From the L2 stage on, *unc-85*

FIG. 2. Divisions in part of ventral cord of (a) wild type and (b) *lin-6(e1466)*. First divisions of the lineage of P8 are indicated. Left lateral view, Nomarski optics. Bar, 10 μm.

animals are uncoordinated. Adults often appear to lack the hsn cell bodies. In the ventral cord, blocks have been seen to arise either from failure of nuclear divisions at anaphase or from fusion of sister nuclei at interphase; the fusion of sister nuclei has been observed for the two daughters of P2.aaa, P3.aaa, P6.aaa, and P7.aaa. Sister nuclei that in wild type are equal in size are sometimes markedly dissimilar in *unc-85*. Such an asymmetric distribution of cellular material might be related to subsequent abnormalities in differentiation, such as the failure to form a dopaminergic neuron in individuals with a normal lineage. Some cell divisions are unusually prolonged.

2. *Nuclear Migration Mutants*

unc-83 V and unc-84 X. No qualitative distinction can be made between mutants in these two genes. In the newly hatched L1, many of the syncytial hypodermal nuclei, which normally lie dorsolaterally, are found in the dorsal cord (Fig. 4). These nuclei can be seen in the dorsal cord by about 7 hr after fertilization (the

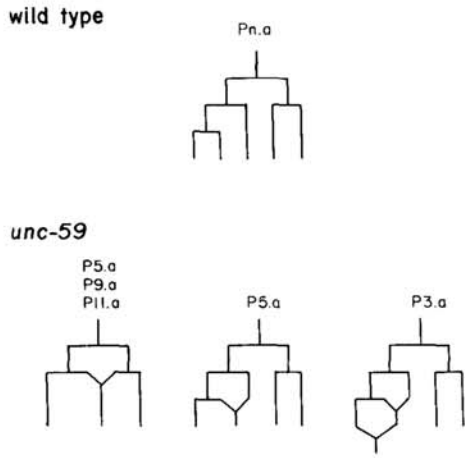

FIG. 3. Examples of nuclear fusions that have been observed in *unc-59* animals. Fusions are indicated by the joining of two progeny nuclei, such as P9.aap and P9.apa. Lineages are drawn as in Sulston and Horvitz (1977), with anterior daughters represented by left branches and posterior daughters represented by right branches (see Materials and Methods for a further explanation of cell nomenclature). The wild-type Pn.a lineage is from Sulston and Horvitz (1977).

"comma stage"); in normal embryos of this age, they lie in the lateral hypodermis. The origin of this difference was investigated by observing developing embryos using Nomarski optics. Most of the cells that will form the large hypodermal syncytium of the juvenile are generated in two irregular longitudinal rows on the dorsal side. Each cell extends a cytoplasmic process through the opposite row and around the outside of the embryo to the contralateral side. In the wild type, each nucleus moves along its process, crossing the midline and a dorsal band of developing body muscle, and so comes to lie in the lateral hypodermis. In *unc-84* animals, the cytoplasmic processes seem to extend normally, but the nuclei move more slowly and only reach the midline; possibly this limited movement is merely a result of pressure from the adjacent muscle cells.

A similar defect in nuclear migration occurs postembryonically in *unc-83* and *unc-84* animals. During the middle of the L1 stage of wild-type individuals, the ventral cord precursor nuclei (Pn) migrate along cytoplasmic protrusions from ventrolateral positions to the ventral cord where they begin to divide (Sulston and Horvitz, 1977). In *unc-83* and *unc-84* animals, a proportion of the nuclei fail to migrate, although the cytoplasmic protrusions of the Pn cells appear to enlarge normally. The enlarged cytoplasmic protrusions along the ventral midline persist, often into the adult, as granular, slightly refractile minicells. Most of the nuclei that remain laterally eventually disappear without dividing; in an occasional animal, however, a group of neuronal-like nuclei can be seen lying ventrolaterally, indicating that a Pn cell has divided. That incorrectly

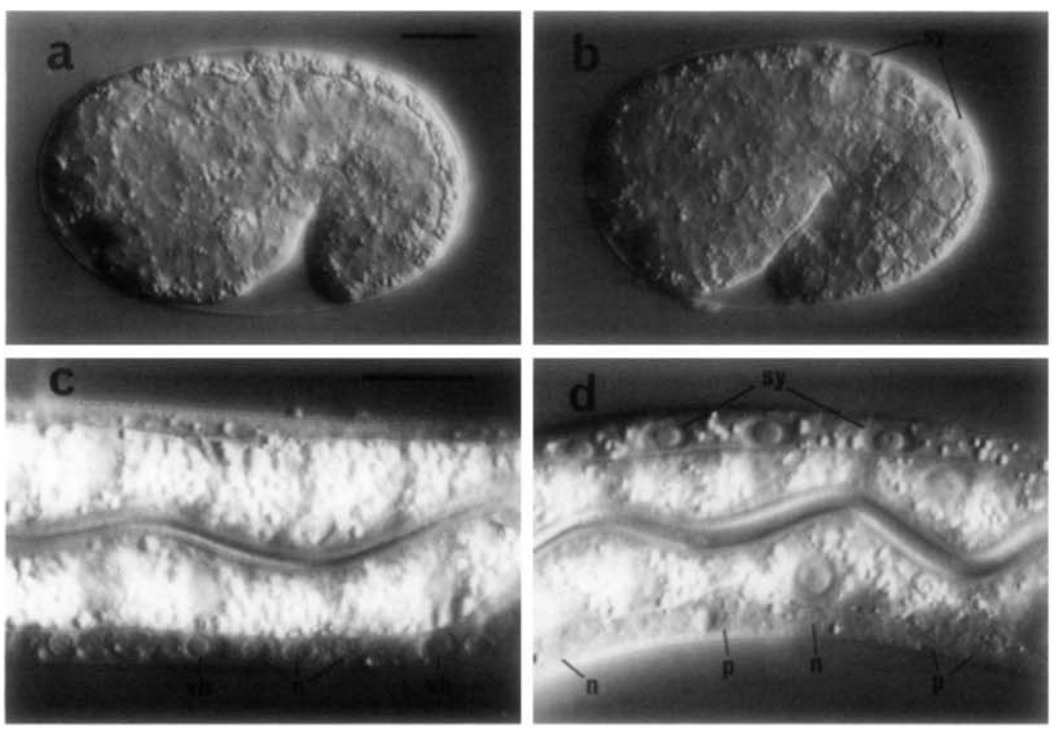

FIG. 4. Above: left lateral view of egg at comma stage, about 7 hr postfertilization. (a) Wild type. (b) *unc-84(e1410)*. Below: left lateral view of L2, midplane, immediately anterior to gonad. (c) Wild type. (d) *unc-84(e1410)*. n, neurons in ventral cord; p, cytoplasm of P cells whose nuclei have failed to enter ventral cord; sy, syncytial hypodermal nuclei lying in dorsal cord; vh, ventral hypodermal nuclei.

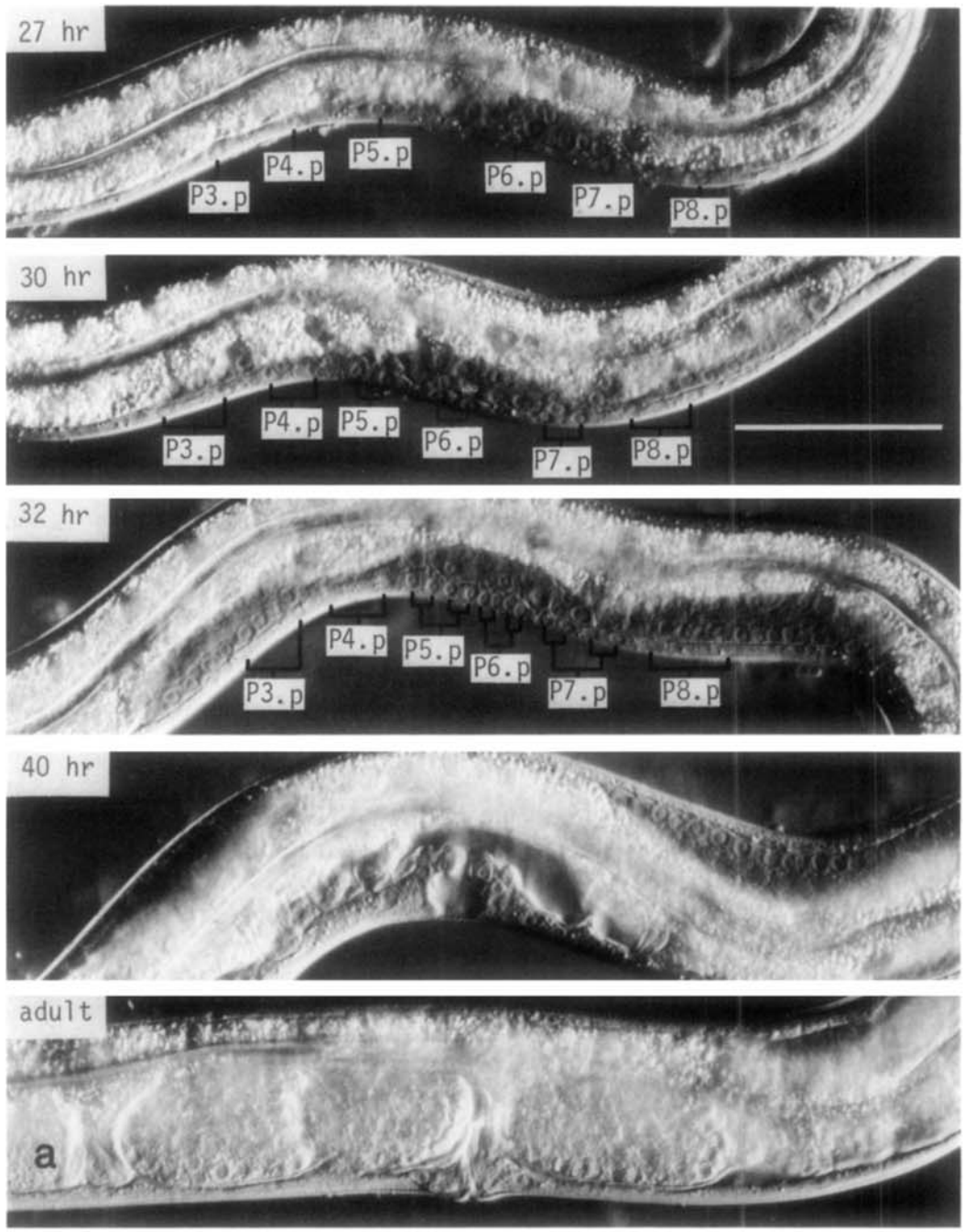

FIG. 5. Development of vulva, left lateral view. Nomarski optics; bar, 50 μm. (a) Wild type. (b) Vulvaless, *lin-3(e1417)*. (c) Multivulva, *lin-8(n111); lin-9(n112)*.

positioned Pn nuclei generally do not divide suggests that Pn cells must be located in the ventral cord for division to occur; alternatively, a single defect intrinsic to the Pn cells might prevent both migration and division.

The loss of some Pn cell lineages leads to variable defects in the hermaphrodite vulva, the male preanal ganglion and the motor neuron complement of the ventral cord. From the L2 stage onward, animals are uncoordinated. Older animals often appear to lack the hsn cell bodies.

Surprisingly, all seven alleles of *unc-83* and *unc-84* are temperature sensitive, showing more mutant phenotypes at 25°C than at 20 or 15°C. It is possible that this temperature sensitivity is not caused by an altered gene product that specifically fails to function at high temperature but instead reflects a temperature-sensitive process revealed or induced by eliminating the ac-

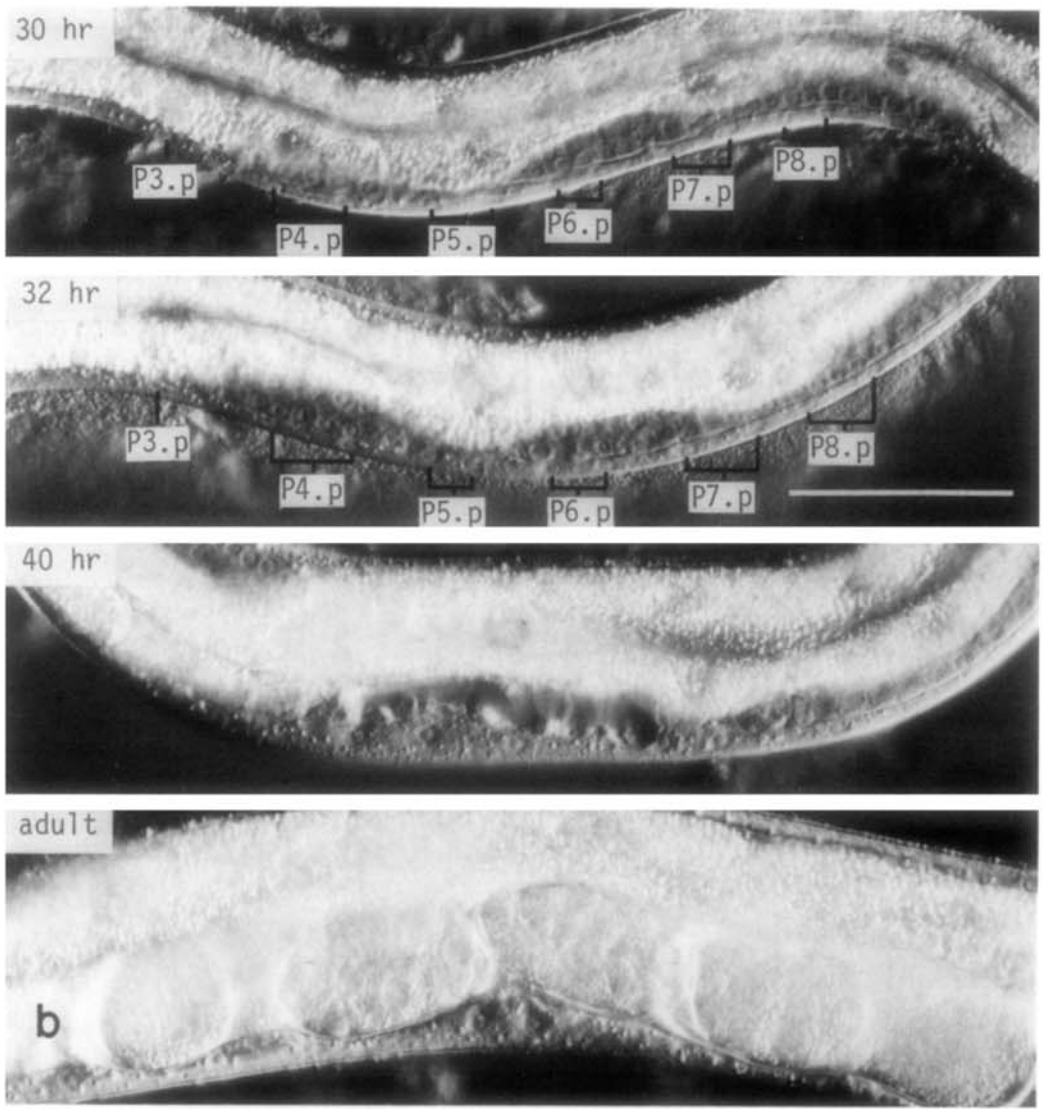

FIG. 5. (*Continued*)

tivity of the mutated gene; in other words, perhaps in the absence of *unc-83* or *unc-84* gene activity, the process of Pn nuclear migration is heat sensitive.

3. Mutants Affecting the Vulva

The cell divisions that lead to vulva development in the wild-type hermaphrodite have been described (Sulston and Horvitz, 1977). We now know that these divisions occur in two distinct stages, of which the second but not the first is dependent on the presence of the gonad (J. White and J. Kimble, personal communication). In the first stage, six (occasionally five) ventral hypodermal cells (P3.p–P8.p) divide once. In the second stage, the six progeny of P5.p–P7.p continue dividing so that four produce four descendants each and two produce three descendants each; the 22 cells so generated then develop into the vulva (Fig. 5a). The remaining six daughters (derived from P3.p, P4.p, and P8.p) fuse with the large hypodermal syncytium.

Two classes of mutants abnormal in vulval cell lineages have been identified (Horvitz and Sulston, 1980). In vulvaless mutants (*lin-2*, *lin-3*, *lin-4*, *lin-7*), vulval cell divisions are abnormal and no vulva is formed (Fig. 5b). In multivulva mutants (*lin-1* and the double mutant *lin-8; lin-9*), extra cell divisions generate multiple pseudovulvae along the ventral side of the animal (Fig. 5c).

lin-4 has a complex phenotype and will be discussed in the next section, which concerns mutants with reiterative cell lineages. The other vulvaless mutants (*lin-2*, *lin-3*, *lin-7*) are affected specifically in vulval cell lineages. In these three mutants, first-stage divisions are normal, but second-stage divisions generally fail; those

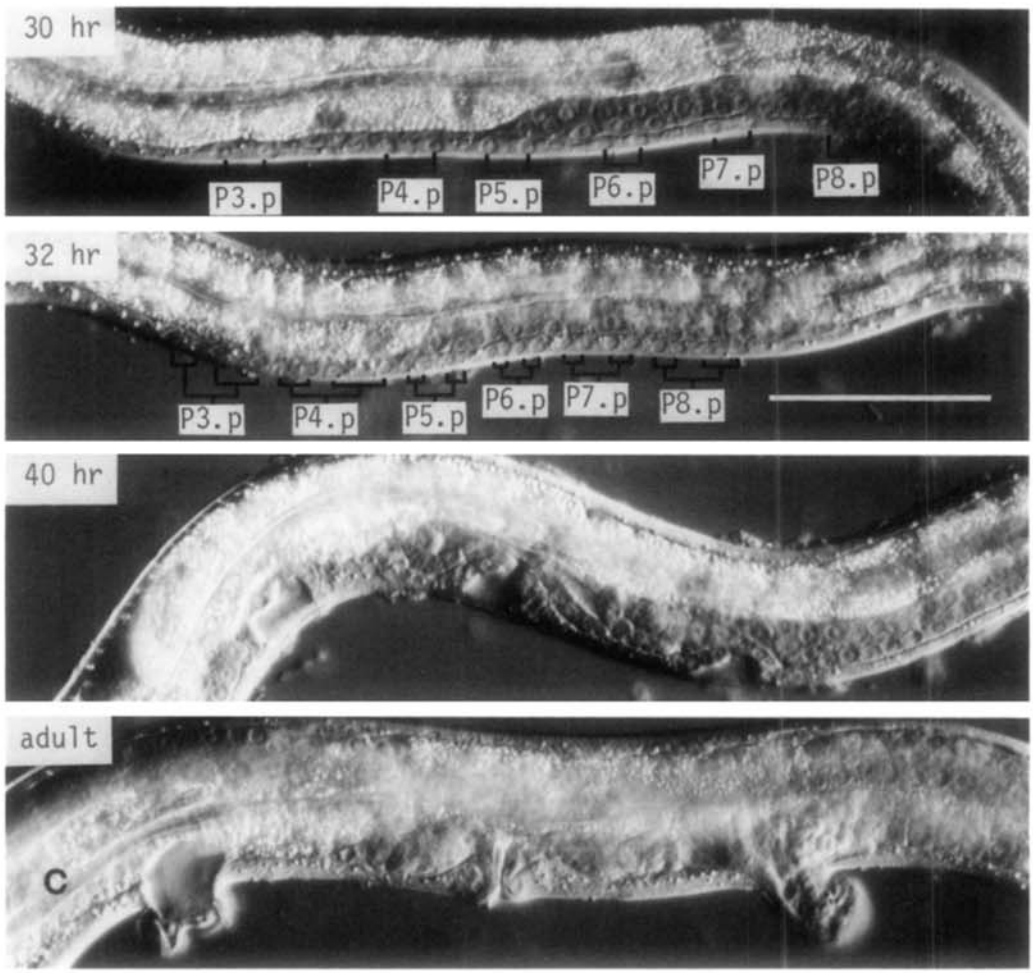

FIG. 5. (*Continued*)

first-stage daughters that divide yield at least three and usually four descendants, as in wild type, indicating that it is the initiation of second-stage divisions that is abnormal in these mutants.

lin-2 X. In about 80% of the animals, no second-stage divisions occur. In many of the remainder, progeny from more than one precursor divide, often generating close to the normal complement of 22 cells. This phenotype is suggestive of either a threshold effect or cooperativity, as multiple second stage divisions occur more frequently than would be expected if each event were independent. In animals with second stage divisions, a functional vulva is often made, even when fewer than 22 cells have been generated.

lin-3 IV. Of 10 animals examined, 4 had no second stage divisions; in 4 others, P6.pp gave rise to 4 progeny, and in a further 2, P6.pa and P6.pp together gave rise to a total of 8 progeny. In the animals with second-stage divisions, the division directions and behavior of the progeny appeared to be the same as in wild-type animals. In the 2 animals with 8 P6.p descendants, a functional "mini-vulva" was formed by these 8 daughter cells.

lin-7 II. All seven animals examined displayed some second-stage divisions; the number of first-stage daughters involved ranged from one (P6.pp) up to the normal six. The progeny from these divisions appeared to be less cooperative than usual, in that the normal pattern of aggregation and differentiation often failed. However, a normal vulva does occasionally develop. Initiation of second stage divisions in one first-stage cell but not in its sister is common. One "bivulva" animal developed as a result of its second-stage divisions occurring in two regions separated by a nondividing cell.

lin-1 IV. Both hermaphrodites and males show excessive proliferation in Pn.P cell divisions in the ventral hypodermis. In the hermaphrodite, all six of the ventral hypodermal cells that normally undergo first-stage divisions (P3.p–P8.p) also undergo second-stage divisions; most produce eight daughters. Groups of these cells aggregate to form multiple cavities similar in morphology to normal intermediates in vulva development.

Each of these cavities everts at L4 ecdysis. so that the adult hermaphrodite has a series of protrusions along its ventral side. The vulva itself often contains excess cells and can be oversized and asymmetric; it frequently ruptures at L4 ecdysis.

In the *lin-1* male, three ventral hypodermal cells (P9.p–P11.p) undergo essentially three rounds of division; in contrast, in the wild-type male, P9.p divides once or not at all. Lineages were followed in one *lin-1* male: P10.p and P11.p behaved normally, and P9.p generated eight descendants. The excessive ventral hypodermal cells in the male result in the production of a rudimentary ectopic hook and, sometimes, a vestigial hook sensillum; these supernumerary structures are located just anterior to the normal hook (Fig. 6). In wild type, the hook and hook sensillum are derived from P10.p and P11.p (Sulston *et al.*, 1980). Thus, in both sexes, *lin-1* causes certain ventral hypodermal cells to acquire characteristics normally associated with other ventral hypodermal cells.

lin-1 displays a slightly protruding excretory pore (the opening of the excretory duct located ventral to the rear pharyngeal bulb; Sulston and Horvitz, 1977). The lineages of G1 and G2 are sometimes blocked. The other multivulva mutant studied, the double *lin-8; lin-9*, shares these characteristics, suggesting that homologies may exist among the various cells affected.

In L1 larvae of *lin-1*, the lateral hypodermal cell V1 is tiny; in the wild type, V1 is the same size as the other V cells. In some *lin-1* animals, V1 divides late and yields small hypodermal cells, while in others it seems to disappear entirely. In the wild type, V1 is distinctive in that it lies more laterally than V2–V6 in the young L1. This effect on V1 has been observed in all three alleles of *lin-1*, indicating that it results from the same mutation that causes the multivulva phenotype; however, V1 appears to be normal in *lin-8; lin-9*.

lin-8 II; lin-9 III. The multivulva phenotype of strain CB1322 results from mutations in two genes, *lin-8 II* and *lin-9 III* (Horvitz and Sulston, 1980). The phenotype of the *lin-8; lin-9* double mutant is similar to that of *lin-1*. In hermaphrodites, P3.p–P8.p all undergo both first- and second-stage divisions, and groups of their progeny aggregate, differentiate, and evert at L4 ecdysis. In contrast to *lin-1*, *lin-8; lin-9* normally makes a functional vulva, which does not rupture at L4 ecdysis. The adult male generally carries a supernumerary ectopic hook. In addition, in *lin-8; lin-9* males, P3.p–P6.p (but not P7.p or P8.p) often divide, producing pseudovulvae along the ventral side of the animal. Sometimes the linker cell of the male gonad (Kimble and Hirsh, 1979) attaches to one of these pseudovulvae, and the gonad fails to grow posteriorly in its usual way. This observation suggests that the linker cell of the male is analogous to the anchor cell of the hermaphrodite, which normally attaches to the developing vulva (Kimble and Hirsh, 1979).

lin-8; lin-9 displays a slightly protruding excretory pore as well as blocks in the lineages of G1 and G2. In addition, multinucleate intestinal cells are often found; in wild-type animals, intestinal cells are never more than binucleate.

4. Mutants with Reiterated Cell Lineages

unc-86 III. The phenotype of *unc-86* is complex, with a number of superficially unrelated aspects. The newly hatched animal lacks microtubule cells (Chalfie and Thomson, 1979) in both sexes and cephalic companions (Sulston and Horvitz, 1977) in the male, has excess dopaminergic deirid neurons (Sulston *et al.*, 1975), and lacks serotonin in the neurosecretory motor neurons of the pharynx (Albertson and Thomson, 1976). The absence of microtubule cells causes an insensitivity to

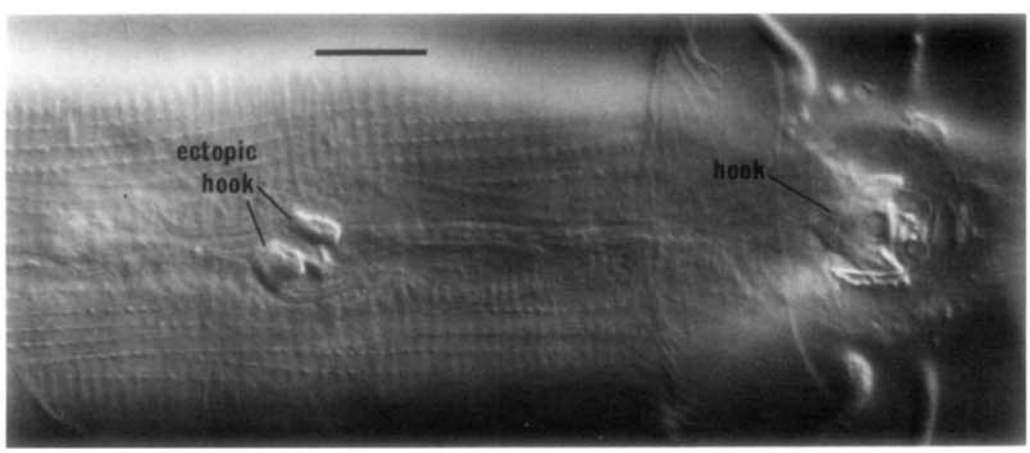

FIG. 6. Ectopic hook in tail of *lin-1(e1275); him-5(e1490)* male. Ventral view of animal anesthetized with 0.5% 1-phenoxy-2-propanol, Nomarski optics. Bar, 10 μm. Anterior to left; fan and sensory rays of copulatory bursa are seen to right.

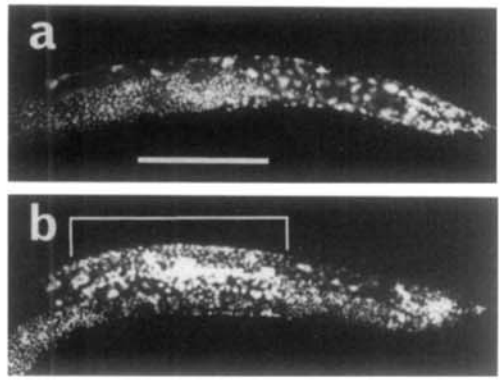

FIG. 7. Hoechst-stained whole mounts (as in Fig. 1). Left lateral views of posterior region of adult males. Bar, 100 μm. (a) Wild-type. (b) *lin-4(e912)*, bracket indicates mass of cells formed by mesodermal proliferation.

gentle mechanical stimulation (Chalfie and Thomson, 1979; M. Chalfie and J. Sulston, unpublished observations). Probably because of the absence of cephalic companion cells, *unc-86* adult males fail to chemotax toward hermaphrodites (J. Sulston and R. Horvitz, unpublished observations). The hsns usually cannot be found in their normal positions in L4 and adult animals and, presumably as a result—it is known that laser ablation of the hsns causes egg retention (R. Horvitz and J. Sulston, unpublished observations)—*unc-86* mutants retain eggs. As has been described previously (Hodgkin *et al.*, 1979), the hermaphrodite produces about 2.3% male self-progeny, as opposed to the 0.3% males produced by virgin wild-type hermaphrodites.

To determine if the serotonin deficiency could be responsible for the other defects of *unc-86*, animals have been grown in the presence of 1 mg/ml serotonin. The exogenous serotonin is accumulated by the neurosecretory motor neurons, but after two generations of exposure, most phenotypic characteristics of *unc-86* are unchanged. Egg-laying ability is restored, consistent with the observation that serotonin induces egg laying by hermaphrodites in which the hsns have been laser ablated (R. Horvitz and J. Sulston, unpublished observations).

In postembryonic development, abnormal reiterative cell divisions occur in three lineages on each side of the animal (Q, T, and V5). In each case, a cell appears to divide to produce one normal daughter and a second daughter like itself in morphology and developmental potential; this second daughter then divides to produce one daughter like that already formed and one once again like itself; such reiterative divisions occur from one to three times. As a result of the reiteration in the Q lineages, the postembryonic microtubule cells (normally Qn.paa; Chalfie and Thomson, 1979; Chalfie *et al.*, 1981) are not formed. The reiterations in the V5 lineages yield multiple dopaminergic postdeirid neurons, which are detected by the technique of formaldehyde-induced fluorescence. The cell lineages displayed by *unc-86* will be described elsewhere (Chalfie *et al.*, 1981). Similar reiterative behavior of embryonic lineages may be the reason for the absence of some cells and the overproduction of others in the newly hatched animal; in particular, it seems likely that the juvenile microtubule cells and the deirids may be homologous with the postembryonic microtubule cells and the postdeirids, respectively.

lin-4 II. The phenotype of *lin-4* also involves the reiteration of cell lineages. Most blast cells are affected, but none of the reiterations characteristic of *unc-86* have been observed in *lin-4*. The newly hatched animal is normal. Many postembryonic lineages are aberrant; arrests, reiterations, and gross proliferations are seen in different lineages. In L4 larvae and older animals of both sexes, when division has ceased in wild-type animals, some tissues continue to proliferate: seam cells of the lateral hypodermis divide in both sexes and, in the male, proliferation of the sex mesoblasts produces hundreds of small cells in the posterior part of the body (Fig. 7). Examination of adult males has indicated that similar proliferation may sometimes occur in the B and Pn.p lineages, but this process has not been observed directly. The gonad is occasionally abnormal; for example, we have observed apparently "loose" cells within the hermaphrodite uterus and limited growth of the male *vas deferens.*

All *lin-4* animals undergo six molts instead of the normal four; a few enter a seventh, which is not completed because of death resulting from internally hatched larvae. To see if additional molts might otherwise occur, the gonad was ablated in a number of individuals using a laser microbeam (Sulston and White, 1980); most animals nonetheless molted six, and a few seven, times. All cuticles are of the single-layered larval type. Starving *lin-4* populations do not form dauer larvae.

Usually, the first stage of vulval divisions occurs essentially normally, as in the vulvaless mutants discussed above. However, the pattern of subsequent divisions is distinctive. Some first-stage daughters divide only once, whereas others undergo three or more rounds of division, often in a stem-cell-like pattern. The ventral hypodermal cells produced seem to be less cooperative than in wild type.

The abnormal cell lineages of *lin-4* will be described elsewhere (Chalfie *et al.*, 1981).

DISCUSSION

Applications of the Mutants

We have described the phenotypes of mutants in 14 genes that affect the postembryonic cell lineages of *C.*

elegans. These mutants are useful in two distinct ways. First, each of them lacks certain cells and thus can help to determine the functions of these cells. Second, certain mutants may directly affect genetic elements that control development and so may reveal the nature of these elements.

Diagnosis of Cellular Function

(1) *lin-6*, which is defective in essentially all postembryonic cell divisions, has been employed to prove that no postembryonically derived cell is required for the neuronal reorganization that occurs during the L2 stage (White *et al.*, 1978). (2) *lin-5* and *lin-6* provide evidence that the seam cells are required for the formation of cuticular alae and for shrinkage during dauer larva formation (Singh and Sulston, 1978). (3) *unc-86* males both lack cephalic companion cells and fail to chemotax toward hermaphrodites, supporting the proposal that these male-specific neurons are involved in chemotaxis (Ward *et al.*, 1975; Sulston and Horvitz, 1977). (4) All mutants defective in ventral cord development (*lin-5, lin-6, unc-59, unc-83, unc-84, unc-85*) can move forward but not backward, indicating that at least some postembryonically derived ventral cord neurons control backward locomotion. (5) These ventral cord mutants also appear to undergo displacement and/or loss of the hsn neurons, which suggests that some ventral cord neurons may be necessary for hsn survival and/or development.

In interpreting the properties of mutants in this way, two points must be kept in mind. (1) The failure of a particular lineage does not necessarily lead to the absence of cells of the types normally generated from that lineage. For example, undivided cells in *lin-5, unc-59,* and *unc-85* animals often display characteristics of the progeny they normally would produce (Albertson *et al.*, 1978; J. White, personal communication). Furthermore, a functional structure may be formed from an abnormal complement of cells; for example, in *lin-3* eight daughters from P6.p can form a functional vulva, which in wild-type animals involves the 22 descendants of P5.p, P6.p, and P7.p. (2) A mutant may have defects of which we are not aware; thus, the phenotype of *unc-86* males suggests but does not prove that cephalic companion cells mediate sexual chemotaxis.

Developmental Boundaries

Regulation in *C. elegans* has been observed only within certain groups of cells that are similar in lineage history and morphology (Kimble *et al.*, 1979; Sulston and White, 1980). Such groups have been termed "equivalence groups," signifying the apparent equivalence in the developmental potential of their members. The cells of an equivalence group are not clonally related. They are, however, related by lineage history as lineally equivalent progeny derived from morphologically similar precursors; for example, the posterior daughters of P3–P8 constitute the vulval equivalence group. As has been noted previously (Kimble *et al.*, 1979), equivalence groups in the nematode may be analogous to polyclones in *Drosophila* (Crick and Lawrence, 1975): in both, nonclonally related groups of cells divide to generate specific regions (termed "compartments" in *Drosophila*) of the animal. In *C. elegans*, the cells of a given equivalence group resemble one another in structure and function and, as far as is known, are of similar lineage history (Sulston and White, 1980).

Some of the mutations leading to abnormal cell lineages appear to specifically affect members of a given equivalence group. For example, in hermaphrodites of multivulva strains only the members of the vulval equivalence group, P3.p–P8.p, are affected; all six of these cells undergo second-stage divisions in multivulva mutants, whereas only P5.p, P6.p, and P7.p do so in wild-type animals. Similarly, in wild-types males, P9.p–P11.p constitute a preanal ganglion equivalence group, and in males of multivulva strains, P9.p behaves like P10.p and P11.p. Thus, in both sexes, multivulva mutants appear to cause certain members of an equivalence group to behave like other members of that equivalence group. Vulvaless mutants similarly cause some members of the vulval equivalence group to behave like other members of the vulval equivalence group; in vulvaless mutants, P5.p–P7.p can fail to undergo second-stage divisions and instead fuse with the syncytial hypodermis, just as P3.p, P4.p, and P8.p normally do. In these cases, then, there is coincidence between the developmental boundaries observed by mutations and those observed during regulation after laser ablation. These boundaries also correlate with the behavior of the ventral hypodermal cells during normal development: in both sexes, those Pn.p ventral hypodermal cells not members of the equivalence groups fuse with the large hypodermal syncytium before the L2 stage (Sulston and White, 1980).

Males of multivulva strain CB1322 are additionally affected in a group of ventral hypodermal cells (P3.p–P6.p) that corresponds to a part of the vulval equivalence group, as defined by laser ablation experiments. Thus, in these animals a novel developmental boundary, not found in the wild type, has been established; Nomarksi observations indicate that the fusion program of these cells is similarly altered. Thus, in both wild-type and mutant animals, only those ventral hypodermal cells that fail to fuse with the hypodermal syncytium are capable of regulative potential, suggesting that fusion and regulative potential, may reflect two aspects of a single determinative event defining hypodermal cell fate. [Since regulative potential is revealed

by laser ablation experiments performed substantially before fusion occurs (Sulston and White, 1980), it is not fusion per se that prevents regulation.] In CB1322 males, the determination of P3.p–P6.p may be altered so that these cells assume fates normally associated with P9.p–P11.p in males and P3.p–P8.p in hermaphrodites.

The pleiotropies of other cell lineage mutants may reveal homologies that are present but not apparent in the wild type. For example, it seems plausible that the embryonic deirids and the postembryonic postdeirids, both of which are multiple in *unc-86*, are formed by similar lineages. Also, *unc-83* and *unc-84* reveal an unexpected identity between the juvenile subdorsal hypodermal cells and the postembryonic ventral cord precursors.

The Genetic Program of Development

The mutants we have described affect different cell lineages and vary substantially in the breadth and nature of their pleiotropies. How might we decide which of these mutants are likely to be affected in genes fundamental to the genetic program for development rather than altered in functions necessary for general cellular maintenance and growth? One possible criterion is specificity: mutants that affect specific lineages or that affect various lineages in a specific way seem to be reasonable candidates. However, apparent cellular or tissue specificity could reflect a differential requirement for a particular function. For example, mutations affecting the *rudimentary* locus in *Drosophila* appear superficially specific for wing development; these mutants have low levels of the enzymes involved in pyrimidine biosynthesis, and their phenotype probably reflects a relatively high requirement for pyrimidines in the development of the wing (Norby, 1973; Jarry and Falk, 1974; Rawls and Fristrom, 1975). Apparent specificity could also result from perdurance effects (Garcia-Bellido and Merriam, 1971): a low level of a general cellular function expressed early in development might become apparent only after cell division and growth reduce its concentration below a critical threshold value; such a lag in phenotypic expression is analogous to the way in which maternal functions in an oocyte can allow development of a genotypically defective zygote (e.g., Hirsh *et al.*, 1977). Thus, although specificity may reveal genes that are involved in the logic of development, it is by no means a rigorous criterion. In fact, so little is known about the partitioning of the genetic program for development that it remains possible that mutations in genes that control development will result in grossly pleiotropic effects.

Besides specificity, the nature of the effect of a mutation offers a possible criterion for choosing which mutants are likely to be affected in fundamental steps in development. For example, homoeotic mutants, which have a replacement of one body structure by another found elsewhere in the animal, are probably altered in specific determinative events (e.g., Morata and Lawrence, 1977). Similarly, any nematode mutant in which a cell (or group of cells) is transformed from its normal fate to a fate normally associated with another cell would be of particular interest.

Based upon these criteria, the mutants with general effects on cell division (*lin-5, unc-59, unc-85*) seem likely to be altered in cellular maintenance or growth, or perhaps, in the mechanics of cell division. *lin-6* could be primarily defective in DNA synthesis. *lin-5* and *lin-6*, which fail in essentially all postembryonic somatic cell lineages, are derived from heterozygotes and may well undergo normal embryogenesis because of maternal effects; their gene products (or at least a consequence of their products) may be present in oocytes and fail to function as they become diluted by postembryonic growth. Together, *lin-5* (which fails in cell division but not in DNA replication) and *lin-6* (which fails in DNA replication but not in cell division) suggest that in *C. elegans* replication and division are independent processes. *unc-59* and *unc-85* display abnormal nuclear fusions as well as abnormal cell divisions. Nonetheless, their primary defect could be in cytokinesis, as these nuclear fusions may occur only because multinucleate cells have been formed by aberrant cell divisions.

The *unc-83* and *unc-84* gene products seem to be specifically required for the movements and divisions of certain hypodermal nuclei. Both in the embryogenesis of the syncytial hypodermis and in the postembryonic development of the ventral cord, the cells affected in *unc-83* and *unc-84* animals develop cytoplasmic protrusions superficial to the body muscles. In the wild type, the nuclei move along these protrusions, whereas in the mutants, cytoplasmic protrusions form normally, but the nuclei fail to move. These nuclear migration mutants display considerable specificity. First, only the movements of two classes of hypodermal nuclei are affected. Second, the seven mutants defective in nuclear migrations define only two genes (Horvitz and Sulston, 1980), suggesting that this phenotype does not result from general alterations in cellular metabolism.

The vulvaless mutants (*lin-2, lin-3, lin-7*) are affected only in the ventral hypodermal cell divisions involved in vulva development in the hermaphrodite. The multivulva mutants (*lin-1* and *lin-8; lin-9*) are affected in ventral hypodermal cell division in both sexes as well as in a few other characteristics. As discussed above, in both vulvaless and multivulva mutants, certain cells are altered to behave like certain other cells. Thus, these mutants display the specificity and other attributes expected from alterations in primary developmental events.

The mutants with reiterative cell lineages (*unc-86, lin-4*) are perhaps the most intriguing of all. Although these mutants affect multiple cell lineages, at least in the case of *unc-86* these effects are all of a single, highly specific nature. Both mutants are altered so that specific cells assume fates normally associated with certain of their own ancestors, leading to a reiterative proliferation of particular cell types. As will be discussed elsewhere (Chalfie *et al.*, 1981), such alterations can be interpreted as changes in specific determinative events; the temporal reiteration of cells and cell groups may be analogous to the spatial reiterations seen in homoeotic mutants of insects. The existence of these mutants suggests that the development of *C. elegans* may normally proceed by a series of switches in cell fate that accompany each cell division during the course of a cell lineage; these mutants appear to fail in executing certain of these determinative switches. Further examination of these mutants and others like them may help reveal more about the organization of the genetic program for development as well as the mechanisms by which genes act to control determination and differentiation in a nematode.

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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 alteratiions 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 nonparasitic 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 hatchee. Or, the precursor cells might

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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 auxillary 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-

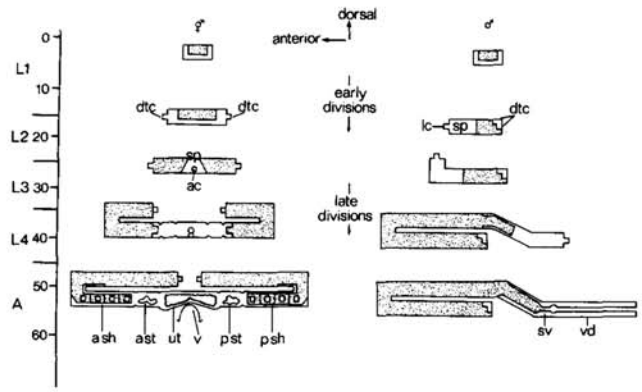

FIG. 1. Postembryonic development of the hermaphrodite (left) and male (right) gonads (based on Hirsh *et al.* (1976) and Kimble and Hirsh (1979)). Coordinates of the animal are indicated at the top. The morphology of the developing gonad is shown at consecutive stages of development from top to bottom 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 constitutent 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

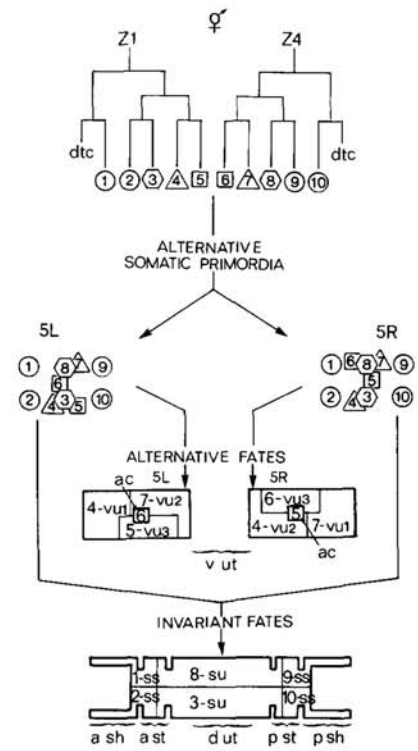

FIG. 2. Ancestry and fates of cells in the hermaphrodite somatic primordium (based on Kimble and Hirsh, 1979). Anterior is to the left for all diagrams. The early divisions of Z1 and Z4 (top) generate 12 cells in hermaphrodites. Ten of these cells, named SP1-SP10 but represented by only the relevent 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).

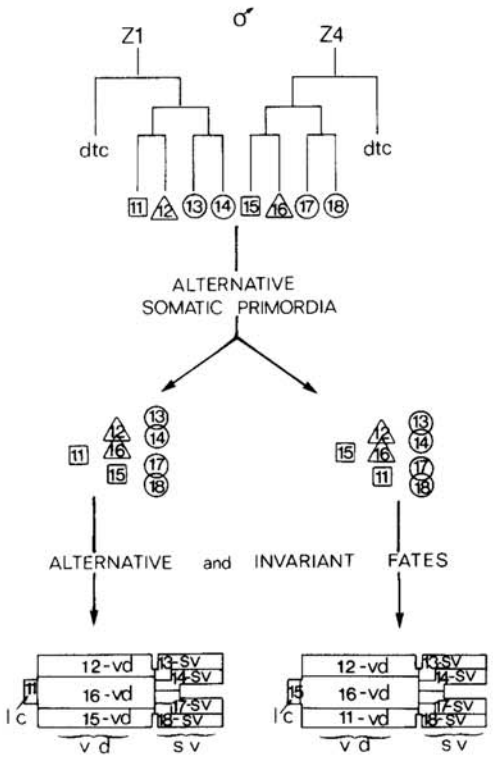

FIG. 3. Ancestry and fates of cells in the male somatic primordium (based on Kimble and Hirsh, 1979). Anterior is to the left for all diagrams. The early divisions of Z1 and Z4 (top) generate 10 cells in males. Eight of these cells, named SP11-SP18 but represented by only the relevent number here, become 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 twofold 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 +	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.

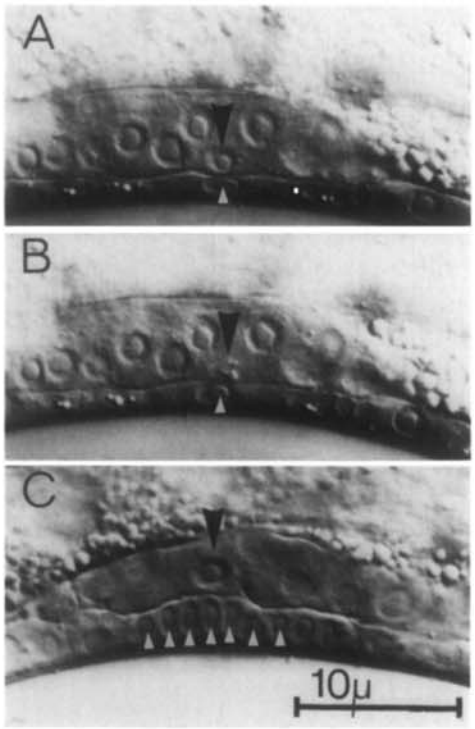

FIG. 4. Anchor cell regulation, Nomarski optics, lateral view. (A) Presumptive anchor cell (black arrowhead) in an unoperated hermaphrodite in L2 lethargus. (B) Debris of same cell as in A (black arrowhead), just after the cell's ablation. (C) Anchor cell (black arrowhead) derived by replacement regulation 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.

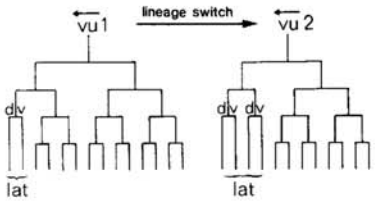

FIG. 5. Lineage switch in the nonanchor cell pair. The cell expected to follow the vul lineage (left) follows the vu2 lineage (right) instead. The vu2 lineage makes two more lateral uterine cells (lat) and contributes four fewer cells to the main body of the uterus. The polarity of the lineage, indicated 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

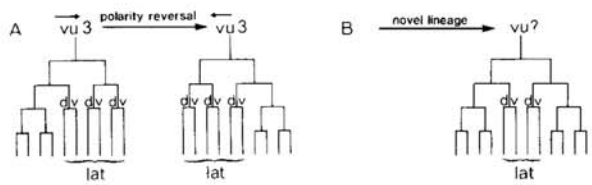

FIG. 6. Two lineage changes observed in SP5 (5L) or SP6 (5R) after ablation of its sister. (A) Vectorial regulation simply reverses the polarity of the vu3 lineage so that the three pairs of lateral uterine cells (lat) are produced in the anterior part of the lineage instead of the posterior part as 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.

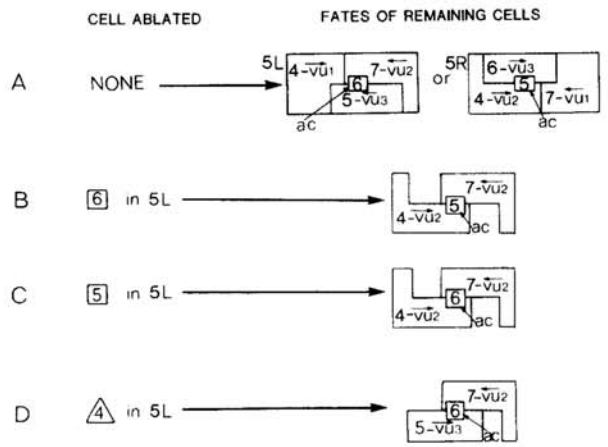

FIG. 7. Summary of regulation in the ventral uterine/anchor cell group of precursors. The ablated cell is shown in the left column; the contribution of each precursor to the ventral uterus is shown in the right column as dorsal views. Fate names: vu1, vu2, and vu3, are three unique ventral uterine lineages (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

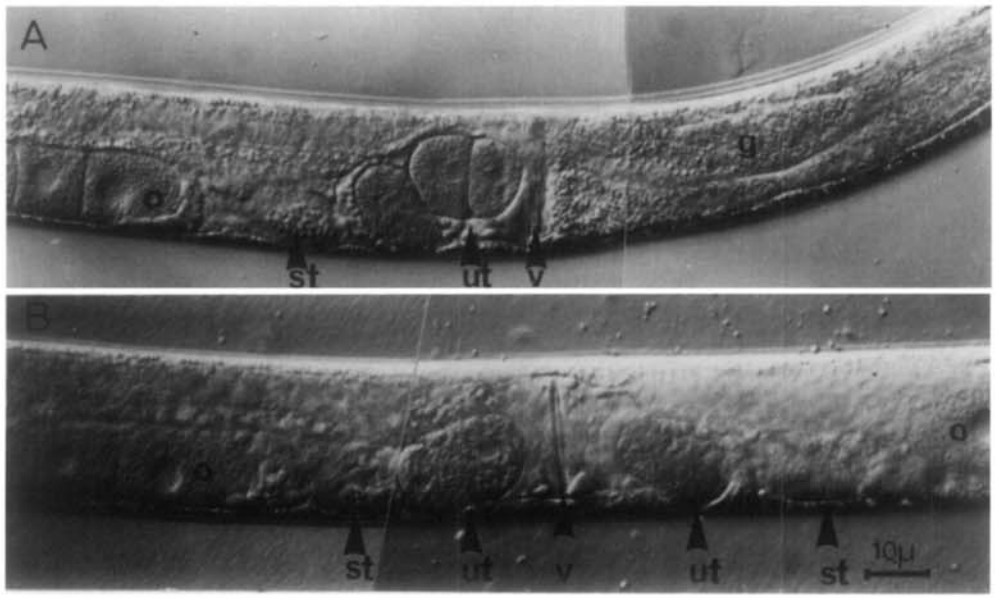

FIG. 8. Formation of an anterior half-gonad after ablation of Z4; Nomarski optics. Adult animals are shown in ventral view; anterior is to the right. (A) Experimental animal. A functional half-gonad is formed anterior to the vulva, whereas the region posterior to the vulva is occupied only by the gut (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 individual 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

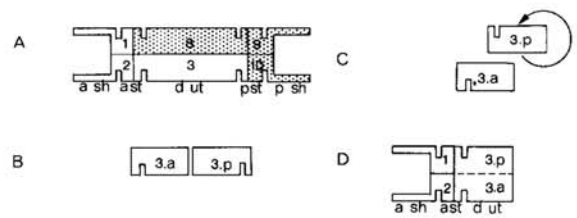

FIG. 9. Vectorial regulation after ablation of Z4. Somatic structures are named as in Figs. 1 and 2. (A) Fates of Z1 (clear) and Z4 (stippled) descendants in the unoperated animal. Removal of Z4 would, without some change, lead to the formation of asymmetric somatic structures. (B) The two daughters 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.

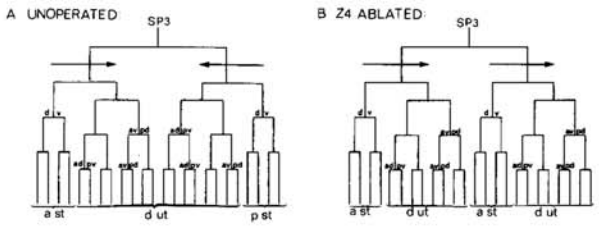

FIG. 10. Lineages followed by SP3 in the unoperated animal (A) and after ablation of Z4 (B). The arrows point to the larger daughter in the asymmetric division of SP3.a and SP3.p. The contribution of the descendant cells to adult structures is indicated below each lineage tree. Somatic structures are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

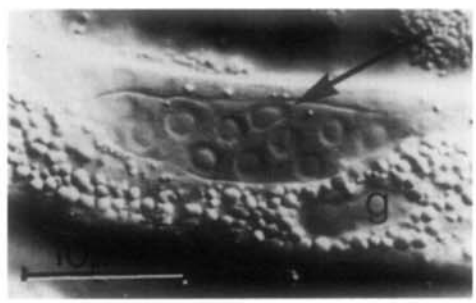

FIG. 11. Isolated sheath-spermathecal precursor; Nomarski optics. Arrow indicates SP1 in a late L2 gonad after ablation of Z4 and Z1.p during L1. The precursor's sister, a distal tip cell, occupies a position at the anterior tip of the developing gonad, and is not seen here. The other nuclei seen in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

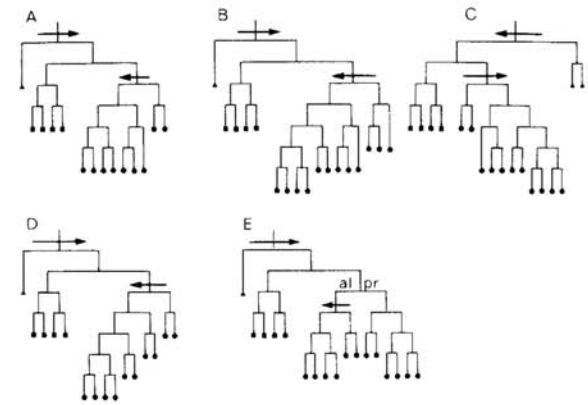

FIG. 12. Lineages of isolated sheath-spermathecal precursors. All lineages have been drawn in the orientation of SP1 for ease of comparison. Arrows point to the larger cell of an asymmetric division. Geometric symbols indicate the fate of each descendant: ●, spermathecal cell; ■, proximal sheath cell; ▲, 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

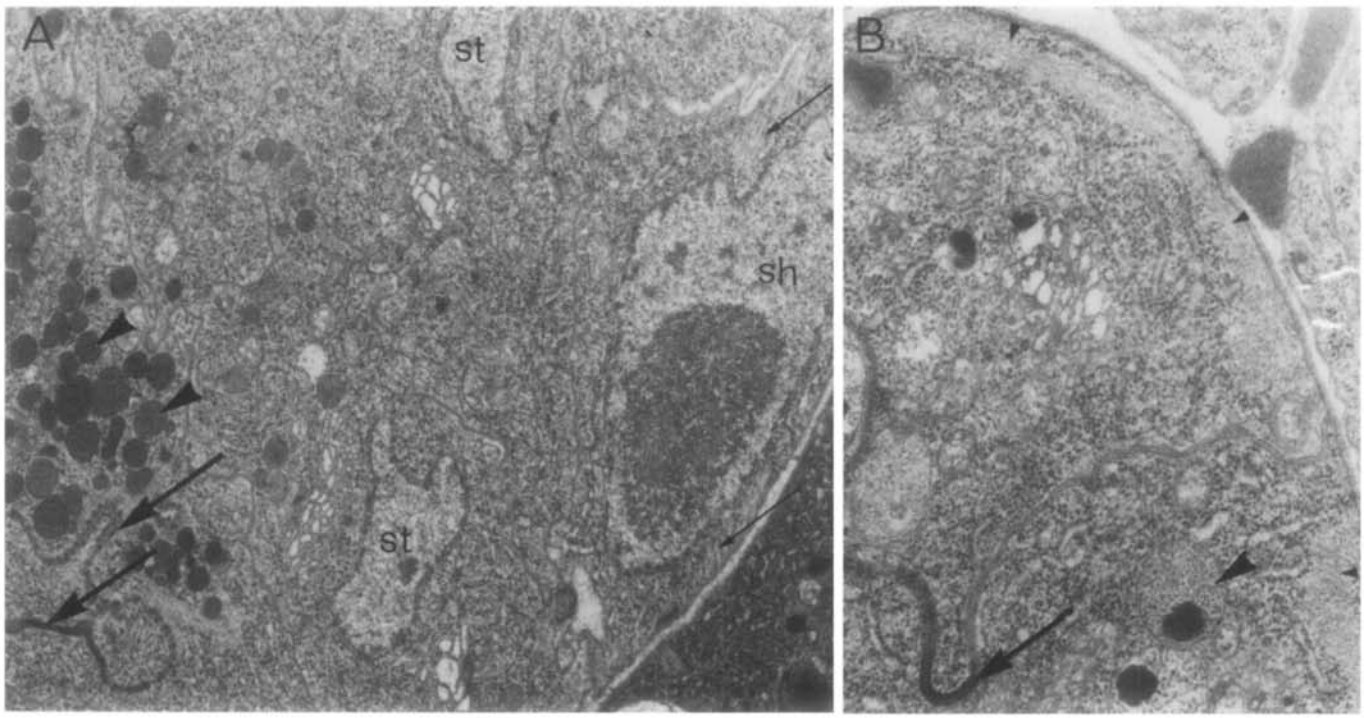

FIG. 13. Electron micrographs of descendants of an isolated sheath-spermathecal precursor (that of lineage shown in Fig. 12C). (A) Cells classified as spermathecal cells by light microscopy exhibit secretory granules (arrowheads) and specialized junctions (large arrows) typical of spermathecal cells 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×. (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×.

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.

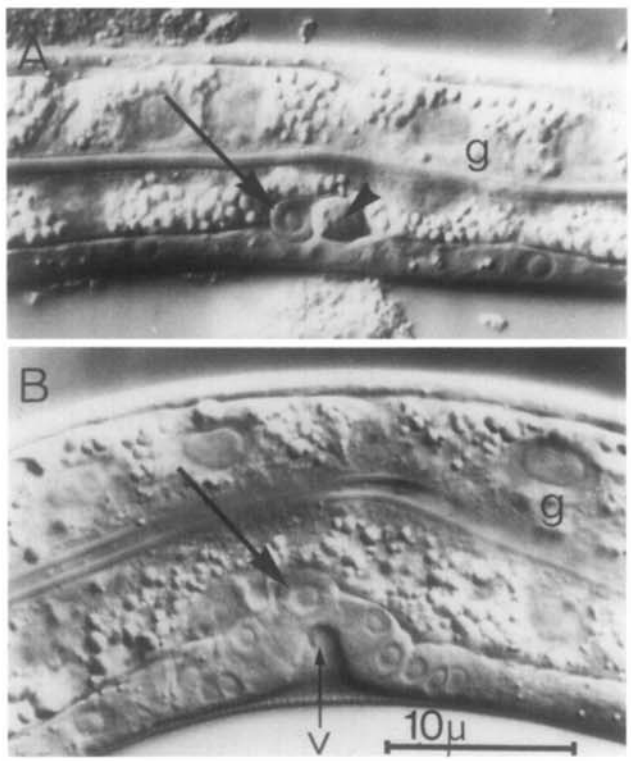

FIG. 14. Isolation of an anchor cell precursor; Nomarski optics. g, Gut. (A) Anchor cell (arrow) is the only remaining cell in the gonad. The debris left by the ablation of six cells (precursors to the rest of the gonad killed in L1 and early L2) is minimal (arrowhead). (B) Isolated anchor cell (large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

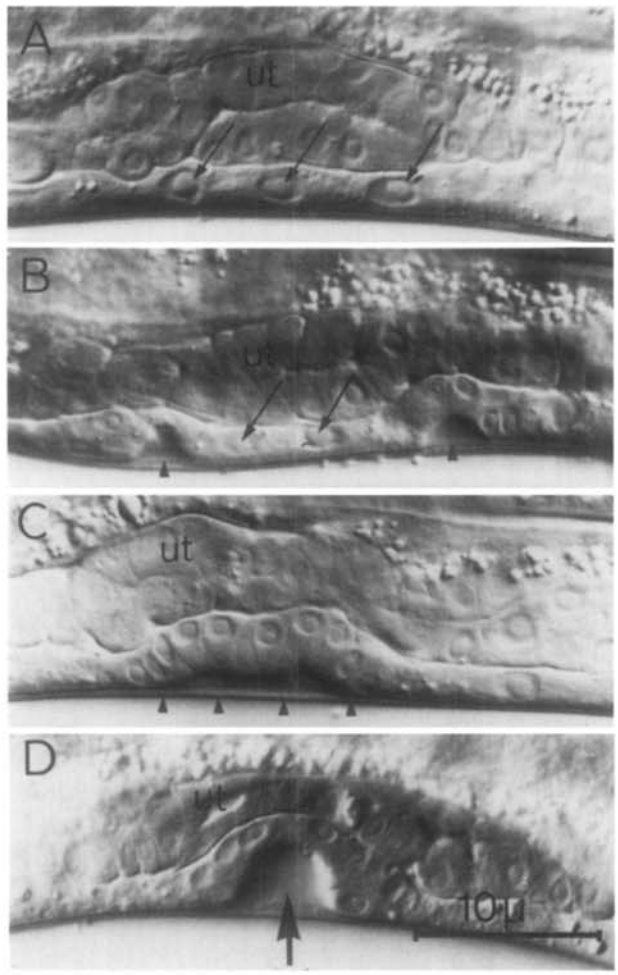

FIG. 15. Morphology of hypodermis in prospective vulval region after ablation of anchor cell; Nomarski optics. All photographs show early L4 animals, lateral view. (A) No induction. Arrows point to descendants of the first-stage division of the normal vulval precursors which, in this case, have not divided 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

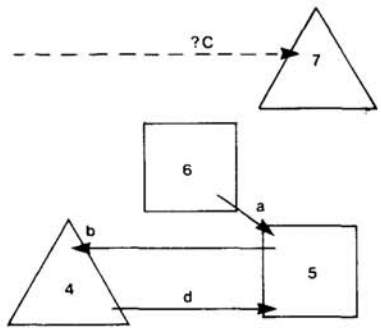

FIG. 16. Proposed cell-cell interactions in the ventral uterine-anchor cell group. The anchor cell pair are squares, and the nonanchor cell pair are triangles; cells are numbered as in Fig. 2. The *proposed* interactions among this group of cells are as follows: The anchor cell(SP6) inhibits its alternative 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 regulatioin, 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 suggests 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).

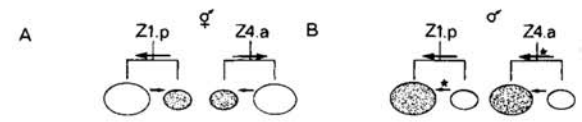

FIG. 17. Alterations in cell polarity during normal development. Z1.p and Z4.a divide asymmetrically in both sexes. The larger arrows point to the larger daughter of the division, and the smaller arrows point to the daughter that produces an anchor cell or linker cell (represented by stippling). The 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.

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 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.

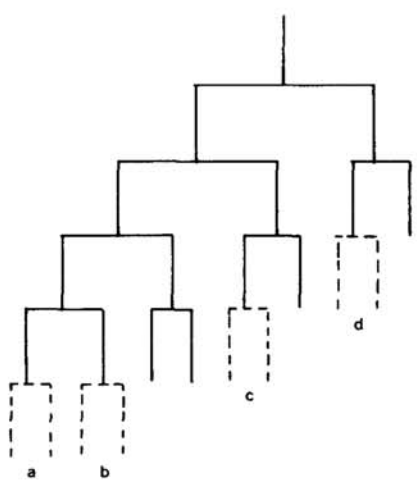

FIG. 18. Backbone of divisions (solid lines) proposed as a simple program of proliferation for the spermathecal precursor (a sublineage of the sheath-spermathecal precursor). The addition of some, or all, of certain divisions (dashed lines) to this backbone generates the lineage observed in the unoperated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

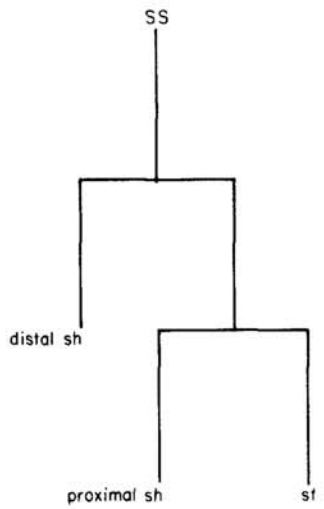

FIG. 19. Generation of distal sheath, proximal sheath, and spermathecal precursors by the sheath-spermathecal lineage. This pattern is invariant in all lineages so far observed in *C. elegans* (see Fig. 12), and has also been observed in *Panagrellus redivivus* (Sternberg and Horvitz, 1981). Since it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

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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Gonadal Cell Lineages of the Nematode *Panagrellus redivivus* and Implications for Evolution by the Modification of Cell Lineage

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To explore the nature of cell lineage modifications that have occurred during evolution, the gonadal cell lineages of the nematode *Panagrellus redivivus* have been determined and compared to the known gonadal lineages of *Caenorhabditis elegans* (J. Kimble and D. Hirsh, 1979, *Develop. Biol.* **70**, 396-417). Essentially invariant lineages generate the 143 somatic cells of the male gonad and at least 326 somatic cells of the female gonad of *P. redivivus*. The basic program of gonadogenesis is strikingly similar among both sexes of both species. For example, the early division patterns of the somatic gonad precursors Z1 and Z4 are almost identical. Later division patterns are more divergent and, in a few cases, generate structures that are species specific. In general, similar cell types are produced after similar patterns of cell divisions. Differences among the Z1 and Z4 cell lineages appear to reflect phylogenetic modifications of a common developmental program. The nature of these differences suggests that the evolution of cell lineages involves four distinct classes of alterations: switches in the fate of a cell to that normally associated with another cell; reversals in the polarity of the lineage generated by a blast cell; alterations in the number of rounds of cell division; and an "altered segregation" of developmental potential, so that a potential normally associated with one cell instead becomes associated with its sister. A number of cell deaths occur during gonadogenesis in *P. redivivus*. The death of Z4.pp, a cell that controls the development of the posterior ovary in *C. elegans*, probably prevents the development of a posterior ovary in *P. redivivus* and hence is responsible for the gross difference in the morphologies of the gonads of the *P. redivivus* female and the *C. elegans* hermaphrodite. As exemplified by the death of Z4.pp, an alteration in the fate of a "regulatory cell" could facilitate rapid and/or discontinuous evolutionary change.

INTRODUCTION

The essentially invariant nature of nematode development was recognized many years ago (e.g., Wilson, 1925; Pai, 1928). This invariance has facilitated the recent elucidation of the complete postembryonic (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979) and most of the embryonic (Deppe *et al.*, 1978; J. Sulston and E. Schierenberg, personal communication) cell lineages of the free-living nematode *Caenorhabditis elegans*. One striking characteristic of these lineages—a strong correlation between lineage history and cell fate—has led to the suggestion that a specific pattern of cell divisions may be necessary for the generation of a particular cell type. Animals in which the normal cell lineages have been perturbed, either by mutation (Horvitz and Sulston, 1980; Sulston and Horvitz, 1981; Chalfie *et al.*, 1981) or by physical ablation using a laser microbeam (Sulston and Horvitz, 1977; Kimble *et al.*, 1979; Sulston and White, 1980) have provided additional evidence that history and fate are causally related.

The relationship between lineage history and cell fate could be explored further by examining cell lineages in other nematode species. For example, the morphologies and functions of lineally equivalent cells in different species can be compared. Such comparative studies might also reveal aspects of the genetic control of cell lineage (differences in lineages presumably would reflect differences in the underlying genetic programs) as well as indicate how divergence in cell lineage may relate to the evolution of different nematode species.

The "sour paste nematode" *Panagrellus redivivus* (order: Rhabdita; family: Panagrolaimidae) is an appropriate organism for comparison with *C. elegans* (order: Rhabdita; family: Rhabditidae) (Goodey, 1963). Much is known about the development, anatomy, and behavior of *P. redivivus* (e.g., Samoiloff and Pasternak, 1968; Yuen, 1968; Leushner and Pasternak, 1975; Pollock and Samoiloff, 1976; Duggal, 1978a-d). Hechler (1970, 1971) has described its gross morphology, life history, and aspects of its postembryonic development. Sulston and Horvitz (1977) observed patterns of postembryonic cell division in *P. redivivus* similar to those seen in *C. elegans*.

Despite many similarities in anatomy and behavior, *P. redivivus* and *C. elegans* differ in a number of respects. *P. redivivus* adults are about twice as large as *C. elegans* adults. *P. redivivus* is dioecious, with males and females both needed for reproduction; *C. elegans* is a self-fertilizing hermaphrodite that occasionally generates males capable of mating with hermaphrodites but not essential for reproduction. The *P. redi-*

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vivus female has a single ovary directed anteriorly from the vulva; the *C. elegans* hermaphrodite has two ovaries located symmetrically about the vulva. *P. redivivus* females do not lay eggs, but instead release first-stage larvae from their uteri; *C. elegans* hermaphrodites lay fertilized eggs.

Since the gross differences in gonad morphology and function between *C. elegans* and *P. redivivus* might reflect differences in cell lineage, we decided to examine the postembryonic cell lineages of *P. redivivus*. In this paper, we report the gonadal cell lineages of the *P. redivivus* female and male, and compare these lineages to the known gonadal lineages of the *C. elegans* hermaphrodite and male (Kimble and Hirsh, 1979).

MATERIALS AND METHODS

Nematode strains and handling. P. redivivus was obtained from D. J. Hooper (Rothamsted Experimental Station, Harpenden, Hertfordshire, England). Nematodes were grown on lawns of *Escherichia coli* OP50 on NG agar at 20°C as described for *C. elegans* (Brenner, 1974). Stocks were maintained by crossing several males and females. Nematodes were handled as described by Brenner (1974) and Sulston and Horvitz (1977).

Observation of living specimens. For long-term observation the technique of Sulston and Horvitz (1977) was used, as modified by Sulston *et al.* (1980): coverslips (18 × 18 mm) were sealed with silicon vacuum grease; S medium (Brenner, 1974) contained cholesterol (5 mg/liter) without added polyvinyl pyrrolidine. For short observations (up to 1 hr) nematodes were mounted according to Sulston and Horvitz (1977), but the agar was not trimmed and coverslips not sealed (rapid mount). Newly hatched L1's (first stage larvae) were obtained as described below.

Nematodes were observed using a Zeiss Universal microscope equipped with a Plan 100 objective and Nomarski differential interference contrast optics, including a heat filter and a narrow band (546 ± 2nm) green interference filter. Air temperature was maintained at 20°C. Cell lineages were determined by continuous observation of nuclei as they divided, migrated, differentiated, or died during postembryonic development. Lineages reflect the behavior of nuclei, as cell boundaries are often not visible with Nomarski optics. Sketches were made as often as necessary, as described by Sulston and Horvitz (1977) and Kimble and Hirsh (1979). Approximately 350 animals were observed during the course of this study. Of these, extensive lineages were followed in 82. Lineages were compiled from overlapping observations in many animals and verified by following the complete lineage at least twice. We believe that, except where otherwise noted, the lineages represent all the postembryonic somatic cell divisions in the gonad.

Most individuals were observed for several days; worms were routinely refrigerated overnight for 5–16 hr to retard development. Nematodes were always refrigerated on a fresh petri plate seeded with OP50. If transfer was rapid, fewer than 45 min of development were missed after 12 hr of refrigeration.

Timing and sexing. A synchronous population (±2 hr) could be obtained by cutting open several adult females with two syringe needles and releasing eggs and larvae into 20 μl of M9 buffer. The buffer was pipetted onto a petri plate, and the larvae were removed. Larvae from eggs that hatched within 3 hr were transferred to a fresh plate and maintained at 20°C. Several individuals were observed with Nomarski optics every 6 hr to ascertain stages.

L1, L2, L3, and L4 refer to the first through fourth larval stages, respectively. The L*n* ecdysis is the moment the head breaks free of the L*n* cuticle. Ecdyses are shown by horizontal lines inside the time axes on lineage charts. Lethargus (denoted by stippling along the time axis) is the quiescent period preceding each ecdysis. The molt refers to lethargus and ecdysis. L1, L2, L3, and L4 ecdyses are at 27, 41, 56, and 80 hr posthatching for the female and at 27, 41, 55, and 74 hr posthatching for the male.

Newly hatched larvae, dissected from the uterus or hatched *in utero*, can be sexed by criteria similar to those used to distinguish young *C. elegans* hermaphrodites and males (Sulston and Horvitz, 1977): the presence of a female-specific neuron—analogous by sex specificity and position to the hermaphrodite-specific neuron of *C. elegans*—in the female and the presence of an enlarged B cell in the male.

Laser microsurgery. Nematodes for laser microsurgery were placed into 1% (v/v) 1-phenoxy-2-propanol in M9 (Brenner, 1974). When the nematode stopped moving (0.5–3 min for L3 or younger), it was transferred using a pulled capillary to a rapid mount without bacteria. Animals were thus recovering from the anaesthetic as microsurgery was performed.

The laser microbeam system used in this study was similar to that described by Sulston and White (1980). Differences were as follows. The beam from a Phase-R (New Durham, N. H.) Model DL-2100A tunable coaxial flashlamp pumped dye laser with a 1-μsec pulse duration and maximum energy output of about 1 J/pulse was directed into a Zeiss standard microscope through a IV Fl epi-illuminator. The beam was focussed onto the plane of the field diaphragm of the epi-illuminator by an American Optical 8× eyepiece and was reflected by a Zeiss FT510 dichroic reflector to the Plan 100 ob-

jective. The laser was aligned and centered in the microscope using a coaxial auxillary 0.5 mW Spectra-Physics Model 155 He/Ne laser. The beam from the He/Ne laser was directed to coincide visually with a point marked on an eyepiece micrometer. The cell to be ablated was brought into focus and centered under the predetermined point on the eyepiece. Several pulses of the dye laser (15–17 kV) generally caused visible damage to the desired cell. The state of the cell and that of surrounding tissue was verified 1–3 hr after surgery. The dye used was coumarin 480 (Blue 6; Phase-R) at 1.5×10^{-4} *M* in methanol.

Photography. Nomarski photomicrographs were obtained using a Zeiss Universal microscope as described above, except that no heat or green interference filters were used. Nomarski photographs were taken on Kodak Technical Pan 2415 film with a Zeiss microflash illuminator. Low-power, bright-field photomicrographs were taken on Ilford FP4 film with a Planapo 10 objective and standard illumination from a 12-V halogen bulb.

Camera lucida. Camera lucida drawings were made using Nomarski optics with a Zeiss camera lucida and Plan 100, Planapo 40 or Plan 16 objectives as appropriate. Measurements were obtained using a calibrated ocular micrometer. Nematodes were anesthetized as described above.

Nomenclature. Nomenclature and cell lineage charts are as described by Sulston and Horvitz (1977), as modified for the gonad by Kimble and Hirsh (1979). In the lineage diagrams anterior is drawn to the left and posterior to the right, except where otherwise indicated. Abbreviations used are: a, anterior; p, posterior; d, dorsal, v, ventral; l, left; r, right; i, internal (medial); and e, external (lateral). Oblique division axes are indicated by a combination of letters. As the gonad reflexes posteriorly, the axes of divisions are labeled as they would be if the gonad continued to grow anteriorly, even at the point of reflex. Consequently, a cell in the male vas deferens may divide anterioposteriorly but will be recorded as a posterioanterior division if the gonad is reflexed at the location of the dividing cell. Thus, anterior is proximal in the male and distal in the female (except in the postvulval sac). Proximal and distal refer to the end of a gonadal arm nearest and farthest, respectively, from the opening to the outside, i.e., from the vulva in the female and cloaca in the male. Cells are named according to the sequence of divisions of their ancestral progenitors. Some blast cells are redefined by two capital letters for ease of reference. Other cells—e.g., anchor cell (ac), distal tip cell (dtc), linker cell (lc)—are named as in *C. elegans* based on functional and morphological criteria, as seen with Nomarski optics. Generally, structures are named conforming to the usage established for *C. elegans* (Hirsh *et al.*, 1976; Kimble and Hirsh, 1979). However, to distinguish the epithelium of the ovary from the oviduct we define the sheath as the epithelium of the ovary not including the oviduct.

RESULTS

GROSS ANATOMY OF THE *P. redivivus* FEMALE AND MALE GONADS

Adult Female

The repoductive system of the adult female consists of a uterus, spermatheca (seminal receptacle), oviduct, and ovary directed anteriorly from a vulva situated somewhat posterior to the center of the animal (Fig. 1). A postvulval sac extends posteriorly from the vulva halfway to the anus. The main gonadal arm reflexes dorsally and posteriorly at the spermatheca. Oocytes move proximally (toward the vulva) through the oviduct, are fertilized in the spermatheca and pass into the uterus (Duggal, 1978d). Eggs hatch *in utero*, and L1 (first larval stage) larvae are ejected through the vulva.

Adult Male

The adult male reproductive system includes a ventrally located testis, which reflexes dorsally and posteriorly and is continuous with a seminal vesticle (Fig. 1). The seminal vesicle connects with a long vas deferens. The cloaca-proximal part of the vas deferens is a distinct ejaculatory duct. The ejaculatory duct and the lumen of the intestine join at the cloaca. The male tail contains sensory papillae, spicules, and a gubernaculum (Hechler, 1971).

GENERAL OUTLINE OF GONAD DEVELOPMENT

The gonad primordium consists of four cells arranged anterioposteriorly (Fig. 2). The primordium is located ventrally and mostly on the right side of the animal. These four cells are named Z1, Z2, Z3, and Z4, from anterior to posterior, respectively, by analogy with those in the *C. elegans* male and female. As in *C. elegans*, Z1 and Z4 generate the somatic structures of the gonad by essentially invariant lineages and Z2 and Z3, which are germ cell precursors, divide in a variable manner. We will concentrate on the somatic lineages.

There are four stages in the development of the somatic gonad. First, Z1 and Z4 divide to generate a total of 12 descendants (late L1 to middle L2). Second, most of these cells rearrange to form a structure called the somatic primordium (Kimble and Hirsh, 1979)(late L2). Third, cells of the somatic primordium divide further (early L3 through middle L4). Fourth, the gonadal cells

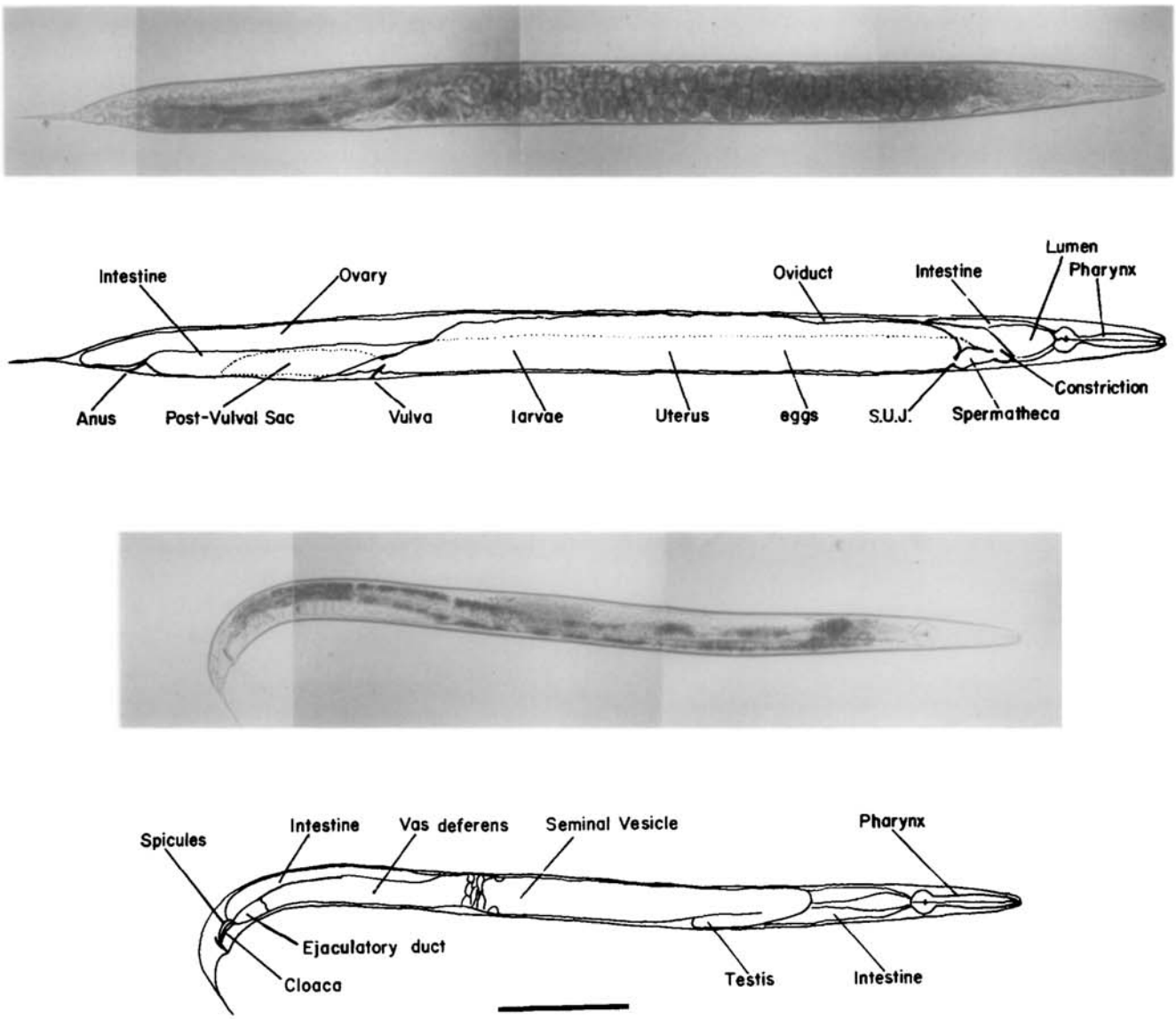

FIG. 1. Gross anatomy of the female (above) and male (below), right lateral views. Bright field photomicrographs with tracings. Bar = 0.2 mm. S.U.J., spermathecal/uterine junction. The two rows of dark granules visible behind the male and female gonads are intestinal. Dotted lines represent the boundary of the ovary or postvulval sac obscured by the intestine or uterus. The *P. redivivus* gonads lie mainly on the right side; thus most photographs and drawings are right lateral views, with anterior to the right; unfortunately, the convention for lineage diagrams (Sulston and Horvitz, 1977) has anterior to the left.

differentiate to form the adult structures (L4 to early adult). The morphological changes during gonad development are shown in Fig. 3. Although the timing of gonad divisions varies slightly from animal to animal, the gonad provides a convenient way to determine the stage and sex of larval animals.

FEMALE Z1 AND Z4 LINEAGES

Early Period

Beginning in late L1, Z1 and Z4 divide and produce six descendants each by the middle L2 (Figs. 4 and 5). Although Z1 and Z4 undergo similar patterns of division, the divisions of Z1 and its descendants occur before those of Z4 and its descendants. Z1.aa remains at the anterior-most tip of the primordium, while the other Z1 descendants move posteriorly past Z2 and Z3 toward the Z4 descendants. The division of Z4.p is asymmetric, producing a very small posterior daughter, Z4.pp, which becomes refractile and dies soon after it is formed (Fig. 6a). Its death follows the same series of events that characterizes the programmed cell deaths that occur in *C. elegans* (Sulston and Horvitz, 1977).

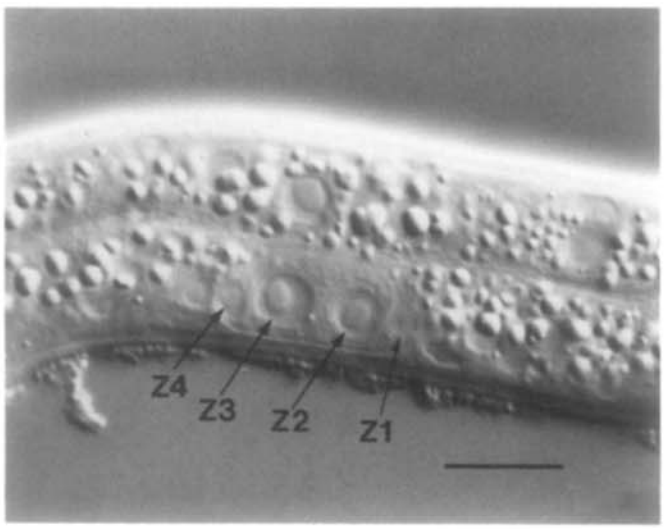

FIG. 2. Gonad primordium in anesthetized young L1 larvae, right lateral view, Nomarski optics. Bar = 0.01 mm. Anterior is to the right.

Somatic Primordium

In late L2, all surviving descendants of Z1 and Z4 except Z1.aa form the female somatic primordium at the posterior end of the gonad (Fig. 5g). Z1.aa remains at the anterior end and becomes the distal tip cell (dtc), which leads the growing ovary and has a small and distinct nucleolus. Z4.aaa, which is formed at a position to the right and anterior (Fig. 5), moves central and posterior to lie just anterior to Z1.ppp on the ventral edge of the primordium. In all six females studied, Z4.aaa became the anchor cell (ac). (In *C. elegans*, Z4.aaa does not always become the anchor cell.) The other nine Z1 and Z4 descendants divide further.

Late Period

At the L2 ecdysis, all cells in the somatic primordium except the ac begin a series of divisions that generate the somatic structures of the female gonad. Figure 7a indicates the positions of these cells and their names based upon these positions. The descendants of these cells maintain the relative distoproximal order of their progenitors (Fig. 7b).

AR and AL lineages. Two precursors, AR (Z1.ap) and AL (Z1.paa), each generate 44 descendants that form the sheath, oviduct, constriction between the oviduct and spermatheca, and half of the spermatheca (Fig. 8a).

AR.a and AL.a each divide in the pattern of a posterior parental reiteration (Chalfie *et al.*, 1981), i.e., each posterior daughter produces an anterior daughter like its sister and a posterior daughter like itself; each reiterated anterior daughter migrates distally over the germ nuclei and divides twice. The axes of these divisions vary from animal to animal. In general, an anterioposterior first division is followed by oblique divisions or vice versa. Each anterior daughter of the posterior reiterative divisions generates two dorsal and two ventral nuclei. These nuclei, which make up the sheath, move distally as the ovary grows and spread evenly over it.

AR.p and AL.p each divides asymmetrically, generating a larger anterior daughter. The anterior daughters (AR.pa and AL.pa) divide before the posterior daughters, but the posterior daughters undergo up to five rounds of division as opposed to four for the anterior daughters. As the gonad twists at the region of the developing oviduct during L3 lethargus, the axes of the last two rounds of division of AR.paa and AL.paa vary from dorsoventral to left-right; the axes are perpendicular to the longitudinal axis of the gonad. The 16 cells that will form the oviduct remain compressed in what Hechler (1970) has described as a "plicated structure" proximal to the ovary and distal to the spermatheca (Fig. 9A). A slight constriction separates the oviduct from the ovary. The oviduct expands greatly in

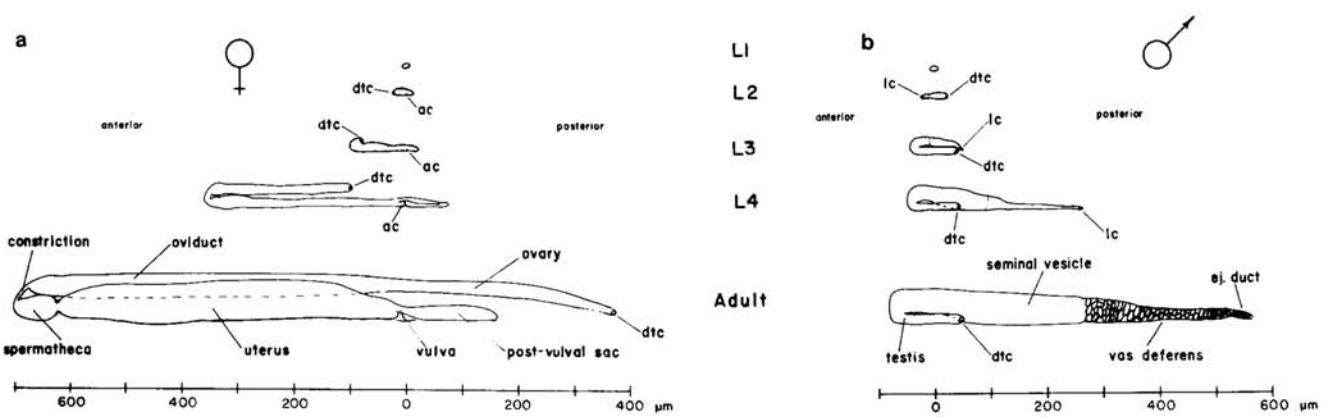

FIG. 3. Overview of (a) female and (b) male gonad development, left lateral views, adapted from camera lucida drawings. Gonads depicted are from typical individuals approximately in the middle of each larval stage and from young adults.

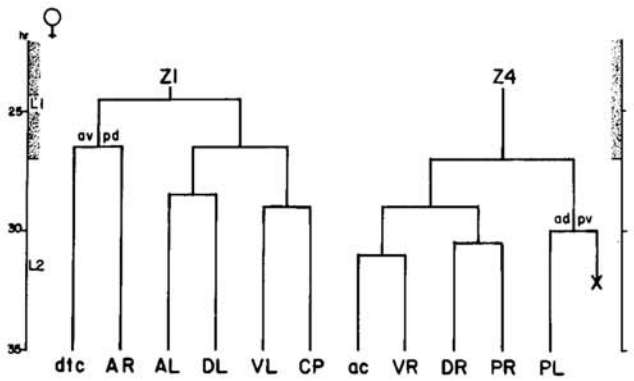

FIG. 4. Female early Z1 and Z4 lineages. Vertical scale indicates hours from hatching. Stippling denotes the period of lethargus. See Materials and Methods for abbreviations. dtc, Distal tip cell; AR, AL, DL, VL, CP, VR, DR, PR, and PL, blast cells that generate most of the somatic cells of the gonad are named by their relative positions in the somatic primordium (see Fig. 7); ac, anchor cell; X, programmed cell death.

the adult, the extent depending on the number of unfertilized oocytes (e.g., Duggal, 1978c).

AR.pap and AL.pap generate eight descendants each. The anterior six descendants of each are arranged in two distoproximal rows and form the cells of the constriction that connects the oviduct with the spermatheca (Fig. 9A). The posterior two descendants of each form the distal-most cells in the spermatheca.

AR.pp and AL.pp divide in a pattern similar to that of a posterior parental reiteration. The anterior daughter from the first division divides twice, whereas those from the next three divisions divide only once. The 24 descendants constitute the distal half of the spermatheca and the spermathecal/uterine junction (Fig. 9B).

DL, DR, VL, and VR lineages. Four cells in the somatic primordium (DL,DR,VL,VR) divide repeatedly to each generate a distoproximal line of descendants (Figs. 8b–c). Each line essentially forms a quadrant of a tube with modifications at its ends. The timing of divisions follows a pattern of dorsal (e.g., DL,DR) before ventral (VL,VR). The rounds of mitosis begin in the center of each quadrant and move distally and proximally, i.e., DL.ap and DL.pa divide before DL.aa and DL.pp, etc.

DL.a, DR.a, VL.a, VR.a: The division pattern of the anterior daughters is a proliferation (i.e., repeated symmetric divisions) (Chalfie *et al.*, 1981) with more divisions in the center of the tissue. For example, after six rounds of division in each quadrant, DL.aaa and DL.app have generated eight nuclei each while DL.aap and DL.apa have generated 16 nuclei each; descendants of the latter two cells divide at least once more. Although the lineages and fates of all of these cells have not been determined, many of the anterior eight cells in each quadrant appear to form the proximal spermatheca and spermathecal/uterine junction. The other cells (at least 40 for each quadrant) form the uterus.

During the L4 molt, the four lines of cells separate in the center and retract to form a lumen (Fig. 9C). The uterus elongates and stretches from the vulva to the flexure of the gonad (Figs. 1 and 3).

DL.p and DR.p: DL.pa and DR.pa each generate four descendants, which contribute to the dorsal uterus. DL.pp and DR.pp each generate two cells of the postvulval sac. These cells appear to be distinct in morphology from those generated by DL.a and DR.a but similar to those generated by PR.a and PL.a (see below). A lumen forms in this region in the middle L4.

VL.p and VR.p: VL.p and VR.p each generate four descendants. The daughters of VL.pa and VR.pa connect the ventral half of the uterus (VL.a and VR.a progeny) to the vulval/uterine junction (vuj). VL.pp and VR.pp both divide dorsoventrally. Their daughters form 4 of the 11 cells in the vuj (see Fig. 10). The 11 vuj cells, 10 ventral uterine cells, plus the anchor cell, are arranged around the cone-shaped invagination of the ventral hypodermis that will form the vulva.

Anchor cell. Z4.aaa, the anchor cell (ac), does not divide. It has a characteristic morphology with a condensed nucleolus and clear nucleoplasm. It is positioned along the ventral midline and adjoins the center of the invaginating hypodermis that forms the vulva (Fig. 10).

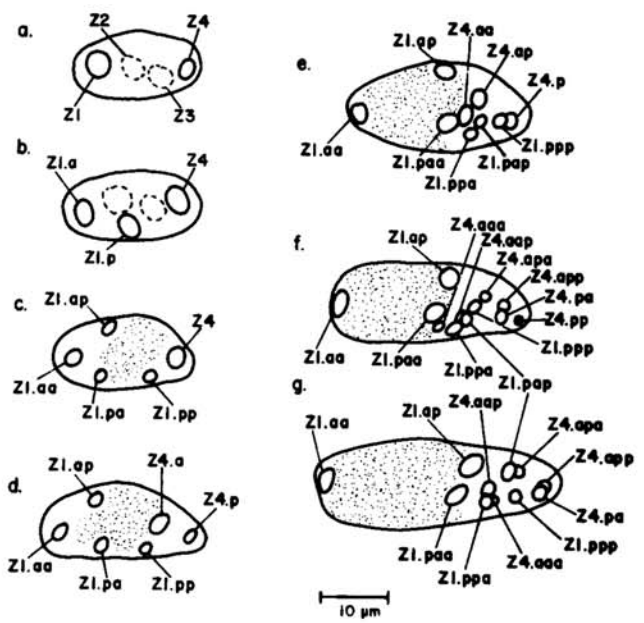

FIG. 5. Spatial arrangements of nuclei during the early period of female Z1 and Z4 divisions. Camera lucida drawings of left lateral views of individual larvae. Solid nucleus represents programmed cell death. Dashed circles and stippling indicate regions occupied by germ nuclei. Anterior is to the left, and dorsal at the top. Approximate age of larvae (hr): (a) 23; (b) 25; (c) 26; (d) 27; (e) 29; (f) 32; and (g) 35.

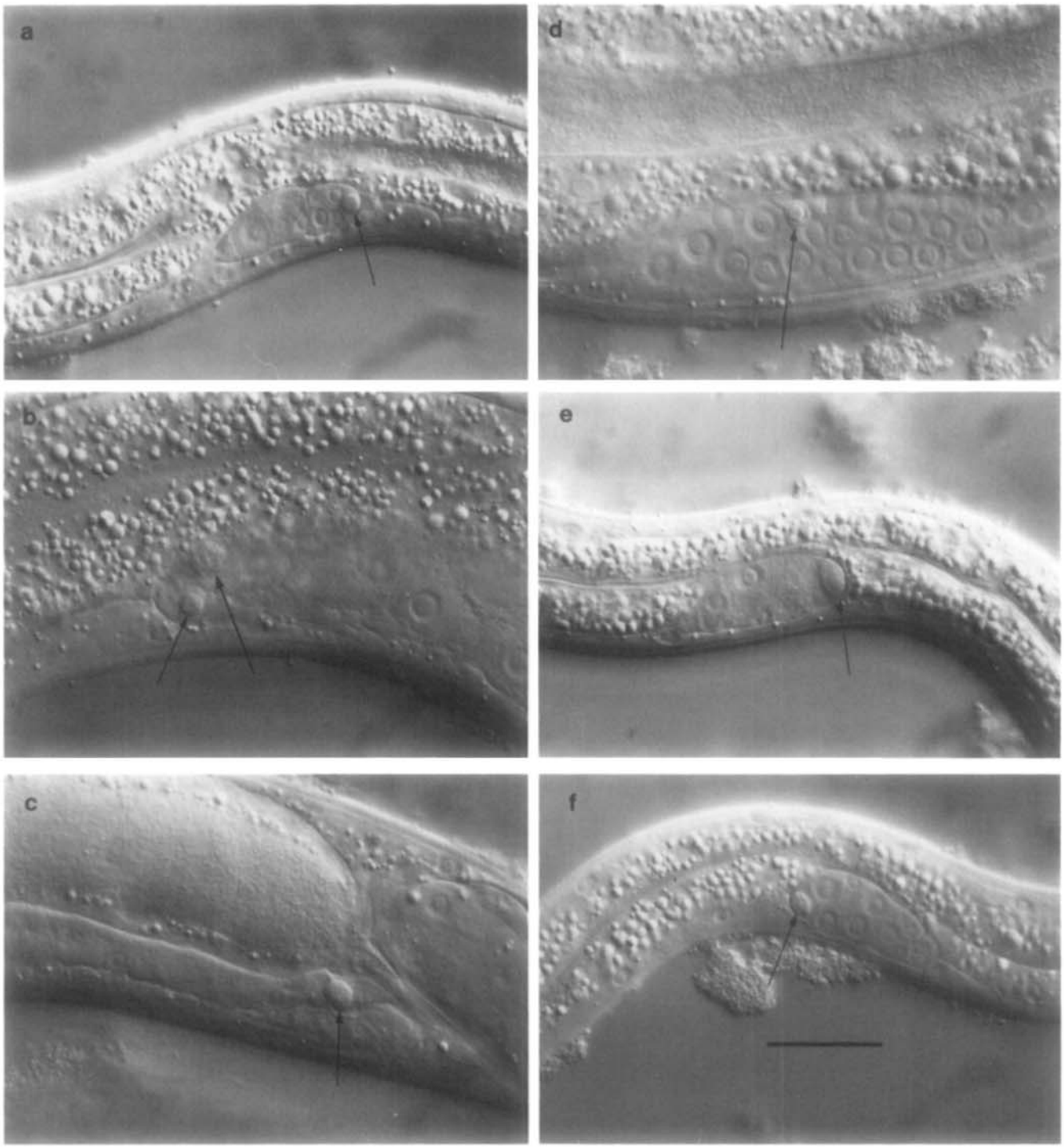

FIG. 6. Cell deaths, Nomarski optics. Bar = 0.02 mm. Arrows point to dying cells. (a) Programmed death of Z4.pp in the L2 female. Left lateral view (anterior is to the left). (b) Death of PR.p and PL.p in an L4 female postvulval sac. Right lateral view (anterior to the right). (c) Linker cell death in the L4 male. Right lateral view (anterior to the right) of cloaca and ejaculatory duct with refractile lc. (d) Germ nucleus death in an L4 male testis. Left lateral view (anterior to the left). (e) Germ nucleus death in an L2 male. Left lateral view (anterior to the left) of gonad primordium with dying nucelus at posterior tip. (f) Right lateral view (anterior to the right) of L2 male primordium with laser-ablated dtc.

The ac nucleus becomes obscured during the L4 molt; it appears to be absent in the adult.

CP lineage. CP divides transversely twice (Fig. 8d), generating four progeny in a left-right line posterior and dorsal to the anchor cell. The lateral granddaughters divide transversely, and the medial granddaugh-

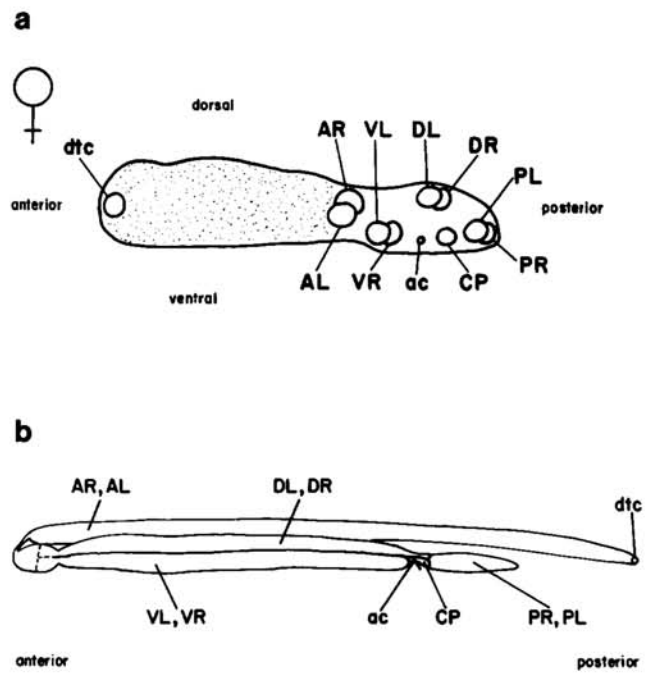

FIG. 7. (a) Female L2 molt somatic primordium, left lateral view, camera lucida. Stippling denotes region of Z2 and Z3 (germ line) descendants. Ancestry of cells is shown in Fig. 4. dtc, Distal tip cell; ac, anchor cell. Right side: AR, DR, VR, and PR. Left side: AL, DL, VL, and PL. Center: CP and ac. Names of blast cells refer to their positions in the primordium (A, anterior; P, posterior; L, left; R, right; D, dorsal; V, ventral; C, central). (b) Schematic left lateral view of adult female gonad with the progenitor of each region labeled. See Fig. 3a for the corresponding anatomy. The anchor cell is labeled at its L4 position as it is not present in the adult.

ters divide longitudinally. The lateral-most great-granddaughters, CP.lll and CP.rrr, form the ventral portion of the postvulval sac closest to the vulva (Fig. 10). The other six great-granddaughters, with the anchor cell and the four VL.pp and VR.pp daughters described above, constitute the vuj.

PR and PL lineages. PR and PL divide asymmetrically, producing a small posterior daughter and a large anterior one (Fig. 8d). Each anterior daughter divides obliquely, and both of its progeny divide twice, generating 16 cells of the postvulval sac. In about one-third of the females observed, one of the posterior daughters (PR.p, PL.p) divided. In most animals neither cell divides. In three animals, four PR.p- and PL.p-like nuclei were present. In one of these, both PR.p and PL.p were seen dividing; in the other two, divisions were not observed but it seems likely that the four nuclei were daughters of PR.p and PL.p. The timing and axes of these divisions, when they occur, vary. In the one animal in which both divisions were observed, several hours separated the divisions. The 16 descendants of PR.a and PL.a are arranged in four quadrants. PR.p and PL.p reside at the posterior tip of the developing sac. In the middle L4, a lumen forms as the quadrants separate in the center. PR.p and PL.p (or their daughters) become segregated in a posterior lobe of the sac. Late during the L4 stage, these nuclei increase in refractility, condense, move into the lumen of the sac, and eventually disappear (Fig. 6b). The appearance of these nuclei as they die differs from that of the dying Z4.pp: the nuclei, which are several times the diameter of Z4.pp, never have the flat disk-like appearance of the Z4.pp death (cf. Fig. 6a).

MALE LINEAGES

As in the female, Z1 and Z4 commence division during the late L1 stage to generate a total of 12 progeny (Figs. 11 and 12). Eight of these cells form the somatic primordium during the L2 molt (Fig. 13a). The remaining four do not divide further. The cells in the somatic primordium (except the linker cell, lc) commence division during the early L3 stage and generate 138 descendants, which maintain the relative distoproximal order of their progenitors (Fig. 13b). These progeny form the ejaculatory duct, the vas deferens, and part of the seminal vesicle.

Early Period

The Z1 and Z4 nuclei move from the anterior and posterior poles of the primordium prior to the L1 lethargus. Z1 usually adopts a position ventral and left while Z4 adopts a position dorsal and right, but these positions may vary. Z1 and Z4 each divide to generate six nuclei by the middle of the L2 period. The division patterns of Z1 and Z4 are almost identical (Figs. 11 and 12). The Z1 and Z4 daughters are located with roughly mirror symmetry about an oblique plane. Two differences between the division patterns of Z1 and Z4 are apparent. First, Z1.aa divides slightly asymmetrically with Z1.aaa larger than Z1.aap, while Z4.aa divides symmetrically with Z4.aaa and Z4.aap intermediate in size between the two Z1.aa daughters. Second, Z1.p is usually anterior to Z4.p when it divides. Consequently, the order from anterior to posterior of their daughters is Z1.pa, Z4.pa, Z1.pp, and Z4.pp.

Somatic Primordium

By the L2 molt, the twelve Z1 and Z4 progeny assume characteristic positions in the developing gonad (Fig. 13a). The eight Z1.a and Z4.a progeny form the somatic primordium at the proximal (anterior) end of the gonad, while the four Z1.p and Z4.p progeny surround the dividing germ nuclei. Z4.pp becomes the distal tip cell, remaining at the distal tip of the testis through adult-

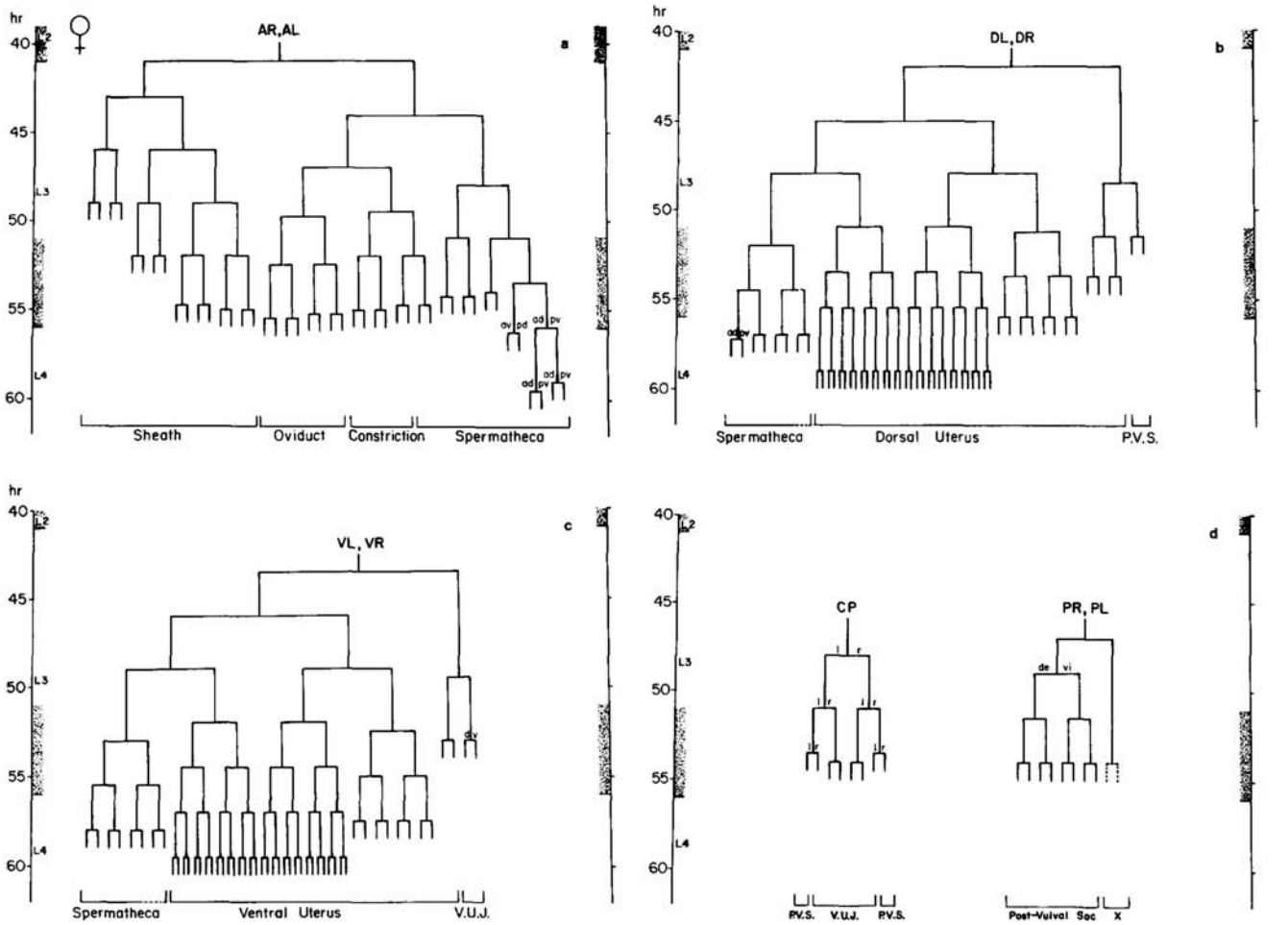

FIG. 8. Female late lineages. Ancestry of blast cells is shown in Fig. 4. Fates of cells are indicated underneath brackets: sheath, epithelium of ovary; oviduct, epithelium covering maturing oocytes; constriction, narrow region between oviduct and spermatheca; spermatheca, spermatheca and/or spermathecal/uterine junction. Dorsal uterus, cells of the dorsal uterine quadrants; ventral uterus, cells of ventral uterine quadrants; V.U.J. (vulval/uterine junction), cells connecting the uterus to the vulva; X, die in the L4 period. Dashed brackets refer to an uncertainty in the fates of specified cells. (a) AR and AL. (b) DL and DR. (c) VL and VR. (d) PR and PL; CP. Dashed lines in lineage refer to a variable division.

hood. Z4.pp became the dtc in all eight males studied. Z1.pp is between Z4.pp and the more anterior located Z1.pa and Z4.pa. These three flat "sv" cells (Z1.pp, Z1.pa, and Z4.pa) are peripheral to the germ line nuclei. The Z1.ap and Z4.ap daughters (abbreviated DS) are proximal to the germ line nuclei and are arranged with fourfold rotational symmetry about the longitudinal axis of the gonad. Three nuclei (Z1.aap, Z4.aaa, and Z4.aap; abbreviated ED), are arranged with threefold symmetry about the longitudinal axis. Z1.aaa is the most anterior (proximal) cell and becomes the linker cell (lc). Z1.aaa became the lc in all seven males studied. (In *C. elegans*, Z1.aaa does not always become the lc.)

Late Period

Seminal vesicle cells. The three sv cells (Z1.pp, Z1.pa, and Z4.pa) spread anteriorly along the outer surface of the germ line nuclei. Z1.pp reaches the flexure by the L4 molt, and Z1.pa and Z4.pa move to halfway between the flexure and the vas deferens. These cells continue to move proximally with respect to the testis. In the adult, these (and four DS.pp nuclei; see below) constitute the seminal vesicle.

DS lineage. The four DS cells (Z1.apa, Z1.app, Z4,apa, and Z4.app) generate 30 descendants each by middle L4. These cells form the vas deferens and contribute to the seminal vesicle and ejaculatory duct (Fig. 14). Six of the DS divisions are asymmetric (Fig. 20, Discussion). In five of these cases, the larger daughter divides before the smaller daughter, which divides only once. In the sixth case, DS.p divides to generate a large daughter DS.pp that becomes a part of the seminal vesicle, and a small daughter DS.pa that remains at the distal end of the vas deferens. DS.a undergoes three successive asymmetric divisions. The anterior (proximal) daughter

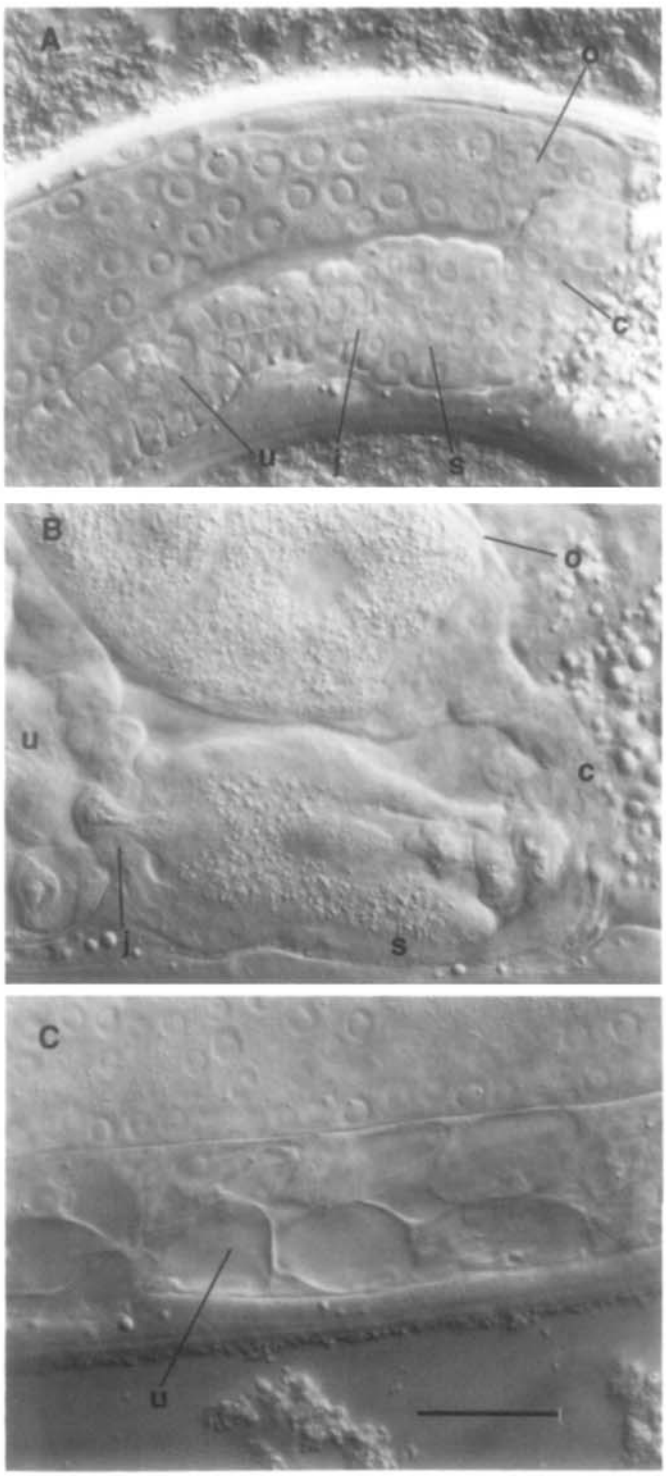

FIG. 9. Morphology of female gonadal structures in the late L4, right lateral views; Nomarski optics. Oviduct (o), constriction (c), spermatheca (s), spermathecal/uterine junction (j), and part of the uterus (u) in: (A) L4 and (B) adult. Note the sperm passing through the junction in the adult. (C) Uterus in adult, showing the right dorsal and ventral uterine quadrants. Bar = 0.02 mm.

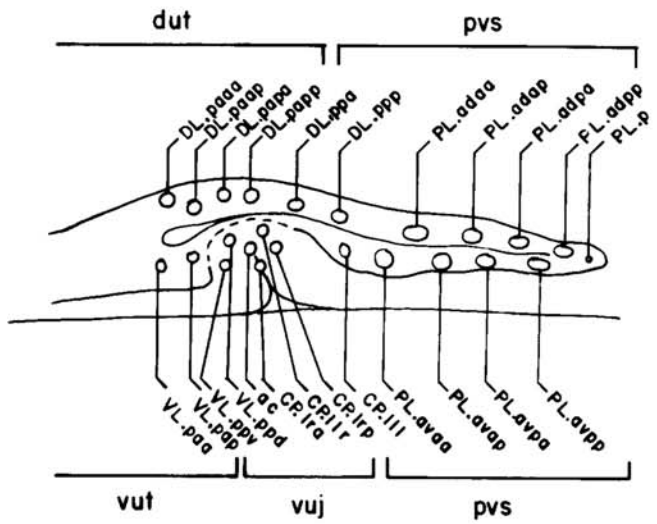

FIG. 10. Schematic diagram of the positions of nuclei in the vulval region. Left lateral view of early L4 female. Anterior is to the left, and dorsal at the top. pvs, Postvulval sac; vuj, vulval/uterine junction; dut, dorsal uterus; vut, ventral uterus.

is smaller in each case. The first anterior daughter (DS.aa) generates two cells of the ejaculatory duct. The second and third anterior daughters (DS.apa and DS.appa) each contribute two cells to the vas deferens. The anterior (proximal) daughter of DS.appp generates eight cells; the posterior (distal) daughter of DS.appp divides apparently symmetrically once, but its anterior daughter (DS.appppa) divides asymmetrically.

Most of the DS divisions are distoproximal. However, some of the axes of the divisions of the DS.appppp granddaughters are oblique to the longitudinal axis of the gonad. The distal portion of the vas deferens (consisting of the DS.appppp descendants and DS.pa) is approximately six cells in circumference. The proximal

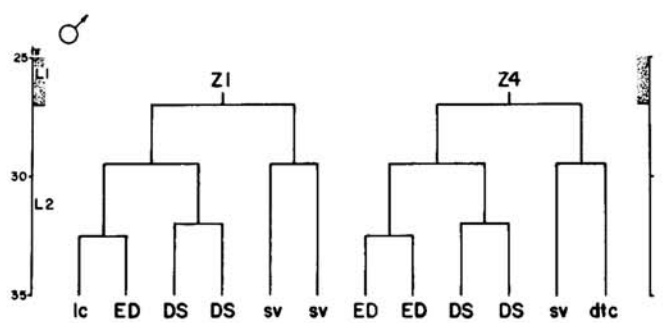

FIG. 11. Male early Z1 and Z4 lineages. lc, Linker cell; sv, seminal vesicle cells; dtc, distal tip cell; ED, generates ejaculatory duct cells; DS, generates cells of distal structures (ejaculatory duct, vas deferens, and seminal vesicle).

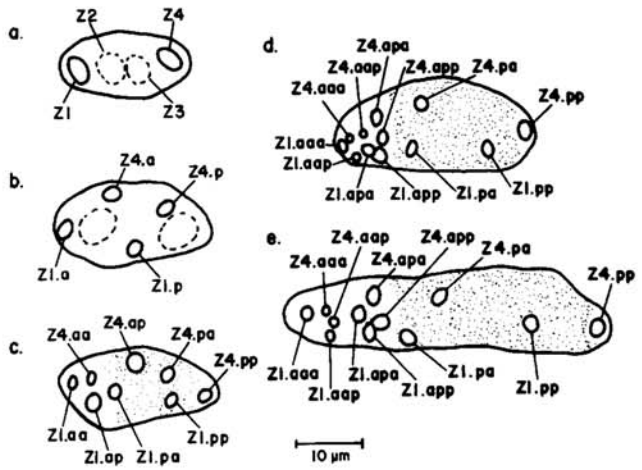

FIG. 12. Spatial arrangements of nuclei during the early period of male Z1 and Z4 divisions. Camera lucida drawings of left lateral views of individual larvae. Dashed circles and stippling indicates the regions occupied by germ nuclei. Approximate age of larvae (hr): (a) 25; (b) 28; (c) 31; (d) 34; and (e) 37. Anterior is to the left, and dorsal at the top.

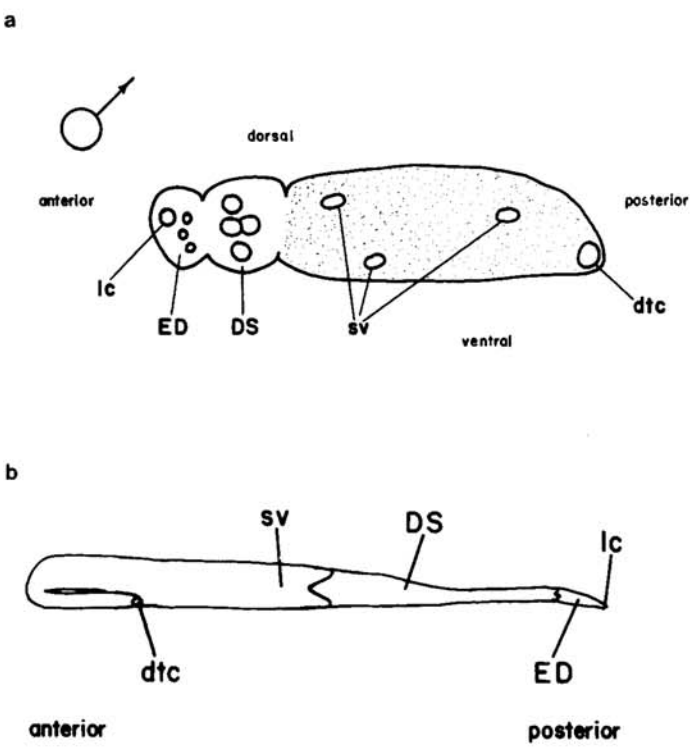

FIG. 13. (a) Male L2 molt somatic primordium, left lateral view, camera lucida. Stippling denotes region occupied by Z2 and Z3 descendants. Ancestry of cells is shown in Fig. 12. Differentiated cells (lc, sv, and dtc) and blast cells (ED and DS) are as defined in Fig. 11. (b) Schematic left lateral view of adult male gonad with the progenitor of each region labeled. See Fig. 3b for corresponding anatomy. The linker cell is labeled at its L4 position as it is not present in the adult.

part of the vas deferens is two cells in circumference. The cells of the dorsal and ventral quadrants on each side of the developing vas deferens tube intercalate, resulting in a tube that is essentially two cells in circumference rather than four cells in circumference. During the late L4, small "vacuoles" appear in the center of the vas deferens as a lumen forms. These vacuoles increase dramatically in size as the nuclei move to the outside of what becomes a hollow circumferentially ribbed tube (Fig. 15).

ED lineage. The three ED cells (Z1.aap, Z4.aaa, and Z4.aap) arranged around the longitudinal axis divide to generate six daughters each (Fig. 14). During ejaculatory duct morphogenesis the threefold rotational symmetry of the ED daughters is converted into the approximately fourfold rotational symmetry of the mature ejaculatory duct. The 18 ED and the 8 DS.aa descendants intercalate along the longitudinal axis (i.e., become compressed longitudinally) to form four lines of cells, which differentiate into the ejaculatory duct. Like those of the vas deferens, these nuclei move to the outside during late L4. Two sizes of vacuoles appear inside the differentiating ejaculatory duct—small vacuoles appear outside the larger vacuoles (Fig. 16)—which contrasts with the single type of "vacuole" in the vas deferens. The smaller vacuoles do not appear to form part of the lumen.

Linker cell. The lc leads the gonad as it reflexes and grows posteriorly. In the late L4 period the lc and the

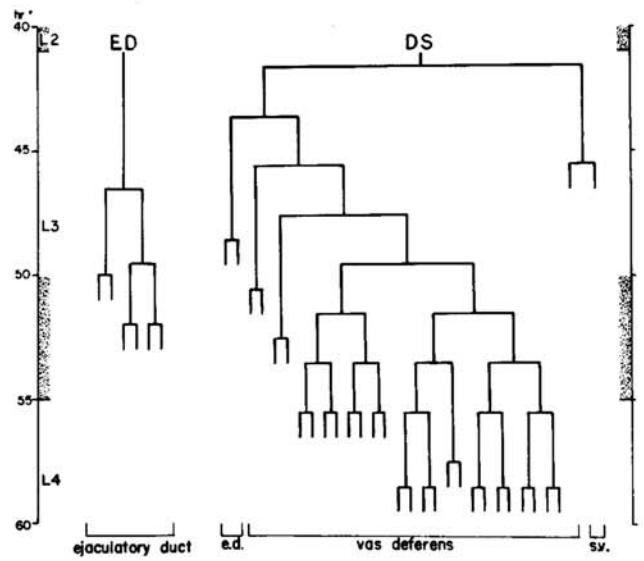

FIG. 14. Male late lineages. Ancestry of blast cells is defined in Fig. 11. Fates of cells are indicated underneath brackets: e.d., ejaculatory duct; s.v., seminal vesicle.

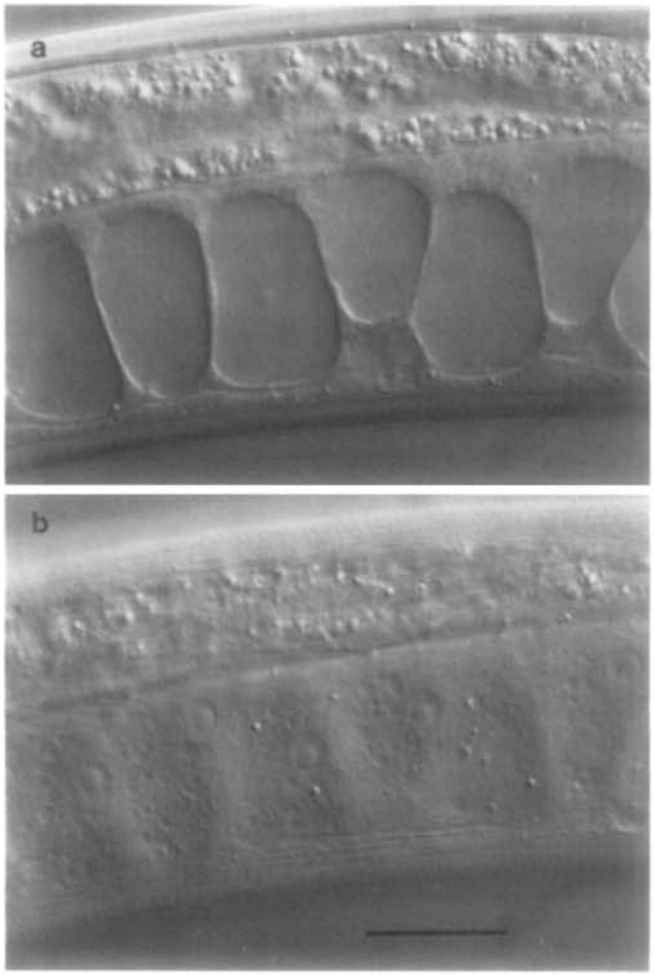

FIG. 15. L4 male vas deferens, left lateral view; Nomarski optics. (a) Focal plane through lumen of vas deferens. (b) Focal plane through cytoplasm and nuclei of cells on the left side of the vas deferens in the same animal as a. Bar = 0.02 mm.

growing gonad reach the cloaca, and the lc dies (Fig. 6c), connecting the ejaculatory duct lumen with the cloacal lumen.

GERM LINE (Z2, Z3)

The lineages of the germ line precursors Z2 and Z3 were followed sufficiently to determine that they are variable. The axes and timing of germ line divisions vary among animals. Sister nuclei tend to undergo mitoses more synchronously than nonsister nuclei. In the male, apparent deaths (Fig. 6d) of germline nuclei (or cells) occur from as early as the L2 to at least the L4 ecdysis. Essentially every L4 male gonad observed had at least one cell death. No correlation with lineage can be made from our present data. In one animal, Z3 did not divide and died during the L3. In two animals, cell deaths occurred at the posterior part of the gonad during the L2 (e.g., Fig. 6e). In at least one of these animals, a germ line nucelus located at the position normally occupied by the dtc died. The dtc assumed the posterior position as the dying cell disappeared. Germ cell deaths have been observed in occasional females.

LASER ABLATIONS

In *C. elegans* hermaphrodites and males, Z1 and Z4 follow equivalent cell lineages (Kimble and Hirsh, 1979). In *P. redivivus* females and males, there are differences between the Z1 and Z4 lineages. It is possible that Z1 and Z4 in *P. redivivus* may nonetheless be equivalent in developmental potential. A cryptic developmental potential may be revealed after laser ablation. For example, in the *C. elegans* hermaphrodite, P4.p, which normally generates two ventral hypodermal nuclei, can generate seven vulval nuclei after laser ablation of P6.p (Sulston and White, 1980); thus, P4.p has the potential to generate vulval nuclei.

In the *P. redivivus* male, when either Z1 or Z4 was ablated (six animals of each), the remaining somatic precursor divided in the early period as usual, but generated both a lc and a dtc. (Normally, Z1 generates the lc, and Z4 generates the dtc.) Z1.pp was observed to become the dtc after Z4 ablation. One of the Z4.aa daughters (most likely Z4.aaa) became the lc after Z1 ablation. A vas deferens and ejaculatory duct similar in size and morphology to those of an unperturbed animal were formed from fewer cells; the testis appeared normal as it had the usual morphology; divisions of Z2 and Z3 appeared to be normal. Thus, Z1 and Z4 may be of equivalent potential in the male.

In the *P. redivivus* female, when Z1 was ablated (four

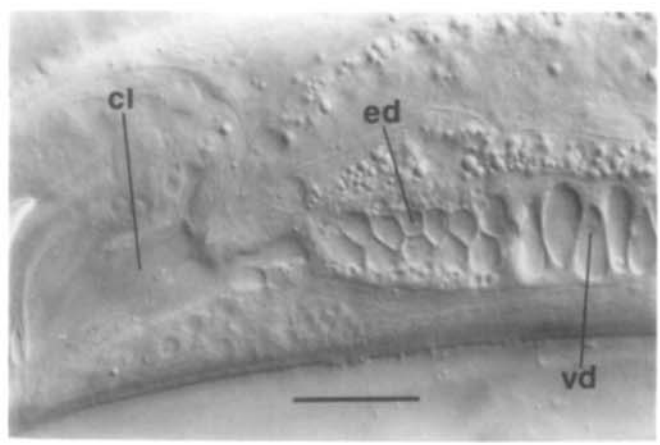

FIG. 16. Ejaculatory duct in adult male, right lateral view (anterior is to the right); Nomarski optics. Note two sizes of "vacuoles." Bar = 0.02 mm. ed, Ejaculatory duct; cl, cloaca; vd, vas deferens.

animals), Z4 generated five surviving descendants. In the one animal followed, Z4.pp died by the L2 molt, as it does normally. The dtc, ovary (including the germ line (Z2 and Z3) descendants), sheath, and oviduct appeared to be missing, and spermathecal and uterine tissue appeared to be present. When Z4 was ablated (eight animals), Z1 generated six descendants by the L2 molt; most of the postvulval sac cells, the ac, and approximately one-half of the uterine cells were missing; a normal number of germ line descendants appeared to be present. Thus Z1 and Z4 may not be of equivalent potential in the female.

DISCUSSION

The general program of gonadogenesis is very similar in *P. redivivus* females, *P. redivivus* males, *C. elegans* hermaphrodites, and *C. elegans* males (cf. Results and Kimble and Hirsh (1979)). In both sexes of both species, the gonad primordium of the newly hatched animal consists of two germ line precursors (Z2 and Z3) flanked by two somatic gonad precursors (Z1 and Z4). Z1 and Z4 undergo similar patterns of cell division during the L1 and L2 stages to generate a total of 10–12 descendants. Most of these descendants rearrange to form a somatic primordium, which primarily consists of cells that divide further; a few cells generated by the early lineages—the distal tip cells (dtc), anchor cell (ac), and linker cell (lc)—do not divide further and play special roles at the distal or proximal ends of the developing gonad. The 7–9 blast cells of the somatic primordium divide during the L3 and L4 stages to generate the cells that constitute the adult gonad.

LINEALLY EQUIVALENT CELLS HAVE SIMILAR FATES

The uniformity of this developmental program allows a comparison of the fates of developmentally homologous cells. In general, lineally equivalent cells become or generate cells of similar function. This relationship is most apparent among developmentally homologous cells produced by the early (L1 and L2) divisions of Z1 and Z4. The late (L3 and L4) lineages of Z1 and Z4 are more divergent and, in a few cases, generate structures that are species specific. Nonetheless, in most cases similar cell types are generated by similar patterns of cell division. Specific aspects of the homologies of the Z1 and Z4 cell lineages both between the species and between the sexes are discussed in detail below.

Homologies among Early Lineages

Both the early Z1 and Z4 division patterns and the fates of their lineally equivalent descendants are strikingly similar among the two sexes and the two species (Fig. 17). The relationships among the *C. elegans* and *P. redivivus* early Z1 and Z4 lineages can be summarized as follows. Each early lineage can be characterized by the differences between it and that of the *C. elegans* hermaphrodite, as defined by which cells become the dtc, ac, or lc and by the order along the distal–proximal axis of the structures generated by the blast cells (Fig. 17). (The lc of males and the ac of females and hermaphrodites appear to be analogous functionally, as both act transiently, plug the lumen of the proximal end of the gonad preventing its connection to the external environment, and are absent in the adult; furthermore, in "multivulva" males the lc can connect to a developing pseudovulva (Sulston and Horvitz, 1981).)

The lineages of the *P. redivivus* female differ from those of the *C. elegans* hermaphrodite by a change in the fate of Z4.pp from a dtc to a cell that undergoes programmed death and by the invariance of the origin of the ac and CP. Also, PR and PL generate cells of the postvulval sac in *P. redivivus* but generate structures of the posterior gonadal arm in *C. elegans*. The lineages of the *C. elegans* male differ from those of the *C. elegans* hermaphrodite by a reversal in the polarity of the Z1.p lineage (e.g., Z1.paa is the lc in the male, but Z1.ppp is the ac in the hermaphrodite), and by the lack of Z1.a and Z4.p divisions in the male. The lineages of the *P. redivivus* male differ from those of the *C. elegans* hermaphrodite by a reversal in the polarity of the Z1 lineage; e.g., Z1.aaa is the lc in the *P. redivivus* male, while Z1.ppp can be the ac in the *C. elegans* hermaphrodite. Each of the males differs from the *C. elegans* hermaphrodite by a reversal in the polarity of the lineage of a specific blast cell (Z1.p in *C. elegans* and Z1 in *P. redivivus*). The lineages of the males of the two species differ by two polarity reversals, as well as by the lack of Z1.a and Z1.p divisions in the *C. elegans* male. It is interesting that two ways of developing a monopolar male gonad are used in the two species; again, in comparison with the *C. elegans* hermaphrodite, the *P. redivivus* male gonad develops by directly reversing the polarity of Z1, whereas the *C. elegans* male gonad develops by effecting an equivalent polarity reversal of Z1 in two steps, i.e., the posterior migration of the dtc (Z1.a) and the polarity reversal of Z1.p.

No variation in the fates of the descendants of the early Z1 and Z4 lineages have been observed in *P. redivivus*. In contrast, in the gonadal development of *C. elegans* hermaphrodites and males, pairs of apparently lineally equivalent cells exhibit a natural variability in cell fate: in the hermaphrodite, either Z1.ppp or Z4.aaa becomes the ac; in the male, either Z1.paa or Z4.aaa becomes the lc (Kimble and Hirsh, 1979). The seeming

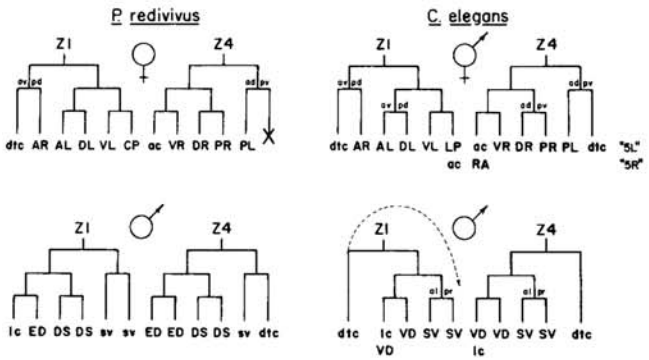

FIG. 17. Comparison of the early Z1 and Z4 lineages of the somatic gonad in both sexes of *P. redivivus* and *C. elegans*. The L1–L2 division patterns of Z1 and Z4 are shown schematically. For correct timing of divisions see Figs. 4 and 11 for *P. redivivus* and Kimble and Hirsh (1979) for *C. elegans*. By analogy with the nomenclature used for the *P. redivivus* female (Fig. 7a) cells in the *C. elegans* hermaphrodite are named based upon their positions in the somatic primordium; this nomenclature is used to facilitate comparison and differs from that employed by Kimble (1981). Cells in the *C. elegans* male are named by the fates of their descendants. Lower case, differentiated cell types; upper case, blast cells; X, programmed cell death. dtc, Distal tip cell; ac, anchor cell; lc, linker cell; sv, outer layer seminal vesicle cell. Dashed arrow depicts the migration of Z1.a with respect to other cells from the lineage. Cells known to display natural variability in their fates are indicated by dual labels. There are two types of hermaphrodite somatic primordia in *C. elegans*: in the "5L" primordium Z4.aaa is the ac; in the "5R" primordium Z1.ppp is the ac (Kimble and Hirsh, 1979). In all *P. redivivus* females observed, Z4.aaa was the ac. Female and hermaphrodite: AR and AL, generate right and left sides (respectively) of the anterior arm sheath, oviduct, and spermatheca; DL and DR, generate left and right sides (respectively) of the dorsal uterus, anterior and posterior spermatheca, and spermathecal/uterine junction in *C. elegans*, and the dorsal uterus, spermatheca, and/or junction, and postvulval sac in *P. redivivus*; VL and VR (VL and VR are on the left and right sides, respectively, of the somatic primordium) generate anterior and posterior junction, ventral uterus, and ventral/uterine junction (vuj) in *C. elegans*, and left and right ventral uterus, vuj, and spermathecal/uterine junction in *P. redivivus*. CP generates vuj and postvulval sac in *P. redivivus*. LP (located posterior to VL) in a "5L" primordium or RA (located anterior to VR) in a "5R" primordium generates vuj and ventral uterus. PR and PL in *P. redivivus* generate the right and left sides (respectively) of the postvulval sac. PR and PL in *C. elegans* generate right and left (respectively) of the posterior arm sheath, oviduct, and spermatheca. Males: Although division patterns in the *P. redivivus* and *C. elegans* males differ, analogies can be drawn. After the first division of Z1 and Z4 in males of both species, one daughter (Z1.a and Z4.p in *C. elegans*; Z1.p and Z4.p in *P. redivivus*) is (in *C. elegans*) or can generate (in *P. redivivus*) a dtc. The other daughter (Z1.p and Z4.a in *C. elegans*; Z1.a and Z4.a in *P. redivivus*) follows identical lineages in both species to generate cells of the somatic primordium (lc, ED, DS in *P. redivivus*, or lc, VD, SV in *C. elegans*); lineally equivalent cells of this sublineage display some similar characteristics. Thus, the anterior-most granddaughter (Z1.paa and Z4.aaa in *C. elegans*; Z1.aaa and a daughter of Z4.aa, probably Z4.aaa, in *P. redivivus*) can become a lc; Z1.pap and Z4.aap in *C. elegans* and Z1.aap and Z4.aap in *P. redivivus*, along with the anterior-most daughter when it is not a lc, are three blast cells (arranged with threefold rotational symmetry) that generate the more proximal structures of the gonad. The remaining granddaughters (Z1.apa, Z1.app, Z4.apa, and Z4.app in *P. redivivus* and Z1.ppa, Z1.ppp, Z4.apa, and Z4.app in *C. elegans*) are four blast cells (arranged with fourfold rotational symmetry) that generate the more distal structures of the gonad. The descendants of the Z1 and Z4 early lineages in both species (with the exception of Z1.a in *C. elegans*; see text) are generated in the distoproximal order later assumed by their descendants in the adult gonad. In the *P. redivivus* male, the somatic cells in the gonad primordium are or can generate a series of structures arranged from distal to proximal as follows: dtc; outer layer seminal vesicle (sv); outer layer seminal vesicle, vas deferens, and ejaculatory duct (DS); ejaculatory duct (ED); and lc. In the *C. elegans* male, the homologous cells are or can generate: dtc; no cell (Z1.a and Z4.p do not divide); inner layer seminal vesicle (SV); outer layer seminal vesicle, vas deferens, and ejaculatory duct (VD); and lc.

invariance of gonadogenesis in *P. redivivus* might reflect an underlying nonequivalence of Z1 and Z4. However, in the male Z4.aa can produce a lc (probably Z4.aaa) after ablation of Z1, and Z1.ppp can become the dtc after ablation of Z4. Thus, Z1 and Z4 are of equivalent developmental potential in the *P. redivivus* male. It is possible that in an occasional animal, Z4.aaa naturally becomes the lc, and Z1.ppp becomes the dtc. In contrast, it seems less likely that Z1 and Z4 are equivalent in the *P. redivivus* female, as Z1.ppp has not been seen to become an ac after Z4 ablation. Other indications of the nonequivalence of Z1 and Z4 in the *P. redivivus* female are the spatial compression and temporal delay of the Z4 lineage with respect to the Z1 lineage.

Homologies between Late Lineages

A comparison of the late lineages suggests two generalizations. First, the potential to generate specific cell types appears to be segregated similarly by the first few divisions of homologous blast cells during the late period. (Figs. 18 and 19). Second, similar patterns of cell division generate similar cell types (e.g., Fig. 20). Some of the differences between the late lineages are discussed below.

Female and hermaphrodite late lineages. AR.pp and AL.pp undergo different division patterns in *P. redivivus* females and *C. elegans* hermaphrodites. However, AR has been isolated from other somatic cells of the gonad by laser ablation of Z4 and Z1.p in the *C. elegans* hermaphrodite (Kimble, 1981); AR.pp (Z1.appp) in such animals can undergo a pattern of divisions very similar to that normally followed by AR.pp in *P. redivivus* females. This observation suggests that the AR.pp cells in the two species are more similar in developmental potential than indicated by the unperturbed lineages and that in *C. elegans* interaction of AR.pp with other somatic cells changes its lineage from that of AR.pp in *P. redivivus*.

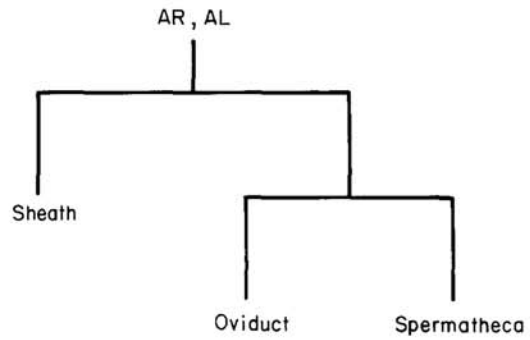

FIG. 18 The first divisions of AR (Z1.ap) and AL (Z1.paa) in *P. redivivus* females (Fig. 8a) and *C. elegans* hermaphrodites (Kimble and Hirsh, 1979) result in cells that have, or generate cells of, similar function. The sheath sublineage generates 16 descendants in *P. redivivus* and one in *C. elegans*. The oviduct sublineage generates oviduct, the constriction between the oviduct and spermatheca, and two spermathecal cells in *P. redivivus*. These structures seem to be myoepithelial as oocytes appear to be moved by contractions of the oviduct and constriction. In *C. elegans*, the oviduct sublineage consists of two rounds of divisions producing myoepithelial oviduct cells (Hirsh, *et al.*, 1976). The spermatheca sublineage generates 12 descendants in *P. redivivus* and 9 in *C. elegans*.

DL.a, DR.a, VL.a, and VR.a generate more descendants and DL.p, DR.p, VL.p, and VR.p generate fewer descendants in *P. redivivus* than do the homologous cells in *C. elegans* (Figs. 8b–c, 19a–b) (Kimble and Hirsh, 1979; Kimble, 1981). Thus, there is an anterioposterior asymmetry within the *P. redivivus* DL, DR, VL, and VR lineages, just as there is between the Z1 and Z4 lineages.

The *P. redivivus* CP lineage may involve a mirror-image duplication of the *C. elegans* LP lineage, with a subsequent reduction in the number of rounds of cell division (Fig. 19c). The monopolar ventral uterus of *P. redivivus* might have evolved from a bipolar ventral uterus (like that of *C. elegans*) by a reversal in the polarity of the VR lineage (which is of opposite polarity to the VL lineage; see Fig. 19a). Duplication of the CP lineage concomitant with a 90° rotation of its division axes (longitudinal to transverse) could have restored the bilateral symmetry of the ventral uterus.

PR (Z4.app) and PL (Z4.pa) generate most of the postvulval sac in *P. redivivus*. The lineally equivalent cells in *C. elegans* (RP and LP, AR and AL) as well as the lineally equivalent cells from the Z1 lineage in *P. redivivus* (AR and AL) generate sheath, oviduct, and spermatheca. Thus, based upon their similar developmental origins, one might expect homologies in lineage, cell type, and/or function between the cells of the postvulval sac and nonuterine gonadal tissue. Although classically the postvulval sac has been regarded as uterine in nature (e.g., Chitwood and Chitwood, 1974), ultrastructural studies of the *Aphelencoides blastophthorus* gonad are ambiguous, as postvulval sac cells appear similar to uterine cells in their flattened morphology but different in their lack of "secretory droplets" (Yuen, 1971).

Male late lineages. While the gonads of the *P. redivivus* and *C. elegans* males are of the same general morphology, their detailed characteristics are quite distinctive (cf. Results and Kimble and Hirsh (1979)). These differences are reflected in the late lineages. There is no obvious counterpart in *C. elegans* to the ED lineage of *P. redivivus*; perhaps it is this difference that results in the longer ejaculatory duct of *P. redivivus*. Similarly, there is no obvious counterpart in *P. redivivus* to the SV lineage of *C. elegans*, which leads to the lack of the inner layer of the seminal vesicle in *P. re-*

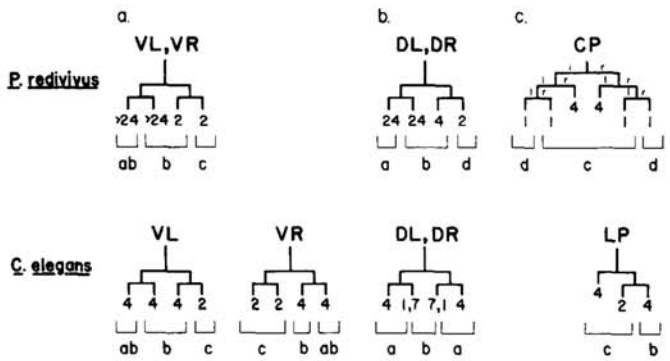

FIG. 19. Comparison of the uterine lineages of the *P. redivivus* female and the *C. elegans* hermaphrodite. Numbers refer to number of descendants generated by that branch of the lineage tree. Fates are indicated underneath brackets: a, spermatheca and/or spermathecal/uterine junction cells; b, uterine cells; c, includes vuj cells; d, postvulval sac cells. *C. elegans* VL (Z1.ppa), VR (Z4.aap), and LP (Z1.ppp) lineages are from hermaphrodites with the "5L" type of somatic primordium (Kimble and Hirsh, 1979), which is comparable to the somatic primordium of *P. redivivus* females, e.g., in both, Z4.aaa becomes the ac (see Fig. 17). (a) Ventral uterine lineages. The first division in both species segregates the potential to generate spermathecal and/or spermathecal uterine junction cells and to generate vuj cells. The spermathecal cells are descendants of VL.a in both species; the vuj cells are descendants of VL.p in both species. In *P. redivivus* VR follows the same lineage as VL; in *C. elegans* VR follows a lineage that is almost a rotationally symmetrical version of VL. (b) Dorsal uterine lineages. DL.a and DR.a in *P. redivivus* and *C. elegans* generate cells of the spermatheca, spermathecal/uterine junction, and dorsal uterus. *P. redivivus* DL.pa and DR.pa granddaughters are anterior to the vulva, but differentiate at the same time and with similar appearance as the DL.pp and DR.pp descendants of the postvulval sac. DL.pa and DR.pa in *C. elegans* each generate one spermathecal/uterine junction cell and seven dorsal uterine cells. (c) Ventral uterine vuj lineages. The *P. redivivus* CP lineage generates postvulval sac cells rather than ventral uterine cells like that of the *C. elegans* LP lineage. The first two divisions of CP.r and LP segregate the potential to generate vuj from that of postvulval sac (*P. redivivus*) or ventral uterus (*C. elegans*). In *P. redivivus* the vuj cells are posterior to all other ventral uterine cells. In *C. elegans*, the vuj cells flank the non-vuj ventral uterine cells.

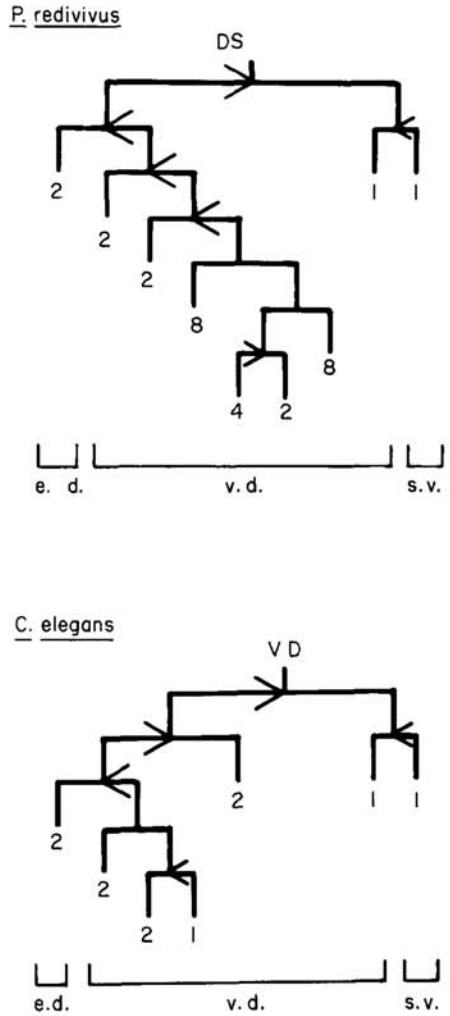

FIG. 20. Comparison of *P. redivivus* DS and *C. elegans* VD lineages. Arrowheads on branches of lineage trees point to the smaller of the two daughter cells after an asymmetric division. Numbers refer to the number of descendants generated by each branch. e.d., Ejaculatory duct; v.d., vas deferens; s.v., seminal vesicle. Note that the first division of DS or VD separates e.d. from s.v. Both lineages have a characteristic pattern of a series of asymmetric divisions in which the smaller daughters usually divide once before differentiating.

divivus. However, the lineages that generate cells of the ejaculatory duct, vas deferens, and outer layer of the seminal vesicle (DS in *P. redivivus* and VD in *C. elegans*) have common features (Fig. 20). DS and VD generate functionally similar structures by similar cell lineages in the two species, but are not themselves lineally equivalent (Fig. 17); instead, DS and VD are derived from adjacent branches of the lineage. This apparent "altered segregation" of the potential to generate different cell types between *C. elegans* and *P. redivivus* males is reminiscent of the lack of correlation between cell lineage and fate seen in the *C. elegans* male mesoderm (Sulston *et al.*, 1980): the fates of some lineally equivalent cells generated by sex myoblasts on the left and right sides of the animal are not identical; where different, the right member of the lineally equivalent pair has the fate of a more anterior cell on the left side, e.g., SM2.pap on the right has the same fate as SM2.paa on the left. We will discuss "altered segregation" in more detail below.

PATTERNS OF CELL DIVISION

Symmetric and Asymmetric Divisions

Many cell divisions that occur during the development of both *P. redivivus* and *C. elegans* yield daughters unequal in size. Such an inequality invariably is followed by a nonequivalence in the fates of the daughters, as evidenced by the number of descendants, the timing of divisions, and/or the types of cells produced. For example, such asymmetric cell divisions occur in the DS lineage of the *P. redivivus* male. In each of the four identical DS lineages (Figs. 14 and 20), there are six asymmetric divisions. In all cases the fates of daughters of asymmetric divisions differ.

Generally, the larger daughter generates more descendants. For example, in the DL, DR, VL, and VR lineages of the *P. redivivus* female, the first division is asymmetric; the larger anterior daughter generates at least 48 descendants, while the smaller posterior daughter generates 4 to 6 descendants. However, in the *P. redivivus* male, Z1.aaa, which is larger than Z1.aap, becomes the lc while its sister is an ED blast cell. The lc probably differentiates and functions (by leading the reflexion of the gonad) before ED prepares for division. Perhaps an asymmetry reflects the immediate activity of a nucleus as well as the ultimate extent of divisions.

Proliferative and Reiterative Patterns of Cell Division

Two simple patterns of cell division occur in many *P. redivivus* and *C. elegans* lineages (Sulston and Horvitz, 1977; Deppe *et al.*, 1978; Kimble and Hirsh, 1979; Chalfie *et al.*, 1981); repeated symmetric cell divisions (proliferations), e.g., the *P. redivivus* female VL.a lineage (Fig. 8c); or repeated asymmetric cell divisions in which a cell generates one cell like itself and one cell of a different type (parental reiterations). Both patterns may be combined within a single lineage. For example, the AR.a and AL.a lineages of the *P. redivivus* female (Fig. 8a) are posterior parental reiterations with the anterior daughter and final posterior daughter executing the same sublineage (see below), which consists of two rounds of symmetrical cell division (a proliferation) followed by differentiation into sheath cells.

A proliferation leads to a geometric increase in cell number, as in the *P. redivivus* female uterine lineages,

while a parental reiteration leads to an arithmetic increase, with a rate dependent upon the number of descendants produced by the novel, nonparental-like cells. That both proliferations and parental reiterations could result in comparable increases in cell number raises the question of why a particular lineage pattern may be utilized. In the case of AL.a and AR.a, a reiterative lineage adds four cells per reiterated division to cover the growing ovary; the reiteratively dividing posterior cell (e.g., AL.a, AL.ap, AL.app, etc.) remains at the proximal end of the ovary and acts as a source of additional cells. Perhaps a requirement for a particular spatial or temporal distribution of cells results in the utilization of a reiterative rather than a proliferative cell lineage.

Sublineages

Complex cell lineages appear to consist of modular sublineages (e.g., Chalfie *et al.*, 1981). In many cases, multiple precursor cells undergo identical patterns of cell division (sublineage), e.g., the 13 ventral nerve cord precursors (P*n*.a) in the *C. elegans* hermaphrodite and the 18 ray precursors (R*n*) in the *C. elegans* male (Sulston and Horvitz, 1977; Sulston *et al.*, 1980). Similarly, blast cells with identical division patterns are involved in gonadogenesis in *P. redivivus*, e.g., the four DS or three ED cells as well as Z1 and Z4 in the male, and the pairs AR and AL, DL and DR, VL and VR, and PR and PL in the female. These common sublineages suggest that the blast cells that generate them are identical in nature; e.g., the four DS cells are probably equivalent cell types.

CELL DEATHS

The cell deaths occurring during gonadogenesis in *P. redivivus* (Fig. 6) differ in a number of respects. The deaths of Z4.pp, PR.p, and PL.p (or their daughters) in the female and of the lc in the male are invariant with respect to lineage and time; the germ cell deaths in the male testis appear to be random in lineage and time. Of these deaths only that of Z4.pp behaves like the cell deaths described in *C. elegans* by Sulston and Horvitz (1977), i.e., it dies immediately following its formation; the other cells die one or more larval stages after their formation. The lc death, as in *C. elegans* (Sulston *et al.*, 1980) appears to serve two functions: elimination of a cell that is no longer needed to lead the developing gonad, and connection of the lumen of the vas deferens to the cloaca. The deaths of PR.p, PL.p and Z4.pp may be involved in the morphogenesis of the monodelphic gonad (see below). The function or cause of the germ cell deaths is unclear. Germ cell deaths occur in other nematodes (Roman and Hirschmann, 1969). All dying cells, including those killed by laser ablation, are generally similar in appearance (Fig. 6).

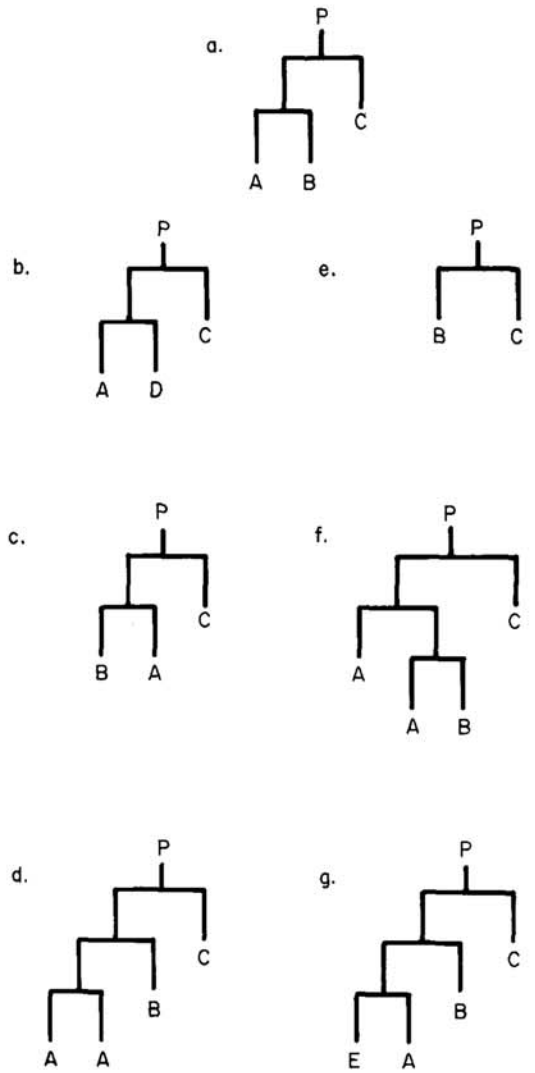

FIG. 21. Transformations of cell lineages. Six types of cell lineage transformations are illustrated by the difference between the lineages "b–g" and the canonical lineage "a". (a) Canonical lineage. (b) Switch in the fate of P.ap from B to D. (c) Polarity reversal of P.a. (d) Extra symmetric division of P.aa, leading to a tandem duplication of the A sublineage. (e) Suppression of the division of P.a, which might result in a cell of type B, type A, or mixed characteristics. (f) Extra asymmetric division like that of P.a, leading to a posterior parental reiteration. (g) A novel asymmetric division of P.aa, revealing a cryptic cell type E.

EVOLUTION OF CELL LINEAGES

As discussed above, the Z1 and Z4 cell lineages are remarkably similar among the two sexes and the two species. The differences among these lineages seem likely to have resulted from phylogenetic modifications of a common developmental program. The nature of

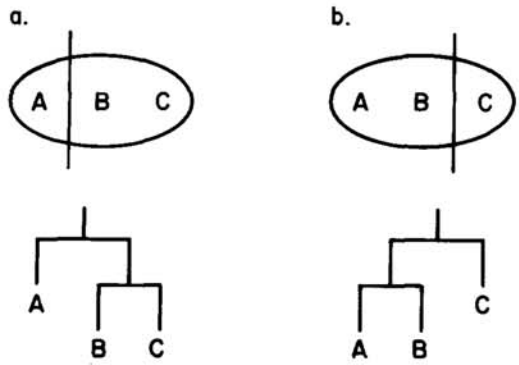

FIG. 22. Altered segregation of developmental potential. "Altered segregation" involves a transfer of the potential to generate a particular cell type from one cell to its sister. In this example, the potential to generate B is segregated with the posterior daughter (as in other lineage trees, anterior daughters are indicated by left branches) in a and with the anterior daughter in b. A second division segregates B from C in a or A from B in b, generating the lineage shown. One mechanism that might underlie this phenomenon involves asymmetrically distributed determinants specifying the potential to generate cells of types A, B, and C; the plane of the first division (indicated by the vertical line) could differentially segregate these determinants. As drawn (and as in the example of the *P. redivivus* and *C. elegans* male early lineages described in the text) the posterior daughter is larger than its sister in a, and smaller than its sister in b. Altered segregation could occur without a reversal in the size of the daughters, depending on the distribution of determinants and the planes of division.

these differences suggests that the evolution of cell lineages involves a few distinct classes of alterations that lead to specific transformations of one cell lineage into another. Many of the types of transformations observed among the wild-type Z1 and Z4 lineages of both sexes and both species have also been seen in cell lineages of *C. elegans* after perturbation by mutation or laser ablation. The apparent ease with which such transformations can be generated experimentally indicates that they might be induced by relatively simple changes in the genetic program.

We propose the existence of four classes of cell lineage transformations (Figs. 21 and 22):

(1) Switches in the fates of specific cells to fates normally associated with other cells (Fig. 21b). In the *C. elegans* hermaphrodite, Z4.pp becomes a dtc; in the *P. redivivus* female, Z4.pp undergoes programmed cell death. Similar switches in cell fates have been found to be a common feature of *C. elegans* cell lineage mutants (Sulston and Horvitz, 1981; Chalfie *et al.*, 1981; W. Fixsen, V. Ambros, P. Sternberg, C. Ferguson, and R. Horvitz, unpublished observations) and to occur during regulation after laser ablation (Sulston and White, 1980; Kimble, 1981). In many of these examples, the cells involved are blast cells characterized by the division patterns and cell types they generate.

(2) Reversal in the polarity of a sublineage (Fig. 21c) (i.e., the anterior-most descendant derived from a blast cell acquires the properties normally associated with the posterior-most descendant, etc.). For example, the Z1 lineage is of opposite polarity in the *P. redivivus* male and female. Similar polarity reversals have been observed during regulation ("vectorial regulation") after laser ablation (Kimble *et al.*, 1979; Kimble, 1981).

(3) Alterations in the number of rounds of cell division. Additional cell divisions (either symmetric or asymmetric) are added, or else certain cell divisions are suppressed (Figs. 21d–g). For example, a single extra symmetric division (followed by a polarity reversal) would account for the major difference between the CP lineages of *P. redivivus* and *C. elegans*. An extra symmetric cell division has been induced by laser ablation of V1–V4 in a *C. elegans* male (Sulston and White, 1980). A series of additional symmetric divisions (proliferations) appear to have led to the extensive VL and VR lineages of the *P. redivivus* female. Proliferations have been induced (along with other changes) in *C. elegans* by the mutation *lin-4(e912)* (Chalfie *et al.*, 1981). A repeated asymmetric division would lead to a parental reiteration, as seen in the AR.a and AL.a lineages of the *P. redivivus* female, as well as in a number of lineages in *unc-86* mutants (Chalfie *et al.*, 1981); in this case a daughter cell acquires the fate of its parent, and thus such a change could be considered an example of the first class of transformations described above. A novel asymmetric division could lead to the generation of new cell types.

(4) A developmental potential normally associated with one cell instead becomes associated with its sister ("altered segregation," Fig. 22). As discussed above, a comparison of the gonad lineages of *P. redivivus* and *C. elegans* males indicates an apparent altered segregation of the potential to generate different cell types between the two species. Such altered segregation also seems to relate the right and left sex myoblast lineages of the *C. elegans* male (Sulston *et al.*, 1980). These apparent exceptions to the correlation of lineage and fate can be reconciled by a simple model for how lineage determines fate: determinants may be differentially segregated to progeny cells. The altered segregation of potential suggests that different division planes can lead to differential apportionment of such determinants. For example, in *P. redivivus* the DS nuclei are larger than the ED nuclei, but in *C. elegans* the VD nuclei are larger than the SV nuclei; i.e., in *P. redivivus* Z4.ap is larger than Z4.aa, whereas in *C. elegans* the converse is true. If there are asymmetrically distributed determinants specifying ejaculatory duct, vas deferens, and seminal vesicle, then different division planes cutting Z4.a in *P. redivivus* and *C. elegans* could result

both in the observed asymmetry in size and in the different fates of Z4.aa and Z4.ap in the two species.

EVOLUTION OF MONODELPHY

The differences between the lineages of the monodelphic gonad of the *P. redivivus* female and the didelphic gonad of the *C. elegans* hermaphrodite might illuminate the evolutionary relationship between monodelphy and didelphy. Triantaphyllou and Hirschmann (1980), reviewing cytogenetic and morphological studies of nematodes of the orders Tylenchida and Dorylaimida, have suggested that (1) monodelphic species have evolved from didelphic ancestors, (2) loss of a gonad arm has occurred several times during evolution, and (3) monodelphy results from the absence or degeneration of germ cells in one gonadal arm.

A mechanism that could eliminate most germ cells from the posterior ovary as well as transform a posterior ovary to a voluminous postvulval sac is suggested by experiments in which a dtc of the *C. elegans* hermaphrodite has been ablated with a laser microbeam (Kimble and White, 1981). Ablation of the posterior dtc (Z4.pp) leads to a cessation of growth of the posterior gonadal arm. Germ cells stop mitotic divisions and instead initiate meiotic divisions, while somatic cell divisions continue apparently normally (Kimble and White, 1981; J. Kimble, personal communication). Thus, the growth of the posterior gonadal arm, usually driven by the mitosis of germ line nuclei, arrests. The gonad of a *C. elegans* hermaphrodite in which Z4.pp has been ablated is monodelphic; it also has a structure similar to a postvulval sac (although it includes a few vestigial germ cells), as a result of the continuation of the Z1 and Z4 divisions in the absence of germ line proliferation.

By analogy, the death of Z4.pp in the *P. redivivus* female appears to be sufficent to account for its developing a monodelphic gonad. It is possible that this death results from some earlier divergence in the developmental programs of Z1 and Z4. There are other differences between Z1 and Z4: the divisions in the Z1 lineage precede those in the Z4 and the PR and PL lineages are distinct from the AR and AL lineages.

Changes in lineage affecting the length of the postvulval sac may have occurred after the primary event that caused loss of the germ line in the posterior gonadal arm. The variability of the PR.p and PL.p divisions in *P. redivivus* supports this view. Considerably more variability occurs in *C. elegans* cell lineage mutants than in wild-type animals (Horvitz and Sulston, 1980). If genetic perturbation increases variability in cell lineage, then a variable lineage in a wild-type strain may reflect an evolutionarily recent alteration in the developmental program. In the gonad of the *P. redivivus* female, the only such variability involves the divisions of PR.p and PL.p, suggesting that the postvulval sac is of recent evolutionary origin.

Since monodelphy probably evolves from didelphy, it is likely that the *P. redivivus* postvulval sac is shrinking. The deaths of PR.p and PL.p (or their daughters) may facilitate this process by eliminating vestigial structures. The degeneration of cells in the postvulval sac during the L4 also occurs in species of the monodelphic genus *Pratylenchus* that by a number of criteria appears to be undergoing rapid evolutionary change (Roman and Hirschmann, 1980). The deaths of PR.pa and PR.pp or of PL.pa and PL.pp, when they exist, provide the only known case in the development of *P. redivivus* and *C. elegans* in which two sister nuclei die; as in other instances of variable cell divisions (e.g., P3.p in *C. elegans* hermaphrodites and P9.p in *C. elegans* males) (Sulston and Horvitz, 1977; Sulston *et al.*, 1980), the fates of the daughters are identical to the fate of the undivided parental cell.

In summary, monodelphic species appear to have evolved from didelphic species. The programmed cell death of Z4.pp is a sufficient mechanism to account for the monodelphy of the *P. redivivus* female. The other differences between the cell lineages of Z1 and Z4 may reflect either (a) the initial steps in programming the death of Z4.pp (the temporal asymmetry of the early lineages) or (b) secondary modifications to reduce the size of the resulting vestigial tissue (modification of the PR and PL lineages or the posterior lineages of DL, DR, VL, and VR) or to fix the fates of cells that may have once been of equivalent alternative potentials (Z1.ppp and Z4.aaa).

The evolution of monodelphic species in diverse taxa suggests that monodelphy may be selectively advantageous. Monodelphy allows a nematode to develop a longer gonadal arm, which could cause eggs and/or larvae to be retained longer in the uterus. Indeed, didelphic *C. elegans* lays eggs, while monodelphic *P. redivivus* releases larvae. The ecological significance of this difference in life history is unclear.

"REGULATORY CELLS" AND EVOLUTION

It has been suggested that mutations affecting regulatory genes are more likely to lead to rapid evolutionary change than are mutations affecting structural genes (e.g., King and Wilson, 1975). Similarly, an alteration in the fate of a cell that exerts control over other cells—a "regulatory cell"—is a potential source of rapid and/or discontinuous evolutionary change. (Such an alteration in fate could reflect either a failure of normal differentiation or a switch to a fate normally

associated with another cell.) As outlined above, the programmed death of a posterior dtc, which in the *C. elegans* hermaphrodite is necessary for germ line proliferation in the posterior gonadal arm (Kimble and White, 1981) would lead to the loss of the posterior ovary in *C. elegans*. Such an alteration in the fate of Z4.pp may have occurred during the phylogeny of *P. redivivus*.

Mutants of *C. elegans* have established that mutations in a single gene can switch the fates of specific cells to fates normally associated with other cells (Sulston and Horvitz, 1981; Chalfie *et al.*, 1981; W. Fixsen, V. Ambros, P. Sternberg, C. Ferguson, and R. Horvitz, unpublished observations). The nature of the development of the monodelphic gonad of the *P. redivivus* female reveals that a change in the fate of a specific (regulatory) cell can have dramatic morphological consequences. Thus, single genetic events that change the fates of specific cells may provide a mechanism for saltatory evolution.

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

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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.

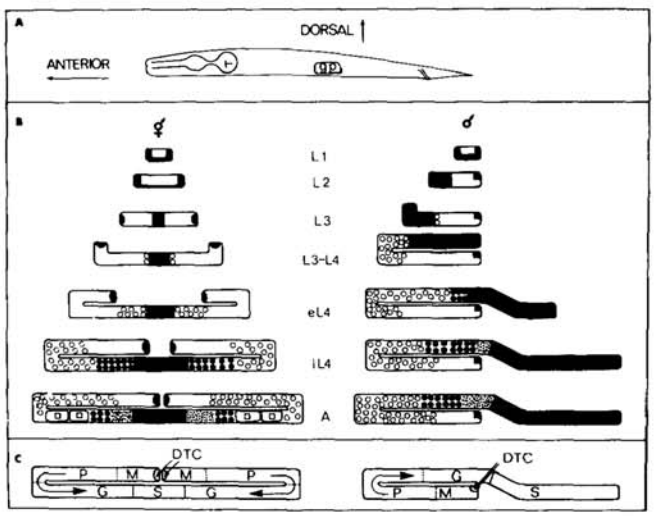

FIG. 1. Gonadogenesis and adult anatomy in hermaphrodites (left) and males (right). (A) The midventral position of the gonadal primordium (gp) is the same in both sexes. The anterior-posterior and dorsal-ventral coordinates indicated here are used for all the diagrams in the paper. (B) Morphology of 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).

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.

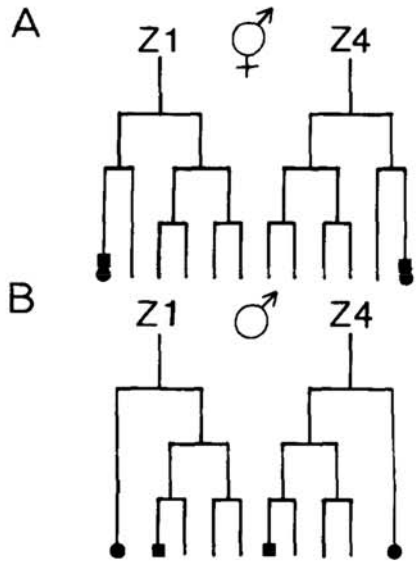

FIG. 2. Ancestry of distal tip cells (●) and cells with leader potential (■) in hermaphrodites (above) and males (below). Each vertical line represents a cell, and each horizontal line represents a cell division. The left-hand branch indicates the anterior and the right-hand branch the posterior daughtor 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.

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

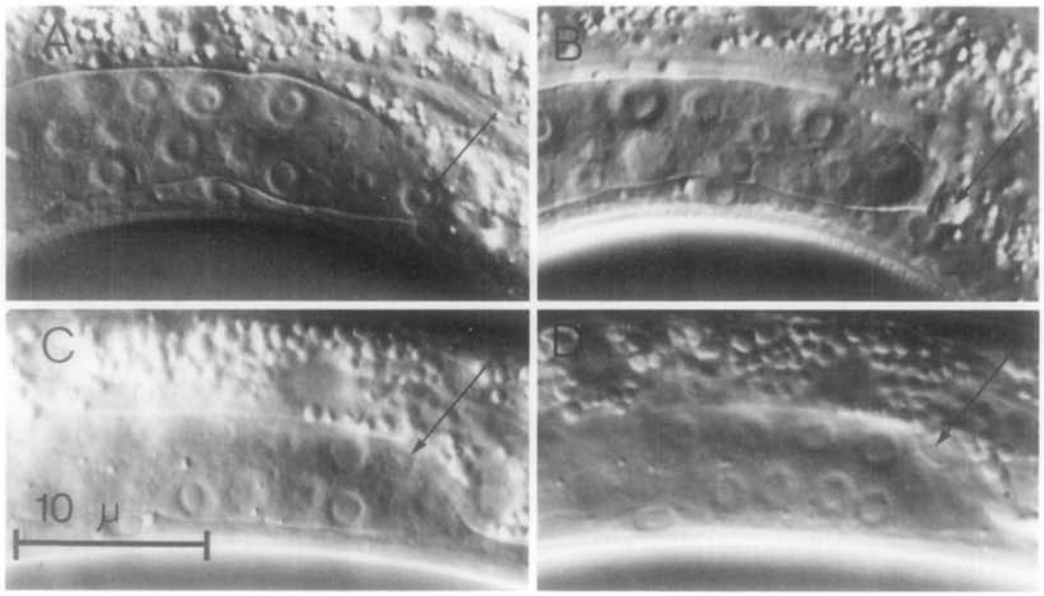

FIG. 3. Nomarski micrographs of distal tip cells (arrows). A posterior distal tip cell is shown in an L3 hermaphrodite before (A) and after (B) ablation. A posterior distal tip cell is shown in an L3 male before (C) and after (D) ablation.

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 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.

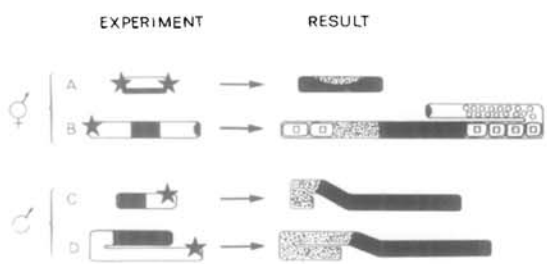

FIG. 4. Effects of laser ablation (depicted by stars) of distal tip cells on germ cell development. Representation of different cell types is the same as in Fig. 1B. In hermaphrodites, if both distal tip cells are ablated (A) development of both anterior- and posterior-half gonads is blocked. If one 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.

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

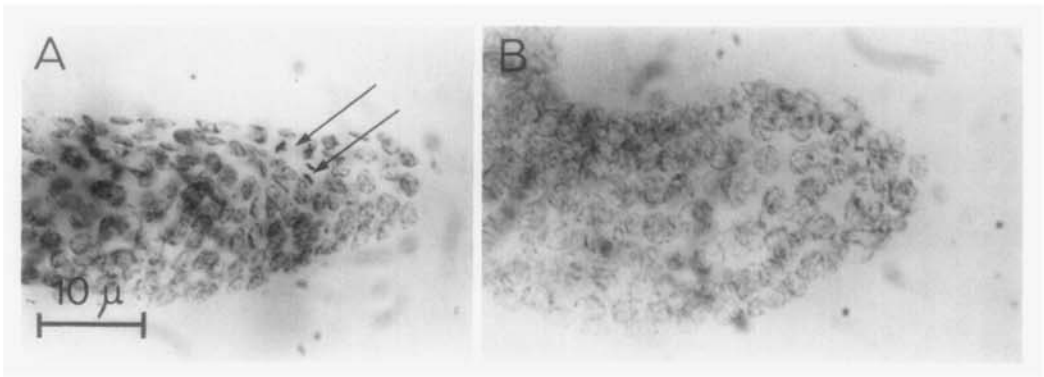

FIG. 5. Chromosome morphology of nuclei before and after distal tip cell ablation observed in Feulgen-stained preparations of dissected gonads. (A) Nuclei in the distal end of an unoperated hermaphrodite gonad are not meiotic. Most are in mitotic interphase and two are dividing (arrows). (B) Nuclei in 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.

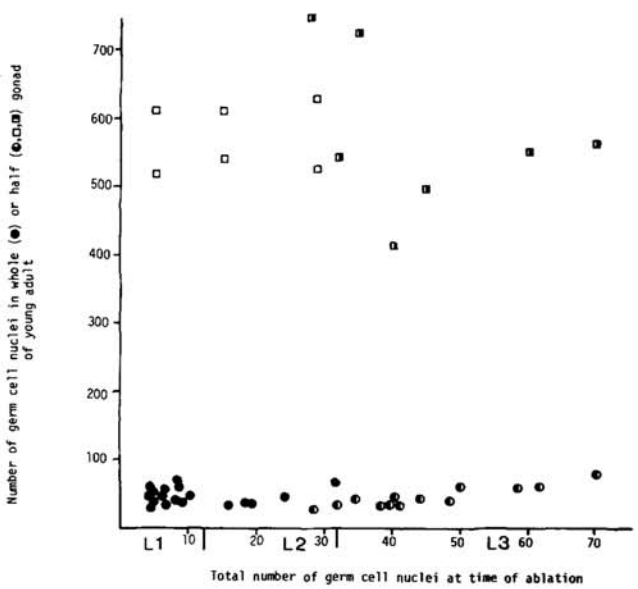

FIG. 6. Effect of laser ablation of hermaphrodite distal tip cells on number of germ cell nuclei produced. The number of germ cells present at time of ablation (abscissa) was counted using Nomarski optics. The number of germ cells present in the young adult (ordinate) was determined by counting nuclei 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

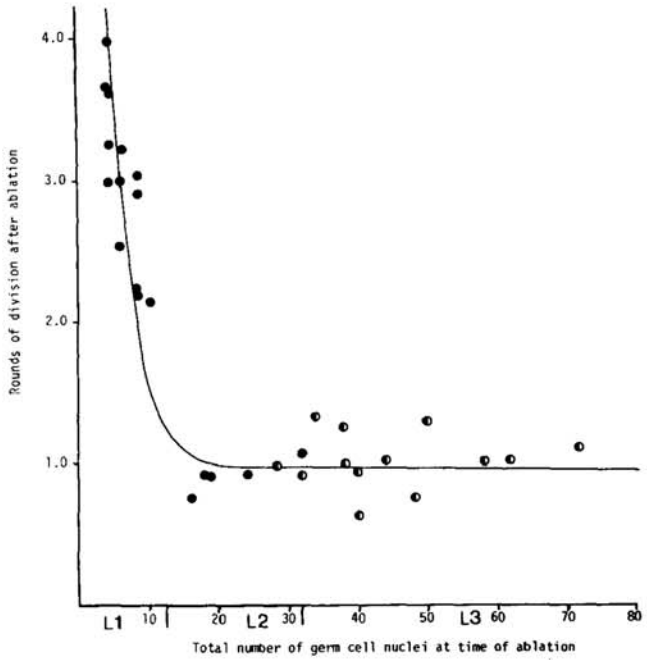

FIG. 7. Effect of laser ablation of distal tip cells on number of mitotic divisions following ablation. The number of germ cells present at time of ablation (abscissa) was counted using Nomarski optics. The number of rounds of division of germ cells after ablation was calculated by solving for n in the 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-

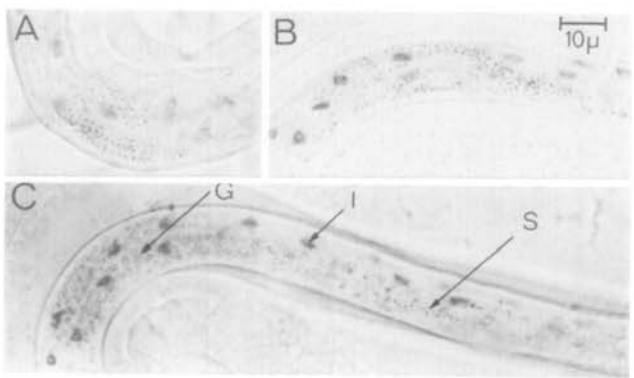

FIG. 8. Gamete differentiation in male gonads after distal tip cell ablation as seen in Feulgen-stained preparations of late L4 animals. (A) Gonad contains a small number of sperm (small dots of Feulgen-positive material) after ablation in L1. (B) Gonad contains a larger number of sperm after ablation 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.

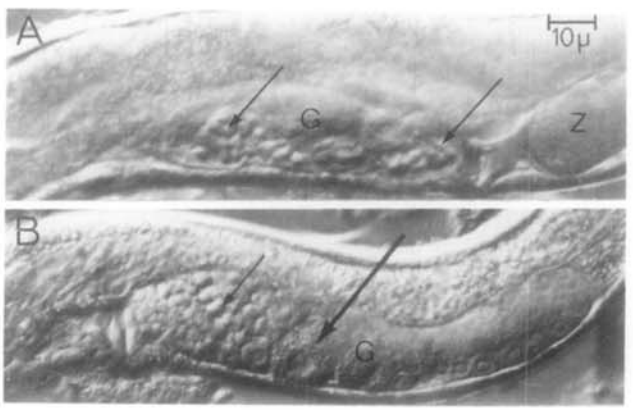

FIG. 9. Gamete differentiation in hermaphrodite gonads after ablation of a single distal tip cell as seen with Nomarski in young adult animals. (A) Half gonad (G) contains sperm only (arrows) after ablation of one distal tip cell during L2–L3 lethargus. A zygote (Z) is seen in the spermatheca of the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

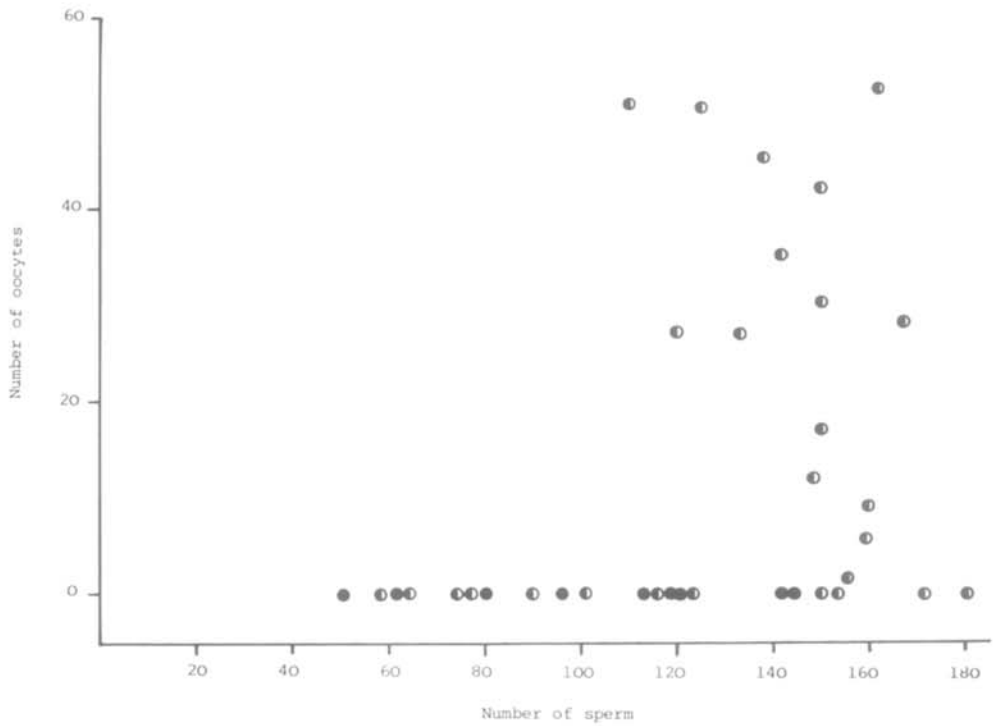

FIG. 10. Number of sperm and oocytes made in the half gonads of individual ablation experiments. In the intact animal, each half gonad makes about 140–160 sperm. In distal tip cell-ablated animals, each half gonad makes the normal complement of sperm before any oocytes are produced. ●, both distal tip 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 hermaphrodite	29-32	6	0	
	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 killed in L1 hermaphrodite	25-28	3	0	
	29-32	5	0	33-34
	33-34	5	5	
	35-37	5	5	
D. Z3 killed in L1 hermaphrodite	35-37	4	0	
	38-39	5	1	38-39
	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

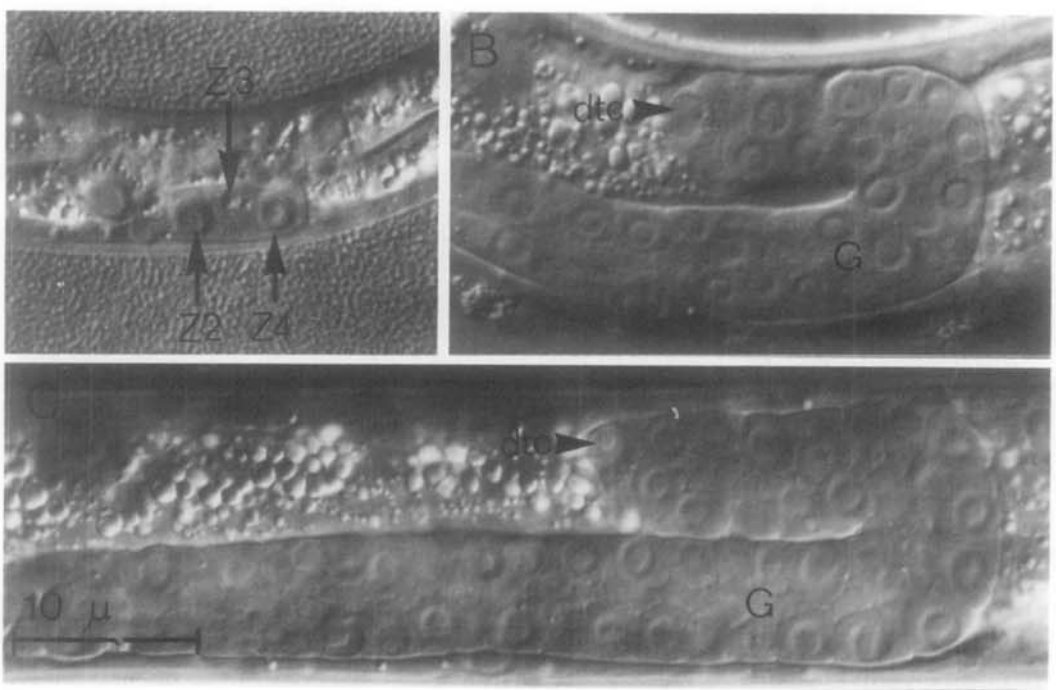

FIG. 11. Nomarski micrographs showing (A) ablation of Z3 in the gonadal primordium, (B) size of a half gonad (G) in a young L4 worm after ablation of Z3, and (C) size of a half gonad (G) in an unoperated animal of the same age as (B). All pictures are at the same magnification. dtc, distal tip cell.

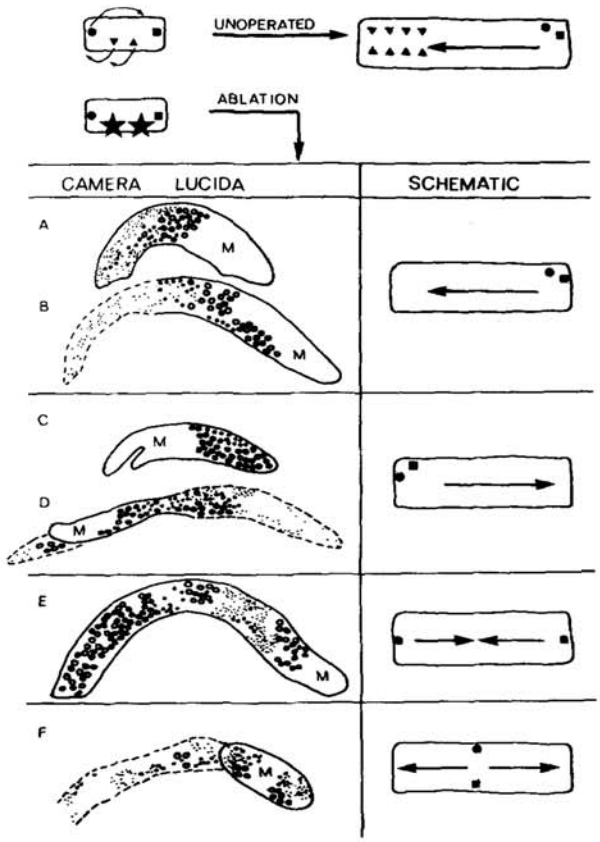

FIG. 12. Change in distal tip cell position corresponds to a change in polarity in the gonad. The anterior-posterior and dorsal-ventral coordinates are the same as shown in Fig. 1. In the unoperated male gonad (top diagram) somatic gonadal cells, Z1.a, ●, Z1.p, ▼; Z4.a, ▲; and Z4.p, ■ undergo rearrangement 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; ○, 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

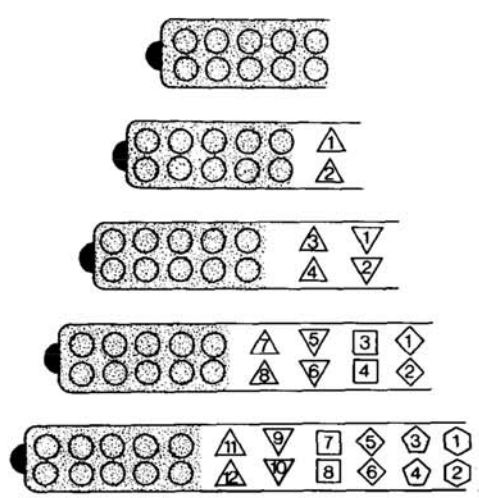

FIG. 13. Generalized model for establishing a gradient of maturation based on the distal tip cell control over meiosis. The control element (solid half circle) is localized on one end of a growing tube, and the controlling activity acts over a distance (stippled area). Due to proliferation of the target 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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Post-embryonic Cell Lineages of the Nematode, *Caenorhabditis elegans*

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The number of nongonadal nuclei in the free-living soil nematode *Caenorhabditis elegans* increases from about 550 in the newly hatched larva to about 810 in the mature hermaphrodite and to about 970 in the mature male. The pattern of cell divisions which leads to this increase is essentially invariant among individuals; rigidly determined cell lineages generate a fixed number of progeny cells of strictly specified fates. These lineages range in length from one to eight sequential divisions and lead to significant developmental changes in the neuronal, muscular, hypodermal, and digestive systems. Frequently, several blast cells follow the same asymmetric program of divisions; lineally equivalent progeny of such cells generally differentiate into functionally equivalent cells. We have determined these cell lineages by direct observation of the divisions, migrations, and deaths of individual cells in living nematodes. Many of the cell lineages are involved in sexual maturation. At hatching, the hermaphrodite and male are almost identical morphologically; by the adult stage, gross anatomical differences are obvious. Some of these sexual differences arise from blast cells whose division patterns are initially identical in the male and in the hermaphrodite but later diverge. In the hermaphrodite, these cells produce structures used in egg-laying and mating, whereas, in the male, they produce morphologically different structures which function before and during copulation. In addition, development of the male involves a number of lineages derived from cells which do not divide in the hermaphrodite. Similar postembryonic developmental events occur in other nematode species.

INTRODUCTION

The development of a multicellular organism from a unicellular egg involves a complex pattern of repeated cell divisions. Classical observations of nematode embryogenesis (reviewed by Chitwood and Chitwood, 1974) revealed that in these organisms early development follows a rigidly fixed program, i.e., an invariant pattern of cell divisions produces specific progeny cells which, in turn, give rise to particular parts of the organism. These and similar studies led to the concept of "cell lineages," in which the ancestry of different organs can be traced back to specific progenitor cells and, ultimately, to the egg (e.g., Wilson, 1925).

In this paper, we extend these observations from the embryonic to the postembryonic period. Many cell divisions occur in the nematode *Caenorhabditis elegans* after hatching. As in embryogenesis, the pattern of these divisions is rigidly determined; essentially invariant postembryonic cell lineages generate fixed numbers of neurons, glial cells, muscles, and hypodermal cells of rigidly specified fates. These lineages reveal the ancestral relationships among specific cells of known structure and function; they thus complement the classical embryology, which defined the ancestral relationships among different organs. We have determined the postembryonic cell lineages by direct observation of living nematodes.

C. elegans is an excellent organism for the study of cell lineages. It is small, easily cultured, and readily amenable to genetic manipulations (Brenner, 1973, 1974). Like other nematodes (e.g., Chitwood and Chitwood, 1974), *C. elegans* is anatomically simple (Fig. 1). Its tubular body, consist-

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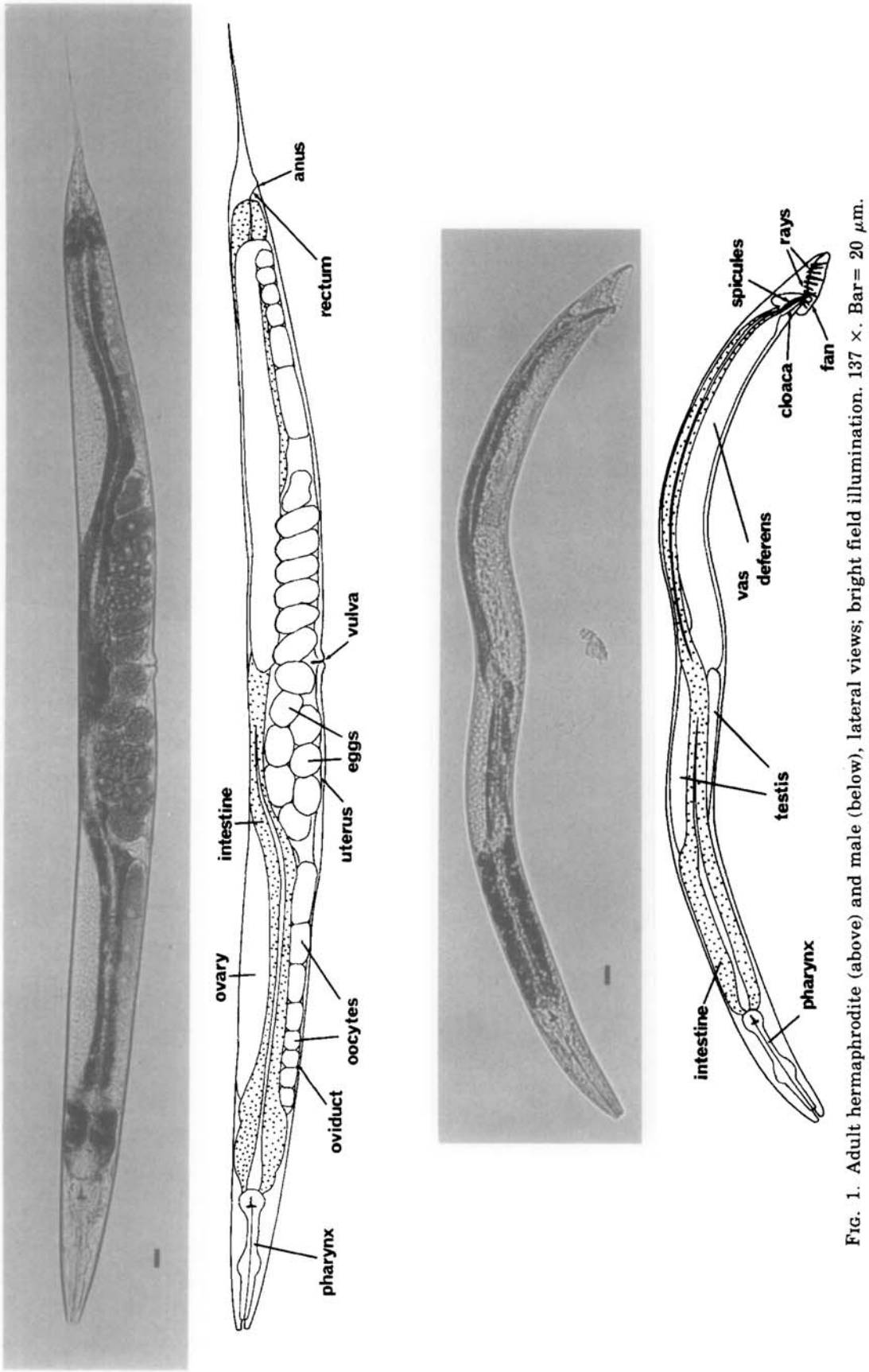

FIG. 1. Adult hermaphrodite (above) and male (below), lateral views; bright field illumination. 137 ×. Bar= 20 μm.

ing of a hypodermal wall and an underlying musculature, encloses its digestive and reproductive systems. Although it has most major differentiated tissue types (nerve, muscle, hypodermis, intestine, and gonad), *C. elegans* consists of relatively few cells; as we show below, the adult contains fewer than 1000 nongonadal nuclei.

The life cycle of *C. elegans* is rapid; in 3.5 days (at 20°C) it develops from a fertilized egg through four larval stages to a mature adult. Superficially, the newly hatched larva appears to be quite similar to the adult. The most obvious developmental change is in the size and complexity of the gonad, which contains 4 nuclei in the young larva and increases to about 2500 in the mature adult (Hirsh *et al.*, 1976). Gross morphological differences are also apparent in the nongonadal sexual structures of the hermaphrodite and male; these structures are not present in young larvae (Figs. 1 and 2).

Recently, one of us (Sulston, 1976) described a technique with which it is possible to determine cell lineages by observing living nematodes. When appropriately mounted on a thin block of agar on a microscope slide, a nematode proceeds through its normal life cycle. Observation of such animals under Nomarski differential interference contrast optics in the light microscope allows one to directly follow the migrations, divisions, and deaths of individual cells.

Using this technique, we have extended the earlier study (Sulston, 1976) of development in the ventral nervous system of *C. elegans*. In this paper, we report the complete postembryonic nongonadal cell lineages of *C. elegans*. These lineages produce the accessory sexual structures of the hermaphrodite and male and lead to other significant developmental changes in the neuronal, muscular, hypodermal, and digestive systems.

To provide a framework for describing the anatomical changes which occur during larval development, we begin with a detailed account of the cellular anatomy of the young nematode. This anatomy has been determined by a combination of Nomarski and electron microscopy.

Readers who do not wish to become submerged in the detailed descriptions under Materials and Methods and Results will find a summary of our observations and conclusions under Discussion.

MATERIALS AND METHODS

(A) Nematode Strains

Caenorhabditis elegans var. Bristol (strain N2) was obtained from Sydney Brenner and grown on *Escherichia coli* OP50 on petri dishes at 20°C, as described previously (Brenner, 1974). Hermaphrodite stocks were propagated by self-fertilization; male stocks were propagated by crossing with hermaphrodites.

Aphelencoides blastophthorus, Longidorus macrosoma, Panagrellus redivivus (*Panagrellus silusiae*), and *Turbatrix aceti* were obtained from David J. Hooper, Rothamsted Experimental Station, Harpenden, Hertfordshire, England. *Ascaris lumbricoides* var. *suis* was obtained from the local slaughterhouse; eggs were squeezed out of adult uteri and development was initiated by incubation in 0.1 *M* sulfuric acid at 25°C (Rogers, 1960); at appropriate intervals, samples were washed with insect tissue culture medium (Shields *et al.*, 1975) and larvae were released from the eggs by gently rolling the tip of a Pasteur pipet over them.

(B) Techniques

(1) Study of Living Specimens

(a) Mounting. An agar slab about 0.5 mm thick was prepared by flattening a drop of 5% agar on a microscope slide with a second, siliconized slide placed across it; the siliconized slide was supported by "spacer" slides raised by one or two thicknesses of adhesive tape. After the agar had hardened, the siliconized slide was removed and a small drop (about 2 μl) of 10%

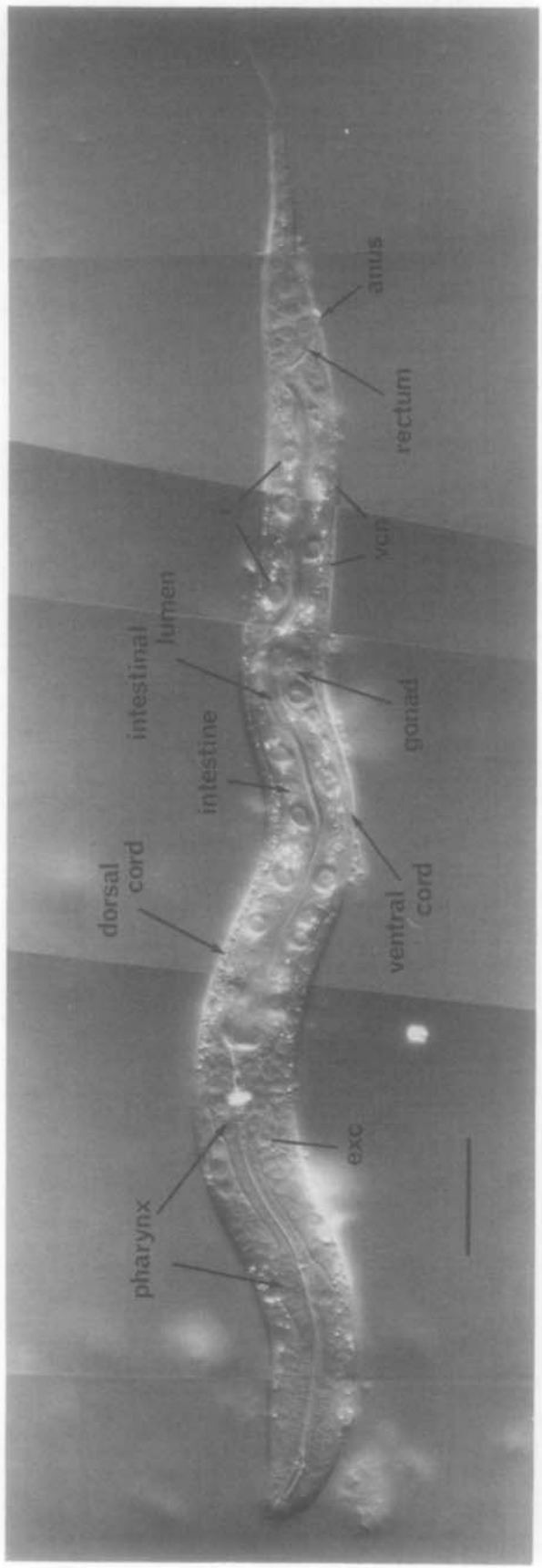

FIG. 2. Young L1 hermaphrodite, lateral view; Nomarski optics. Montage of photographs of a single living animal. exc, excretory cell; i, intestinal nuclei; vcn, ventral cord neurons. Bar = 20 μm.

polyvinylpyrrolidone (molecular weight 44,000; BDH Chemicals, Ltd., Poole, England) in S medium (Sulston and Brenner, 1974) was placed on the agar, care being taken not to break its surface. A selected nematode was transferred to this drop, using either a sharpened wooden stick or a human eyelash attached with Plasticine to a stick. An animal successfully transferred would thrash wildly. The center of a 12 × 12-mm coverslip was very thinly coated with *E. coli* OP50 scraped from a petri dish with a wire loop. The coverslip was lowered gently onto the agar, care again being taken not to break the agar surface. The volume of liquid was such that the pressure of the coverslip prevented the worm from thrashing without excessively inhibiting its movement. All of these operations were performed at a room temperature of about 20°C.

Excess agar protruding beyond the coverslip was removed with a razor blade. The edges of the remaining agar block were sealed with silicone grease or Vaseline to prevent dehydration. Air bubbles which formed when the coverslip was positioned were gradually absorbed by the agar. After a period of quiescence (less than 1 hr), the nematode generally moved into the bacterial lawn and started to feed.

Nematodes mounted for observation in the light microscope could be safely removed afterward for other types of study (e.g., electron microscopy).

(b) Observation. Nematodes were observed in either a Zeiss Universal or a Zeiss Standard RA microscope equipped with a Plan 100 objective and Nomarski differential interference contrast optics. To reduce heating, illumination was kept as low as possible; a heat filter was routinely used and, occasionally, a broadband green interference filter was also employed. Air temperature was maintained between 19 and 22°C.

Because *C. elegans* is small and transparent, internal structures in intact living specimens could be readily examined. Nuclei and large nucleoli were clearly resolved by Nomarski optics, which permits visualization of changes in refractive index and has a very shallow depth of field (e.g., Fig. 2). Cell boundaries, however, were not always visible. The nuclei of different types of cells were generally distinguishable and are described under Results.

This technique is nondestructive and allows the nematode to live under reasonably natural conditions. While mounted for observation, the nematode moved freely between the coverslip and the surface of the agar block. Attracted by the bacterial lawn, it generally stayed near the middle of the coverslip. If the nematode strayed out from under the coverslip, it could be transferred to a petri dish and remounted.

C. elegans can only flex in a dorso-ventral direction. Hence, because it was restricted to the plane between the agar and the coverslip, the nematode was observed lying on its side. A properly mounted animal usually remained lying on the same side. However, for a period just before molting, nematodes actively and repeatedly invert their positions with a rapid twisting movement. On occasion, such "flips" proved very inconvenient, as they made it difficult to continue observing cells which had moved from the side nearest the coverslip to the side nearest the agar slab (because of interference introduced by the body of the nematode).

(c) Cell lineages. Lineages were determined by continually observing given nuclei as they migrated, divided, and died during the course of postembryonic development. Because cell boundaries were often not visible, these lineages depict the behavior of nuclei and not necessarily the behavior of cells; it is possible that some nuclear divisions (or movements) are not accompanied by concomitant cellular divisions (or movements). During periods of nuclear activity, sketches of those nuclei of interest and of appropriate adjacent landmarks (usually other nuclei) were made as

frequently as possible. During periods of nuclear quiescence, observations were recorded at intervals of 0.5–2 hr. When lineages in a given nematode were to be followed for more than 1 day, the animal was refrigerated overnight at 6–8°C during a suitable quiescent period. Nematodes were generally chilled during the early period of a given larval stage. (Individuals refrigerated just prior to or during lethargus – a 2-hr period preceding each of the four larval molts during which there is little pharyngeal pumping or body movement – often did not recover.) When returned to 20°C, most individuals recommenced development after a 0.5- to 2-hr lag. Generally, nematodes were refrigerated for no more than 15 hr.

When appropriately mounted, healthy nematodes developed as rapidly as on petri dishes. Some individuals, particularly after refrigeration, developed more slowly. Occasionally, a younger worm was damaged during the mounting process and barely developed at all; such individuals were discarded. To obtain a standard time scale for the lineage charts presented below, observed time intervals were normalized according to the observed length of the appropriate intermolt period.

On the lineage charts, the time of a molt is defined as the moment when the head of the nematode breaks through the old cuticle after lethargus. Lethargus is indicated by dotted regions along the time scales. The time of a cell division reflects the time a metaphase plate is visible (see Results, Cell Division). The time of a cell death indicates the time of maximal nuclear refractility (see Results, Programmed Cell Death).

(d) Photography. Photomicrographs were taken with a Zeiss microflash illuminator. All photomicrographs are of living specimens.

(2) Cell Assignments

Nuclei observed with Nomarski optics were assigned to specific cell types by tracing them through to the adult stage, in which they have been identified by reconstruction from serial section electron micrographs available in this laboratory and prepared as described by Ward *et al.* (1975).

(3) Camera Lucida Drawings

Drawings were made using a Zeiss camera lucida. Specimens were mounted on slabs of 5% agar as described above, except (1) no bacteria were added, and (2) the molten agar contained 1-phenoxy-2-propanol (Bird, 1971) as an anesthetic. The concentrations of phenoxypropanol used were 0.2% v:v for L1's, 0.3% for L2's and L3's, and 0.5% for L4's and adults. (L*n* denotes animals of the *n*th larval stage.)

The line drawings of nematodes presented below are based upon camera lucida drawings. They show nuclei, nucleoli (when visible), and other appropriate morphological landmarks. These drawings depict typical individuals, but, as there are minor variations from animal to animal, they cannot always be used to identify nuclei in animals in which lineages have not been traced directly. Also, minor distortions in nuclear position sometimes are evident in anesthetized nematodes.

(4) Feulgen Staining

A small drop of 10% ovalbumin was placed on a glass slide coated with a thin layer of collagen prepared according to Bornstein (1958). A large number of nematodes were spread evenly over the surface of the slide with a paper strip; alternatively, single worms were transferred with a wooden stick or platinum wire. When necessary to prevent evaporation, the slide was kept cool over ice. The slide was placed in Carnoy's fixative (Pearse, 1968) for at least 1 hr; overnight fixation was preferable. After rehydration through 50 and 30% ethanol (10 min each), the slide was incubated in 1 *N* HCl at room temperature for 10–20 min and then in 1 *N* HCl at 60°C for 10–12 min. It was transferred to

Schiff's reagent for 1.5–2 hr. Schiff's reagent was prepared by bubbling sulfur dioxide gas through 1% basic fuchsin, essentially as described by Rafalko (1946). After staining, the slide was rinsed twice in distilled water and dehydrated through a graded series of alcohols (30, 50, 70, and 90%, 10 min each). After two 30-min baths in absolute ethanol and two 30-min baths in xylene, the slide was mounted in Depex mounting medium (Gurrs, London, England). Stained specimens were examined using bright field illumination through a blue filter.

(5) Laser

A coumarin dye laser microbeam system developed by J. G. White was used to kill specific cells in individual nematodes. This system produces a focused spot about 1.5 μm in diameter and can destroy a given cell with no apparent damage to its neighbors. It is based upon a system developed by Berns (1972) and will be described in a future publication by Dr. White.

(C) Nomenclature

In describing the anatomy and development of *C. elegans*, we have adopted the following system to name specific cells (or nuclei) and their daughters. Upper-case letters indicate blast cells of unknown embryonic lineage; for example, M is a mesoblast present in the newly hatched L1. In some cases, a set of blast cells undergoes similar patterns of cleavage and produces morphologically similar groups of daughters; each member of such a set is denoted with a common upper-case letter followed by a specific numeric designation, e.g., six blast cells on each side of the lateral hypodermis are V1–V6. When a blast cell divides, each daughter is named by adding to the name of its mother cell a single lower-case letter representing its position immediately after division relative to its sister cell. A cell which divides along a dorso-ventral axis has its daughters indicated by a "d" and a "v"; e.g., M divides to produce M.d and M.v. Left–right divisions are indicated by "l" and "r"; e.g., M.d divides to produce M.dl and M.dr. Anterior–posterior divisions are indicated by an "a" and a "p"; e.g., M.dl divides to produce M.dla and M.dlp. As indicated in these examples, a period separates the name of the original blast cell from the labels which define specific progeny of subsequent divisions.

Within the lineage trees, "d," "l," and "a" daughters are represented by left branches, and "v," "r," and "p" daughters are represented by right branches; these branches are labeled accordingly (except in lineages with most divisions anterior–posterior, in which the labels a and p are omitted from the branches). An oblique division is indicated on the branches by labels with two (or three) characters; the first character from these labels defines the branch assignments. For example, E.r divides along an anterior/ventral–posterior/dorsal axis; hence, the left branch is labeled "av," and the right branch is labeled "pd" (Fig. 22). The daughters of such a division are named by using only the first of the characters which indicate the division axis, i.e., E.ra and E.rp. Thus, the number of characters which follow a blast cell name directly implies the number of divisions which generated the progeny cell; e.g., E.rap was produced by the third division of E.

In the B lineage, the precise fates of certain progeny cells are not predetermined. For example, B.alaa and B.araa, generated on the left and right sides respectively, recentralize. Their relative anterior–posterior position after recentralization is indeterminate. The anterior cell ("Bα") follows one lineage program, whereas the posterior cell ("Bβ") follows another. In other words, either B.alaa or B.araa can become Bα. Thus a greek letter following a blast cell name indicates a cell derived from that blast cell via one of a number of alternative lineage routes.

Entirely lower-case names are used as

abbreviations for cell types in lineage charts, camera lucida drawings, and photographs. For example, "bm" identifies a body muscle. In the lineage charts, this name follows the systematic name based on the lineage history of the cell, e.g., M.dlpp; bm. The abbreviations used are: bm, body muscle; cc, coelomocyte; dep, anal depressor muscle; exc, excretory cell; exc gl, excretory gland; g, neuron or glial cell; hsn, hermaphrodite-specific neuron; i, intestinal nucleus; im, intestinal muscle; plg, posterior lateral ganglion; rect gl, rectal gland; se, seam cell; set, tail seam cell; sph, anal sphincter muscle; sy, syncytial nucleus; um1, type 1 uterine muscle; um2, type 2 uterine muscle; vcn, ventral cord neuron; vh, ventral hypodermal nucleus; vm1, type 1 vulval muscle; vm2, type 2 vulval muscle.

The nomenclature used in this paper is somewhat different from that used previously (Sulston, 1976) to describe the cell lineages of the ventral nervous system of *C. elegans*. We believe that the revised nomenclature is easier to use in referring to the relatively large number of cells described in this paper.

RESULTS

(A) Anatomy

(1) Gross Morphology

Like other nematodes (e.g., Chitwood and Chitwood, 1974), *C. elegans* has an elongated cylindrical body with tapered ends (Figs. 1 and 2). An external cuticle covers the hypodermal body wall. Beneath the hypodermis are four longitudinal rows of body muscles located subventrally and subdorsally. The mouth leads into the bilobed muscular pharynx, which pumps food into the tubular intestine to the rectum and anus. The nervous system consists of a circumpharyngeal nerve ring, dorsal and ventral nerve cords, and a variety of sensory receptors and ganglia.

The reproductive system of the adult hermaphrodite produces both sperm and oocytes. Two reflexed gonadal arms, each of which contains an ovary, oviduct, spermatheca, and uterus, terminate at the vulva, located midway along the ventral side. The reproductive system of the adult male consists of a single testis which empties into the vas deferens; the vas deferens unites posteriorly with the rectum, forming a cloaca. Accessory sexual structures are located in the male tail.

(2) Newly Hatched Hermaphrodite

(a) Ectoderm. The ectoderm, which comprises the majority of cells in the L1, can be broadly divided into the nervous system with its supporting cells and the hypodermis. Embedded in the hypodermis are various blast cells and a number of cells of specialized functions.

The hypodermis essentially consists of four longitudinal ridges (dorsal, ventral, lateral right, and lateral left) joined circumferentially by thin sheets of cytoplasm which separate the muscle cells from the cuticle. Hypodermal nuclei, generally large and flat with large nucleoli (Fig. 3), are located in these four ridges. The dorsal ridge, which is filled with refractile granules in the late embryo and the young L1, normally contains nuclei only in the head. The ventral ridge contains hypodermal nuclei in the head and tail but not along the body in the young L1; as described below (see Cell Lineages, Ectoderm), a number of hypodermal nuclei are produced in the ventral ridge during later development. The lateral hypodermal ridges are almost bilaterally symmetrical and contain nuclei throughout their lengths. Twenty-four of these nuclei are arranged in six similar ventro-lateral pairs located on each side along the body; each pair includes one hypodermal precursor (V) and one precursor (P) of the ventral nervous system (Figs. 4 and 9). Anterior to the first of these pairs on each side there are two hypodermal precursors (H1 and H2). Each side of the tail has a single precursor (T). There are 11 other hypodermal nuclei

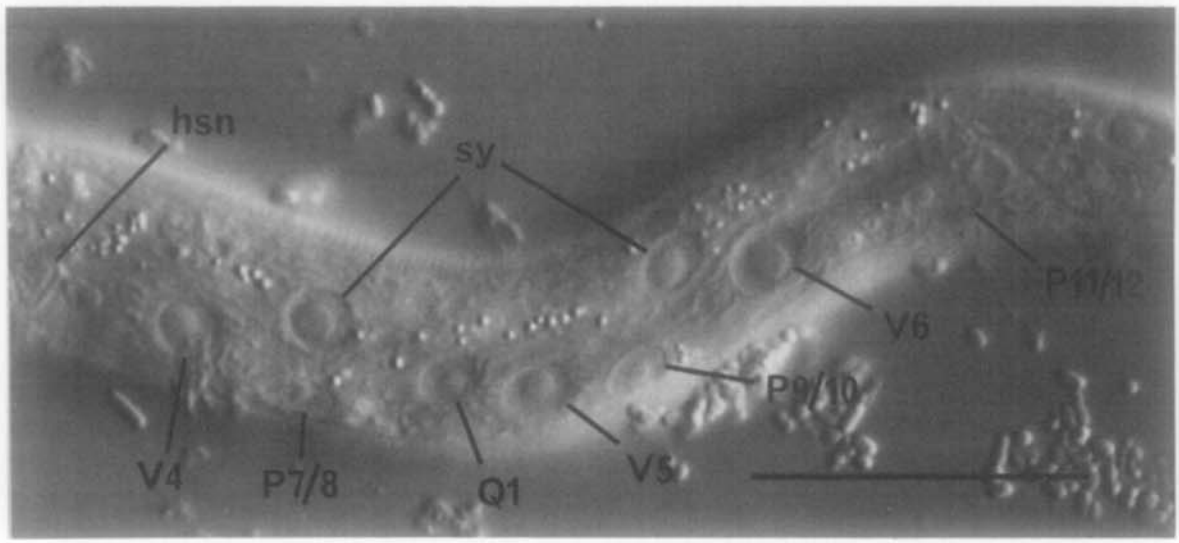

FIG. 3. Left lateral hypodermis, posterior region of young L1 hermaphrodite, lateral view; Nomarski optics. hsn, hermaphrodite-specific neuron; sy, syncytial hypodermal nuclei. Bar = 20 μm.

on each side of the nematode. These nuclei are not bilaterally symmetrical in location; the set on the left appears to be displaced posteriorly relative to that on the right. Except for the precursor cells, almost all of the lateral hypodermal nuclei lie in a single syncytium (White, 1974). Some syncytial hypodermal nuclei do not occupy rigidly defined positions and seem to be moved passively during development.

Three gland cells (rect gl) (Chitwood and Chitwood, 1974) ring the junction between the intestine and the rectum (Fig. 4). Two other cells (B and F) are located dorsal to the rectum; these cells divide only in the male. Another pair of cells is located laterally; the cell on the left (K) divides in both hermaphrodites and males. Two additional cells (C and E) are located ventral to the rectum and posterior to the pre-anal ganglion. C lies more ventrally and further right than E. Both C and E divide only in the male.

The cells of the so-called excretory system (Bird, 1971) lie in close association with the hypodermis. The nucleus of the "H"-shaped excretory cell (exc) lies just left of the midline, ventral to the rear bulb of the pharynx. Immediately anterior to it is the nucleus of the duct cell. Two gland cells (exc gl) which feed into the excretory duct are located just posterior to the posterior-most region of the ventral ganglion. Although normally quiescent, the duct pulsates in dauer larvae. [The dauer larva is a developmental variant specialized for dispersal and/or survival in harsh conditions (Cassada and Russell, 1975)]. The rate of excretory duct pulsation in dauer larvae of other nematodes depends upon the osmolarity of the medium (e.g., Weinstein, 1952), indicating that one function of the "excretory" system is osmoregulation.

In the L1, the nuclei of the nervous system are generally distinct morphologically from those of the hypodermis. Neurons and glial cell nuclei are small and granular with no visible nucleoli (Figs. 2, 7, and 8); in older larvae and adults, these nuclei often acquire visible nucleoli. The adult anterior sensory nervous system has been described in detail (Ward *et al.*, 1975; Ware *et al.*, 1975); we have observed no postembryonic cell divisions in this system. Its cell bodies lie in groups anterior and posterior to the ring of nerve fibers which runs circumferentially around the isthmus of the pharynx. The posterior groups, known as the lateral ganglia, also

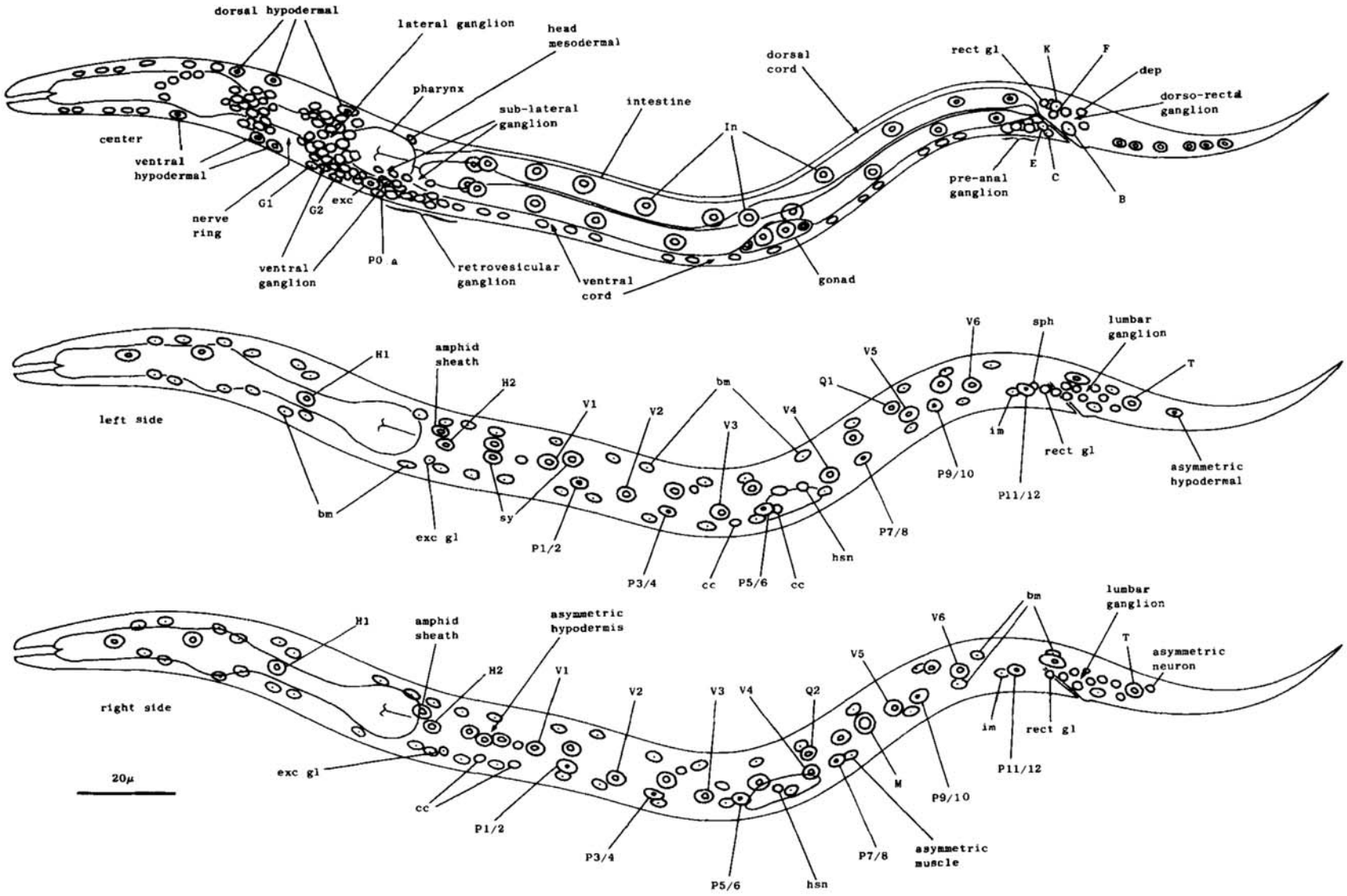

FIG. 4. Young L1 hermaphrodite, lateral views, showing nuclei as seen in central, left, and right optical sections. Not all nuclei in the head ganglia are shown. These figures are somewhat schematic, as nuclei are not arranged precisely into three planes. Left–right pairs of P cells (e.g., P1/2) are not finally named until after their subsequent migration into the ventral cord (see Cell Lineages, Development). bm, body muscle; cc, coelomocyte; dep, anal depressor muscle; exc, excretory cell; exc gl, excretory gland; hsn, hermaphrodite-specific neuron; im, intestinal muscle; rect gl, rectal gland; sph, anal sphincter muscle; sy, syncytial hypodermal nuclei.

include some interneuron and motoneuron cell bodies. In the young L1, the amphid sheath cell bodies (Ward *et al.*, 1975) lie behind the pharynx and are embedded in the hypodermis; during larval development, they gradually move anteriorly and medially (Figs. 4 and 9). The ventral ganglion contains two blast cells (G1 and G2) and lies ventral and posterior to the nerve ring, extending to the excretory glands. The sublateral ganglia lie dorsolateral to the excretory glands. The retrovesicular ganglion, with 12 mature nuclei and 1 neuroblast (P0.a), lies ventral and posterior to the excretory cell and merges with the ventral nerve cord. The ventral nerve cord is the major longitudinal fiber bundle in the nematode and runs along the right side of the ventral hypodermal ridge; the dorsal nerve cord contains fewer fibers and runs along the left side of the dorsal hypodermal ridge. In the L1, 15 motoneuron cell bodies are present in the ventral cord. At the posterior end of the ventral cord is the pre-anal ganglion, which contains six nuclei. Two lumbar ganglia are present in the post-anal region; the ganglion on the left contains eight nuclei, and that on the right contains nine nuclei. A small dorsorectal ganglion with two nuclei is also present in the tail.

On each lateral side of the nematode, four isolated cell bodies with neuronal-like nuclei are embedded in the hypodermis immediately beneath the cuticle (Figs. 4 and 9). The small subventral neuron (hsn) near the gonad primordium in the hermaphrodite is absent in the male (Fig. 5). Two other lateral neuronal-like cells are located (a) just anterior to V1 and (b) subdorsally, near V3. A fourth nucleus with similar morphology is present between V3 and V4, anterior to the hermaphrodite-specific neuron; although this nucelus becomes more hypodermal-like during development, the cell nonetheless appears to be a neuron or supporting cell in the adult.

Also lying subdorsally in the lateral hypodermis between V4 and V5 is a pair of neuroblasts: one on the left side (Q1) and one on the right side (Q2).

(b) Mesoderm. At hatching, the L1 contains 81 body muscle cells (bm) which control its locomotion. The nuclei of these cells are ovoid with a granular nucleoplasm surrounding a spherical nucleolus (Fig. 6). During the L2 stage, the nucleoplasm becomes smooth and remains so throughout the rest of development. The somatic muscle nuclei are distributed almost symmetrically in four longitudinal rows located subventrally and subdorsally (Figs. 4 and 23). Each dorsal quadrant contains 21 somatic muscle cells, the ventral right quadrant contains 20, and the ventral left quadrant contains 19. The ventral asymmetry appears to result from a gap on the left side somewhat posterior to the gonad primordium.

Four specialized muscle cells are present in the body of the newly hatched larva. Two are intestinal muscles (im). These muscles run subventrally along the posterior-most region of the intestine and attach to it and to the thin layer of hypodermis just ventral to the lateral cords; their filaments are oriented longitudinally. The intestinal muscle nuclei are located slightly dorso-laterally to the ventral body muscle rows and are morphologically similar to but somewhat smaller than body muscle nuclei. Slightly posterior to these cells is a single saddle-shaped muscle (sph), which almost surrounds the posterior end of the intestine and probably functions as a sphincter muscle to control defecation. The nucleus of this sphincter muscle is located somewhat left of the central midline. An H-shaped "anal depressor" muscle cell (dep), with its nucleus located centrally in the crossbar of the H, is located posterior to the anus and opens the anus during defecation.

A single mesodermal blast cell (M) is present on the right side of the young L1 somewhat posterior to the gonad primordium (Fig. 6). This cell has a large, relatively flat nucleus and a large nucleolus.

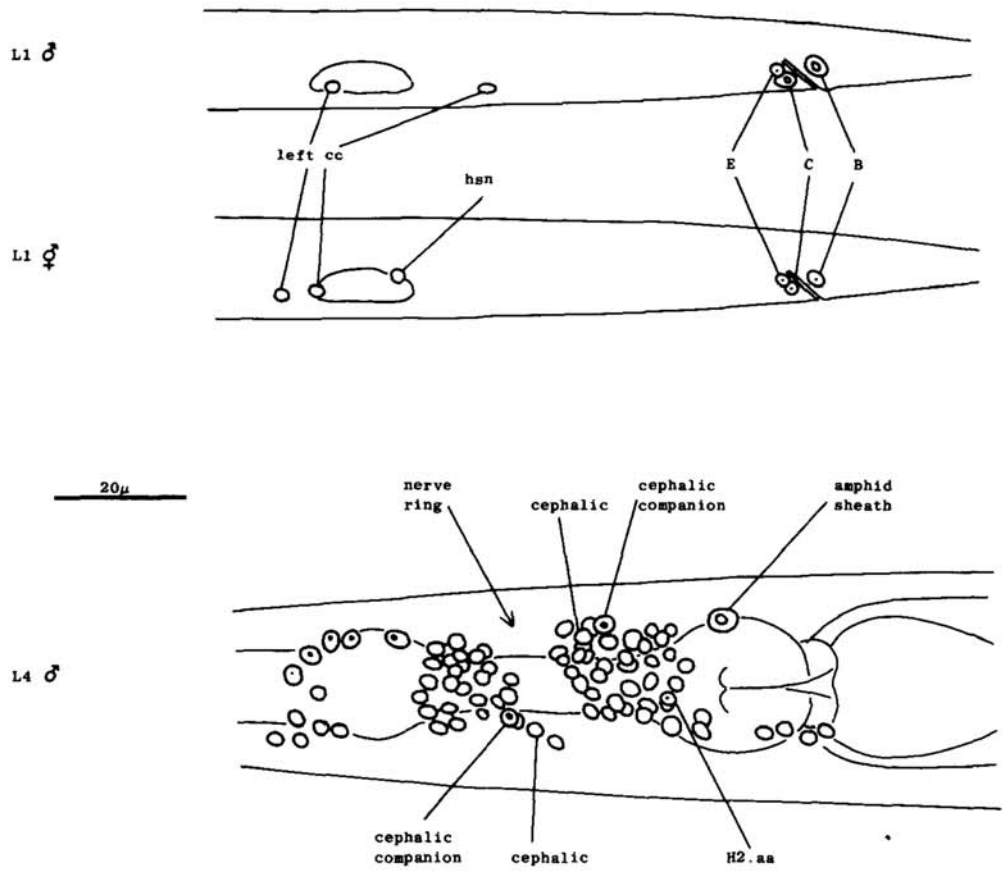

FIG. 5. Anatomical differences between the young hermaphrodite and the young male. The top two figures show lateral views of the posterior halves of a young L1 male and hermaphrodite. The sex-specific characteristics shown are (a) position of a left coelomocyte (cc), (b) presence of hermaphrodite-specific neurons (hsn), and (c) size of the B and C nuclei. Four male-specific cephalic companion neurons, also present at hatching, are best seen during the L4 stage, as shown in the bottom figure (left lateral view). The position of H2.aa in the lateral ganglion is indicated.

Four coelomocytes (cc) (Chitwood and Chitwood, 1974) are also present in the L1. These glandular cells are located in the pseudocoelom adjacent to the somatic musculature. In the young L1, their nuclei are granulated and do not contain visible nucleoli (Fig. 6). During larval development, the cytoplasm of the coelomocytes acquires both granules (of high refractive index) and vacuoles (of low refractive index), giving these cells a very characteristic appearance (Fig. 6). In the L1 hermaphrodite, all four coelomocytes are located subventrally between the pharynx and the gonad primordium; the two on the right are anterior to the two on the left (Figs. 4 and 23). In the male, one of the left coelomocytes is located posterior to the gonad primordium (Fig. 5). We consider these coelomocytes to be mesodermal in origin because, as described below (see Cell Lineages, Development), other coelomocytes are generated by blast cells which produce muscles during larval development.

Located centrally, dorsal to the rear bulb of the pharynx, is a single cell with a muscle-like nucleus and a nucleolus slightly smaller than that of a body muscle. This cell is often squashed in appearance. Electron micrographs reveal that this head mesodermal cell forms gap junctions (White *et al.*, 1976) with adjacent body muscles.

(c) Intestine. The intestine of the L1 is a hollow tube which normally contains 20 large, round nuclei with large nucleoli

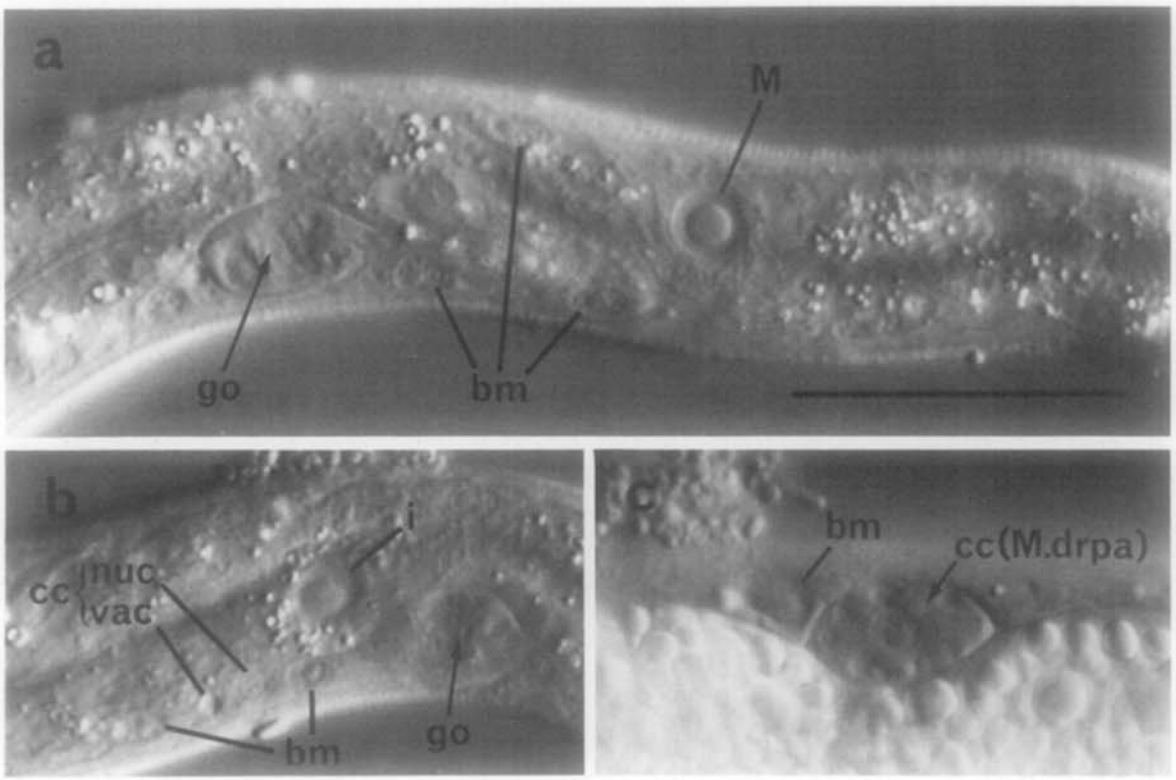

FIG. 6. Mesoderm, left lateral views; Nomarski optics. Bar = 20 μm. (a) Body muscle (bm) and mesoblast (M) nuclei in young L1 hermaphrodite; go, gonad. (b) Ventral left coelomocyte (cc) in young L1 hermaphrodite. i, intestinal nucleus; nuc, nucleus; vac, vacuole. (c) Dorsal right coelomocyte (cc, M.drpa) in L4 hermaphrodite. Note opacity of the well-fed intestine.

(Fig. 2); its cytoplasm is usually filled with refractile granules. Along most of the length of the intestine, nuclei are located dorsal and ventral to the lumen; in the anterior-most region, however, a ring of four nuclei surrounds the lumen. This anterior-most region has a thinner internal boundary than the rest of the intestine; electron micrographs reveal that the microvilli in this region are approximately half the length of those found elsewhere. The number of intestinal nuclei is not rigidly determined, as individuals with 19, 21, and 22 nuclei have been observed. The intestine has a left-handed twist of 180° about the longitudinal axis of the nematode in the region of the primordial gonad. As such a twist can introduce a left-handed superhelical twist, we suspect that the bilateral asymmetry of the position of the intestine—its anterior part is displaced slightly left of the midline, and its posterior part, slightly right—is caused by this rotational twist.

Pale at hatching, the cytoplasm of the intestine becomes filled with refractile droplets as the nematode feeds. Lethargus (or starvation), causes the intestine to again become relatively pale. Subsequent feeding restores the refractile droplets, which are therefore probably involved in energy storage. Because of the opacity of the well-fed intestine, hypodermal nuclei on the lower side of the nematode are difficult to observe between molts.

(d) Gonad. The gonad primordium consists of four cells in the young L1 (Fig. 4). The two larger cells are adjacent; the two smaller cells are slightly anterior and posterior to them, respectively. All four nuclei have relatively large nucleoli (Fig. 2). The gonad primordium lies obliquely, with its anterior cells located slightly right and its posterior ones located slightly left. This

bilaterally asymmetric positioning is probably related to the twist in the intestine (see above, Intestine). The gonad primordium of the male is morphologically identical to that of the hermaphrodite.

(e) Pharynx. The morphology of the pharynx of the adult hermaphrodite has recently been described in detail (Albertson and Thomson, 1976). No postembryonic cell divisions or deaths have been seen in the pharynx.

(3) Newly Hatched Male

At hatching, the L1 male is very similar in structure to the L1 hermaphrodite. We have found three criteria by which the sex of a young nematode can be determined (Fig. 5): (1) In the male, a left coelomocyte lies posterior to the gonad primordium. (2) The male is missing a neuronal-like nucleus embedded in each side of the lateral hypodermis near the gonad primordium. (3) Ectodermal nuclei B and C near the anus enlarge soon after hatching in the male; these nuclei divide only in the male.

In addition, the male head contains four neuronal-like nuclei which are absent in the hermaphrodite. These nuclei are located close to the cell bodies of the cephalic neurons and presumably are the cell bodies of the four male-specific sensory processes in the cephalic sensilla discovered by Ward *et al.* (1975). Although present in the newly hatched larva, these "cephalic companion" cell nuclei are more easily distinguished in the L4, in which they have relatively large distinct nucleoli (Fig. 5), possibly reflecting increased activity at this stage of development.

(B) Cell Lineages

(1) Cell Division

Although the size and shape of different blast cells vary, the overall process of cell division as observed with Nomarski optics during the postembryonic development of *C. elegans* is quite uniform (Fig. 7). For several hours before division, the nucleus of a blast cell generally has a prominent nucleolus and a smooth nucleoplasm. About 10 min before division, the nucleoplasm becomes coarsely granular, often producing a "rosette" around the nucleolus. In rapid succession during the next few minutes, the nucleolus disappears, the nuclear membrane breaks down, and the metaphase plate forms. During early anaphase, the plate splits and the two halves begin to move toward the poles; individual chromosomes are usually not visible. For a short time, nuclear components cannot be seen; the daughter nuclei then gradually appear. Finally, in some cells, daughter nucleoli are formed.

(2) Programmed Cell Death.

In a number of the ectodermal cell lineages, certain cells undergo a series of morphological changes which we interpret as programmed cell death (Saunders, 1966) (Fig. 8). Blobs appear at the perimeter of the nucleus. The nucleoplasm becomes less granular and somewhat more refractile. There is then a marked increase in the refractility of the nucleus, giving it a characteristic flattened appearance. The internal refractility diminishes slightly, leaving a refractile shell at the surface of the nucleus. The nucleus shrinks and it gradually disappears. Generally, other nuclei completely obliterate the site, although in some cases a permanent gap remains. Either way, no subsequent trace of the dead cell can be detected either by Feulgen staining or by electron microscopy.

Most cell deaths are of posterior daughters from anterio-posterior divisions.

As described below (see Cell Lineages), the pattern of cell deaths in the male differs from that in the hermaphrodite.

(3) Development

To the best of our knowledge, the results described in this section include all nongonadal cell divisions and deaths that occur during the postembryonic develop-

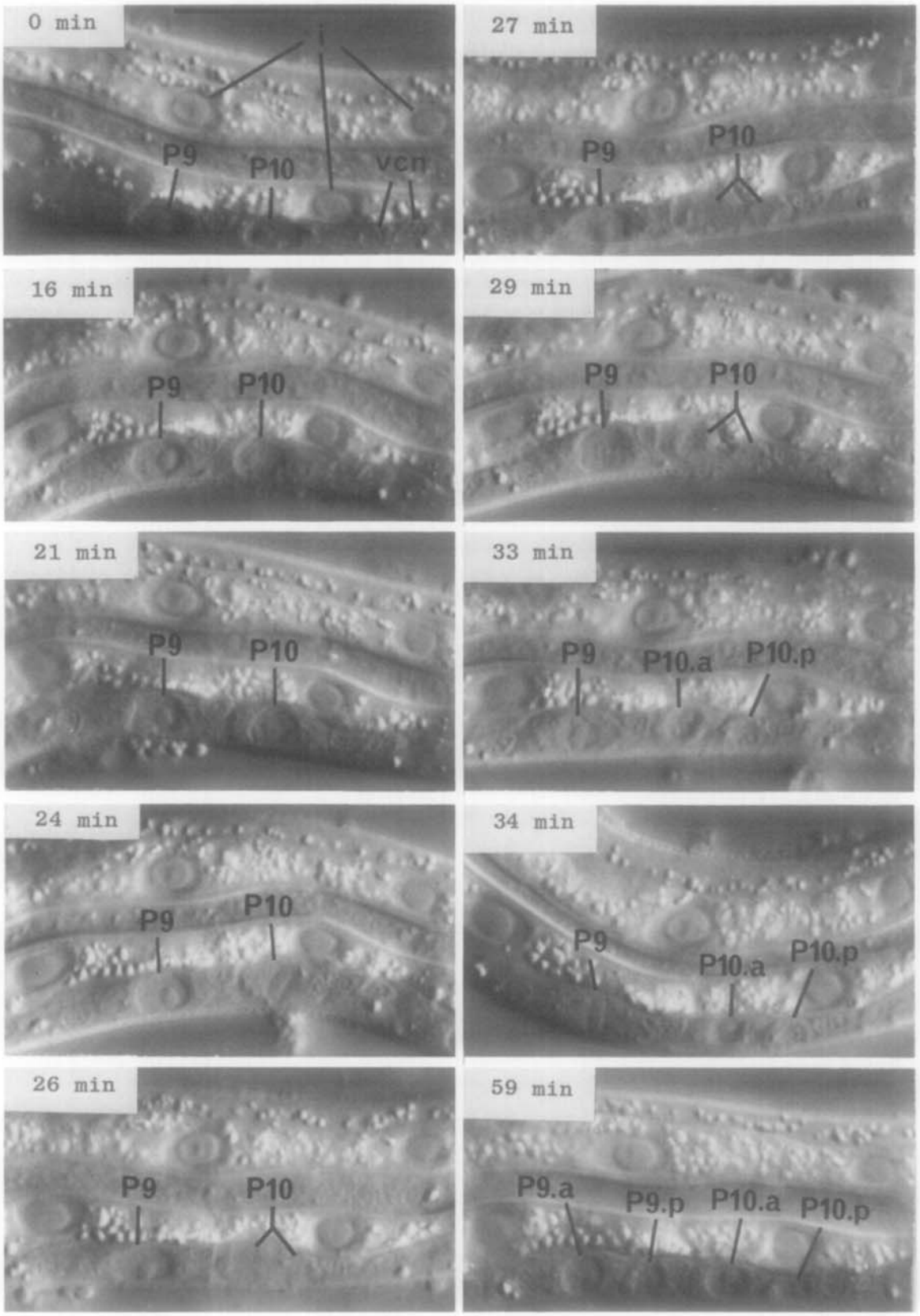

FIG. 7. Cell division. Sequential photographs of an L1 hermaphrodite, lateral view; Nomarski optics. vcn, ventral cord neurons. 0 min, interphase; 16 and 21 min, P10 prophase; 24 min, P10 metaphase; 26 min, P10 anaphase; 27 min, P10 telophase; 29 min, P9 prophase; 33 and 34 min, P9 metaphase. Bar = 20 μm.

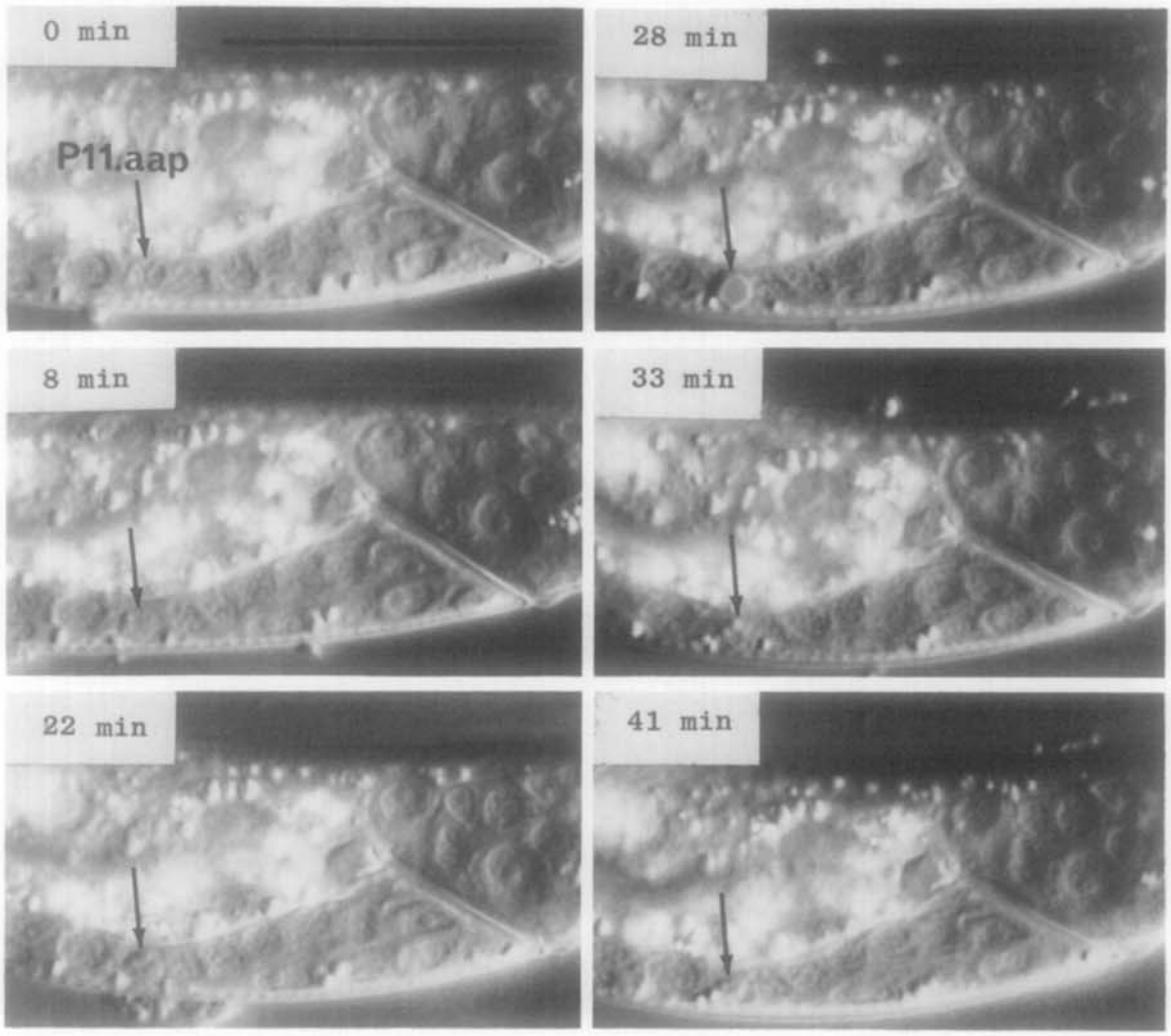

FIG. 8. Cell death. Sequential photographs of an L1 hermaphrodite, lateral view; Nomarski optics. The arrow points to the dying cell, P11.aap. Bar = 20 μm.

ment of *C. elegans*. However, very late developmental events in the male tail could have been missed. Because the detailed anatomy of the adult male tail is not known, many of its nuclei seen with the light microscope could not be assigned to specific cell types.

Cell lineages not specifically described for the male are identical to those which occur in the hermaphrodite.

(a) Ectoderm. V cells, hermaphrodite (Figs. 9 and 10): All 12 of the ventrolateral hypodermal precursor cells (V) divide about midway through the L1 stage. The anterior daughters enter the hypodermal syncytium. The posterior daughters remain separate from the syncytium; together with one head hypodermal cell (on each side) present at hatching, the posterior daughters form a distinct row of cells embedded in each lateral hypodermal ridge. Such cells have been named "seam" cells. Seam cells display a characteristic periodic activity. Toward the end of each intermolt period, the cytoplasm of the seam cells fills with refractile blobs (Fig. 11). This activity continues until midway through lethargus. That this activity is seen prior to the L4 molt (when the seam cells do not divide; see below) as well as prior to the other three molts (when the seam cells do divide) suggests that it is

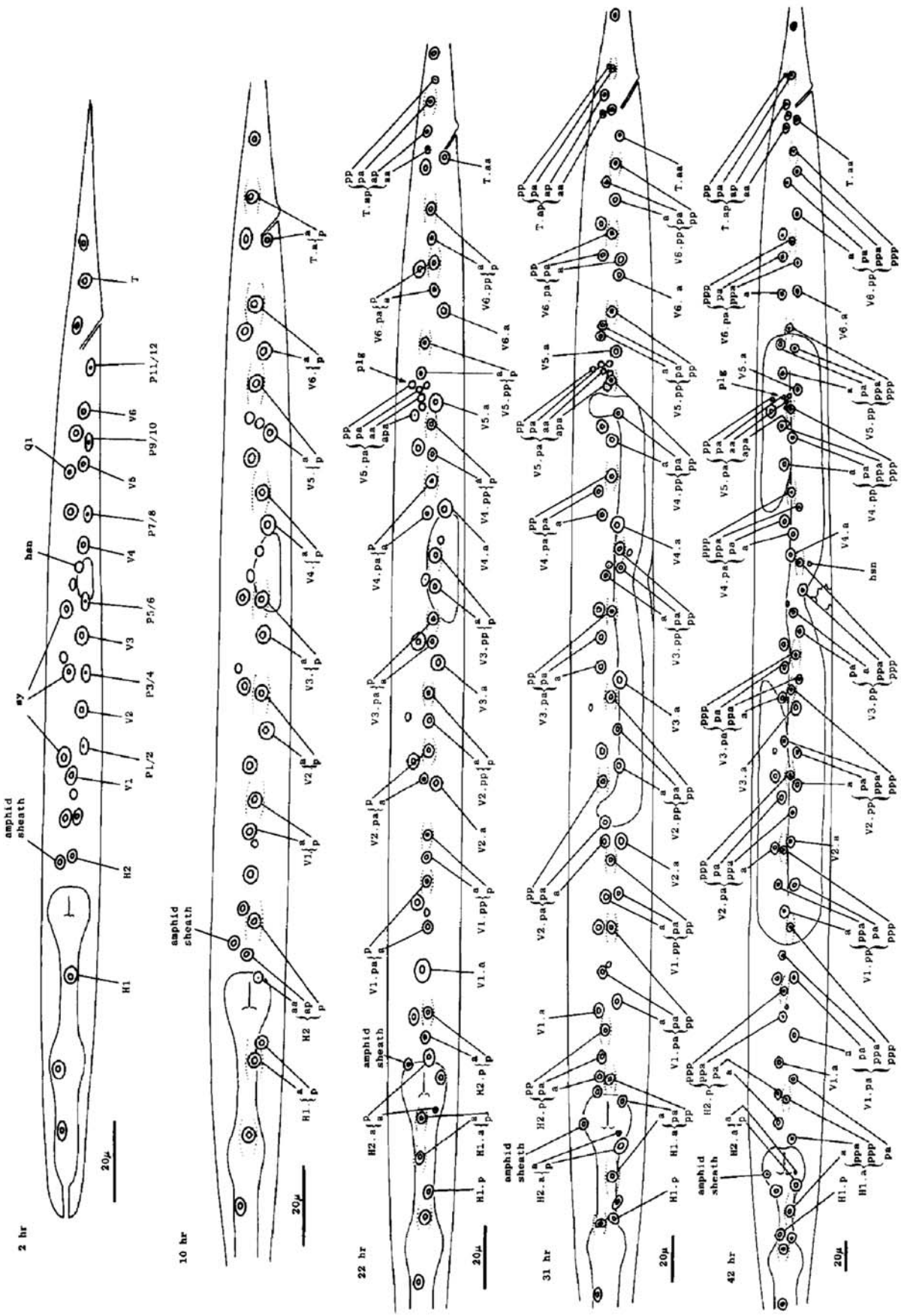

FIG. 9. H, V, and T.a; hermaphrodite, lateral views; development of the left lateral hypodermis. Seam cells are indicated by dotted lines. hsn, hermaphrodite-specific neuron; plg, posterior lateral ganglion.

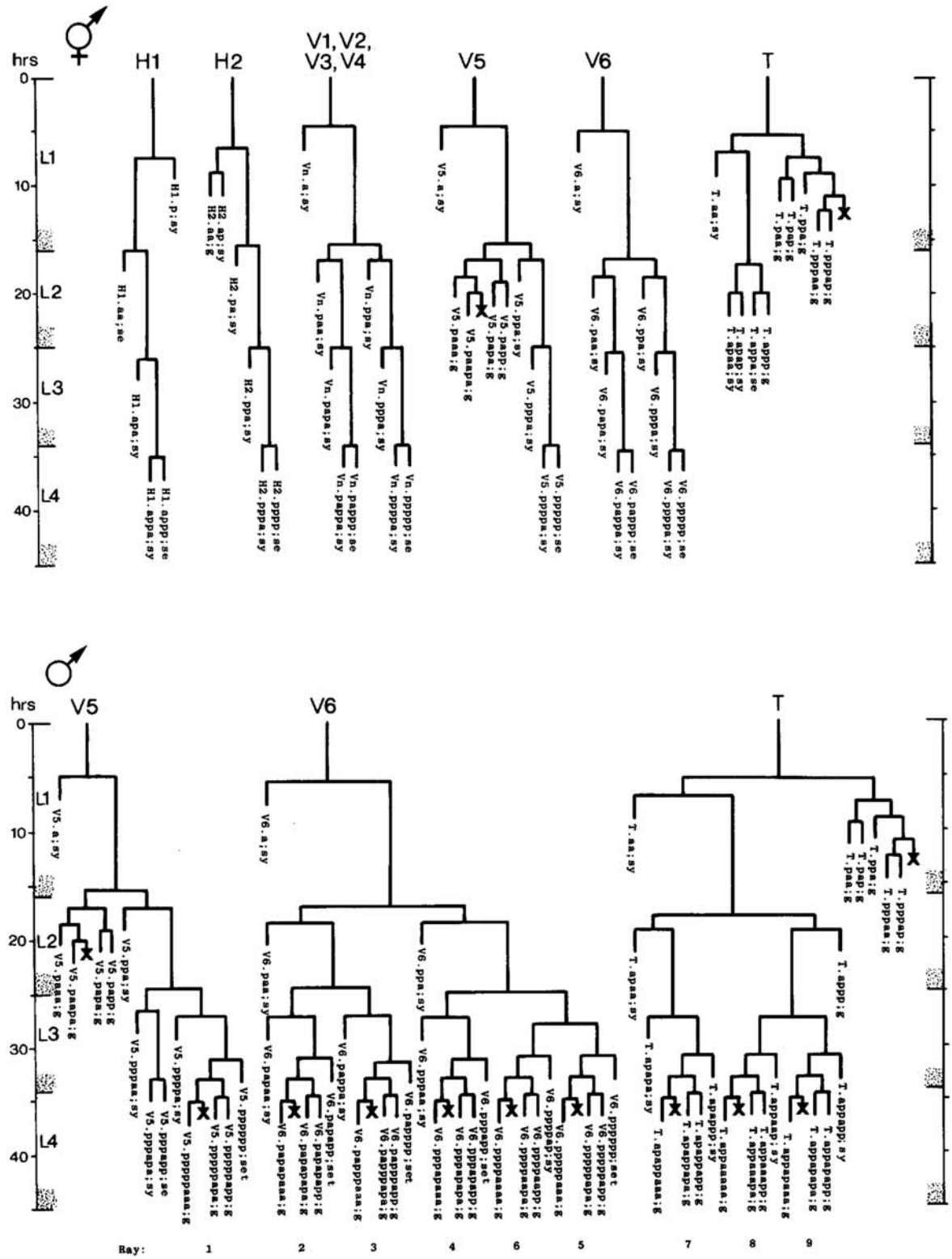

FIG. 10. H, V, and T lineages, hermaphrodite and male; development of the lateral hypodermis. All divisions are anterior–posterior. g, neuron or glial cell; se, seam; set, tail seam (a separate group of cells which shows seam-like cytoplasmic activity in the late L4 male); sy, syncytial.

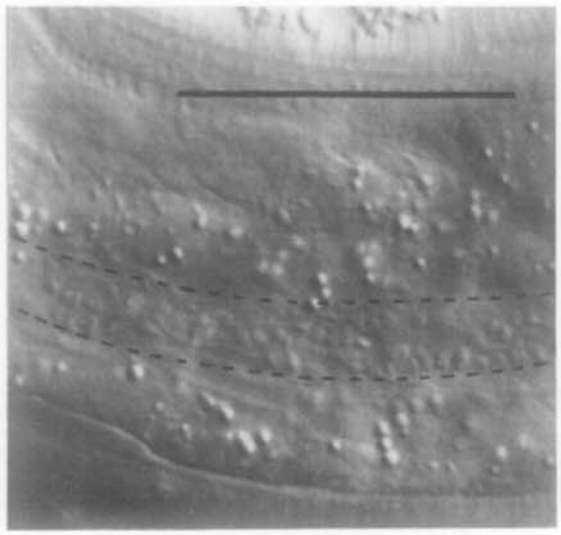

FIG. 11. Seam cell. Cytoplasmic activity in H1.aa of late L4 hermaphrodite, lateral view; Nomarski optics. The seam is outlined with a dotted line. Bar = 20 μm.

related to molting rather than to cell division.

The Vn.p seam cells undergo two rounds of division at about the time of the L1 molt; each produces a longitudinal row of four small nuclei. With the exception of V5.pap, the posterior daughters of the second round of divisions are themselves seam cells that divide at the L2 and L3 molts. The posterior daughter of each of these divisions is itself a seam cell. All of the other hypodermal progeny nuclei become part of the syncytium. In the L4 and adult, the seam cells form a continuous lateral band.

During the L2 stage, the lineage of V5 becomes distinct from that of the other V cells. On each side of the nematode, V5.paa and V5.pap undergo a series of divisions to produce four daughter nuclei. Together with two other nuclei on the left side (Q1.paa and Q1.pap; see below, Q Cells), these nuclei compose the posterior lateral ganglia, which consist of six cells on the left side and four cells on the right side. Ultrastructural studies of the posterior lateral ganglia show that they are positioned beneath the "postdeirids" (Chitwood and Chitwood, 1974) and contain the cell bodies associated with these sensilla. The proposed homology (Chitwood and Chitwood, 1974) between the postdeirids and the deirids in the head is supported by the observation that each of these sensilla has a single neuron containing the neurotransmitter dopamine (Sulston *et al.*, 1975). We have now identified the dopaminergic cell in each posterior lateral ganglion as being V5.paaa. This assignment was suggested by correlating the formaldehyde-induced fluorescence of the dopaminergic cell with the position of the V5.paaa cell body as visualized by Feulgen staining, using methods described previously (Sulston *et al.*, 1975); it was confirmed by killing selected cells of known lineage with a laser microbeam and showing that the death of V5.paaa or of any of its ancestors (but not of any other cell) leads to loss of the formaldehyde-induced fluorescence. These experiments failed to reveal any regulative capacity of the cells involved in the development of the posterior lateral ganglia; no extra divisions occurred to replace those cells destroyed with the laser.

H cells, hermaphrodite (Figs. 9 and 10): The lineages of the hypodermal precursors of the head (H1 and H2) appear to be related to those of the V cells. Both generate seam cells as well as hypodermal nuclei in the syncytium, and both utilize similar stem cell patterns of cell division. However, there is an inversion of the pattern of the divisions of H1 compared to that of the V cells; the anterior daughter H1.a is the blast cell of the H1 lineage, whereas the posterior daughter Vn.p is the blast cell of the V lineage. It should be noted that H1.a and H1.p slowly reverse their anterior–posterior positions; however, this positional reversal is probably not the cause of the inversion of the pattern of divisions, because H1.a has the morphological appearance of a blast cell before any movement of its nucleus occurs. H1.aa has the appearance of a seam cell; unlike the "equivalent" Vn.pa cells, however, H1.aa

does not divide. The lineage of H2, which is related to those of H1 and the V cells, produces seam cells, syncytial hypodermal nuclei, and one neuron-like cell (H2.aa), which is positioned in the mass of cell bodies posterior to the nerve ring (Figs. 5 and 9). That H1 and H2 together produce only half the number of syncytial hypodermal nuclei expected from two V cells may relate to the fact that postembryonic growth of the head is much less than that of the body (Hodgkin, 1974).

T cell, hermaphrodite (Figs. 9, 10, and 12): The tail precursor-cell T generates both hypodermal and nerve cells. Its anterior daughter T.a produces a lineage initially like that of a V cell; however, this similarity in the pattern and timing of cell divisions is not reflected in the morphology of the progeny. In the neuronal lineage of T.p (Figs. 10 and 12), there are two anterior–posterior positional inversions. First, about 1 hr after it is formed, T.pp begins to move slowly past T.pa. This migration appears to be an active process; neighboring cells seem to hinder rather than assist the movement. Later, T.pppap moves anterior to its sister; its nucleus becomes considerably deformed as it squeezes past other cells to reach its final position.

The fates of the progeny of the T cells in the adult have not yet all been established; at least some of these progeny appear to be associated with the phasmids, post-anal sensilla located on each side of the nematode (Chitwood and Chitwood, 1974).

Q cells (Figs. 13 and 14): The left and right lateral neuroblasts, Q1 and Q2, display bilaterally symmetrical patterns of cell division. However, their progeny are not symmetrical in their movements: Q2.a, Q2.p, and Q2.ap all migrate anteriorly, while Q1.ap migrates posteriorly.

The fate of Q2.pp is variable. In hermaphrodites, it becomes less and less distinct and usually disappears, although in one individual it was observed to survive into the adult. In two males, Q2.pp underwent typical programmed cell death. Perhaps its fate is weakly sex specific.

P cells, hermaphrodite (Figs. 12, 15, 16, 17, and 18): The P precursor cells are responsible for the postembryonic development of the ventral nerve cord and its associated ganglia. One of us has described the development of these systems in detail elsewhere (Sulston, 1976). For completeness, we summarize these observations below.

About the middle of the L1 stage, the ventro-lateral P cells appear to be "loosened" from their hypodermal neighbors. Cytoplasmic extensions of the P cells grow into the ventral cord. The nuclei of P1 and P2 migrate into these extensions; the residual cytoplasm soon follows (Fig. 15). After these movements of P1 and P2, similar migrations of successively more posterior pairs occur roughly in order along the cord; P11 and P12 migrate into the preanal ganglion.

There is some variability in the anterior–posterior order of a given left–right pair of P cells after they have entered the ventral cord; in at least some cases, the subsequent development of a cell depends not upon its side of origin, but rather upon its anterior–posterior position. For this reason, we number the P cells after their migration into the cord (Fig. 16). We have determined the extent of this variability for the following pairs. P1 generally arrives from the right side (in 14 out of 16 individuals examined), P11 arrives from the left (7 out of 7), and the entries of P3 and P5 are approximately random.

Soon after its migration, each P cell divides. All 12 P cells follow identical initial programs of division, each producing five neurons and one hypodermal cell (Fig. 17). In addition, a single neuroblast in the retrovesicular ganglion follows a program identical to that of the anterior daughters of the P cells (Pn.a); by analogy, we have named this neuroblast P0.a.

As cell divisions continue, the neuroblasts and their progeny move freely past the juvenile cells and the new hypodermal

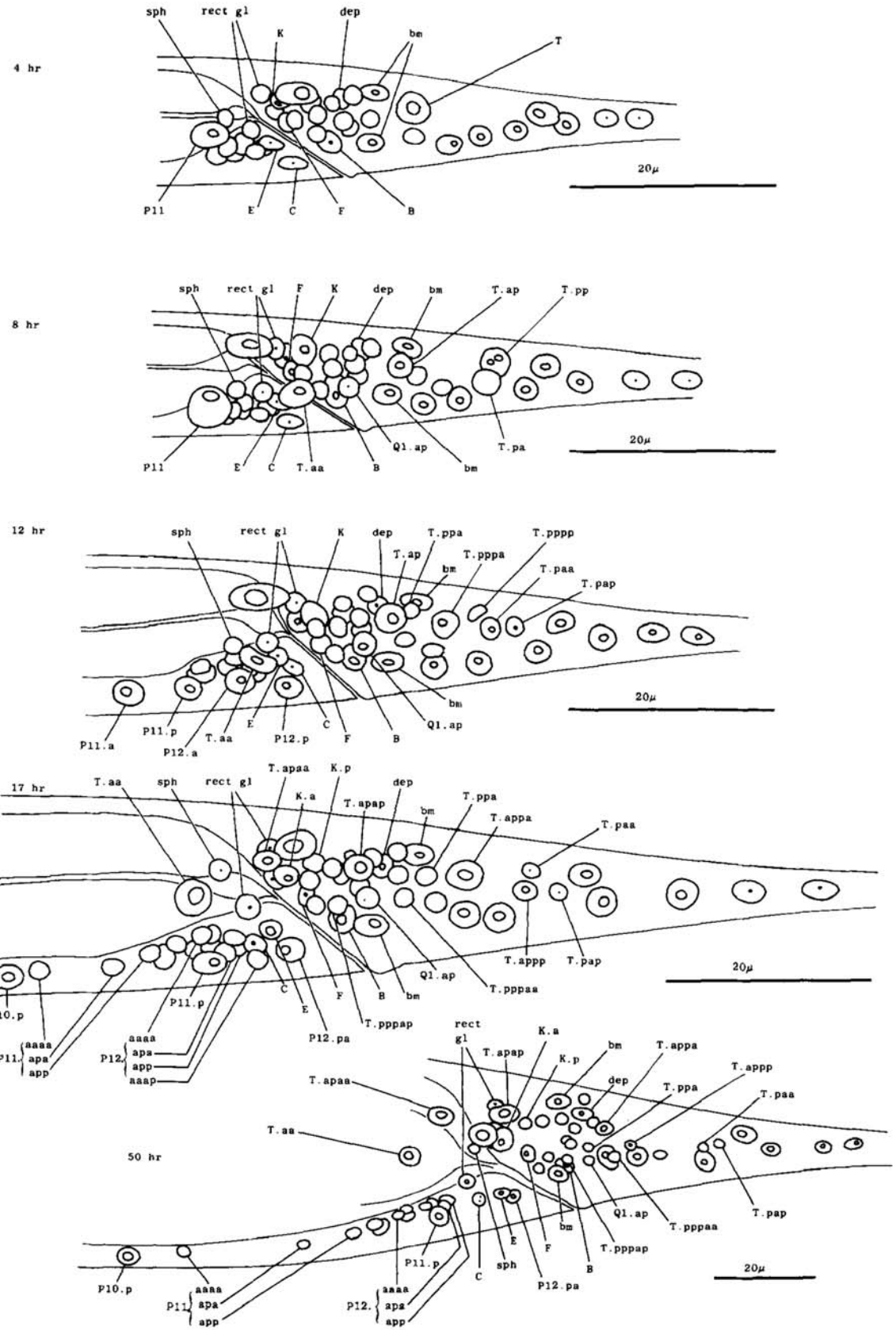

FIG. 12. T, K, P11, and P12; hermaphrodite, left lateral views; development of the hermaphrodite tail. Only left and central nuclei are drawn.

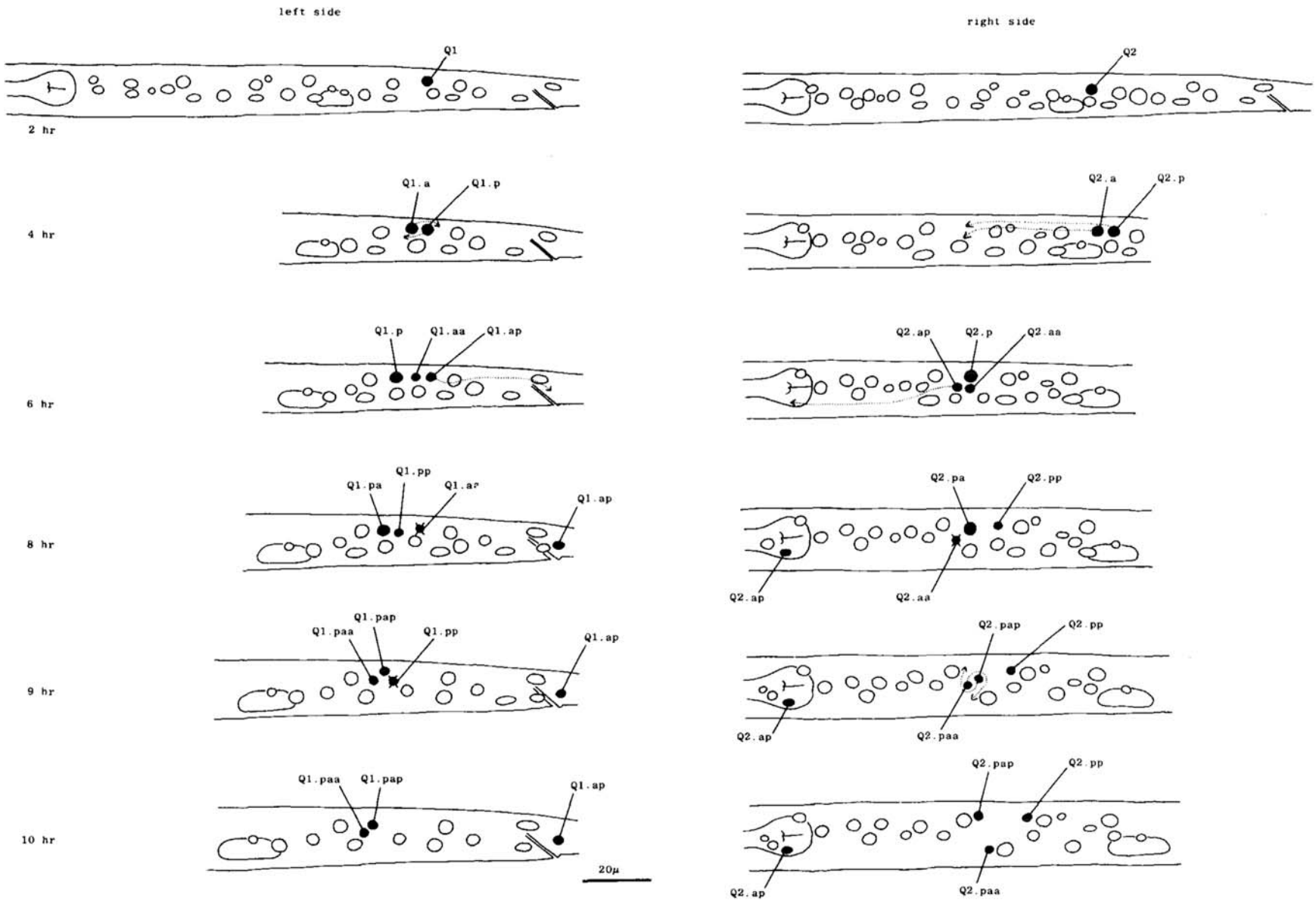

FIG. 13. Q, left lateral views; development of the lateral neuroblasts. Sometimes, Q2.paa and Q2.pap move directly to their final positions, without circling around one another.

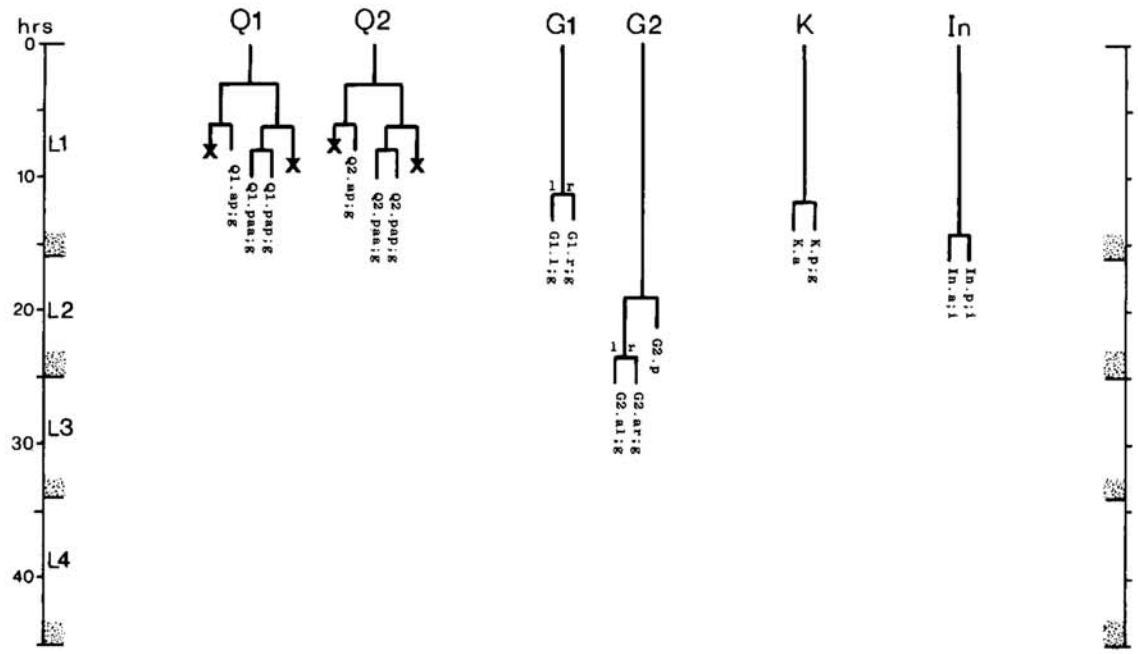

FIG. 14. Q, G, K, and I lineages. Q1 and Q2 are lateral neuroblasts; G1 and G2 are in the ventral ganglion; K is in the tail; In are intestinal nuclei. Divisions are anterior-posterior unless otherwise indicated. g, neuron or glial cell; i, intestine.

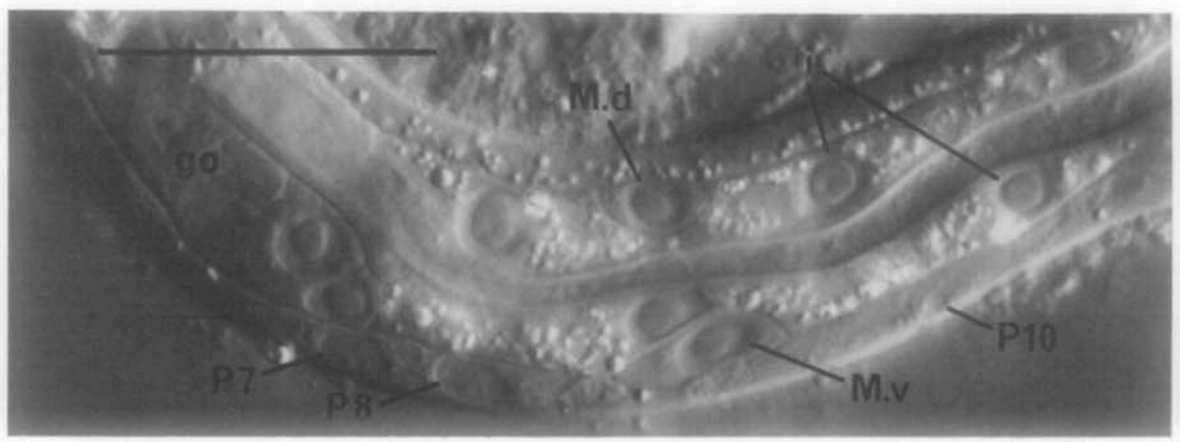

FIG. 15. Ventral cord and muscle development. L1 hermaphrodite, left lateral view, central plane; Nomarski optics. The nucleus of P10 is just entering the cord. The intestine is pinched between M.d and M.v. Bar = 20 μm.

cells (Pn.p); the extent of this movement is variable, leading to different patterns of intercalation of new neurons with the juvenile and hypodermal cells in different individuals. Gradually, the ventral cord becomes evenly filled with nuclei. Toward the end of the period of division, the pattern of nerve cells is modified by a fixed program of cell deaths in the anterior and posterior regions of the cord. There is also an extra division of the most posterior hypodermal cell (P12.p), followed by the death of its posterior daughter (P12.pp).

Late during the L3 stage, the six ventral hypodermal nuclei (P3.p, P4.p, P5.p, P6.p, P7.p, P8.p) in close proximity to the developing gonad (Fig. 18) divide. The daughters of P5.p, P6.p, and P7.p move closer together and continue to divide to produce the 22 cells which form the vulva. The

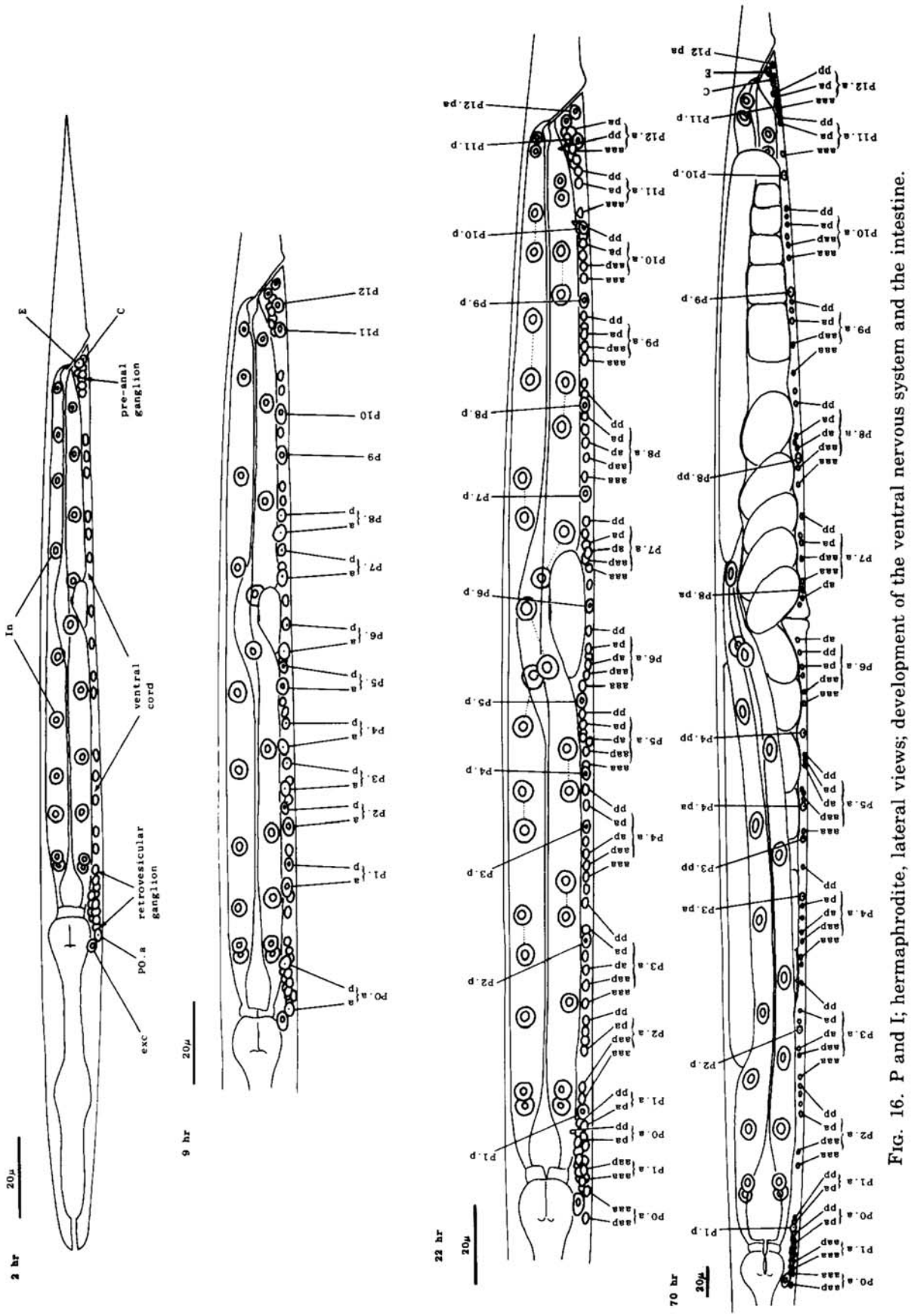

FIG. 16. P and I; hermaphrodite, lateral views; development of the ventral nervous system and the intestine.

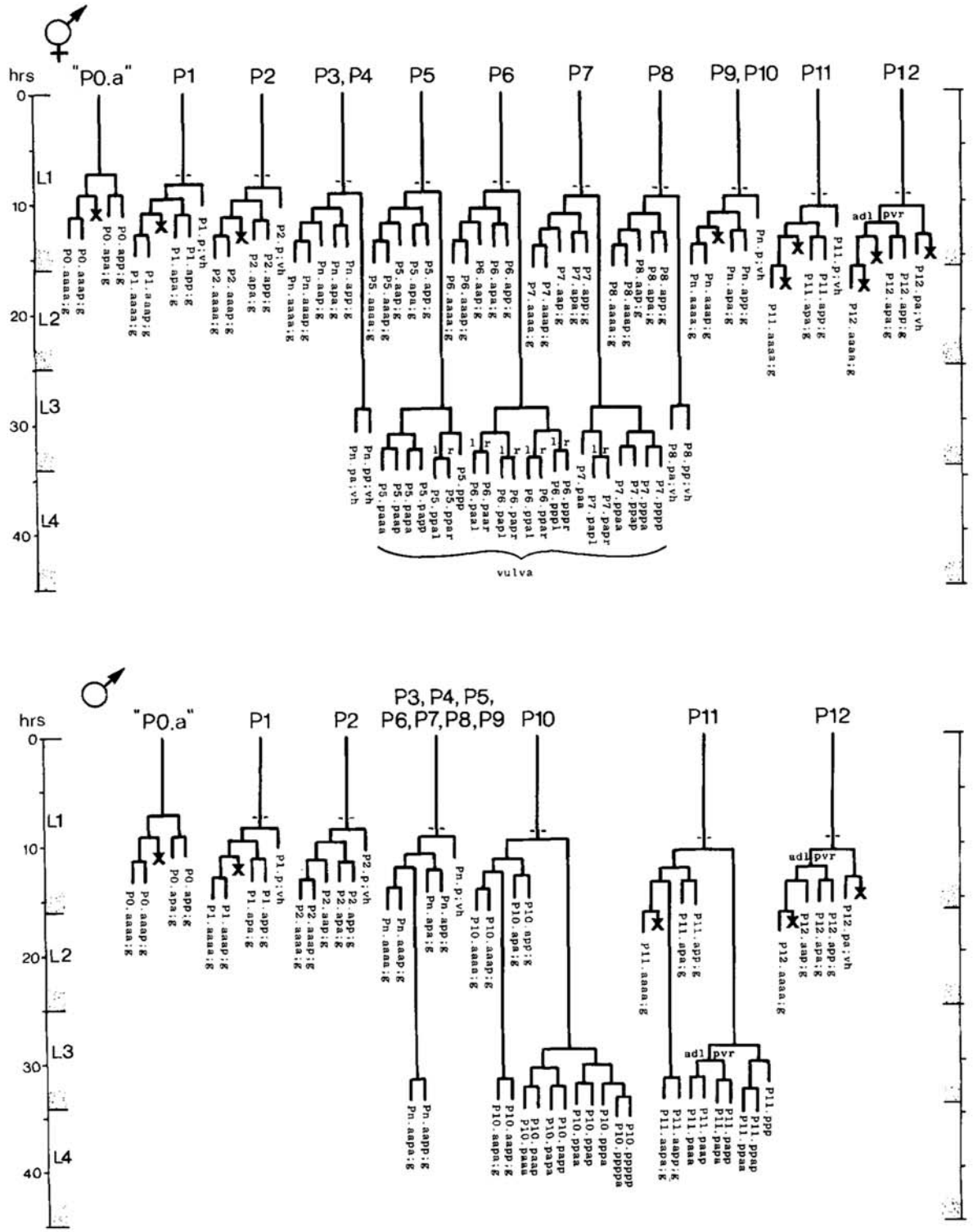

FIG. 17. P lineages, hermaphrodite and male; development of the ventral nervous system. Dotted lines indicate the times nuclei migrate into the ventral cord. P0.a is so named for convenience; its actual ancestry is unknown. Divisions are anterior–posterior unless otherwise indicated. g, neuron or glial cell; vh, ventral hypodermal cell.

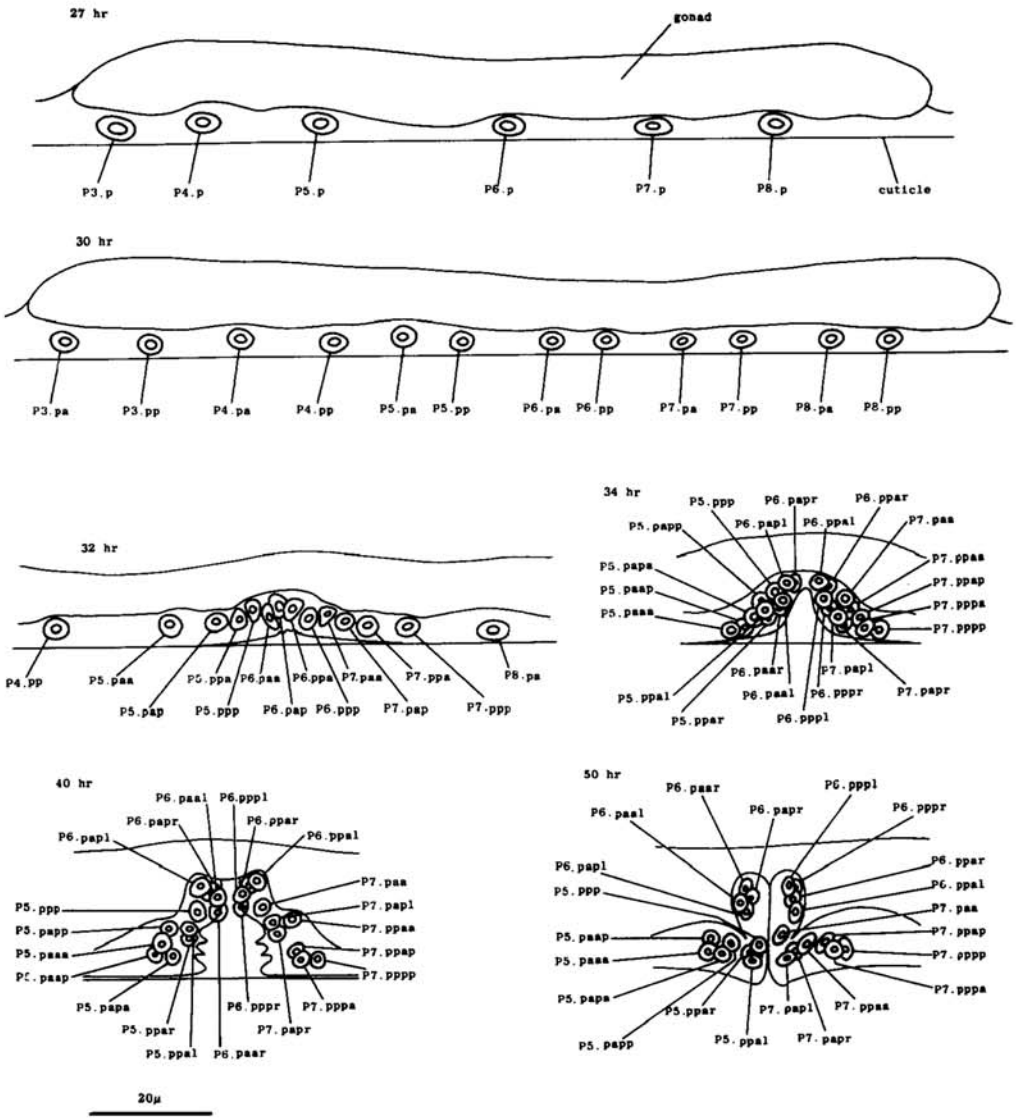

FIG. 18. P3.p–P8.p; hermaphrodite, left lateral views midway along ventral side; development of the vulva.

daughters of P3.p, P4.p, and P8.p become ventral hypodermal nuclei. In the adult, the ventral hypodermal nuclei formed during the development of the ventral cord lie in the hypodermal syncytium (White *et al.*, 1976).

The fine-structure neuroanatomy of the ventral cord of the adult *C. elegans* hermaphrodite has been elucidated by White *et al.* (1976). The neurons in the ventral cord can be assigned to morphologically distinct classes which recur periodically along the length of the cord. The number of cells of each class is constant in different individuals, but slight variations in the relative positions of different classes occur. These anatomical observations can be explained by the developmental processes described above according to the following model. Each morphological class consists of those cells with an equivalent lineage history (Table 1); the positional variations result from the differential migrations which occur during development. The correlation of

TABLE 1

CORRELATION OF MORPHOLOGICAL CLASS WITH LINEAGE HISTORY OF MOTOR NEURONS IN THE VENTRAL NERVOUS SYSTEM OF THE ADULT HERMAPHRODITE [a]

(1) Morphological class	(2) Lineage history (model)	(3) Confirmed by EM assignment
VA	Pn.aaaa	**P2, P3, P4, P5, P6**
VB	Pn.aaap	**P0, P1, P2, P3, P4, P5, P6**
VC	Pn.aap	**P3, P4, P5, P6**
DAS	Pn.apa	**P1, P2, P3, P4, P5, P6**
VD	Pn.app	**P0, P1, P2, P3, P4, P5, P6**
DA, DB, DD	Juvenile	Eight anterior-most juvenile cells of ventral cord (three anterior-most established in individuals of known lineage).

[a] Column 1 indicates morphological classes as defined by White *et al.* (1976). Column 2 shows the predicted lineage history of cells of each morphological class according to the model. Column 3 indicates neurons of each morphological class which, by their positions and neighbors, are consistent with having the lineage listed; to date, morphological assignments have been completed only for the anterior ventral cord (containing the progeny of P0.a-P6). Assignments for neurons in boldface in Column 3 have been established in individuals of known lineage. Three neurons located in the retrovesicular ganglion are not allocated as expected from this model: P0.aaaa and P1.aaaa are nonmotor neurons, and P0.apa is of morphological class VA. Three progeny (P0.aap, P1.aap, P2.aap) undergo programmed cell death and thus are not found in the adult. All electron microscopy was done by White *et al.* (1976).

cell type with lineage history has been directly confirmed by using serial-section electron micrographs to reconstruct the retrovesicular ganglion and part of the ventral cord of individuals of known lineage (Table 1). However, three daughters located in the retrovesicular ganglion are not allocated as expected from their lineage history. As discussed below (see Discussion, Mechanisms of Determination), they may reflect end effects which seem to affect a number of aspects of the postembryonic development of *C. elegans*.

G cells (Figs. 4 and 14): G1, located near the anterior end of the ventral ganglion, divides transversely late during the L1 stage to produce daughters with neuronal-like nuclei. Also in the ventral ganglion, G2 divides during the L2 stage. Like G1, G2.a then divides transversely and produces two daughters with neuronal-like nuclei.

K cell (Figs. 12 and 14): The solitary K precursor cell in the tail divides once about 3.5 hr before the L1 molt. K.p joins the two other neurons in the dorso-rectal ganglion. K.a is a hypodermal cell which completes a bilaterally symmetrical pair with a cell located right of the midline and present in the young L1.

Other cell deaths: On two occasions, other cell deaths were observed early during the L1 stage. One was immediately anterior to the excretory cell and the other was in the dorsal region of the lateral ganglion. These two cell deaths may normally be embryonic events which, in some individuals, are delayed until after hatching.

Male: Below, we first describe those ectodermal lineages which occur in the hermaphrodite and are modified in the male (V, T, P); we then describe male-specific lineages derived from cells present in the newly hatched L1 which do not divide in the hermaphrodite (B, F, C, E).

V and T cells, male (Figs. 10 and 19): The V and T lineages of the male are identical to those of the hermaphrodite until L2 lethargus; thus, the male carries all tail and posterior lateral cell bodies found in the hermaphrodite. Further divisions in the V5, V6, and T lineages then generate nine similar sets of neuronal-like nuclei on each side of the nematode as well as a number of larger hypodermal-like nuclei. Numerically, these nine sets correspond to the nine "rays" found on each side of the male tail (Figs. 1 and 20). These rays lie in a noncellular cuticular webbing, which forms a leaf-shaped fan. The entire structure, consisting of fan and rays, probably functions as a mechanosensory organ during copulation.

Preliminary experiments with the laser microbeam suggest how the V and T cell

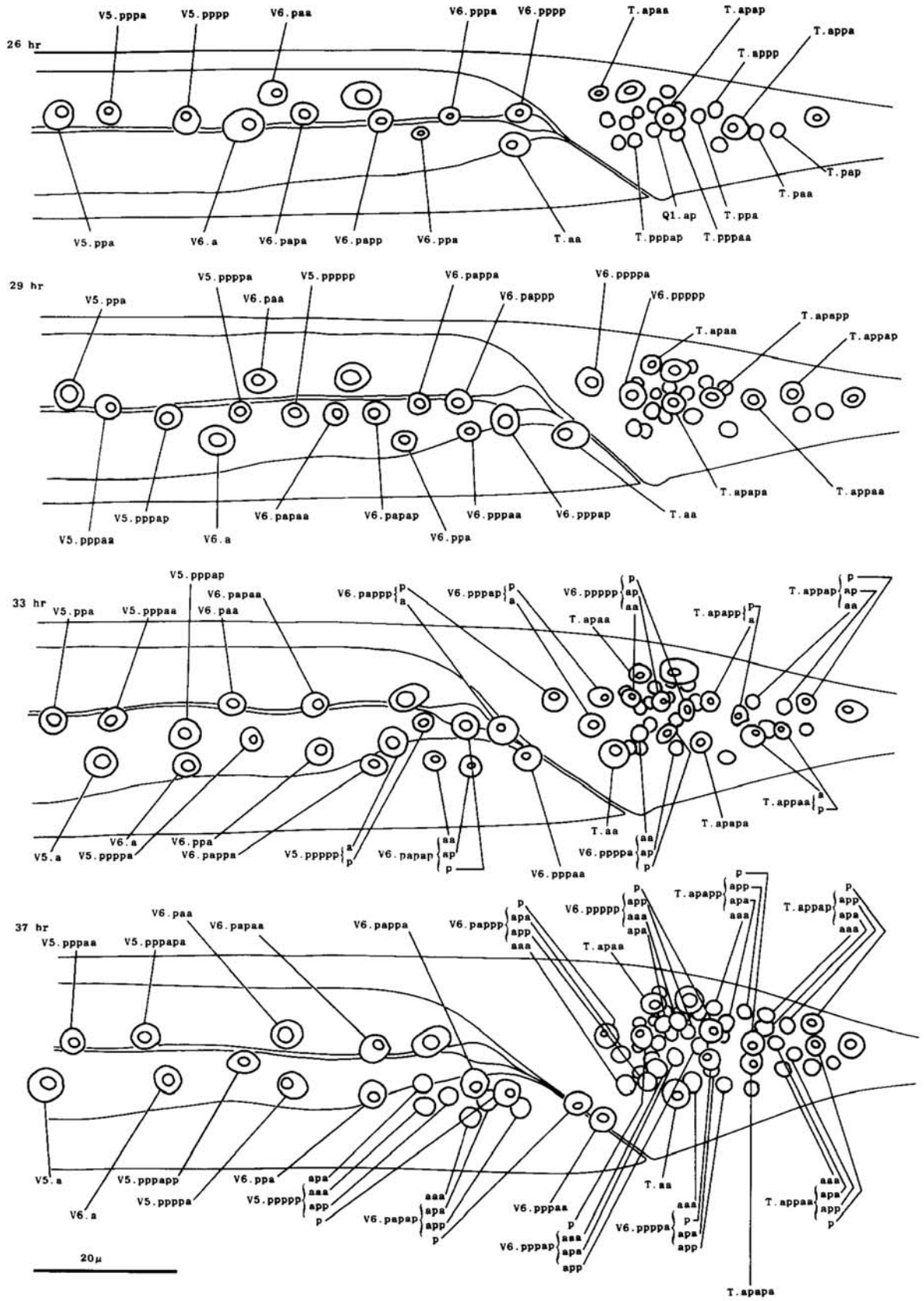

FIG. 19. V5, V6, and T; male, left lateral views of the left side; development of the rays of the male tail. There is extensive rearrangement of the cells later, during L4 lethargus.

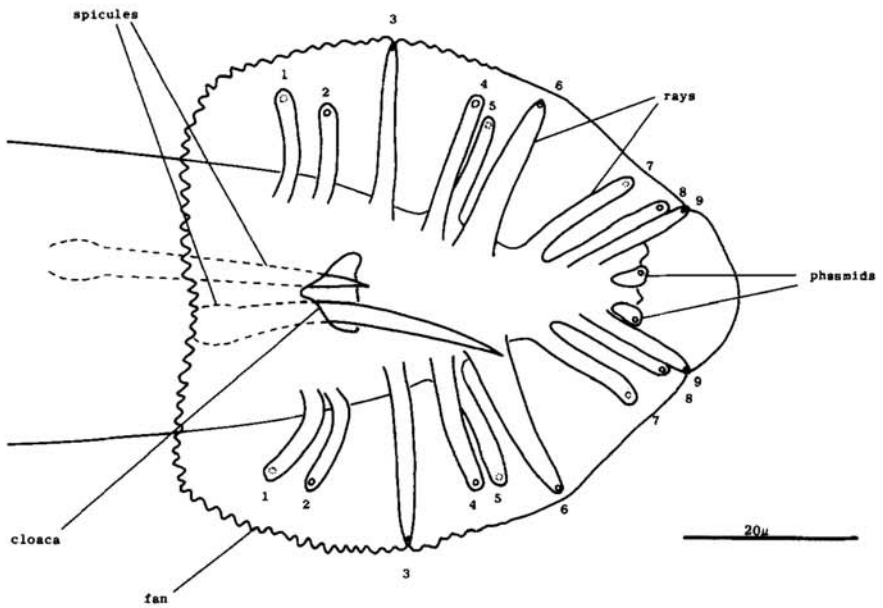

FIG. 20. Male tail, ventral view. One spicule is extended and one is retracted. As indicated, the sensory tips of the rays lie either on the ventral (inside) surface (indicated by a solid circle) or on the dorsal (outside) surface (dotted circle).

lineages lead to the development of the rays. Destruction of the precursor (e.g., V6.ppppp) of one of the nine sets of progeny cells with the laser results in the loss of a particular ray. In this way, the ancestry of the nine rays has been assigned (Fig. 10). Destruction of the precursor of ray 5, 7, or 9 also resulted in the loss of one of the three dopaminergic cells on that side of the male tail (Sulston *et al.*, 1975). That only 6 of 18 morphologically similar rays have dopaminergic neurons suggests why mutant males lacking dopamine are still capable of mating (Sulston *et al.*, 1975): at least some of the 12 other rays may act as alternative sensory elements.

The precise lineages of the six dopaminergic cells were then determined by destroying some of the progeny of the precursors of rays 5, 7, and 9. Usually, destruction of "aaa" (e.g., V6.pppppaaa) eliminated the dopaminergic cell, but did not eliminate the ray; destruction of "apa" (e.g., V6.pppppapa) eliminated neither the dopaminergic cell nor the ray; and destruction of "app" (e.g., V6.pppppapp) eliminated the ray, but did not eliminate the dopaminergic cell. These results suggest that, in each set, aaa is the neuron and app forms the structural element. Destruction of any one of the hypodermal nuclei "p" (e.g., V6.pppppp) associated with these sets produced no lesion detectable in the light microscope. As in the development of the posterior lateral ganglia (see above, V cells, hermaphrodite), laser experiments failed to reveal any potential for regulation.

Although 18 similar lineages generate 18 morphologically similar rays, lineally equivalent daughters do not all differentiate identically: Only six of the aaa progeny are dopaminergic. It will be interesting to determine if the other aaa progeny are indeed neurons and, if so, to identify what transmitter(s) they employ.

P cells, male (Figs. 17 and 21): In the development of the male ventral nerve cord, there are fewer programmed cell deaths during the L1 stage and extra cell divisions during the L3 stage; both aspects of this "reprogramming" involve only the Pn.aap daughters. In these ways, the male produces 14 extra cells in the ventral cord.

In the male, ventral hypodermal nuclei P3.p–P8.p (which generate the vulva in the hermaphrodite) do not divide; instead, P10.p and P11.p divide to produce 16 male-specific nuclei in the region of the pre-anal ganglion. We do not know the fate of these nuclei in the adult male; preliminary observations suggest that some may be neurons. In 4 out of 17 individuals observed, P9.p divided once, yielding two ventral hypodermal nuclei.

B and F cells, male (Figs. 21 and 22): Some of the descendants of the B and F cells form the cloaca; others produce the copulatory spicules, elongated cuticular structures used to open the vulva of the hermaphrodite during mating (Figs. 1 and 20). The specific fates of the daughters of these lineages have not been established. Most of these progeny cells are probably structural, as they have clear nucleoplasms with prominent nucleoli; a few, designated "g" in Fig. 22, are more compact with granular nucleoplasms and seem to be more like neurons or glial cells. However, unlike the neurons of the ventral cord and hermaphrodite tail, these cells often contain visible nucleoli soon after they are formed (possibly reflecting relatively rapid growth). Because of these ambiguities, any distinctions made between neurons and structural cells for these lineages are rather subjective.

B divides obliquely late during the L1 stage. During the L2 stage, its anterior-dorsal daughter (B.a) divides transversely; B.al and B.ar divide twice each, producing a row of four nuclei on the right and left sides of the tail. Both the anterior-most (B.alaa and B.araa) and posterior-most (B.alpp and B.arpp) of these nuclei recentralize. One member of each of these pairs becomes located anterior to the other, but there is no fixed pattern as to the order these cells assume; all four possible final configurations have been observed. The subsequent lineage of each of these four cells is determined not by its ancestry (whether it was derived from B.al or B.ar), but rather by its newly assumed position (anterior or posterior). In other words, positional influences play a determining role in the developmental fates of these cells.

E cell, male (Figs. 21 and 22): E.lp and E.rp are associated with the vas deferens where it joins the cloaca. E.rap and E.raa are in the pre-anal ganglion. Usually, E.la remains posterior to the preanal ganglion, but, in two individuals, it behaved like E.ra and migrated anteriorly and divided.

C cell, male (Figs. 21 and 22): The C cell generates compact, neuronal-like nuclei. C.a remains in the pre-anal ganglion, while C.p divides transversely and ultimately produces a pair of five-celled ganglia which flank the cloaca.

(b) Mesoderm. M cell, hermaphrodite (Figs. 6, 23, 24, and 25): All of the postembryonic mesodermal cell lineages derive from a single mesoblast, M. M divides dorso-ventrally about midway through the L1 stage (Fig. 15). Three subsequent divisions generate 16 daughter cells, 4 in each of the muscle quadrants. Two of the ventral cells (M.vlpa and M.vrpa) divide longitudinally once more, so that by the L1 molt 18 progeny cells are present. During the L2 stage, two of the dorsal cells (M.dlpa and M.drpa) differentiate into coelomocytes. It is interesting that all four of these "specialized" cells are lineally equivalent daughters from the four muscle quadrants. Two of the daughters of the "extra" ventral divisions (M.vlpaa and M.vrpaa) migrate anteriorly. The other 14 progeny cells (6 dorsal, 8 ventral) become normal body muscles and are used for locomotion. They intercalate with juvenile body muscles posterior to the gonad primordium. The precise pattern of this intercalation varies among different individuals. A typical arrangement is shown in Fig. 24. The mature adult thus contains 95 body muscle cells; the ventral right quadrant and each dorsal quadrant contains 24 of these cells, and the ventral left quad-

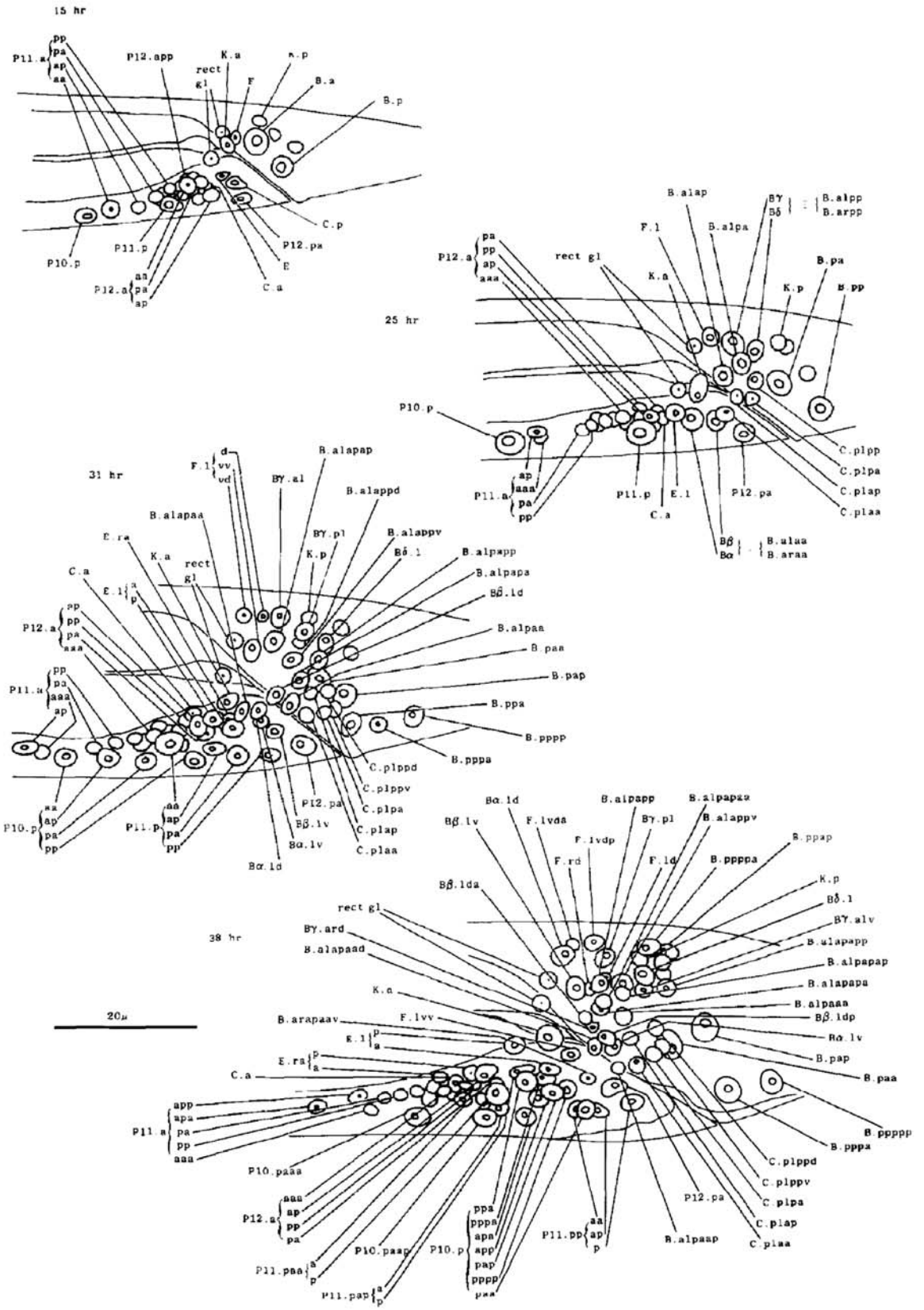

FIG. 21. B, C, E, F, P10.p, and P11.p; male, lateral views; ectodermal development in the male tail. There is extensive rearrangement of the cells later, during L4 lethargus. Only left and central cells are shown.

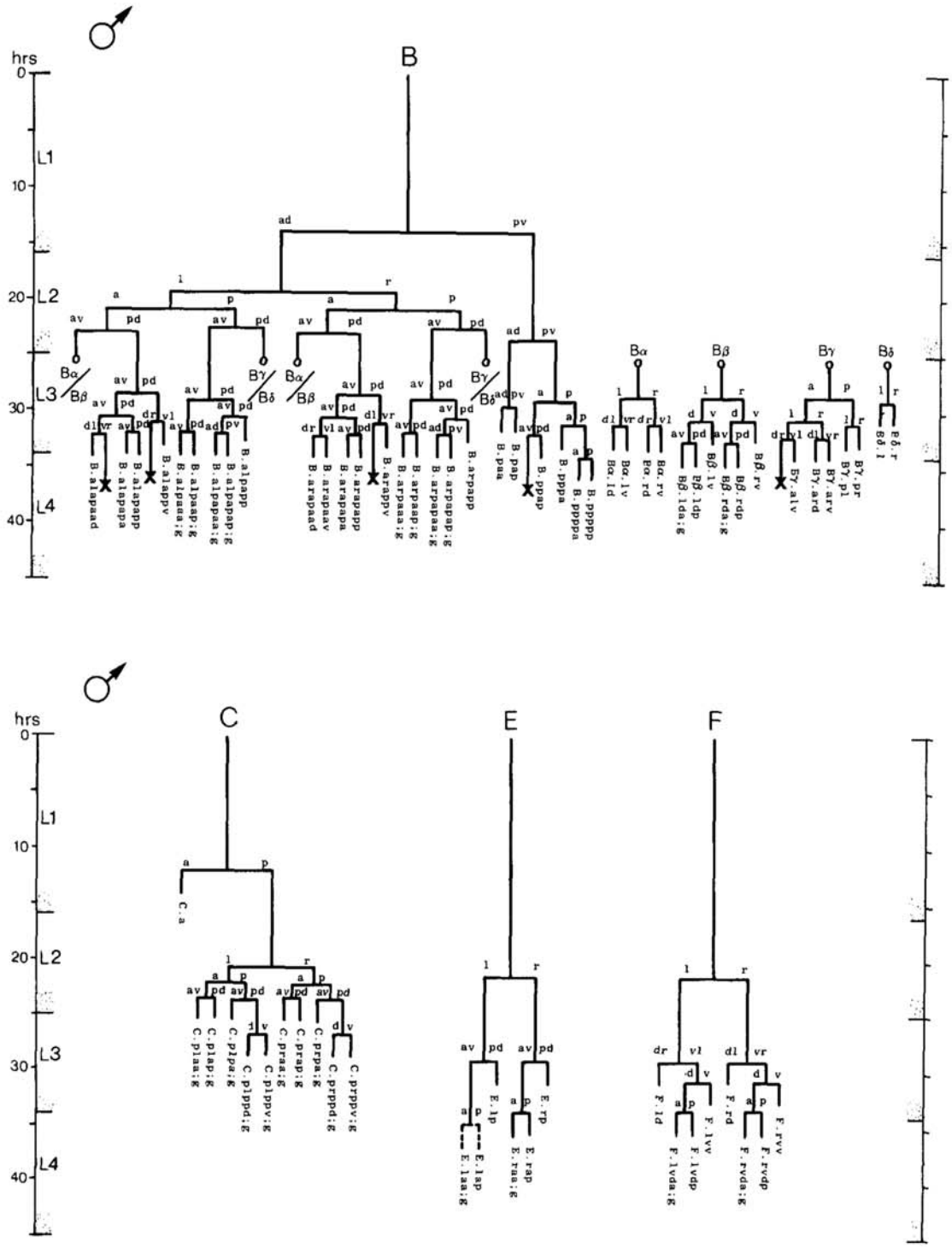

FIG. 22. B, C, E, and F lineages, male; ectodermal development in the male tail. g, neuron or glial cell.

rant contains 23.

M.vlpaa and M.vrpaa continue their movement until late during the L2 stage, stopping when they reach a position midway along the developing gonad. Late during the L3 stage, these sex myoblasts begin a series of three longitudinal divisions. Each cell produces 2 sets of 4 daughters by the L3 molt, generating a total of 16 new cells located in 4 sectors around the dorsoventral axis of the developing vulva. In each set, the cell nearest the vulva (e.g.,

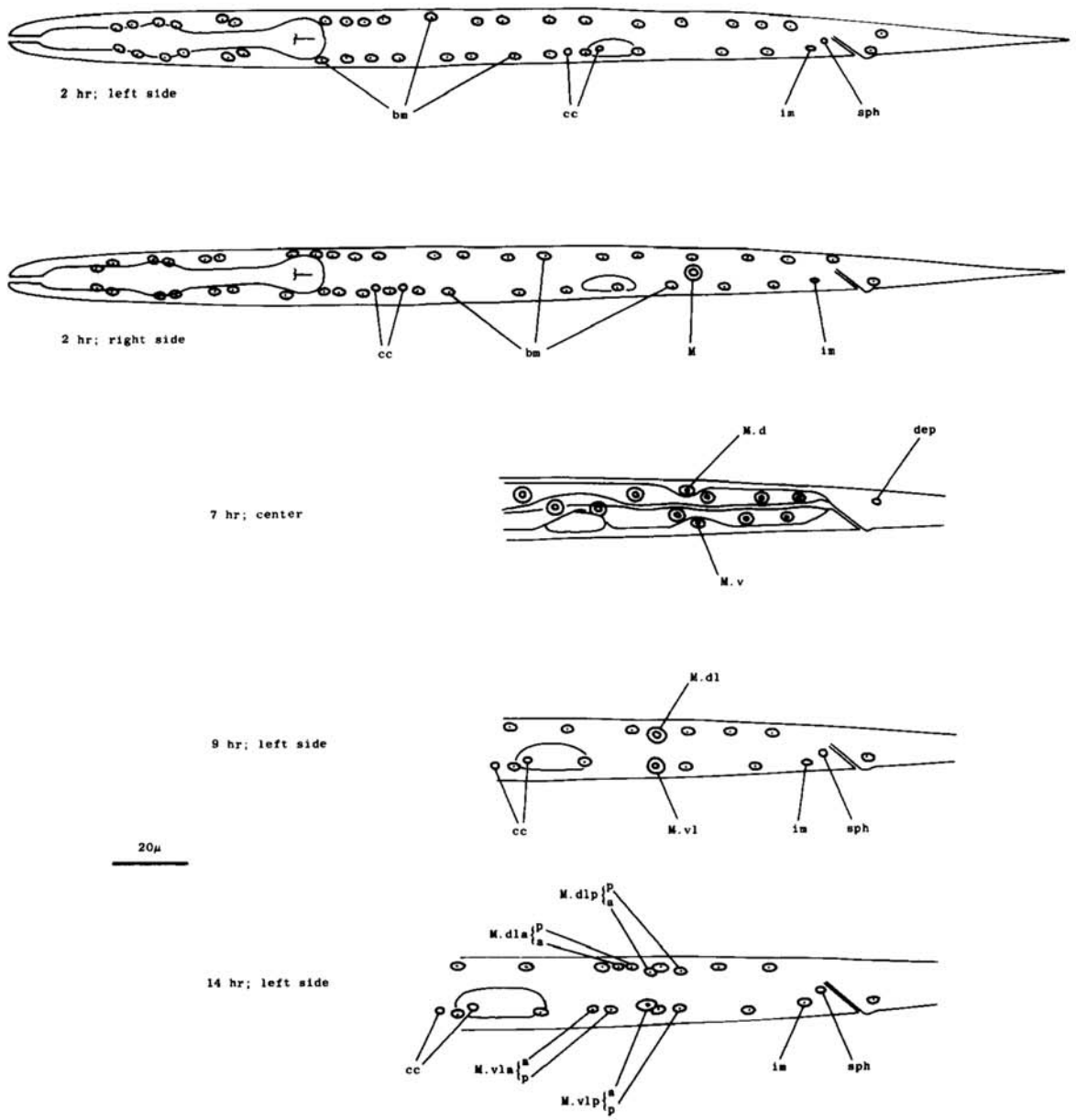

FIG. 23. M; hermaphrodite, lateral views; mesodermal development during the L1 stage. bm, body muscle; cc, coelomocyte; dep, anal depressor muscle; im, intestinal muscle; sph, anal sphincter muscle.

M.vlpaaapp) becomes a type 1 vulval muscle (vml); its sister (M.vlpaaapa) becomes a type 2 vulval muscle (vm2). vm1 attaches to the vulva more ventrally than does vm2 and attaches to the body wall more dorsally than vm2; vm1 is located more superficially than vm2. The cell farthest from the vulva in each set (e.g., M.vlpaaaaa) becomes a type 2 uterine muscle (um2); its sister (m.vlpaaaap) becomes a type 1 uterine muscle (um1). Both um1 and um2 form thin sheets with most of their filaments in a circumferential orientation. um1 attaches to the ventral side of the uterus close to the vulva and to the lateral ridge of the body wall. um2 attaches solely to the uterus in a region further from the vulva; in contrast to um1, um2 extends to the dorsal side of the uterus.

During the L4 stage, the nuclei of the sex muscles assume their final positions around the developing gonad. During the L4 molt, all of the 16 sex muscle cells

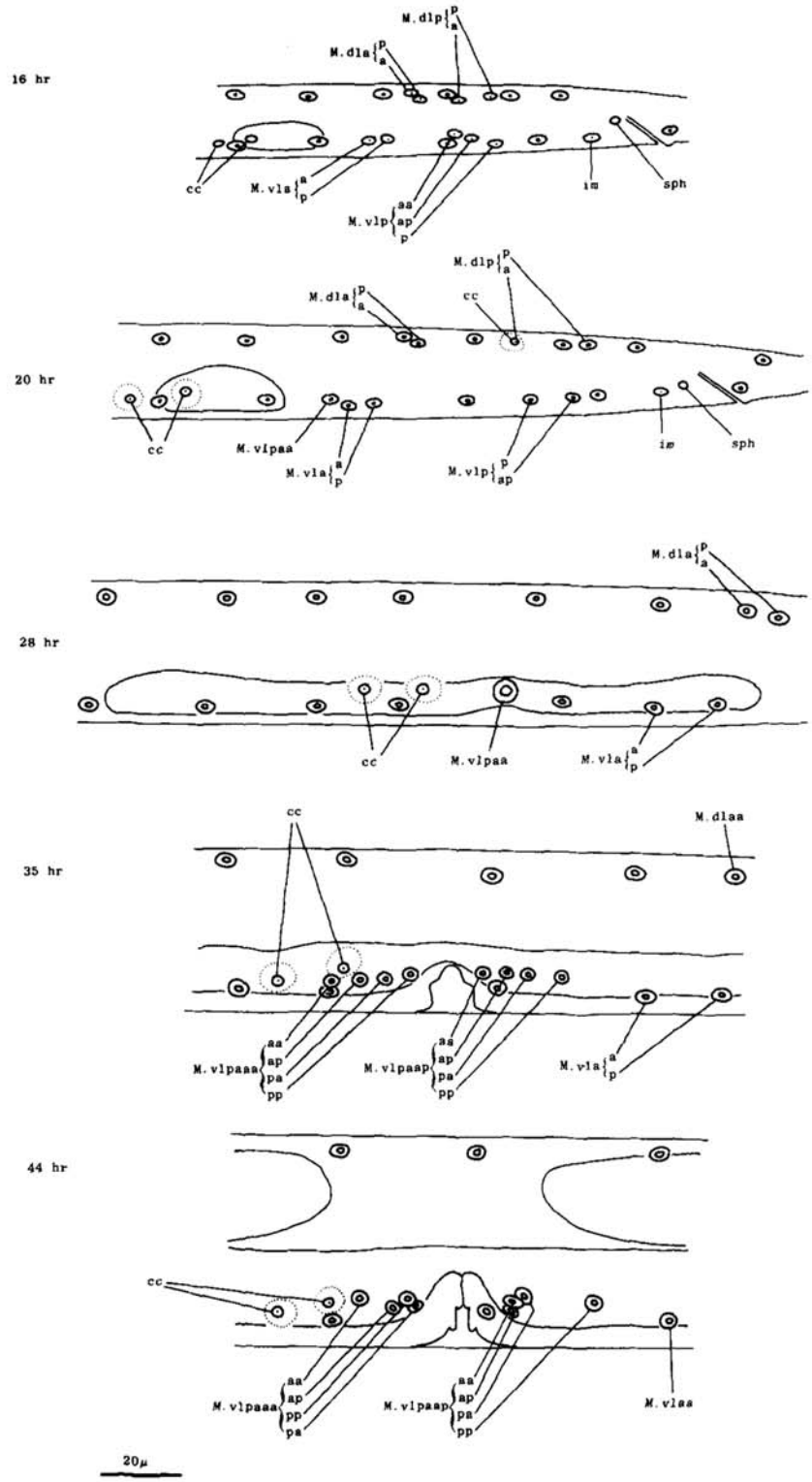

FIG. 24. M; hermaphrodite, left lateral views of the left side; mesodermal development. cc, coelomocyte.

twitch in characteristic directions. In the adult, these muscles are used in laying eggs.

M cell, male (Figs. 25 and 26): The mesodermal cell lineage in the male begins exactly as in the hermaphrodite. However, cells homologous with those which in the hermaphrodite differentiate into coelomo-

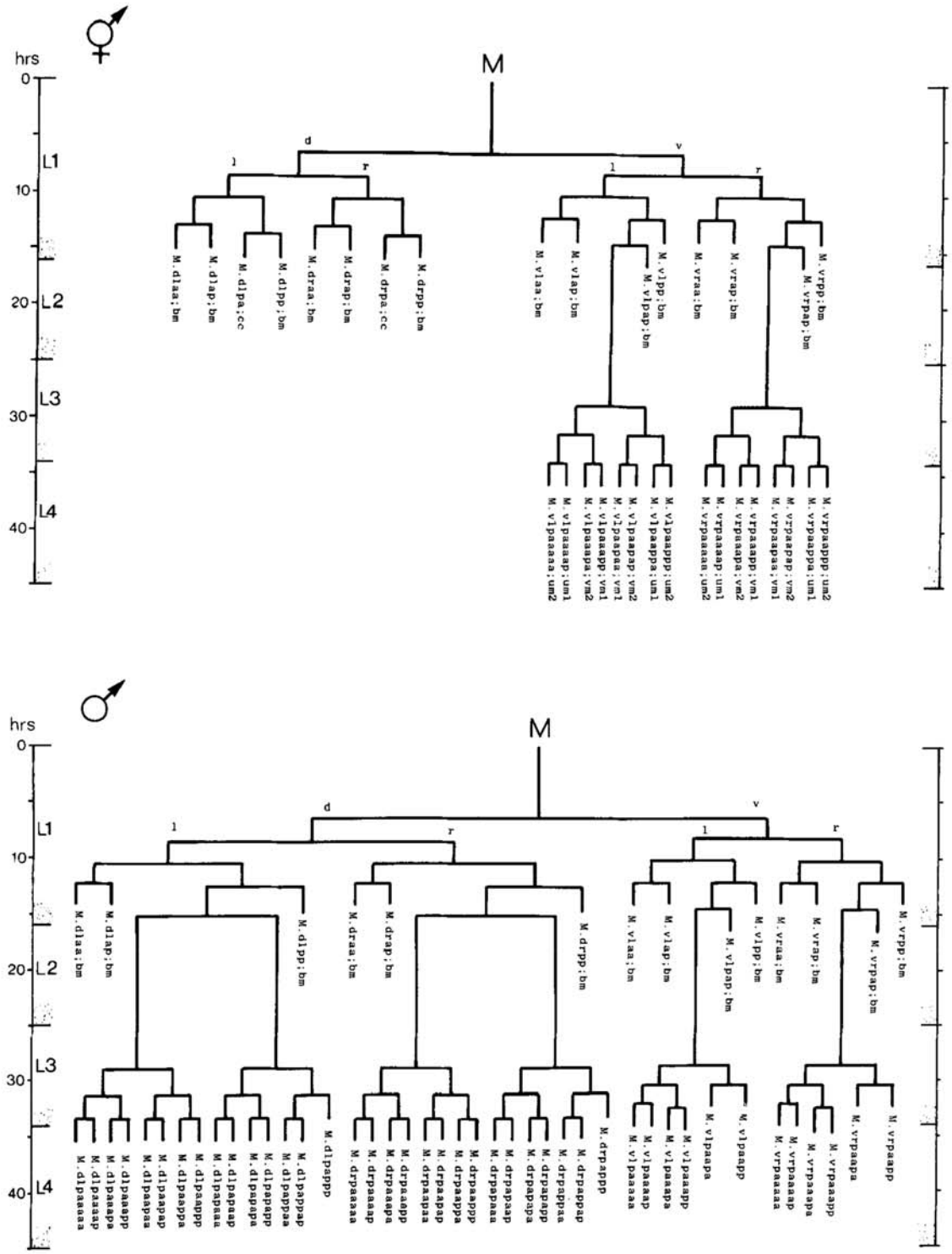

FIG. 25. M lineage, hermaphrodite and male; mesodermal development. Divisions are anterior–posterior unless otherwise indicated. bm, body muscle; cc, coelomocyte; um1, type 1 uterine muscle; um2, type 2 uterine muscle; vml, type 1 vulval muscle; vm2, type 2 vulval muscle.

cytes (M.dlpa and M.drpa) are blast cells in the male. The six male sex mesoblasts grow in size and, during the L3 stage, migrate posteriorly. Late during the L3 stage, these 6 cells begin a series of divisions, producing a total of 42 daughters. Most of the progeny of the ventral cells move to positions along the ventral side of

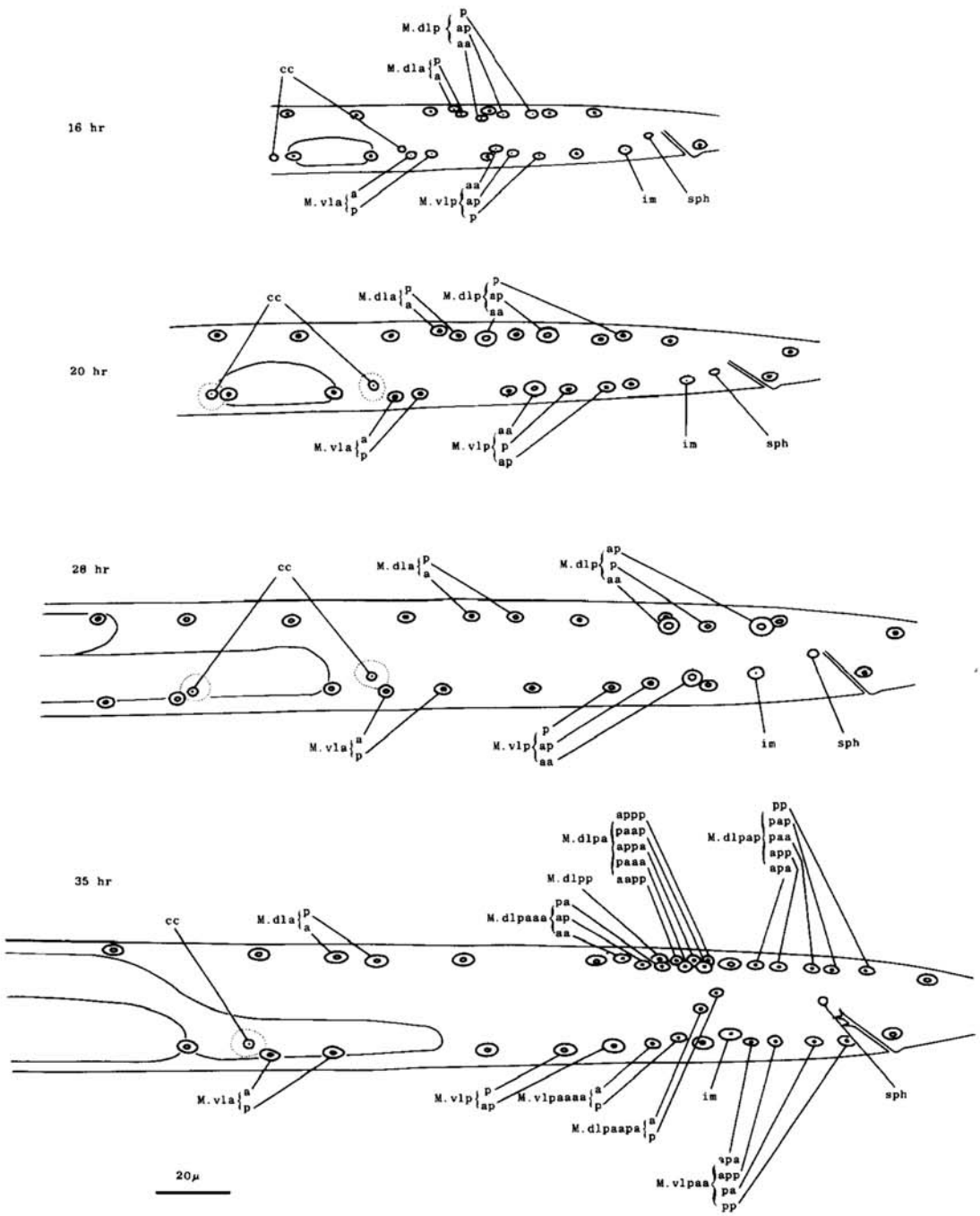

FIG. 26. M; male tail, left lateral views of the left side; mesodermal development. cc, coelomocyte. The muscles continue to migrate in the L4.

the nematode. Some of the daughters of the posterior dorsal cells migrate posteriorly into the post-anal region of the tail; some of the others move ventrally. Most of the daughters of the anterior dorsal cells migrate to the ventral side; most then continue their movements, some in an anterior direction and some in a posterior direction. These migrations, which have not yet been followed in detail, often occur concomitantly with the divisions which generate these cells; there is substantial variation among individuals regarding the precise order of events as well as the orientation of the division planes of the migrating cells. For example, sometimes a

given cell will migrate from the dorsal to the ventral side and then divide, whereas sometimes this same cell will divide on the dorsal side and then both of its daughters will migrate to the ventral side.

We have not yet identified these 42 daughter cells in the adult male, but we believe they must include a variety of male-specific muscles located in the tail and, usually, one coelomocyte (located near the dorsal midline somewhat anterior to the anus). Approximately 16 of the muscles are located anterior to the anus and run diagonally in a ventral-posterior to lateral-anterior direction. Other male-specific muscles control the movements of the spicules.

(c) Intestine. I cells (Figs. 14 and 16): Fifteen minutes after the beginning of L1 lethargus, the intestinal divisions take place. Normally, the six anterior-most nuclei do not divide; any of the four posterior-most nuclei may also fail to divide. Thus, division of 10–14 of the 20 juvenile intestinal nuclei produces the 30–34 nuclei normally found in the adult.

Other patterns of division occasionally occur. Once, for example, an intestinal nucleus located immediately behind the anterior six appeared to die during division; after anaphase, no daughter nuclei were formed. Concomitantly, the adjacent anterior nucleus divided, the only time this nucleus has been observed to do so.

(d) Gonad. The cell lineages of the gonad have not been determined. As suggested by Hirsh *et al.* (1976), the morphology of the gonad offers a convenient means of staging the postembryonic development of *C. elegans*. For comparison with the timing of other postembryonic events, various stages of gonad development in both the hermaphrodite and the male can be seen in Figs. 9, 24, and 26.

(4) Other Nematodes

Postembryonic developmental events similar to those we have observed in *C. elegans* also occur in other nematodes. We have studied the postembryonic development of a variety of nematode species in two ways: (1) observation of Feulgen-stained specimens to determine whether or not a postembryonic increase occurs in the number of cell bodies in the ventral cord, and (2) examination of living specimens using Nomarski optics to determine whether or not cell divisions could be observed directly. Based upon these criteria, postembryonic nongonadal cell divisions occur in all nematodes we have studied.

Panagrellus redivivus (the "sour-paste nematode") and *Turbatrix aceti* (the "vinegar eelworm") are free-living nematodes in a different family from that of *C. elegans* (de Coninck, 1965). *Aphelencoides blastophthorus* is plant parasitic and in a different order (de Coninck, 1965). All three of these species showed development of their ventral nerve cords and cell divisions in other tissues that appeared to be very similar to these events in *C. elegans*. *A. blastophthorus* may contain slightly more ventral cord neurons than *C. elegans*.

Ascaris lumbricoides var. *suis*, an intestinal parasite of pigs, is in an order different from both *C. elegans* and *A. blastophthorus* (de Coninck, 1965). It also showed development in its ventral cord similar to that seen in *C. elegans*. For microscopy, embryos and L1 larvae were artificially released from eggs at different developmental stages as described under Materials and Methods. (Normally, *A. lumbricoides* larvae do not hatch until the L2 stage.). The first cleavage of the egg occurred on Day 4; by Day 14, the embryos had reached the "comma" or "tadpole" stage (Chitwood and Chitwood, 1974). At Day 17, the eggs contained moving larvae with nuclei sparsely distributed in the ventral cord. By Day 21, the number of nuclei in the ventral cord had increased severalfold to a number comparable to that in *C. elegans* L2 larvae. Some specimens with intermediate cord counts contained cells in the ventral cord in the process of division. Because of difficulties in

visibility, cell divisions in *A. lumbricoides* were not observed using Nomarski optics.

Longidorus macrosoma, a raspberry ecto-parasite, is in a different subclass from the other nematode species examined (de Coninck, 1965). Its ventral cord was quite distinctive, containing about 140 neuron-like nuclei in the L1. As in the other nematodes, substantial development occurs after the L1 stage, increasing the number of ventral cord neurons to about 1000 by the beginning of the L3 stage. Cell division was not observed using Nomarski optics.

We have not traced lineages in any of these other nematodes.

Postembryonic increases in the number of cells in the ventral cord, hypodermis, and intestine of the free-living nematode *Rhabditis anomala* have been described by Wessing (1953). The increase in the number of ventral cord nuclei in *P. rediviVus* has been previously reported by Hechler (1970).

DISCUSSION

(A) Summary

The number of nongonadal nuclei in the hermaphrodite of *C. elegans* increases from about 550 at hatching to about 810 in the mature adult (Table 2). Essentially invariant cell lineages generate a fixed number of progeny cells of rigidly determined fates. These postembryonic cell lineages range in length from one to eight sequential divisions.

A number of these lineages produce the accessory sexual structures of the hermaphrodite: the vulva (through which eggs are laid), vulval and uterine muscles, and neurons which innervate these muscles. New neurons are also added to the motor nervous system. Almost one-third of all postembryonic progeny nuclei become part of the hypodermal syncytium of the nematode body wall. The number of intestinal nuclei almost doubles. There is a small increase in the number of body muscles. Few cell divisions occur in the head; both the anterior sensory nervous system and the pharynx appear to be complete at hatching.

In the development of the hypodermis, eight blast cells on each lateral side (H1, H2, V1–V6) follow similar programs of cell division to generate a total of 92 syncytial hypodermal nuclei and 28 seam cells. (Seam cells form a distinct cellular band embedded in the lateral hypodermal ridge.) These hypodermal cell divisions occur around the time of the first three larval molts. Two lateral tail blast cells (T) produce two seam and six syncytial nuclei. Twelve other syncytial hypodermal nuclei are produced in the ventral cord by precursors (P1–P4, P8–P12) primarily responsible for neuronal development. The other three ventral cord precursors (P5–P7) generate the 22-cell vulva, the hypodermal structure through which eggs are laid. In the young larva, the ventral cord precursors form an integral part of the hypodermal body wall.

Most of the neuronal development occurs in the ventral nerve cord and its associated ganglia. Identical patterns of division during the first larval stage of 12 precursor cells (P1–P12) and 1 neuroblast (P0.a) followed by a fixed program of cell deaths add 56 neurons to the ventral motor nervous system. Two of the lateral hypodermal blast cells (V5) produce small ganglia as well as the hypodermal nuclei mentioned above. Two other lateral hypodermal blast cells (T) and the lateral neuroblasts (Q1, Q2) also generate neurons and/or glial cells.

Mesodermal development derives exclusively from a single cell (M) present in the newly hatched larva. A series of divisions during the first larval stage produces 14 body muscles, 2 coelomocytes, and 2 myoblasts. These myoblasts migrate anteriorly until they reach the position where the vulva will form. They divide shortly before the third molt to produce 16 sex muscles, which function in egg-laying.

Most of the intestinal nuclei (In) divide

TABLE 2

NUCLEAR COUNTS, HERMAPHRODITE

	Present in young L1		Derived from postembryonic lineages		
	Nondividing nuclei	Blast cells	Surviving nuclei	Cell deaths	Present in adult
Lateral hypodermis					
Seam	2	–	30	–	32
Syncytial	20	–	98	–	118
Neuronal or glial	8	–	28	–	36
Total	30	20 (H,V,T,Q)	156	8	186
Ventral cord and associated ganglia					
Neuronal or glial	33	–	56	9	89
Hypodermal	0	–	12	1	12
Vulva	0	–	22	0	22
Total	33	13 (P)	90	10	123
Mesoderm					
Body muscles	81	–	14	0	95
Sex muscles	0	–	16	0	16
Coelomocytes	4	–	2	0	6
Digestive tract muscles	4	–	0	0	4
Head mesodermal cell	1	–	0	0	1
Total	90	1 (M)	32	0	122
Intestine	6[a]	14[a] (I)	28	0	34
Head					
Neuronal, glial, small structural	201[b]	–	4	0	205[b]
Hypodermal (dorsal, ventral)	15	–	1	0	16
Pharynx	80	–	0	0	80
Pharyngeal-intestinal valve	6	–	0	0	6
Excretory system	4	–	0	0	4
Total	306	2 (G)	5	0	311
Tail					
Neuronal or glial	19	–	1	0	20
Hypodermal	7	–	1	0	8
Rectal glands	3	–	0	0	3
Total	29	1 (K)	2	0	31
Other tail ectoderm (B,C,E,F)	4	0	0	0	4
Total, excluding gonad	498	51	313	18	811

[a] The L1 intestine contains 20 nuclei. Of these, 6 never divide; in a given individual, 10–14 of the others divide.

[b] Estimate.

once, before the first molt.

A number of other cell divisions also occur in the hermaphrodite.

The postembryonic development of the male differs from that of the hermaphrodite. In the male, the number of nongonadal nuclei increases from about 550 to about 970 (Table 3). Most of the additional, male-specific cells are located in the specialized structures of the tail used by the male during copulation. Many cell lineages are similar in the male and hermaphrodite; the two sexes display virtually identical changes in the lateral hypoder-

TABLE 3

NUCLEAR COUNTS, MALE

	Present in young L1		Derived from postembryonic lineages		
	Nondividing nuclei	Blast cells	Surviving nuclei	Cell deaths	Present in adult
Lateral hypodermis					
Seam	2	–	36	–	38
Syncytial	20	–	104	–	124
Neuronal or glial	6	–	28	–	34
Rays	0	–	54	–	54
Total	28	20 (H,V,T,Q)	222	26	250
Ventral cord and associated ganglia					
Neuronal or glial	33	–	70	4	103
Hypodermal	0	–	10	1	10
Unknown	0	–	16	0	16
Total	33	13 (P)	96	5	129
Mesoderm					
Body muscles	81	–	14	0	95
Sex muscles	0	–			
Coelomocytes	4	–	42	0	46
Unknown	0	–			
Digestive tract muscles	4	–	0	0	4
Head mesodermal cell	1	–	0	0	1
Total	90	1 (M)	56	0	146
Intestine	6[a]	14[a] (I)	28	0	34
Head					
Neuronal, glial, small structural	205[b]	–	4	0	209[b]
Hypodermal (dorsal, ventral)	15	–	1	0	16
Pharynx	80	–	0	0	80
Pharyngeal-intestinal valve	6	–	0	0	6
Excretory system	4	–	0	0	4
Total	310	2 (G)	5	0	315
Tail					
Neuronal or glial	19	–	1	0	20
Hypodermal	7	–	1	0	8
Rectal glands	3	–	0	0	3
Total	29	1 (K)	2	0	31
Other tail ectoderm	0	4 (B,C,E,F)	66	5	66
Total, excluding gonad	496	55	475	36	971

[a] The L1 intestine contains 20 nuclei. Of these, 6 never divide; in a given individual, 10–14 of the others divide.

[b] Estimate.

mis, body musculature, and intestine. Some male-specific cells are produced by lineages which are initially identical to those seen in the hermaphrodite. For example, extra divisions in the lateral hypodermal lineages (V5, V6, T) produce the 18 rays of the male tail. The mesodermal lineage (M) generates the male-specific sex muscles instead of the vulval and uterine muscles of the hermaphrodite. The de-

velopment of the male ventral nerve cord involves extra cell divisions as well as fewer programmed cell deaths. Two posterior ventral cord hypodermal cells (P10.p, P11.p) divide to form part of the male tail, whereas the three cells homologous with those which produce the vulva in the hermaphrodite (P5.p, P6.p, P7.p) do not divide in the male. The other male-specific structures (the cloaca, spicules, and associated ganglia) are derived from cells (B, C, E, F) that are present in all L1 larvae but divide only in males.

The postembryonic development of *C. elegans* involves the sequential addition of new structures onto a preexisting larval edifice; no destruction of larval-specific cells occurs. However, there are structural modifications of larval cells: The "dorsal D" motoneurons of the ventral cord reverse their polarity during postembryonic development; in the L1, they receive innervation from the dorsal cord and synapse onto ventral body muscles, whereas in the adult they receive innervation from the ventral cord and synapse onto dorsal body muscles (J. G. White, personal communication).

(B) Functions of Postembryonic Cell Divisions

A number of the postembryonic cell lineages are related to sexual maturation. At hatching, the hermaphrodite and the male are almost identical with respect to cell number, position, and (presumably) function. Yet, by the adult stage, gross anatomical differences are obvious in nongonadal tissues. Most of the cell divisions which give rise to these sex-specific structures occur shortly before the third molt. In the hermaphrodite, ectodermal lineages produce the 22 cells which form the vulva, the structure through which eggs are laid. Mesodermal lineages generate the 16 muscle cells which control the vulva and uterus during egg-laying. Neurons which innervate at least some of these muscles (White *et al.*, 1976) are also formed postembryonically from the neuronal lineages of the ventral cord. In the male, related ectodermal, mesodermal, and neuronal lineages generate some of the structures of the tail which function during copulation. The other male-specific structures are produced by lineages derived from cells which in the hermaphrodite do not divide.

Some of the postembryonic cell divisions may relate to growth. For example, four rounds of hypodermal cell divisions during the first three larval stages substantially increase the number of hypodermal nuclei. The increases in the numbers of intestinal nuclei and body muscle cells may similarly reflect growth, although it is unclear why there are cell divisions in these tissues only during the first larval stage.

Explanations for other postembryonic cell divisions are not obvious. For example, the L1 moves quite adequately using its juvenile locomotory ventral nervous system; yet, the number of neurons in this system is almost tripled during early larval development. Perhaps for mechanical reasons the larger adult requires a more intricate nervous system to control its locomotion. Alternatively, the adult may be capable of behaviors more complex than those of the L1.

(C) Invariance

The postembryonic somatic cell lineages of *C. elegans* are generally invariant, with a fixed pattern of cell divisions and a fixed developmental program defined for every daughter cell. However, essentially five types of variations in the details of this developmental program have been observed, as follows. (1) Variation in the pattern of cell divisions: The greatest inconstancy appears in the single round of divisions of the intestinal nuclei; the four posterior-most nuclei sometimes divide and sometimes do not. Slight variations occur in the patterns of divisions of the hypodermal cells of the ventral cord. Among males, P9.p occasionally divides; among hermaphrodites, P3.p sometimes fails to divide. Similarly, E.la occasionally di-

vides in males. (2) Variation in the pattern of cell deaths: Q2.pp, which normally appears to die, has once been observed to survive into the adult. (3) Variation in which of two alternative lineage programs a given cell will follow: These cases appear to involve positional effects and are discussed below (see Mechanisms of Determination). (4) Variation in the precise order of specific events: For example, in mesodermal development, sometimes M.vla divides before M.vra, whereas sometimes M.vra divides before M.vla. Similar timing variations occur between lineages as well as within a given lineage. Migrations and divisions also are not rigidly ordered, because sometimes a cell will migrate and then divide, whereas sometimes it will divide and its daughters will migrate (see Results, Mesoderm). (5) Variation in the precise positions of cell nuclei: For example, the development of both the ventral nerve cord and the somatic musculature leads to variable intercalation of new progeny cells with the juvenile cells present at hatching. For this reason, our diagrams cannot be used to identify all cells in an animal which has already developed.

(D) Patterns of Cell Divisions

Two distinguishable types of cell divisions occur during the postembryonic development of *C. elegans*. Some divisions are symmetrical, producing daughters which are equivalent in morphology and, often, in subsequent development. Other divisions are asymmetrical, producing two distinctly different types of daughter cells; in at least some instances, asymmetric divisions generate a posterior daughter that is morphologically like its mother cell and an anterior daughter of a new cell type. Both types of divisions can be seen in the development of the lateral hypodermis (Fig. 10); for example, Vn.p divides symmetrically, whereas Vn.pa and Vn.pp divide asymmetrically. Asymmetric divisions almost always occur along an anterio-posterior axis and could reflect an anterio-posterior asymmetry of developmental determinants located either inside or outside the parent cell. That transverse divisions are symmetrical (see below, Symmetry) suggests that the distribution of such determinants may be bilaterally symmetric. The few dorsal–ventral divisions appear to be asymmetrical. However, the examples in the male-specific B, C, and F lineages are difficult to interpret, because divisions in these lineages generally fail to conform to the orthogonal coordinate system seen elsewhere. The only other example (the first division of the mesoblast, M) produces progeny which follow identical lineages for three further divisions; the subsequent different fates of a few of the lineally equivalent dorsal and ventral cells (e.g., M.dlpa and M.vlpa) could arise from local environmental influences.

Comparison of the various cell lineages reveals that there is no standard type of cell lineage utilized for generating groups of new cells. Although most lineages involve both symmetrical and asymmetrical cell divisions, two exemplify extreme types of lineage logic. In the mesodermal lineage (Fig. 25), a series of symmetrical divisions adds a relatively large number of new cells of a particular type at one time. In the lateral hypodermal lineages (Fig. 10), a series of asymmetrical divisions adds sequentially individual progeny which differentiate into types distinct from the parent cell; in such stem cell lineages, one daughter always maintains the morphology of the original mother cell.

A variation of this "stem cell logic" is thought to produce the ventral nervous system of *Drosophila melanogaster* (Poulson, 1950; Seecoff *et al.*, 1973) and may also be used by *C. elegans*. In *Drosophila*, each of a set of neuroblast stem cells generates another stem cell and a "predifferentiated" cell that divides once before differentiating. The lineage of the *C. elegans* ventral nervous system (particularly in the male; Fig. 17) is similar. Perhaps this type of

lineage is common in neuronal development.

Another type of lineage that has been observed in insects may be utilized by *C. elegans*. In a number of insects, similar cell lineages generate scales, hairs, bristles, and sensilla (Lawrence, 1966); many of these insect lineages involve programmed cell death. In *C. elegans*, we find possible analogs in the development of the rays (probably mechanosensilla), the posterior lateral ganglia (associated with the postdeirid sensilla), and the lumbar ganglia (partially associated with the phasmid sensilla) (Fig. 27). Although there are difficulties in interpretation (see the legend to Fig. 27), we feel that the similarity of these lineages may reflect a fundamental subprogram of development.

Frequently, several blast cells follow the same asymmetric program of divisions. Thus, 13 neuroblasts of the ventral nervous system produce 5 daughters each, 10 lateral hypodermal stem cells produce 9 daughters each, 4 sex myoblasts produce 4 daughters each, and 18 ectodermal blast cells in the male tail produce 5 daughters each. In all of these cases, the lineally equivalent progeny of different blast cells differentiate into functionally equivalent cells. In effect, each precursor cell generates one of a number of repeating "units" present in, in these examples, the ventral cord, lateral hypodermis, sex musculature, and male tail. The logic utilized in these lineages may be contrasted with an a priori plausible alternative in which functionally equivalent cells are all produced from a given blast cell and then repositioned with respect to the daughters from other lineages to form an appropriate final configuration.

The relationship between lineage history and developmental fate suggests that

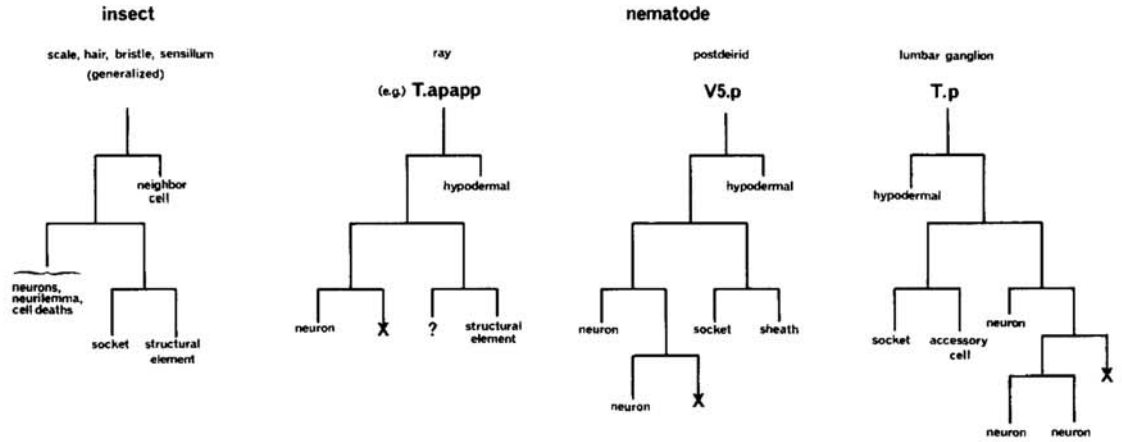

FIG. 27. Comparison of lineages found in insects and *C. elegans*. Insects: Generalized from Lawrence (1966). The lineage tree does not reflect the division axes. Ray: The neuron assignment is based on the identification of dopamine in this cell. The assignment of the structural element is based upon experiments with the laser (see Results, Cell Lineages). Postdeirid: Assignments were made by comparing nuclear morphology and arrangement as seen with Nomarski optics with that observed in serial-section electron micrographs. Because individuals of known lineage have not been examined by electron microscopy, the assignments of the sheath and socket cells (defined by Ward *et al.*, 1975) conceivably could be switched. Lumbar ganglion: assignments were made by tracing nuclei observed with Nomarski optics through to the adult stage and identifying these nuclei in serial-section electron micrographs. The wing-shaped accessory cell and the socket cell are associated with the phasmid. However, the three neurons apparently are not closely associated with this sensillum. Furthermore, the phasmid has another associated accessory cell which is present in the newly hatched larva. In this case, we would propose that the lineage defines the general functions of cells which subsequently assort into different sensory elements. The fates of some sister cells in the lumbar ganglion lineage are reversed compared to the fates of lineally equivalent cells in the ray and postdeirid lineages, possibly reflecting local environmental influences (see Mechanisms of Determination).

specific types of cells may arise only from an appropriate series of divisions of a particular type of blast cell. Hence, when only some progeny cells of a given lineage are needed, it may be necessary to generate and later destroy the other daughters. Such a mechanism provides one explanation for programmed cell death, common in both the nematode (see Results) and other developmental systems (Saunders, 1966).

(E) Mechanisms of Determination

The strong correlation between lineage and function could arise in essentially two distinct (but not necessarily exclusive) ways. On the one hand, the ultimate differentiation of a cell could be determined extrinsically according to its position; alternatively, its fate could be determined intrinsically according to its lineage history. Our observations are consistent with the hypothesis that much of the development of *C. elegans* is based upon a lineal determination of cell function. The high degree of invariance of these lineages suggests such a mechanism. Furthermore, experiments with the laser microbeam system (see Results) suggest that the fates of specific cells in the posterior lateral ganglia, the ventral cord, and the rays of the male tail are autonomously determined; these fates remain invariant despite the fact that the undamaged cells sometimes differentiate in positions which normally would be occupied by cells with different fates. Preliminary experiments (J. White, J. Sulston, N. Thomson, E. Southgate, and R. Horvitz, unpublished results) utilizing mutants with abnormal cell lineages have also confirmed these observations; blocking one branch of the cell lineages of the posterior lateral ganglia or the ventral cord does not alter the developmental fates of cells generated by another branch.

There are, however, some developmental events in which position appears to have an influence. In the male tail—in each of two cases (Bα and Bβ; Bγ and Bδ)—two cells of different lineage histories appear to be equally competent to follow either of two alternative developmental programs; their subsequent lineages are correlated with their relative positions and not with their previous histories. Similarly, ventral cord hypodermal blast cells P5.p and P6.p follow distinct programs in the development of the vulva, despite the fact that either of two cells present in the young L1 can become either P5 or P6; in other words, the developmental program that each of these cells follows is correlated with the position it assumes in the ventral cord. Two observations suggest that the gonad may be responsible for this positional influence on P5 and P6: (a) The lineages of the ventral hypodermal cells Pn.p are arranged essentially symmetrically around the midpoint of the developing gonad (Fig. 18), as if this point were acting as the source of a positional determinant, and (b) the Pn.p cells which divide are those immediately adjacent to the gonad. Other developmental events—e.g., the Pn.aap cell deaths in the ventral cord and the differentiation of the sex muscles—also occur symmetrically around the developing gonad of the hermaphrodite and similarly may reflect its influence.

Other aspects of the postembryonic development of *C. elegans* possibly may be affected by positional influences: (1) Those lineages which utilize sets of "equivalent" blast cells show apparent end effects; such effects include logical inversions (in which the fates of anterior and posterior daughters are reversed) and/or positional inversions (in which daughters migrate past one another), as well as modifications of the pattern of cell divisions itself. For example, such alterations are apparent in the H and T lineages (the head and tail equivalents of the V lineage; Fig. 10) and in the divisions, deaths, and ultimate cell fates at the ends of the ventral cord (Fig. 17; Table 1). These various modifications of cell lineages in the head and tail regions may reflect local environmental influ-

ences. (2) Six apparently equivalent developmental "regions" can be defined longitudinally along the lateral hypodermis of the nematode. In five of these regions, identical lineages are produced by the hypodermal blast cells. However, hypodermal blast cell V5 follows a modified developmental program to generate part of the posterior lateral ganglion. This region of the nematode is also unique in that it contains the lateral neuroblasts (Q) as well as the single mesoblast (M). Perhaps positional factors—possibly defined by one or more of these cells—determine the unique developmental aspects of this area of the nematode.

Although the examples described above indicate that cell lineages may be affected by positional influences, we do not know if the nematode is capable of regulation per se. In experiments involving the posterior lateral ganglia, the rays of the male tail, and the ventral cord, those structures normally derived from a cell which had been eliminated either by the laser or by mutation, were unequivocally absent in the adult; no regulatory processes induced extra divisions to compensate for the laser- or mutation-induced deficiencies. The unique observation of an abnormal death in the intestine followed by a compensatory abnormal division (see Results, Intestine) is a possible example of regulation.

(F) Symmetry

Although the young L1 is essentially bilaterally symmetrical, there are a variety of minor differences between its left and right sides which can be distinguished in the light microscope: (1) The gonad primordium lies obliquely, with its anterior portion displaced to the right and its posterior portion displaced to the left. In the region of the gonad primordium, the intestine is reciprocally displaced (anterior, left; posterior, right). (2) The mesoblast (M) is on the right. (3) The two coelomocytes on the right are anterior to those on the left. (4) There is an extra lateral hypodermal nucleus on the right side near the head and another on the left side in the tail. (5) There is an extra ventral body muscle nucleus on the right side posterior to the gonad primordium. (6) The lumbar ganglion on the right contains one more nucleus than that on the left. (7). The anal sphincter muscle nucleus is located on the left. (8) The excretory cell is displaced left of the midline.

Bilateral symmetry is reflected in the transverse divisions which occur during postembryonic development. Virtually all transverse divisions produce morphologically identical progeny which follow identical programs of development. The only indications of exceptions to this rule—(1) B.alapaav dies, B.arapaav does not; (2) Bγ.ald dies, Bγ.ard does not; (3) E.ra divides, E.la sometimes does not—may well arise from positional influences at the end of identical lineage programs.

The left and right lateral neuroblasts, Q1 and Q2, are bilaterally symmetrical in their initial positions and in their division programs. However, their progeny migrate differently. Q1 produces three progeny in the posterior half of the left side and Q2 produces three (or four) progeny in the anterior half of the right side.

The obliquely oriented gonad primordium of the young L1 shows twofold rotational symmetry around a dorso-ventral axis. The subsequent development of the hermaphrodite gonad, vulva, and sex muscles is also symmetrical about this dorsoventral axis through the midpoint of the gonad.

(G) Migrations

Cellular migrations occur frequently during the postembryonic development of *C. elegans*. Because Nomarski optics does not allow clear visualization of cellular boundaries, we cannot always be certain that whole cells—as opposed to nuclei—are migrating. However, in most migrations, nucleus and cytoplasm do appear to move together. The migrations of the pre-

cursor (P) cells into the ventral cord are distinctive; a cytoplasmic extension moves into the cord and is followed by the nucleus and, finally, by the remaining cytoplasm.

Some migrations – such as those in the posterior half of the ventral cord – appear to be passive events that depend solely on the activities of neighboring cells; often such movements are variable in extent from individual to individual. Other migrations are rigidly determined, with cells traversing relatively long distances from reproducible starting and stopping sites. The most striking of the migrations involve progeny of the lateral neuroblasts (Q1 and Q2) and the mesoblast (M): (1) Three of the progeny of Q2 (Q2.a, Q2.p, Q2.ap) travel rapidly along the left lateral hypodermis; they move about 50 μm (about 16 nuclear diameters) in 2 hr. Q1.ap shows a similar, but somewhat shorter, migration. (2) The two sex myoblasts of the hermaphrodite (M.vlpaa, M.vrpaa) migrate from their origin among the posterior ventral body muscles to the anterior–posterior center of the developing gonad; they move about 65 μm (about 16 nuclear diameters) in 6 hr. (3) Most of the daughters of the dorsal anterior sex mesoblasts of the male (M.dlpaa, M.drpaa) migrate from the region of the dorsal body muscles around to that of the ventral body muscles; they move about 20 μm (about 5 nuclear diameters) in 1 hr. The movements of these male-specific mesoblasts occur independently of the concomitant divisions of these cells. As described above (see Results, Mesoderm), sometimes a given cell will migrate and then divide; sometimes it will divide and then both daughters will migrate; and sometimes it will start to migrate, divide, and then the two daughters will complete the migration.

(H) Prospects

The postembryonic development of *C. elegans* offers a variety of opportunities for further exploration: (1) This development is rigidly determined; specific cells follow precise developmental programs at well-defined times. This system can now be used to obtain a detailed analysis at the fine-structure level of such developmental events as cell migration and synapse formation as well as other types of cellular differentiation. For example, the morphological changes associated with programmed cell death could be studied for a specific cell; the sequence of events could be traced by examining individuals at different stages from the time that cell is born to the time it disappears. (2) The laser system developed by J. G. White will allow exploration of possible intercellular communications during the processes of cell divisions, migration, death, and differentiation. In addition, this laser system can be used to determine additional cell assignments [as described above (see Results) for the posterior lateral ganglia and the rays in the male tail] and cell functions. (3) Mutants which affect the cell lineages of postembryonic development may similarly be used to examine regulative effects and to determine cell assignments and functions. Furthermore, such mutants might provide clues concerning the logical bases of the genetic program for the postembryonic development of *C. elegans*.

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Postembryonic Nongonadal Cell Lineages of the Nematode *Panagrellus redivivus*: Description and Comparison with Those of *Caenorhabditis elegans*

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The postembryonic nongonadal cell lineages of the nematode *Panagrellus redivivus* are described and compared with those of *Caenorhabditis elegans*. The newly hatched larvae of *P. redivivus* females and males and *C. elegans* hermaphrodites and males are very similar. An almost identical set of blast cells divides postembryonically in *P. redivivus* and *C. elegans* to produce similar changes in the neuronal, muscular, hypodermal, and digestive systems. Most of these cell lineages are invariant; however, there is substantial variability in the number of cell divisions in the relatively extensive lineages of the lateral hypodermis of *P. redivivus*. Typically, in *P. redivivus* females, 55 blast cells generate 635 surviving progeny and 29 cell deaths; in *P. redivivus* males, 59 blast cells generate 758 surviving progeny and 35 cell deaths. The lineages generating the cells of the male tails of *P. redivivus* and *C. elegans* are almost identical; thus, the grossly different characteristics of these structures must reflect differences in the morphogenesis of cells equivalent in lineage history. Laser ablation experiments demonstrate that the gonad induces vulva development and that cell–cell interactions are important in specifying the fates of hypodermal precursor cells. The lateral hypodermal lineages provide striking examples of the apparent construction of complex lineages from modular sublineages; one simple pattern of cell divisions and cell fates occurs 70 times in the *P. redivivus* female. The differences in cell lineage between *P. redivivus* and *C. elegans* are relatively minor, and many appear to have involved two types of evolutionary change: the replacement of sublineages, and the modification of sublineages by the four classes of lineage transformations previously proposed based on a comparison of *P. redivivus* and *C. elegans* gonadal cell lineages (Sternberg and Horvitz, 1981). These types of differences suggest that the genetic programming of cell lineage includes instructions specifying where and when a particular sublineage is utilized, and other instructions specifying the nature of that sublineage.

INTRODUCTION

Comparison of the cell lineages of two related species could reveal aspects of both the genetic programming and the evolution of cell lineage. Simple differences in otherwise identical cell lineages would likely result from one or a few mutational events that occurred during the divergence of the two species. Thus the types of differences may reflect the nature of the genetic program that specifies cell lineage. Such a comparison is possible only between species with simple and invariant cellular anatomies and lineages.

Caenorhabditis elegans, a small free-living nematode, has been utilized extensively in the study of cell lineage. The complete embryonic (Deppe *et al.*, 1978; J. Sulston and E. Schierenberg, personal communication) and postembryonic (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston *et al.*, 1980) cell lineages have been determined. The normally invariant cell lineages of *C. elegans* have been perturbed both by mutation (Horvitz and Sulston, 1980; Sulston and Horvitz, 1981; Chalfie *et al.*, 1981) and by physical ablation of specific cells with a laser microbeam (Sulston and Horvitz, 1977; Sulston and White, 1980; Kimble and White, 1980; Kimble, 1981a). These studies have suggested that cell lineage limits the developmental potential of cells, often to a unique fate; for certain cells, cell–cell interactions specify which of a small number of alternative fates is expressed.

Cell lineage mutants should help define the ways in which single gene defects can alter particular cell lineages. A comparative study of cell lineage among related nematode species not only may suggest the possible effects of one or a number of mutations on cell lineage but also should reveal which changes have occurred during evolution.

In a previous paper (Sternberg and Horvitz, 1981) we compared the gonadal cell lineages of *C. elegans* (Kimble and Hirsh, 1979) with those of *Panagrellus redivivus*, another free-living nematode. From this comparison, we defined four distinct types of changes in cell lineage that appear to have occurred during the evolutionary divergence of *C. elegans* and *P. redivivus*: (1) changes in the number of rounds of cell divisions; (2) switches in the fate of a cell to a fate associated with another cell; (3) reversal in the polarity of a lineage; and (4)

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"altered segregation" of the potential to generate specific cell types, so that a cell acquires a developmental potential otherwise associated with its sister. These classes of apparent cell lineage transformations are similar to those that have been observed after genetic or physical perturbation of normal *C. elegans* development, which supports the hypothesis that these transformations reflect basic commands in the developmental program for cell lineage. As genetic studies to date have been concentrated on nongonadal cell lineages, we wished to extend our analysis of evolutionary variation to nongonadal lineages. In this paper we report the complete postembryonic nongonadal cell lineages of the *P. redivivus* female and male and compare them to the known nongonadal lineages of the *C. elegans* hermaphrodite and male (Sulston and Horvitz, 1977).

MATERIALS AND METHODS

Panagrellus redivivus was grown and handled as previously described (Sternberg and Horvitz, 1981). Newly hatched first stage (L1) larvae were obtained by cutting eggs and larvae from the uterus of an adult female and mounting them for observation; we do not know if the process of removing eggs from the uterus affected their time of hatching. L1 larvae were sexed based upon the presence or absence of the two female-specific neurons (FSNs) and by the presence of an enlarged B cell in the male; in late L1 or older larvae, the gonad provided a convenient indicator of sex (Sternberg and Horvitz, 1981). The positions of the coelomocytes were not reliable indicators of sex as they are in *C. elegans* (Sulston and Horvitz, 1977).

Cell lineages were determined by the continual observation of living specimens, using the technique of Sulston (1976) as described for *P. redivivus* by Sternberg and Horvitz (1981). Individual nuclei were followed as they divided, migrated, differentiated, and/or died during postembryonic development. We believe that the lineages presented below represent all postembryonic nongonadal nuclear divisions.

The length of each lethargus (the quiescent period preceding each ecdysis) is denoted by the stippling along the time axis of each lineage diagram. The ecdysis following each of the four larval periods (L1, L2, L3, and L4) is called the L1, L2, L3, and L4 ecdysis, respectively. Ecdysis refers to the moment at which the head emerges from the old cuticle (Singh and Sulston, 1978).

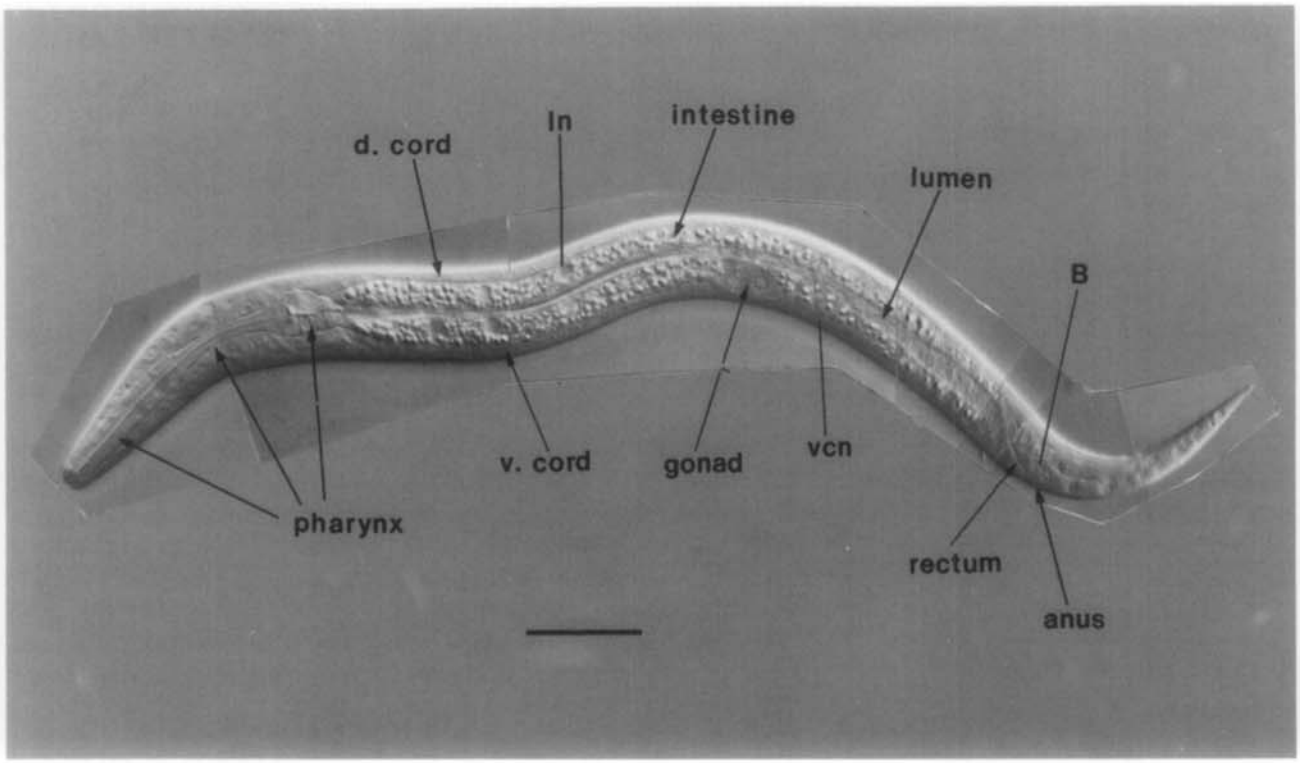
Nomenclature and cell lineage diagrams are as described by Sulston and Horvitz (1977). In the lineage diagrams the left branch indicates anterior (a) and the right branch indicates posterior (p), except where otherwise noted, i.e., by dorsal (d), ventral (v), left (l), or right (r). Oblique divisions are indicated by a combination of letters, e.g., anterior dorsal–posterior ventral (ad–pv). In lineage diagrams, the branch corresponding to the anterior, dorsal, or left daughter is drawn to the left.

Cells, nuclei, tissues, and structures are named as in *C. elegans* (Sulston and Horvitz, 1977). Cells are named according to the sequence of divisions of their progenitors with one letter added for each division. Super- or subscripted letters refer to both progeny cells, e.g., $M.d^{l}_{r}p$ refers to M.dlp and M.drp, $M._{vr}^{dl}$ refers to the four cells M.dl, M.dr, M.vl, and M.vr. Cell or nuclear type is defined by morphology as observed with Nomarski optics similar to that of cells of known type (based upon electron microscopy) in *C. elegans*. Unless there is reason to believe otherwise, we assume that similar types of cells are produced by the same lineage in the two species, e.g., Pn.a is called a neuroblast in *P. redivivus* even though the precise fates of its progeny have not been determined by electron microscopy. Nuclei are called compact if they look like neuronal or glial nuclei, but no *C. elegans* homologue exists. Three blast cells have been renamed to conform to the revised nomenclature for *C. elegans* (J. Sulston, personal communication): "P0.a" is now called W; C is now Y; and E is now U. An "n" following the name of a blast cell refers to any of a number of similar blast cells, e.g., Pn.p refers to the cells P1.p, P2.p, etc. Numbers in parentheses following the first letter in the name of the blast cell refer to the specific cells named, e.g., "P(9–11).p" refers to P9.p, P10.p, and P11.p. Px/y refers to one of the two bilaterally symmetrical cells that can become either Px or Py (the Pn cells are named after their entry into the ventral cord). An R or an L refers to the right and left sides of the animal, respectively, e.g., P5/6R is on the right side, and after migration will become either P5 or P6. In some cases cells are renamed for convenience, e.g., the left female sex myoblast, M.vlpa, is renamed SML; the male sex mesoblasts on each side are renamed SM1, SM2, SM3, and SM4; and the male caudal papillae precursors are renamed Rn.

Photomicrography, camera lucida, and laser microsurgery were performed as described by Sternberg and Horvitz (1981).

RESULTS

The newly hatched larvae of *P. redivivus* (Fig. 1) and *C. elegans* (Sulston and Horvitz, 1977) are essentially identical in gross morphology. Only the shapes of their pharynges and the relative rightward and posterior displacement of the *P. redivivus* gonad primordium are obviously different. Nuclei are readily identifiable by their positions and morphologies as similar to their



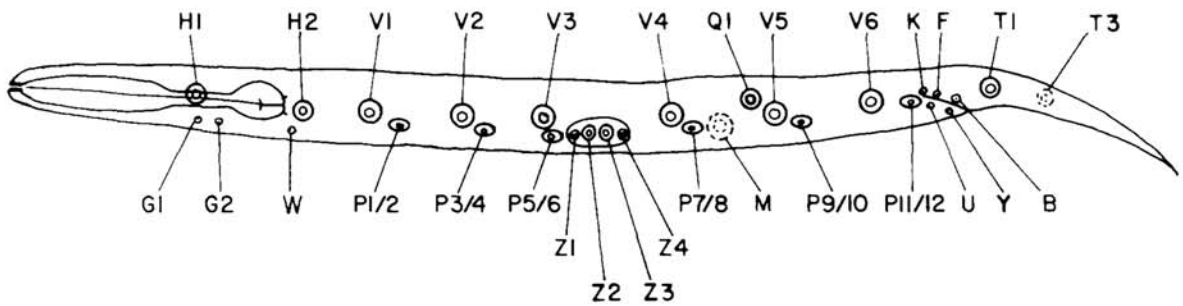


FIG. 1. L1 (15 hr) *P. redivivus* male, montage of photomicrographs of a single living animal, left lateral view, Nomarski optics. B, male-specific ectoblast; d. cord, dorsal cord; v. cord, ventral cord; In, intestinal nucleus; vcn, ventral cord neuron; lumen, intestinal lumen. Bar = 30 μm.

FIG. 2. Schematic drawing of left lateral view of young L1 showing location of blast cell nuclei. Nuclei denoted by dotted lines are contralateral. Bilaterally symmetric blast cells (H1, H2, V1–V6, Px/y, K/K′, Q1/Q2, and T1/T2) are shown only on the left. The intestinal nuclei are not shown (see Fig. 1 for the position of some of the intestinal nuclei). The mesoblast, M, generates body muscles (bm), coelomocytes (cc), and sex mesoblasts (called myoblasts if they produce only muscle cells) (SM); the lateral ectoblasts H(1–2), V(1–6), and T(1–3) generate syncytial hypodermal nuclei, seam cells, and Rn cells (precursors to the male caudal papillae) as well as neuronal cells, including the postdeirid. Q(1–2) generate neuronal cells. P(1–12) and W generate cells of the ventral nervous system and precursors (Pn.p) to the vulva (female) and preanal papillae (male). B, Y, U, and F are male-specific blast cells that generate the spicules, gubernaculum, and associated nervous tissue. In cells are intestinal cells. K and K′ each generate cells associated with the rectum; K′ also generates a neuron. G(1–2) generate neuronal cells; G2 also generates a hypodermal-like nucleus in the head. Z1, Z2, Z3, and Z4 are precursors to the gonad (see Sternberg and Horvitz, 1981).

counterparts in *C. elegans*. The number, morphologies, positions, and sex specificity of blast cells are so similar that we have named cells in *P. redivivus* (Fig. 2) according to the nomenclature used in *C. elegans*. There are four additional postembryonic blast cells in *P. redivivus*: T3, K′, and two of the In cells. The basic similarity of the L1 larvae of both sexes of both species allows a comparison of the lineages of corresponding blast cells. We have determined the anatomy of the *P. redivivus* L1 larvae only so far as to be confident of the identity of all blast cells. Because we do not know the fine structure anatomy of the *P. redivivus* adult, we cannot assign some nuclei to specific cell types.

Unless otherwise stated, information concerning *C.*

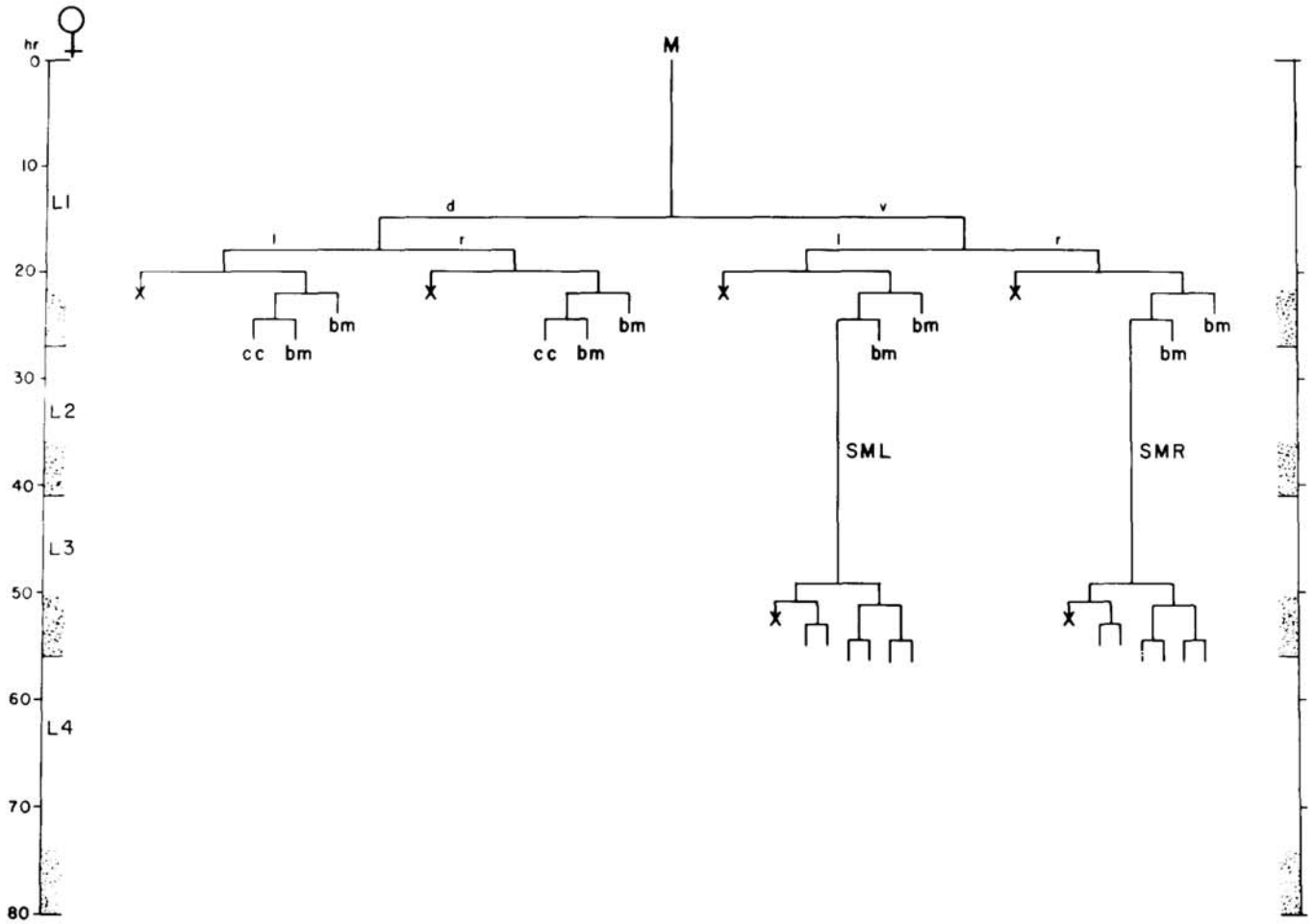


FIG. 3. M lineages. ×, programmed cell death; bm, body muscle; cc, coelomocyte; SML and SMR are the left and right female sex myoblasts, respectively, and SM1–SM4 are the male sex mesoblasts. All unlabeled cells are sex muscles.

elegans anatomy or development is from Sulston and Horvitz (1977).

Cell division and cell death as observed with Nomarski optics appear morphologically identical in the two species.

CELL LINEAGES

Mesoderm

M cell (*Figs. 3–5*). In both sexes of both species the mesoblast (M) divides first dorsoventrally and then transversely (left–right) during the middle of the L1 to generate one blast cell in each of the four muscle quadrants (dorsal left, dorsal right, ventral left, ventral right). All subsequent divisions during the L1 are anteroposterior. In *P. redivivus* the anterior daughter in each quadrant ($M._{vr}^{dl}a$) dies following an asymmetric division of $M._{vr}^{dl}$ (Fig. 4). There are no mesodermal postembryonic cell deaths in *C. elegans*.

The *P. redivivus* female and *C. elegans* hermaphrodite generate body muscles and coelomocytes in each dorsal quadrant and a sex myoblast (SM) and body muscles in each ventral quadrant. The males of both species generate sex mesoblasts and body muscles in each quadrant. The dorsal coelomocytes differentiate during the L2 in the *P. redivivus* female and *C. elegans* hermaphrodite. The sex mesoblasts migrate anteriorly in the *P. redivivus* female and *C. elegans* hermaphrodite and posteriorly in *P. redivivus* and *C. elegans* males during the L2. The sex mesoblasts divide during the L3 in both sexes of both species to generate sex-specific muscles, and, in the male, SM2 generates one (*C. elegans*) or two (*P. redivivus*) coelomocytes.

The *P. redivivus* female sex myoblasts divide twice and the anterior-most daughter on each side (SML.aa and SMR.aa) dies (Fig. 5). The remaining three cells each divide once more to generate a total of six sex muscles per side. In the *C. elegans* hermaphrodite each sex myoblast generates eight sex muscles. The sexual musculatures of the two species are different in arrangement as well as in cell number.

In *P. redivivus* males, the four dorsal sex mesoblasts

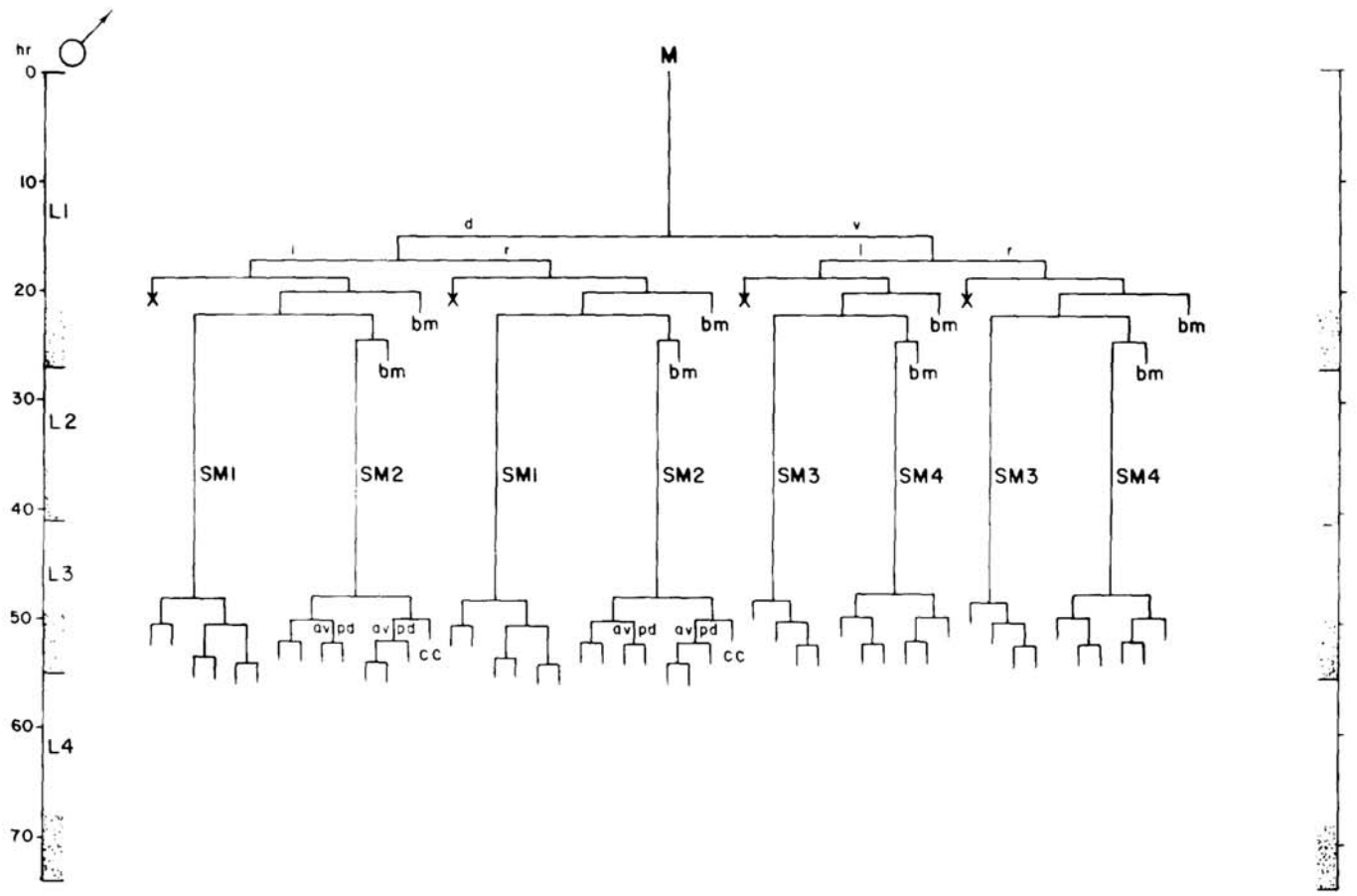


FIG. 3—*Continued.*

generate 28 progeny, and the four ventral sex mesoblasts generate 20 progeny. In *C. elegans* males, the four dorsal sex mesoblasts generate 30 progeny, and the two ventral sex mesoblasts generate 12 progeny. As we have not determined the specific fates of these male specific muscles, a detailed comparison with the fates of the sex mesoblast progeny in *C. elegans* males (Sulston *et al.*, 1980) is not possible. Both SM2.pp cells are coelomocytes in *P. redivivus*. In most *C. elegans* males only SM2.pp on the left side is a coelomocyte (although in one animal studied, both SM2.pp cells became coelomocytes (Sulston *et al.*, 1980)). In *C. elegans* the fates of the sex me-

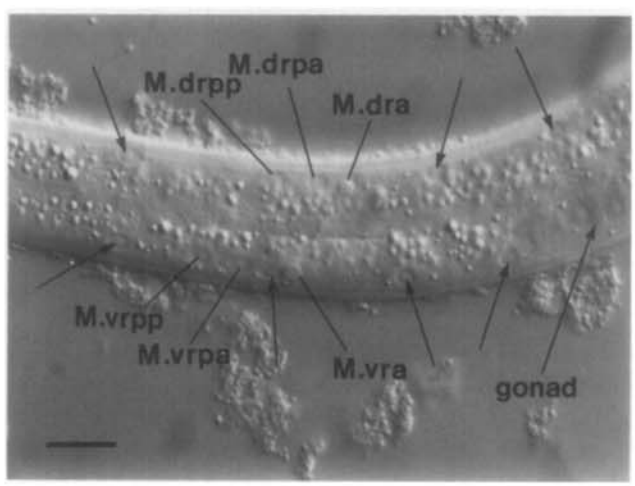


FIG. 4. Nomarski photomicrograph of M.$^{d}_{v}$ra cell deaths and M.$^{d}_{v}$rp progeny in the L1 female (23 hr), right lateral view. The unlabeled arrows point to body muscle nuclei present at hatching. Bar = 10 μm.

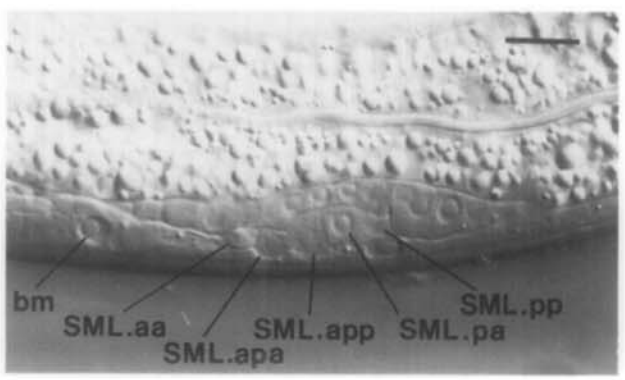


FIG. 5. Development of the female sex mesoderm, Nomarski photomicrograph, left lateral view. SML.aa is dying; SML.ap is undergoing cytokinesis. The SML.pp nucleus is not in the same plane of focus. bm, body muscle. Bar = 10 μm.

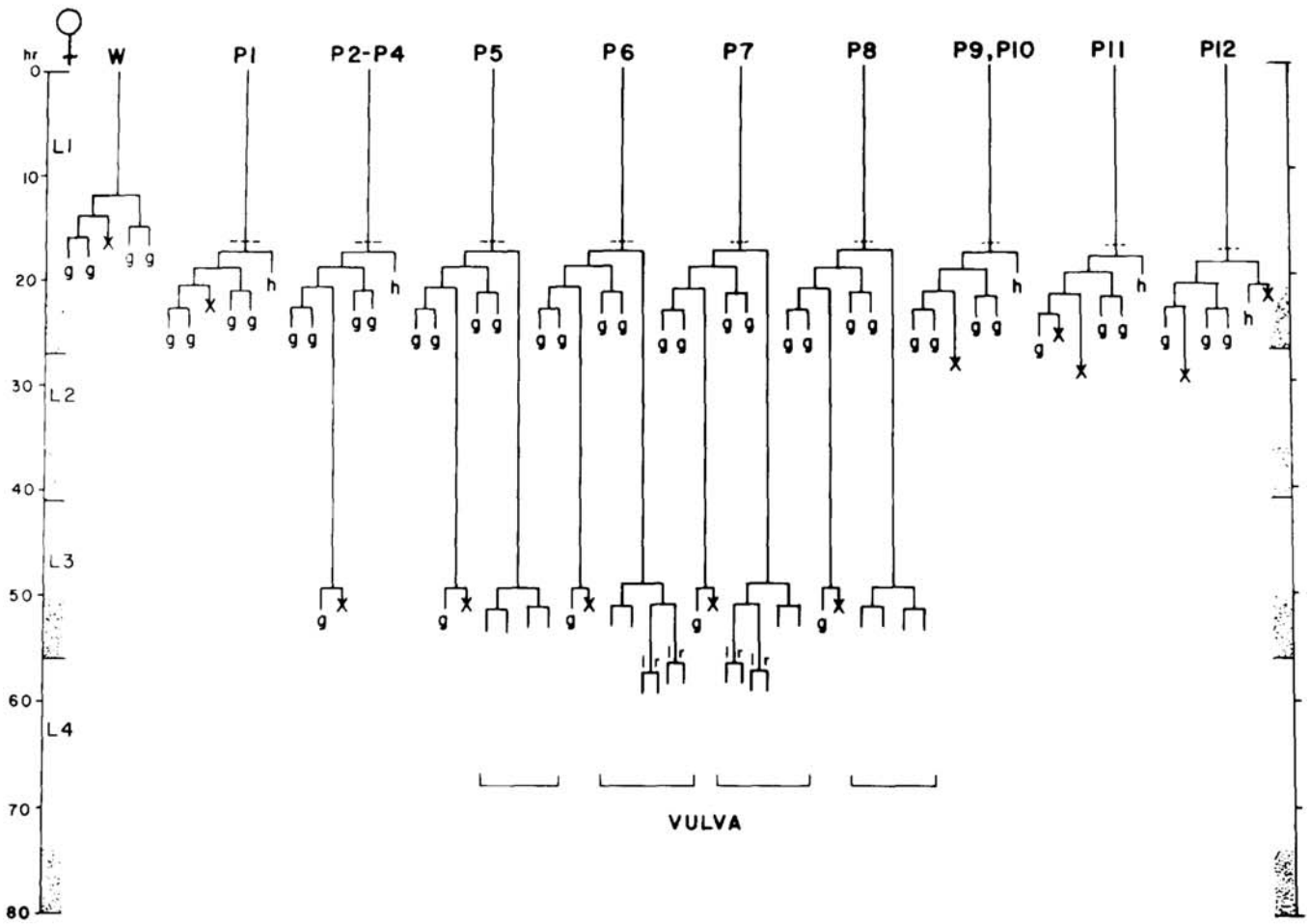


FIG. 6. P and W lineages. g, neuronal or glial cells; h, ventral hypodermal nucleus; ×, cell death. Brackets indicate vulval nuclei. The dashed line crossing the vertical line representing each Pn cell denotes the time of nuclear migration.

soblast progeny differ between the left and right sides, and there is a bilateral asymmetry in the positions of lineally equivalent cells. In *P. redivivus* the progeny of the sex mesoblasts appear to remain bilaterally symmetric; thus it is possible that the fates of lineally equivalent sex mesoblast progeny are bilaterally symmetric in *P. redivivus*.

Ectoderm

P cells (*Figs. 6–11*). In both sexes of both species there are six bilaterally symmetric pairs of ventrolateral nuclei (P1/2L and P1/2R, P3/4L and P3/4R, etc.; Fig. 2) that migrate into the ventral cord during the L1 and intercalate with the 15 juvenile neurons already present in the cord. Because the relative anteroposterior position of the two cells of these pairs varies, these blast cells are renamed after their entry into the cord. The fates of members of many pairs depends on their relative anteroposterior positions. In *P. redivivus*, the only pair for which the probabilities of entry from the two sides appear to be unequal is P11/12L–P11/12R: P11/P12R became P11 in 10 out of 11 animals observed. In the exceptional animal in which P11/12L became P11, T1–T3, and the asymmetric hypodermal nucleus were on the side opposite from normal (this animal was the only 1 out of 22 animals examined that had this arrangement); thus, this individual may have been of at least partially reversed bilateral asymmetry. In *C. elegans*, P11/12L became P11 in all 7 animals observed (Sulston and Horvitz, 1977). Thus, which P11/12 cell becomes P11 and which becomes P12 is usually opposite in the two species.

The P cells divide anteroposteriorly generating an anterior daughter (Pn.a) that is a neuroblast, and a smaller posterior daughter (Pn.p). Of these Pn.p cells, only P12.p divides during the L1; it produces an anterior hypodermal nucleus and a posterior daughter that dies. In *P. redivivus*, all Pn.a cells except P12.a undergo the same characteristic pattern of L1 cell divisions as seen in *C. elegans*, except that P12.aaa does not divide. In *C. elegans*, P12.aaa divides and its posterior daughter dies as does P11.aaap in both sexes of both species. Thus in both species P12.aaa generates a single surviving cell.

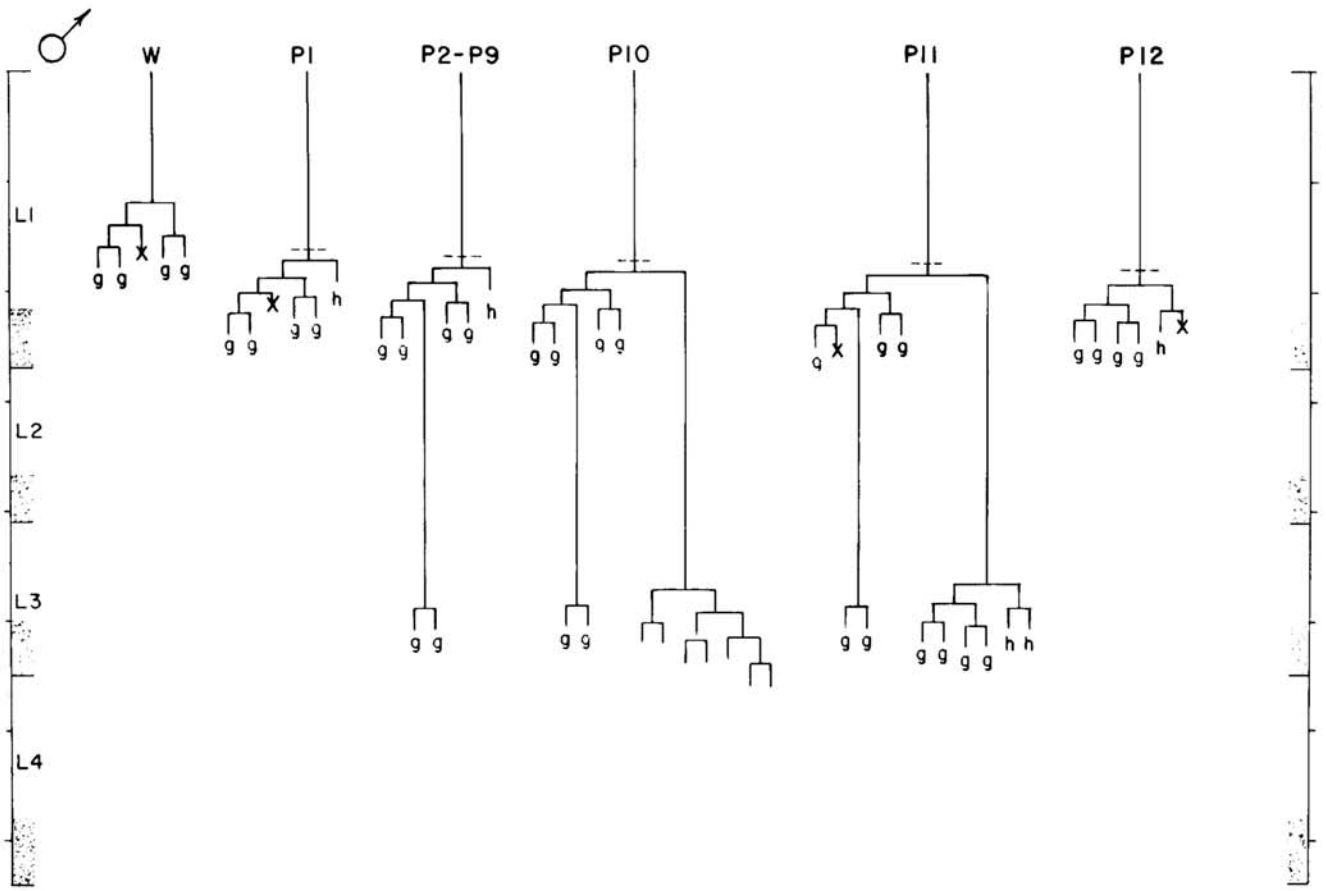


FIG. 6—*Continued.*

This surviving cell could be a VA neuron in *P. redivivus* (as it is in *C. elegans*) (Sulston and Horvitz, 1977); undivided Pn.aaa cells in the posterior ventral cord of the *C. elegans* mutant *unc-59(e1005)* differentiate into VA neurons (White *et al.*, 1982).

The only other differences among the sexes and spe-

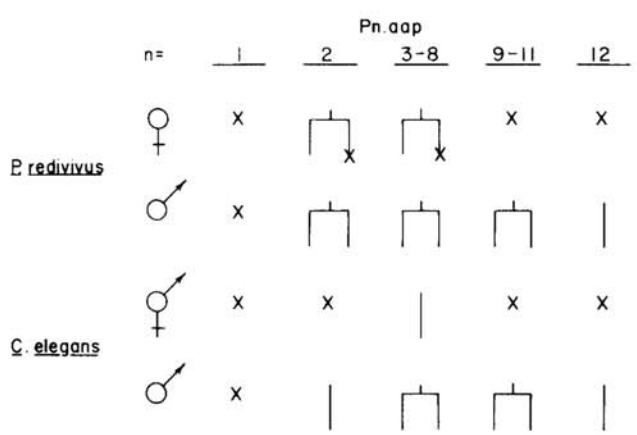


FIG. 7. Comparison of the fates (or sublineages; see Discussion) of Pn.aap cells in both sexes of both species. ×, programmed cell death in the L1; a vertical line represents no death and no division; a branch represents a division in the L3. We consider it likely that the different Pn.aap cell division patterns during the L3 are alternate sublineages because the fates of subsets of Pn.aap cells appear to be coordinately controlled in different sexes and species, and because the divisions of Pn.aap cells occur two larval stages after their generation.

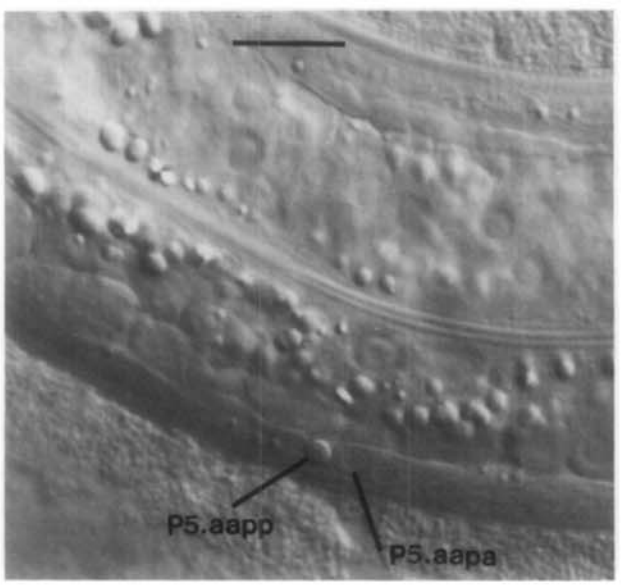


FIG. 8. The death of P5.aapp in the L3 female, right lateral view (anterior at right), Nomarski photomicrograph. Bar = 10 μm.

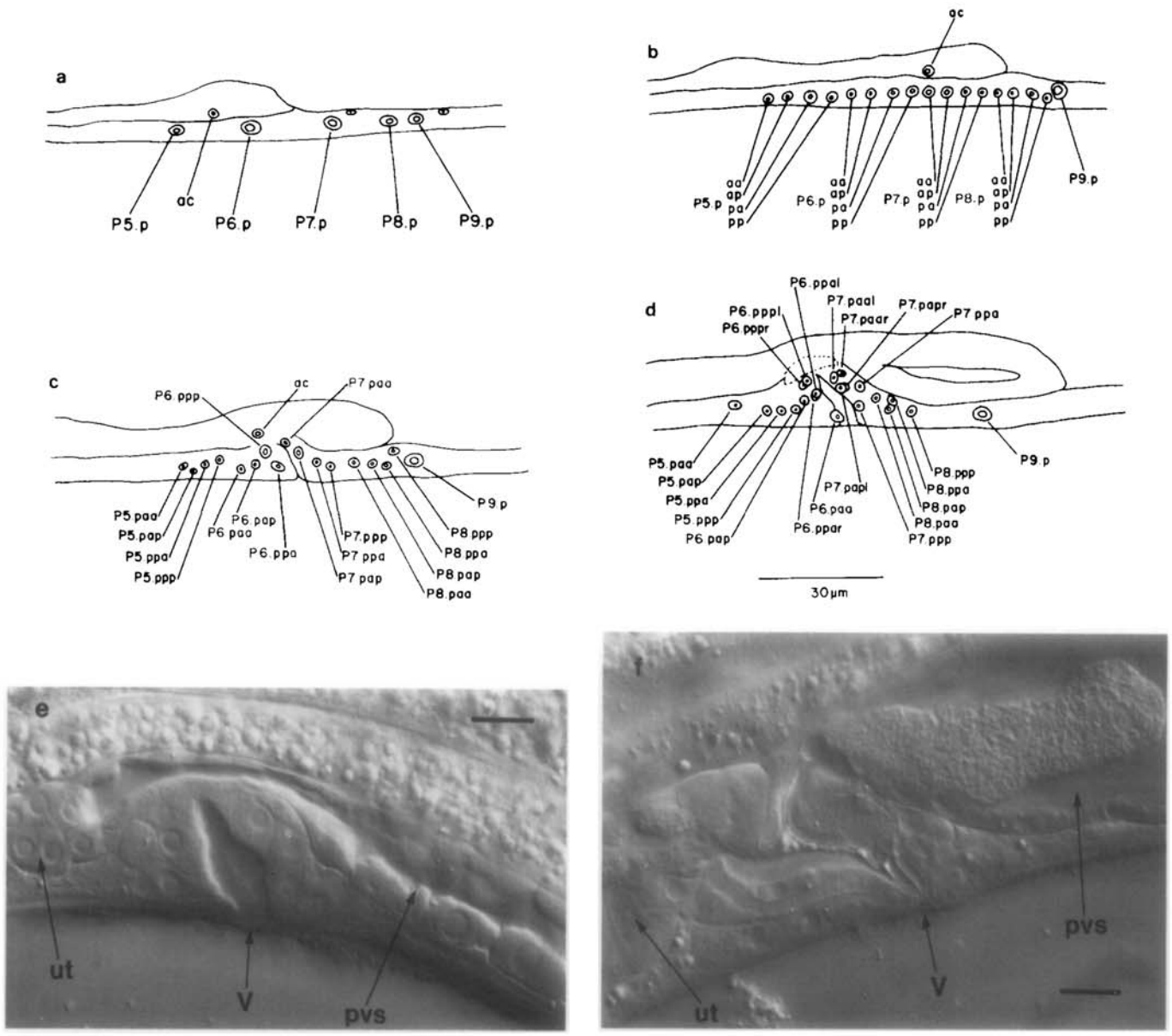


FIG. 9. Development of the vulva, left lateral views, camera lucida drawings (a–d) or Nomarski photomicrographs (e–f). (a) 44 hr, (b) 52 hr, (c) 54 hr, (d) 60 hr, (e) 67 hr. V, vulval invagination. (f) Adult. V, vulval opening. ut, uterus; pvs, postvulval sac. Bar = 10 μm. The Pn.p cells move toward the anchor cell (ac) during the L3; the ac is located approximately between P6.p and P7.p at the time of the first round of divisions. Unlabeled cells in (a) are Pn.aap cells.

cies revealed by observation with Nomarski optics involves the fates of the Pn.aap (Fig. 7) and the Pn.p cells. The fate of P2.aap in the *P. redivivus* female is the same as the fates of P(3–8).aap; the fate of P2.aap in the *P. redivivus* male is the same as the fates of P(3–11).aap. In contrast, in the *C. elegans* hermaphrodite P2.aap dies and P(3–8).aap do not; in the *C. elegans* male P2.aap survives but does not divide and P(3–11).aap divide during the L3. In *P. redivivus* females P(2–8).aap divide during the L3 and the posterior daughter (Pn.aapp) dies (Fig. 8). In *C. elegans* hermaphrodites P(3–8).aap do not divide but are visible as nuclei with prominent nucleoli during the L3.

In *P. redivivus* females, P(1–4, 9–11).p join the hypodermal syncytium, and P(5–8).p divide during the L3 to generate the 20 nuclei of the vulva (Figs. 6 and 9). In *C. elegans* hermaphrodites (Fig. 10), P(1–2, 9–11).p join the syncytium; P(3, 4, 8).p divide once during the L3 to generate two syncytial hypodermal nuclei each, and P(5–7).p divide to generate the 22 nuclei of the vulva. In *P. redivivus* females P5.p and P8.p each divide longitudinally twice. P6.p and P7.p divide twice longi-

tudinally followed by transverse divisions of the four nuclei (P6.ppa, P6.ppp, P7.paa, P7.pap) closest to the anchor cell (ac), a gonadal cell known to be involved in *C. elegans* vulva development (Kimble, 1981a) and which is probably involved in *P. redivivus* vulva development (see below). In both *P. redivivus* females and *C. elegans* hermaphrodites, the first two rounds of vulval divisions are longitudinal and the third round includes transverse divisions (Fig. 10).

In *P. redivivus* males P(1–9).p appear to join the hypodermal syncytium. In *C. elegans* males P(1–8).p and usually P9.p join the syncytium; P9.p sometimes divides to produce two syncytial hypodermal nuclei. In both species P10.p and P11.p divide during the L3 to produce hypodermal nuclei as well as compact nuclei characteristic of neuronal and neuron-associated cells. The patterns of divisions are similar between species except that in *P. redivivus* P10.paa, P10.pap, and P11.ppa do not divide (Fig. 11). In males of both species there is a single ventromedial preanal sensillum embedded in a hypodermal structure: a papilla in *P. redivivus* (see Fig. 14c) and a hook in *C. elegans*. In *C. elegans*, P10.papp forms the hook; in *P. redivivus* no equivalent cell is generated as P10.pap does not divide. P11.pp and P11.pa of *P. redivivus* appear similar in morphology to P11.pp, P11.paa, and P11.pap in *C. elegans*; the two cells in *P. redivivus* are located between the rectum and preanal sensillum as are the three cells in *C. elegans*, as if the two cells in *P. redivivus* are functionally equivalent to the three cells in *C. elegans*. In *C. elegans* these P11.p descendants appear to be necessary for the proper location of the hook and hook sensillum (Sulston and White, 1980). In *P. redivivus* the preanal sensillum (Fig. 14c) is more anterior than that in *C. elegans* raising the possibility that the difference between the P11.p lineages may be responsible for the differences in the positions of the sensilla.

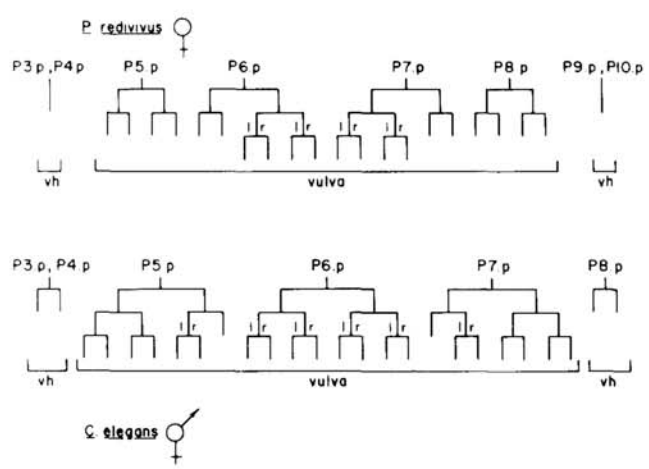


FIG. 10. Comparison of the vulval cell lineages of *P. redivivus* females and *C. elegans* hermaphrodites. The lineages are shown schematically; for correct timing of these divisions see Fig. 6 for *P. redivivus* and Sulston and Horvitz (1977) for *C. elegans*. vh, ventral nucleus that joins the hypodermal syncytium.

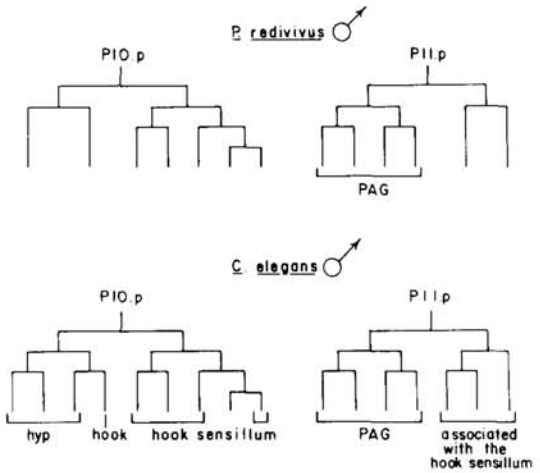


FIG. 11. Comparison of the P10.p and P11.p lineages of *P. redivivus* and *C. elegans* males. PAG, join the preanal ganglion. hyp, hypodermal nuclei. In *C. elegans* P10.ppaa and P10.ppppp are the hook sensillum socket and sheath cells, respectively, and P10.ppppa is a neuron not associated with the hook sensillum. The *C. elegans* lineages are adapted from Sulston and Horvitz (1977) and Sulston *et al.* (1980).

W cell (*Fig. 6*). In both species a neuroblast (formerly called "P0.a" (Sulston and Horvitz, 1977)) undergoes a lineage characteristic of Pn.a cells.

H cell (*Fig. 12a*). Head hypodermal precursors H1 and H2, which are seam cells (Sulston and Horvitz, 1977; Singh and Sulston, 1978), generate syncytial hypodermal nuclei and seam cells. H2 also generates cells with compact nuclei. Both the H1 and H2 lineages of *P. redivivus* differ from those of *C. elegans* by the number of divisions (see Discussion, Fig. 22). Notable features of the *P. redivivus* lineages include: The divisions of H2.aa and H2.aap are both anterior dorsal-posterior ventral suggesting that a posterior parental reiteration (Chalfie *et al.*, 1981) has occurred; the H2.ap, H2.pa, and H2.ppa divisions failed in fewer than one-half the animals examined; there are no H2 divisions after the L3 and no H1 divisions after the L2.

V cells (*Figs. 12–15*). Six seam cells on each side (V1–V6) generate both seam cells and syncytial hypodermal nuclei during rounds of division in each larval period. In addition, V5 generates the four neuronal-like nuclei—including a dopaminergic neuron, as determined by the technique (Sulston *et al.*, 1975) of formaldehyde-induced fluorescence (C. Trent and P. Sternberg, unpublished observations)—and programmed cell death characteristic of postdeirid development during the L2. In males, V6 generates precursors of the caudal papillae, sensory elements in the male tail (Fig. 14). The V

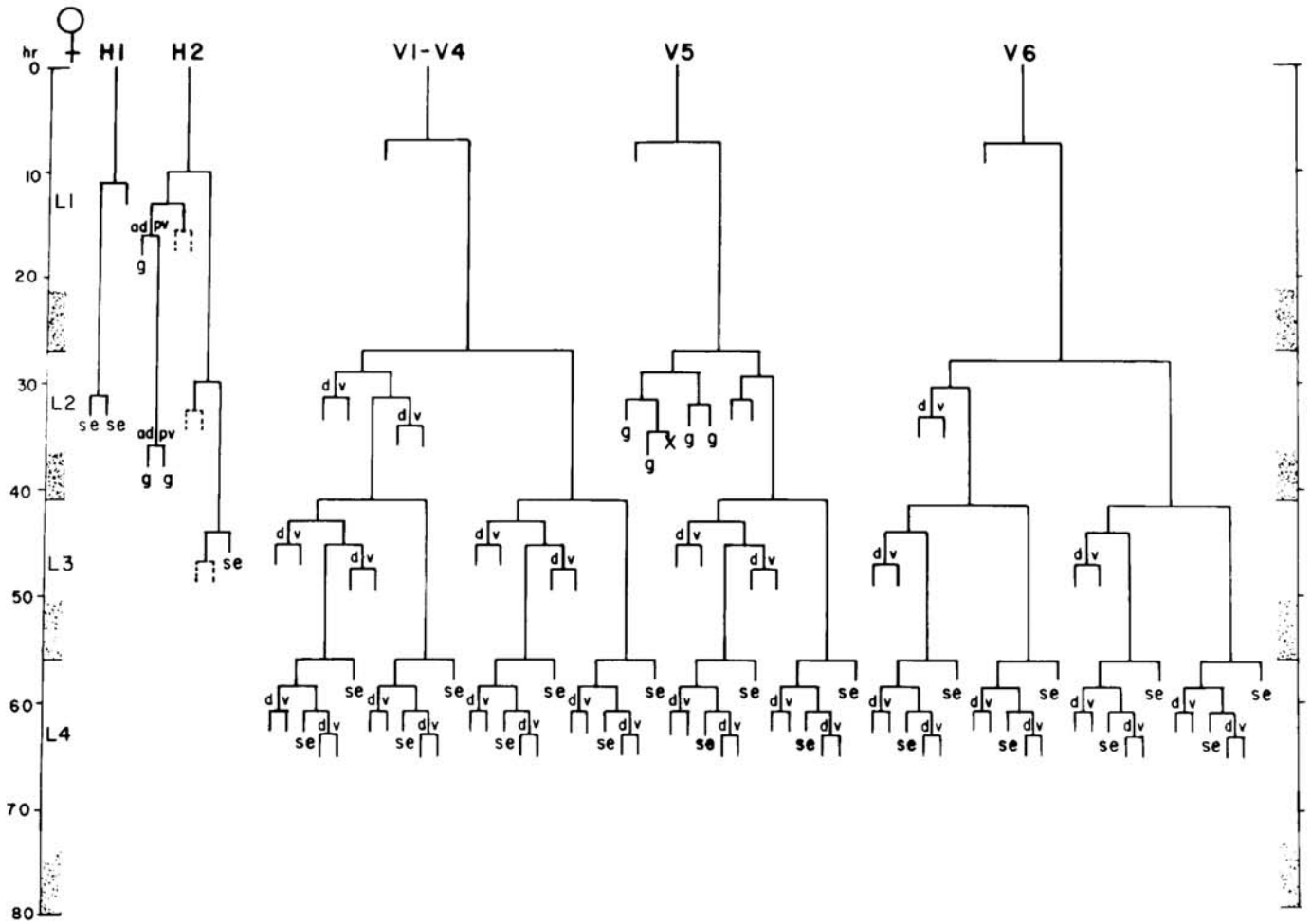


FIG. 12. Lateral hypodermal lineages. (a) H1, H2, V1–V6. (b) V6 and T1.ap and T2.ap in males (the other lineages are identical in the two sexes). g, neuronal or glial nuclei; ×, programmed cell death; se, seam cell; h, hypodermal nucleus. All unlabeled nuclei are syncytial hypodermal nuclei. The precursors to the papillae cell groups are indicated as R1–R7. The papillae cell groups in the male are labeled 1–7. See Fig. 14 for the position of these cells in the L4 and adult. Dashed lines indicate divisions that occasionally fail to occur.

lineages are identical to those of *C. elegans* during the L1 and, as in *C. elegans*, generate syncytial hypodermal nuclei and seam cells, the latter of which are blast cells that divide at all molts but the fourth. However, in later stages the *P. redivivus* V lineages differ from those of *C. elegans*. These differences are discussed in more detail below. In *P. redivivus* males, each V6 generates four caudal papillae, and T1 and T2 (see below) each generate three caudal papillae. In contrast, in *C. elegans* males, each V5 generates one, each V6 generates five, and each T generates three caudal papillae (called "rays" in *C. elegans*). The Rn sublineages, which generate the caudal papillae, are identical in the two species. In contrast to most of the postembryonic cell lineages, the V lineages in *P. redivivus* exhibit considerable variability (Fig. 15). The most commonly observed variant lineage generated an extra asymmetric division of V5.ppp to produce two hypodermal syncytial nuclei (Fig. 15a). Additionally, the plane of the dorsoventral divisions may vary from ad-pv to av-pd.

T1, T2, and T3 cells (*Figs. 12, 16, and 17*). T1 and T2 in *P. redivivus* and the two T cells in *C. elegans* generate hypodermal and compact nuclei. In *P. redivivus*, T1 is on the left side and T2 on the right side, corresponding in position to the two T cells in *C. elegans*. In *P. redivivus* a third blast cell, T3, is posterior to T2 approximately opposite the asymmetric hypodermal nucleus, which is located on the left side, as in *C. elegans*. Although the precise locations of tail nuclei differ between the newly hatched L1 larvae of the two species, the nuclear counts and relative positions of the nuclei suggest that the "asymmetric neuron" (Sulston and Horvitz, 1977) may be the *C. elegans* homologue of T3 in *P. redivivus*. The T1 lineage in *P. redivivus* is similar to the T lineage in *C. elegans*: T1.pa does not divide in *P. redivivus*; T1.apap is a seam cell in the *P. redivivus* female; and T1.appa

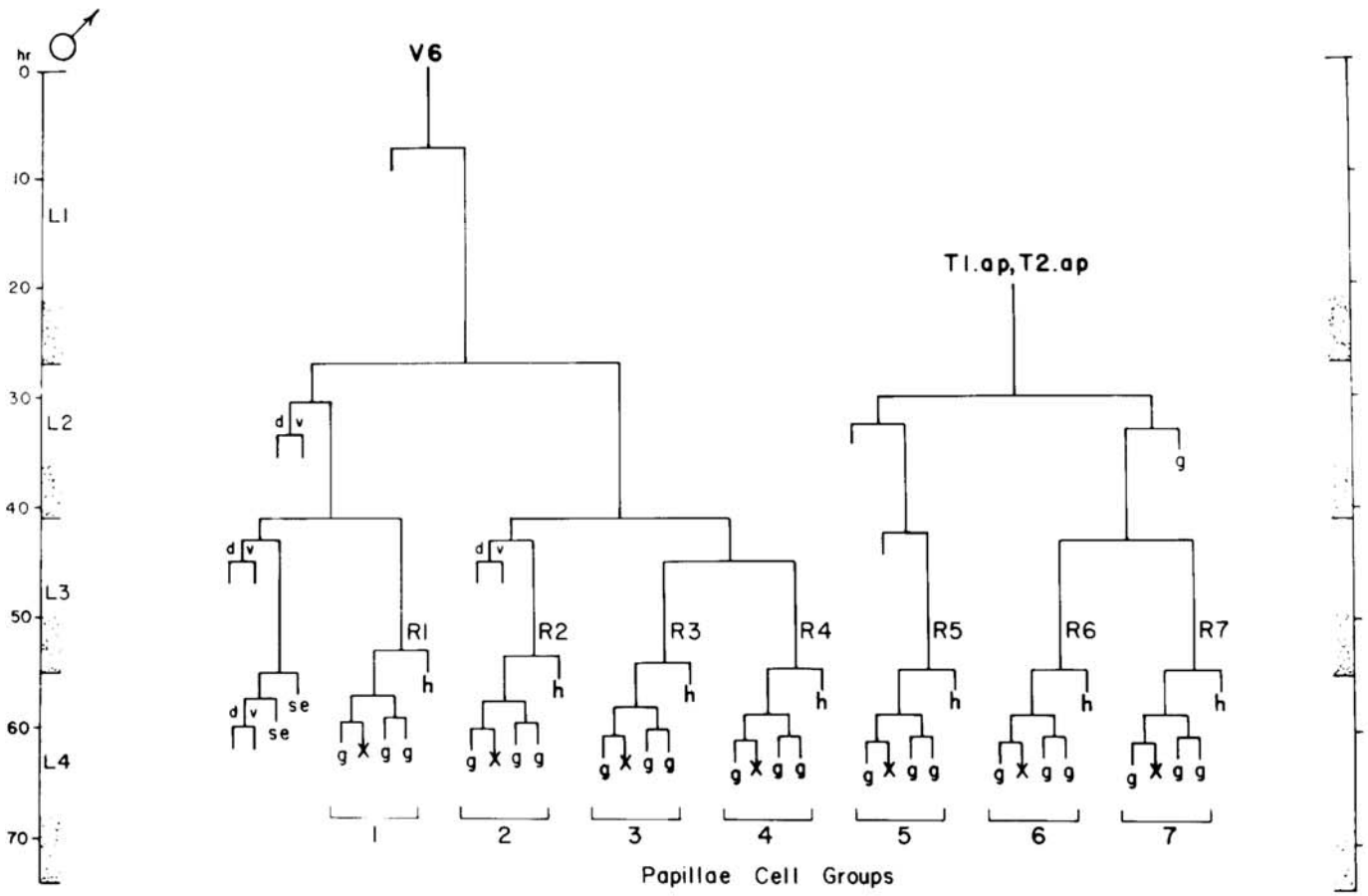


FIG. 12—*Continued.*

is a seam cell at the L2 and L3 molts but not afterward. In *C. elegans*, T.pa generates two socket cells associated with the phasmid, a tail sensory structure (Sulston and Horvitz, 1977); it is possible that in *P. redivivus* T1.pa is a socket cell and serves the same function as T.paa and T.pap together in *C. elegans* (Fig. 17). (There is a single socket cell in most *C. elegans* sensilla (Ward *et al.*, 1975; Sulston and Horvitz, 1977; Sulston *et al.*, 1980)).

T1.a and T2.a in *P. redivivus* and T.a in *C. elegans* behave identically. However, T2.p divides symmetrically to produce two compact nuclei, while T1.p divides asymmetrically and its larger daughter T1.pp undergoes further divisions. Thus, there is a bilateral asymmetry in the T1/T2 lineages in *P. redivivus*. In *P. redivivus* the additional tail blast cell T3 generates three compact nuclei and a programmed cell death in a pattern of asymmetric and symmetric divisions identical to that generated by T1.pp. The *P. redivivus* T3 and T1.pp lineages are identical to the *C. elegans* T.pp lineages (Fig. 17), suggesting that the six cells produced by T1 may be similar to at least some of the seven cells produced by T2 and T3. However, T2 and T3 on the right side generate together one extra compact nucleus compared to T1 on the left side. T1.pp, T1.ppp, T1.pppa, T3.p, and T3.pa are all larger than their sisters; the other divisions yield daughters approximately equal in size.

In one female (mentioned above), T3 was on the left side with T2; T1 and the asymmetric hypodermal nucleus were on the right side. This reversal might reflect either a variability of the fates of these cells or a reversal in the normal bilateral asymmetry in at least the tail.

Q cells (*Fig. 18*). As in *C. elegans*, the lateral ectoblasts Q1 and Q2 divide during the early L1 and generate progeny that appear similar and migrate equivalently in the two species.

K cells (*Fig. 18*). K (on the left side) and K′ (on the right side) are small nuclei present dorsal and posterior to the rectum at hatching. In *P. redivivus* they both divide. In *C. elegans* only K divides. In *P. redivivus*, K.a, K.p, and K′.a remain near the rectum as do K.a and K′ in *C. elegans*. K′.p in *P. redivivus*, like K.p in *C. elegans*, has a compact nucleus and migrates posteriorly to the dorsorectal ganglion. Thus, K′ in *P. redivivus* behaves like K in *C. elegans*; and K in *P. redivivus* generates two

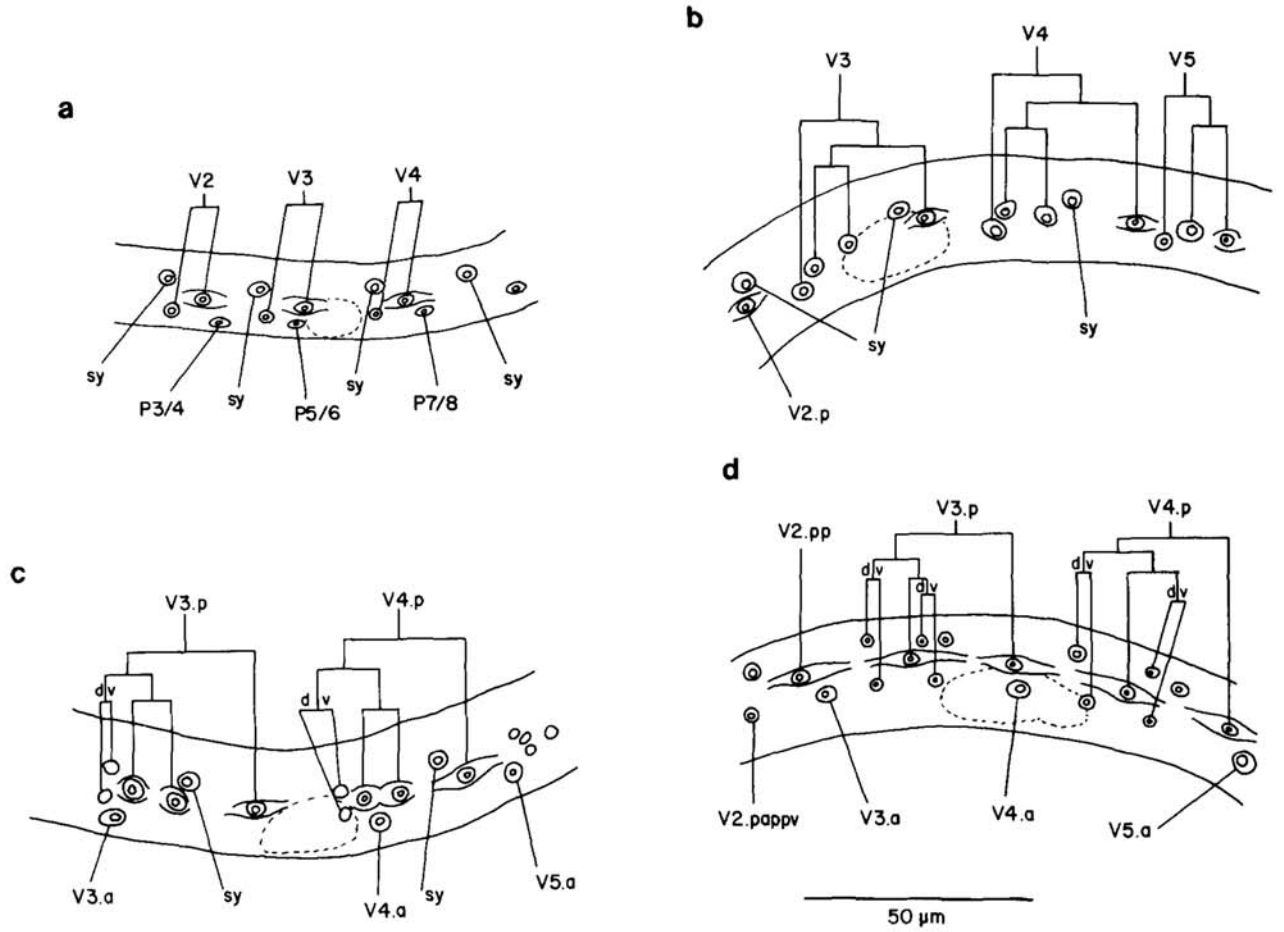


FIG. 13. Development of the lateral hypodermis in the L1 and L2, left lateral views, camera lucida drawings of four separate animals. (a) L1 lethargus, (b) 30 hr, (c) 32 hr, (d) 35 hr. Dashed lines show outline of developing gonad. The outline of the seam cells is indicated. sy, syncytial hypodermal nucleus present at hatching.

cells that appear similar to the undivided K′ cell in *C. elegans*, which suggests that K in one species is like K′ in the other.

G cells (*Fig. 18*). The ectoblasts G1 and G2 divide during the L1 and L2, respectively, to generate apparently similar progeny in both species.

Male-Specific Ectoblasts

B cell (*Figs. 19 and 20*). In males of both species, B generates the same number of ectodermal progeny and programmed cell deaths at equivalent times during development. In both species there is natural variability in the fates of two bilaterally symmetric pairs of cells (B.alaa and B.araa; B.alpp and B.arpp). In addition, only one member of each of the pairs B.alapaaa or B.arapaaa and Bγ.alp or Bγ.arp die, as in *C. elegans*. The planes of divisions in the B lineages differ between the two species, which most likely reflects the different sizes and geometries of the developing proctodea. By light microscopic criteria (nuclear morphology and position) the fates of the B progeny are similar and possibly identical in the two species.

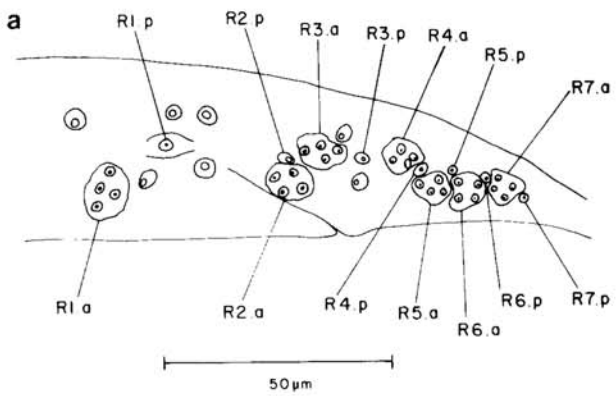
Y cell (*Fig. 19*). Y (formerly called "C") generates the same number and types of nuclei with similar timing in both species. The planes of divisions differ slightly between the species.

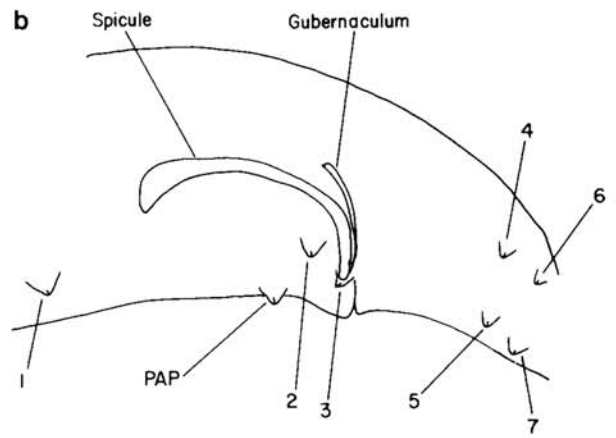
U cell (*Fig. 19*). The U (formerly called "E") lineage is identical in *P. redivivus* and *C. elegans* males, except that U.ra divided in all six *P. redivivus* animals observed. U.la divided in two out of the five animals observed. U.ra may always divide; alternatively, it may rarely fail to divide, as in *C. elegans* (Sulston and Horvitz, 1977; Sulston *et al.*, 1980).

F cell (*Fig. 19*). The F lineage is similar in the two species except that in *C. elegans* F.lvd and F.rvd divide. The planes of the second and third rounds differ by the presence of longitudinal components in *P. redivivus*.

Intestine

In cells (*Fig. 18*). There are 18 nuclei present at hatching in the *P. redivivus* intestine as opposed to 20 nuclei





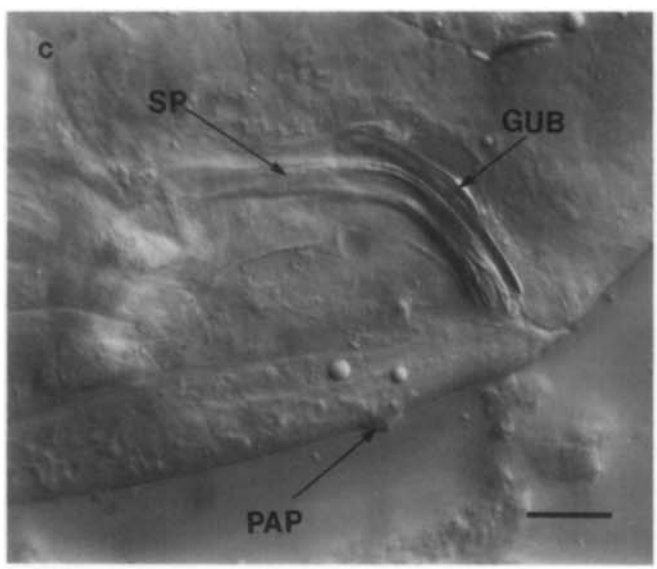


FIG. 14. Development of the male tail, left lateral views. (a) Papillae cell groups in the early L4 male tail, camera lucida drawing. See Fig. 12b for ancestry of Rn cells. The positions of the four nuclei in each papillae cell group are variable at this stage; thus, only the position of the group is shown. Unlabeled nuclei are syncytial hypodermal nuclei. (b) Positions of papillae (1-7) on the left side, spicules, gubernaculum, anus, and preanal ventromedial papillae (PAP) in the adult male. The basis for the assignment is the relative positions of the cell groups. (c) Adult male tail, Nomarski photomicrograph. Bar = 10 μm.

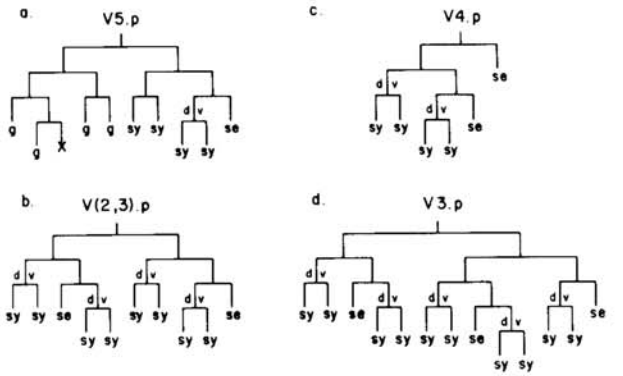


FIG. 15. Variability in the lateral hypodermal lineages. The examples are taken from L2 divisions of Vn.p cells. The lineage diagrams shown here do not accurately depict the timing of cell divisions. g, neuron or glial nucleus; ×, programmed cell death; sy, syncytial hypodermal nucleus; se, seam cell. The following descriptions are interpretations of the rare lineages as modifications by "transformations" (see Discussion) of the typical lineage. (a) An extra asymmetric division of V5.ppp. This pattern has been seen in both sexes. (b) Two asymmetric divisions of Vn.pp seen in both the V2.pp and V3.pp lineages in each of two males. (c) Polarity reversal of the V4.pap sublineage seen in one female. (d) An asymmetric division of V3.pp generating a V3.pa-like sublineage and of V3.pp generating a dorsoventral division seen in one male. Apparently similar patterns have been seen in V2, V3, and V4 in females but lineages were not fully determined. Many other variable lineages were noted but not followed to completion.

in that of *C. elegans*. The anterior two nuclei (one dorsal, one ventral) in *P. redivivus* and the anterior six nuclei in *C. elegans* (three dorsal and three ventral, including the anterior-most "ring of four") do not divide. The lumenal surface of this segment of the intestine appears thinner as observed with Nomarski optics in both species. In *C. elegans*, the microvilli are shorter in this region (Sulston and Horvitz, 1977). The posterior 16 nuclei (8 dorsal, 8 ventral) undergo two rounds of mitosis in *P. redivivus* generating tetranucleate cells; the posterior 14 nuclei (7 dorsal, 7 ventral) undergo one round in *C. elegans* generating binucleate cells. In *P. redivivus*, the second-round divisions of the 2 anterior-most or 4 posterior-most of the 16 nuclei that normally divide may fail; in *C. elegans* the divisions of the 4 posterior-most nuclei may fail. In *P. redivivus*, the two rounds of In divisions generally begin in the center of the animal and proceed anteriorly and posteriorly.

CELL-CELL INTERACTIONS IN VENTRAL HYPODERMAL DEVELOPMENT

In *C. elegans*, the developmental fates of ventral hypodermal (Pn.p) cells are determined by cell-cell interactions. Specifically, cell ablation experiments utilizing a laser microbeam have revealed that the anchor cell

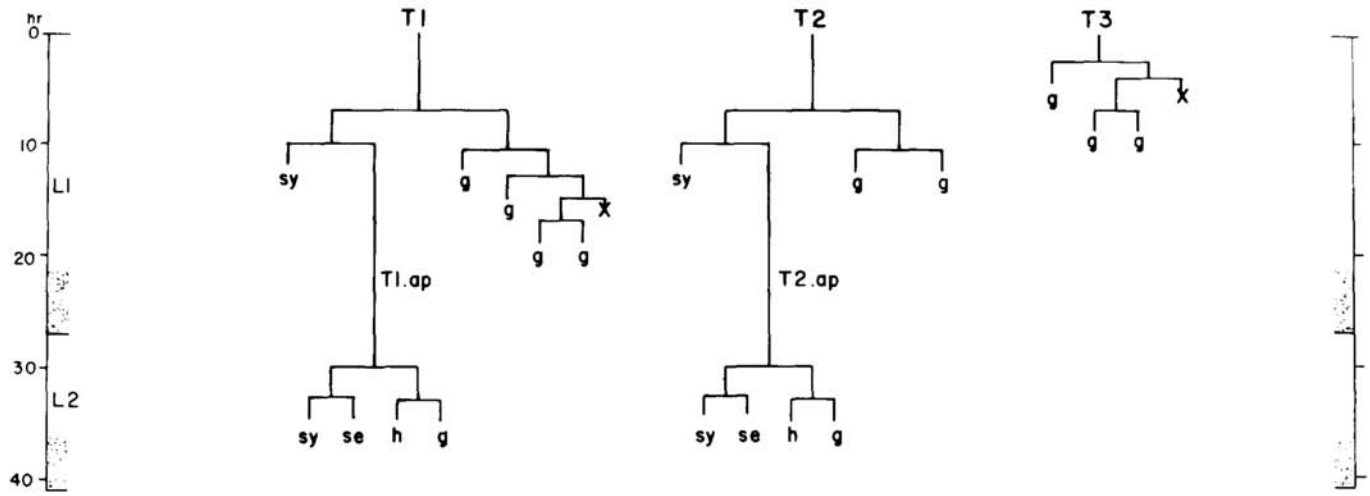


FIG. 16. T1, T2, and T3 lineages. The T1.ap and T2.ap lineages (L2 divisions) are female. The male T1.ap and T2.ap lineages are shown in Fig. 12b. sy, syncytial hypodermal nucleus; se, seam cell; h, hypodermal nucleus; g, neuronal or glial nucleus; ×, programmed cell death. T1.appa and T2.appa are seam cells at the L2 and L3 molts, but later appear to be another type of hypodermal cell.

of the hermaphrodite gonad induces vulva development (Kimble, 1981a) and that, in each sex, certain ventral hypodermal cells influence the fates of other ventral hypodermal cells (Sulston and White, 1980). To test whether Pn.p cell fates in *P. redivivus* depend upon cell–cell interactions, we performed a series of gonadal, Pn, and Pn.p cell ablation experiments.

Female

In *P. redivivus* females, as in *C. elegans* hermaphrodites (Sulston and White, 1980; Kimble, 1981a), Pn.p cell fates are influenced by the gonad (Table 1). After laser ablation of the gonad primordium in *P. redivivus* females, those Pn.p cells that normally undergo vulval divisions instead joined the hypodermal syncytium and no vulva was formed. To determine whether the anchor cell (ac) is responsible for vulva development in *P. redivivus*, we ablated Z4.aaa, which is the ac in *P. redivivus* (Sternberg and Horvitz, 1981). In three of four such experiments no vulva was formed, indicating that in *P. redivivus*, as in *C. elegans*, the ac is involved in vulva development. However, unlike *C. elegans* hermaphrodites, *P. redivivus* females were capable of some vulval cell divisions after ac ablation. Although by light microscopic criteria the ac nucleus appeared to be destroyed in these experiments, it is possible that some ac activity remained (see Sulston and White (1980) for a discussion of caveats concerning laser ablation experiments); if not, vulva development in *P. redivivus* may differ from that of *C. elegans*, i.e., either vulva induction begins prior to the time of Z4.aaa formation (e.g., a precursor to Z4.aaa might have some inductive activity) or else other cells are involved in the inductive process at the same time as Z4.aaa. To explore these alternatives, we ablated either Z4 or Z1 in a number of *P. redivivus* females. More than half of the Z4-ablated females failed in all vulval divisions, just as had gonad-ablated animals; no Z4-ablated and all Z1-ablated animals were normal in vulva development. Thus, the induction of complete vulval cell divisions requires Z4 and/or one or more of its descendants. Since, some Pn.p cells divided in some of the Z4-ablated animals, it is possible that Z1 and/or one or more of its descendants can have a weak inductive effect on the hypodermis.

In *C. elegans* hermaphrodites, the ablation of a vulval precursor cell (P5.p, P6.p, or P7.p) is followed by its

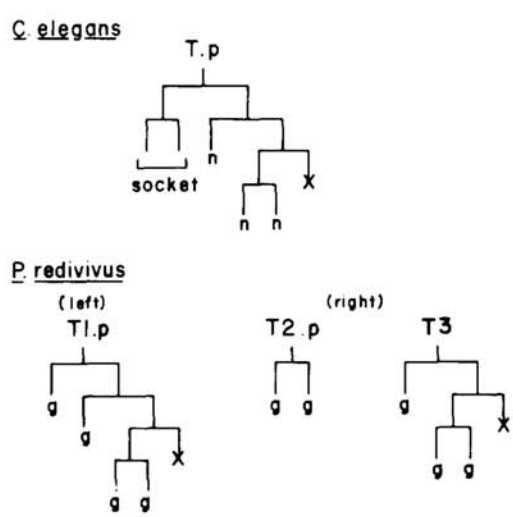


FIG. 17. Comparison of the T1, T2, and T3 lineages of *P. redivivus* with the T lineages of *C. elegans* (Sulston and Horvitz, 1977; Sulston et al., 1980). ×, programmed cell death; n, neuron; g, neuron or glial nucleus.

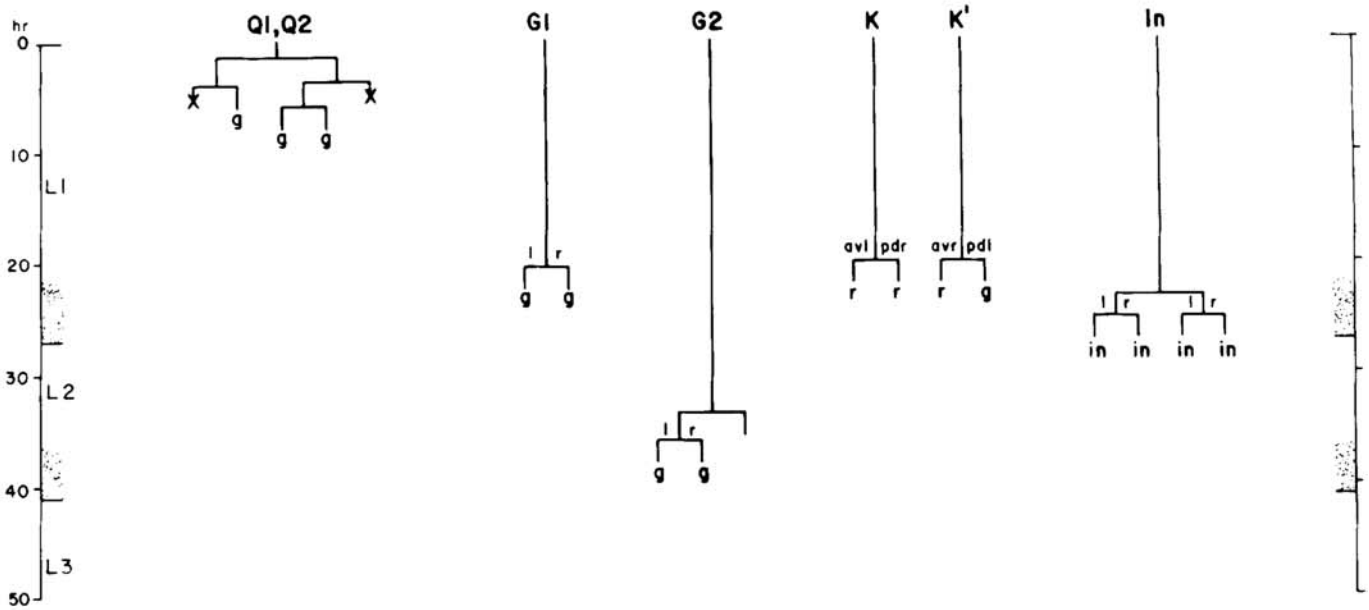


FIG. 18. Q, G, K, and In lineages. Q1 progeny migrate anteriorly and Q2 progeny migrate posteriorly as in *C. elegans*. Some of the second round In divisions may fail. ×, programmed cell death; g, neuronal or glial nuclei; r, rectal cells; in, intestinal nuclei.

replacement by either another vulval precursor or by another Pn.p cell (specifically, P3.p, P4.p, or P8.p) (Sulston and White, 1980). Thus, certain Pn.p cells influence the fates of other Pn.p cells. The effect of eliminating a vulval precursor cell (P5.p, P6.p, P7.p, or P8.p) in *P. redivivus* is similar: either another vulval precursor cell or another Pn.p cell (P4.p or P9.p) can take its place (Table 2). In these experiments, most Pn.p cells that were altered in their behavior as a result of laser ablation expressed fates normally associated with other Pn.p cells, i.e., such cells either joined the hypodermal syncytium (as do P(1–4, 9–11).p normally), or generated four descendants (as do P5.p and P8.p normally), or six descendants (as do P6.p and P7.p normally).

In *C. elegans*, there is a hierarchy of Pn.p cell fates (Sulston and White, 1980): the P6.p fate is primary, as P(4, 5, 7, or 8).p can replace P6.p, but P6.p will not replace P(4, 5, 7, or 8).p; the P5.p and P7.p fates are secondary, as P4.p will replace P5.p, and P8.p will replace P7.p but P5.p will not replace P4.p and P7.p will not replace P8.p; and the P4.p and P8.p fates are tertiary, as these cells will replace but not be replaced (Fig. 21). In *P. redivivus*, the fates of P6.p and P7.p are primary: P5.p can replace P6.p (experiments 4, 5) and P8.p can replace P7.p (experiment 6) but the converse replacements have not been observed (e.g., experiments 3 and 7); also single P5/6.p cells generally behaved like P6.p (experiments 12–14, 16–19), and single P7/8.p cells behaved like P7.p (experiments 15–19). P7.p can replace P6.p after ablation of P(5–6).p (experiments 8, 9); however, in the one experiment performed (experiment 11), P6.p did not replace P7.p after ablation of P(7, 8).p. Although the P6.p fate might be primary to the P7.p fate, it seems likely that P6.p and P7.p are at equivalent levels in the hierarchy and may be able to replace each other.

As discussed above, any of the cells P(3–8).p can participate in vulva development in *C. elegans* hermaphrodites. This finding led to the suggestion that P(3–8).p constitute an "equivalence group" for vulva formation in *C. elegans*, as all six cells seem to be equivalent in developmental potential (Kimble *et al.*, 1979; Sulston and White, 1980; Kimble, 1981b). That P(3–8).p share certain characteristics is supported by the observation that *C. elegans* hermaphrodites carrying certain "multivulva" mutations are affected specifically in P(3–8).p (Horvitz and Sulston, 1980; Sulston and Horvitz, 1981). In *P. redivivus* P9.p as well as P(4–8).p can participate in vulva development, indicating that the vulval equivalence groups differ with respect to at least P9.p.

In *P. redivivus* P3/4L and P3/4R can become either P3 or P4 and P9/10L and P9/10R can become either P9 or P10; thus, members of the pairs P3/4L–P3/4R and P9/10L–P9/10R are probably equivalent in developmental potential. The possible equivalence of P3/4L and P3/4R and of P9/10L and P9/10R suggests that prior to the entry of P cells into the ventral cord, at least eight cells (P3/4L, P3/4R, P5/6L, P5/6R, P7/8L, P7/8R, P9/10L, and P9/10R) are equivalent in potential; after P cell divisions, based upon laser ablation experiments, at least six cells, P(4–9).p, are equivalent in that they all can participate in vulva formation. It remains pos-

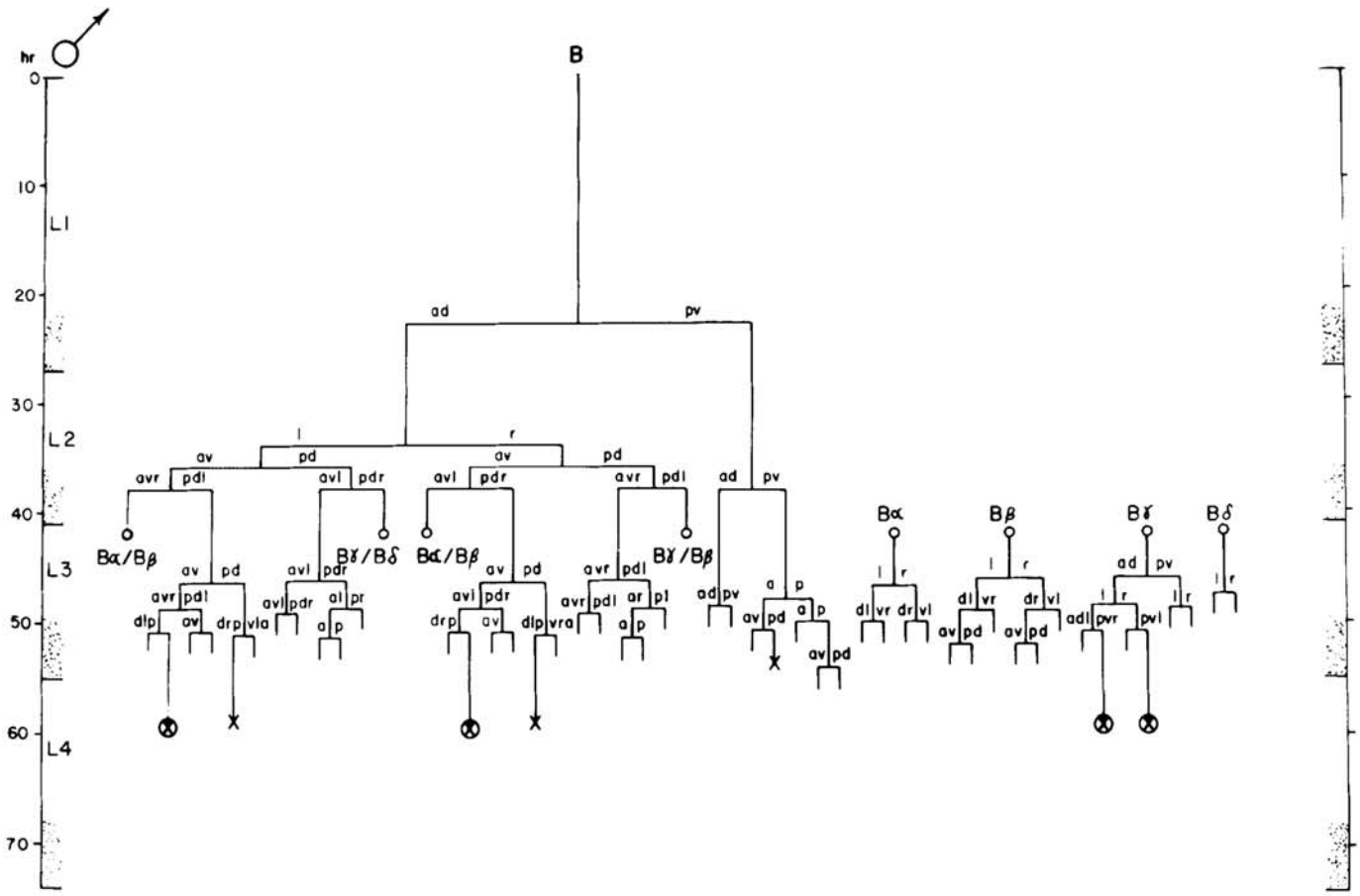


FIG. 19. Male B, Y, U, and F lineages. ×, programmed cell death; circled ×, only one cell of a bilaterally symmetric pair dies in each individual. Circles represent members of bilaterally symmetric pairs of cells with fates that depend on position after both members of each pair move medially. Members of one pair (B.alaa and B.araa) adopt either the Bα or the Bβ fate; members of the other pair (B.alpp and B. arpp) adopt either the Bγ or Bδ fate. The dashed line indicates that the U.la division may fail. The convention of representing oblique divisions by the ranking division plane (anterior over dorsal over left) has been broken in three cases ($B.a^{l}_{r}apaa$, $F^{l}_{\cdot r}$, and $F^{l}_{\cdot r}v$), in which the anterior–posterior component of the division is relatively minor and the convention obscures the similarity of the *P. redivivus* and *C. elegans* lineages.

sible that further ablation experiments may demonstrate that other Pn.p cells (e.g., P3.p and P10.p) can participate in the development of the vulva.

To demonstrate rigorously that cells are equivalent in potential, one must show that each cell can behave like each other cell, i.e., that all cells have the same set of potential fates (Kimble, 1981b). Most, but not all, such equivalences have been demonstrated by ablation experiments in both *P. redivivus* and *C. elegans* (Fig. 21). That cells can express "higher" (more primary) fates in the hierarchy of the vulval equivalence group is demonstrated by Pn and Pn.p ablation experiments (Table 2). That cells can also express "lower" fates, is demonstrated by gonadal cell ablation experiments (e.g., Table 1).

In *P. redivivus*, a functional vulva, as defined by the ability of the female to mate with males and to release larvae, can be formed by as few as 10 hypodermal cells: e.g., the animals described in Table 2, experiments 9, 18, and 19, had functional vulvae. In *C. elegans*, the eight cells generated by P6.p are capable of making a functional vulva (Sulston and White, 1980; Sulston and Horvitz, 1981). In both species, at least one Pn.p with the primary fate (P6.p in *C. elegans* and P6.p or P7.p in *P. redivivus*) may be necessary for the generation of a functional vulva.

Male

In *P. redivivus* males, as in *C. elegans* males, the gonad is not necessary for Pn.p divisions; in all five males in which the gonad primordium was ablated, the Pn.p divisions were normal (Table 3). In *C. elegans* males, P(9–11).p form an equivalence group (Sulston and White, 1980). In *P. redivivus* males, P10.p can replace P11.p and P9.p can replace P10.p, indicating that the

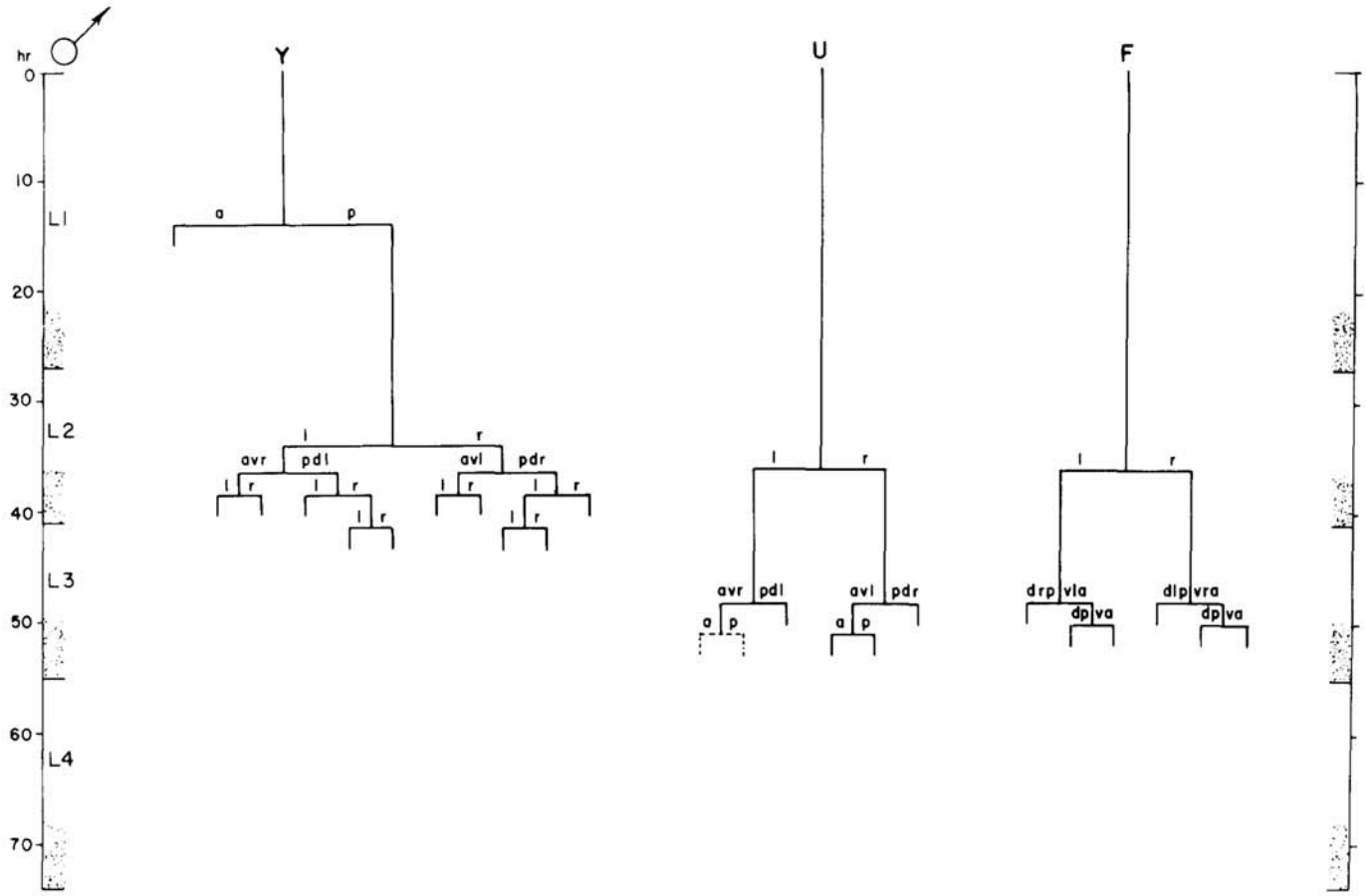


FIG. 19—*Continued.*

equivalence groups for preanal sensilla formation may be the same in the two species. In *P. redivivus* P9.p can participate in preanal papilla formation in the male and in vulva development in the female; in contrast, in *C. elegans* the hermaphrodite and male Pn.p equivalence groups are distinct.

In *P. redivivus* the production of P10.p descendants is necessary for preanal ventromedial papilla (Fig. 14c) formation: the absence of the P10.p lineage led to the absence of a preanal papilla, even when the P9.p and P11.p lineages appeared normal (Table 3). The preanal papilla does not appear to be necessary for mating. Five males lacking papillae after laser ablation were tested for mating and produced progeny.

DISCUSSION

SUMMARY

The newly hatched L1 larvae of *P. redivivus* and *C. elegans* are very similar in morphology as observed with Nomarski optics. In the two species, almost identical sets of precursor cells divide postembryonically to produce cells of the hypodermis, nervous system, intestine, and musculature. In both species, four cells divide only in males to generate the proctodeum and male copulatory apparatus. For each postembryonic cell lineage,

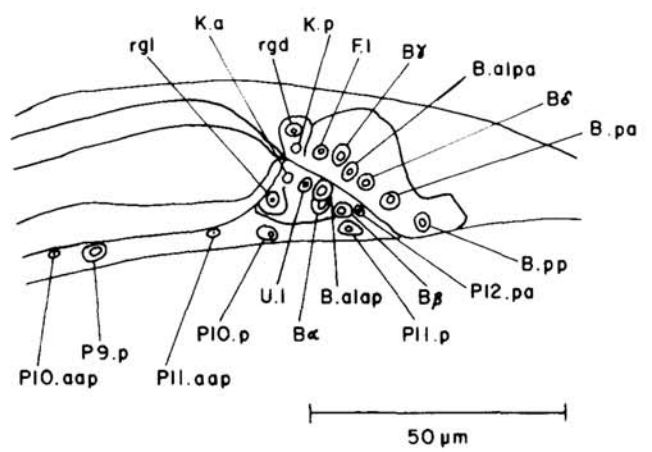


FIG. 20. Spicule primordium in the L3 male tail, left lateral view, camera lucida. Positions of the 14 blast cells are shown, as well as those of relevant P and K descendants. rgl, left rectal gland; rgd, dorsal rectal gland. This configuration of nuclei is highly reproducible and provides a convenient point at which to start following the late (L3) B divisions.

TABLE 1
EFFECT OF GONADAL CELL ABLATION ON VULVA DEVELOPMENT

Approx age (hr)	Ablated	No. of animals	No. of descendants from P4.p	P5.p	P6.p	P7.p	P8.p	P9.p	Vulva[a]
—	None	29	1	4	6	6	4	1	+
9-25	Gonad	8	1	1	1	1	1	1	−
8-26	Z1[b,c]	4	1	4	6	6	4	1	+
10-25	Z4[b]	5	1	1	1	1	1	1	−
10-13	Z4[b,c]	2	1	4	4	4	1	1	−
10	Z4[b]	1	1	4	4	1	1	1	−
23	Z4[b]	1	1	4	1	4	1	1	−
34-39	Z4.aaa[d]	3	1	4	4	4	4	1	o
36	Z4.aaa[d]	1	1	4	6	6	4	1	+

[a] Degree of vulva formation: +, apparently normal vulva; −, no vulva; o, pseudovulva, i.e., an invagination that undergoes morphogenesis but does not connect to the uterus.

[b] Gonadal development in these worms was described in Sternberg and Horvitz (1981).

[c] In these animals lineages were not followed; the number of descendants were determined by the positions of nuclei during the L3 lethargus and early L4.

[d] No anchor cell was present; all other somatic gonadal cells (except Z1.aa) divided during the L3. In the normal gonad, Z4.aaa is the anchor cell and Z1.aa is the distal tip cell (Sternberg and Horvitz, 1981).

TABLE 2
REGULATION IN THE VENTRAL HYPODERMIS OF THE FEMALE

Expt	Approx age (hr)	Ablated	No. of descendants from[a] P4.p	P5.p	P6.p	P7.p	P8.p	P9.p
	—	None	1	2 + 2	2 + 4	4 + 2	2 + 2	1
1	19	P(9-11)	1	2 + 2	2 + 4	4 + 2	2 + 2	1
2	20	P4.p	—	2 + 2	2 + 4	4 + 2	2 + 2	1
3	17	P5	1	—	2 + 4	4 + 2	2 + 2	1
4	22	P6.p	1	2 + 4	—	4 + 2	2 + 2	1
5	33	P6.p	1	2 + 4	—	4 + 2	2 + 2	1
6	20	P7.p	1	2 + 2	2 + 4	—	4 + 2	1
7	18	P8.p	1	2 + 2	2 + 4	4 + 2	—	1
8	18	P5.p, P6.p	1	—	—	2 + 4	2 + 2	1
9	18	P5, P6.p	1	—	—	2 + 4	2 + 2	1
10	23	P6.p, P7.p	1	2 + 4	—	—	4 + 2	2 + 2
11	19	P7.p, P8.p	1	2 + 2	2 + 4	—	—	4 + 2
12	14	P5/6R	1	(2 + 4)[b]		4 + 2	2 + 2	1
13	15	P5/6R	2 + 2	(2 + 4)		4 + 2	2 + 2	1
14	13	P5/6L	1 + 1[c]	(2 + 4)		4 + 2	2 + 2	1
15	14	P7/8L	1	2 + 2	2 + 4	(4 + 2)		1
16	8	P5/6L, P7/8L	1	(2 + 4)		(4 + 2)		2 + 2
17	16	P5/6L, P7/8L	1	(2 + 4)		(4 + 2)		2 + 2
18	17	P5/6R, P7/8R	1	(2 + 4)		(4 + 2)		2 + 2
19	13	P5/6R, P7/8R	1	(2 + 4)		(4 + 2)		1
20	16	P5/6R, P7/8R, P9/10R	1	(2 + 4)		(4 + 4)		(2 + 2)
21	16	P(5-8)	1	—	—	—	—	1

[a] To indicate the polarity of a lineage, the number of descendants is split into the contributions of Pn.pa and Pn.pp, e.g., "2 + 4" indicates that Pn.pa divided once and that Pn.pp divided twice (see Fig. 10 for lineages). In all cases, the second round divisions of Pn.pa or Pn.pp were transverse (left-right).

[b] Parentheses indicate the fates of either P5/6.p, P7/8.p, or P9/10.p.

[c] Both of these cells joined the hypodermal syncytium.

the timing of specific cell divisions relative to the four larval molts is similar in the two species. Most of these lineages are invariant. Typically, in *P. redivivus* females, 55 blast cells divide to produce 635 surviving progeny and 29 cell deaths; in *P. redivivus* males, 59 blast cells generate 758 surviving progeny and 35 cell deaths. In *C. elegans* hermaphrodites, 51 blast cells generate 313 surviving progeny and 18 cell deaths; in *C. elegans* males, 55 blast cells generate 475 surviving progeny and 36 cell deaths.

Like the differences between the sexes, most of the differences between the two species involve initially similar lineages that later diverge. For example, the basic patterns of postembryonic mesodermal (M) cell divisions are almost identical in the two species. The major interspecific difference between the M lineages is the occurrence of programmed cell deaths in *P. redivivus*; there are no postembryonic mesodermal cell deaths in *C. elegans*. The cell lineages of the lateral hypodermis (V1–V6) in *P. redivivus* differ from those of *C. elegans* in two ways: they are more extensive (typically generating 404 surviving progeny in *P. redivivus* females and only 108 surviving progeny in *C. elegans* hermaphrodites) and are considerably variable. This variability appears to consist of extra divisions and polarity reversals (see below) superimposed upon an underlying, relatively simple, pattern of cell divisions. Such variability contrasts with the generally invariant postembryonic development of *P. redivivus* and *C. elegans* and may reflect a tolerance to variability in cell number provided by the relatively more extensive hypodermal lineages of *P. redivivus*; alternatively, this variability may reflect an evolutionarily recent change in the developmental program (see Sternberg and Horvitz (1981) for discussion).

TABLE 3
REGULATION IN THE VENTRAL HYPODERMIS OF THE MALE

Approx age (hr)	Ablated	No. of animals	Lineage pattern generated by		
			P9.p	P10.p	P11.p
—	None	16	Sy[a]	P10.p	P11p
10–15	Gonad	5	Sy	P10.p	P11.p
20–22	P9.p	3	—	P10.p	P11.p
20–23	P10.p	2	P10.p	—	P11.p
25	P10.p	1	Sy	—	P11.p
21	P11.p	1	P10.p	P11.p	—
23	P11.p	1	Sy	P11.p	—
24–25	P11.p	2	Sy	Sy	—
21	P(10, 11).p	2	Sy	—	—
19	P10	1	Sy	—	P11.p
13	P11[b]	1	Sy	P11.p	—
13	P11[b]	1	Sy	Sy	—
18	P(10, 11)	1	Sy	—	—

[a] Sy, joined the hypodermal syncytium.
[b] P11/12R, which usually becomes P11, was ablated before it entered the cord; P11/12L, which usually becomes P12, appeared to undergo the P12 lineage based upon the presence of the P12.pa hypodermal cell.

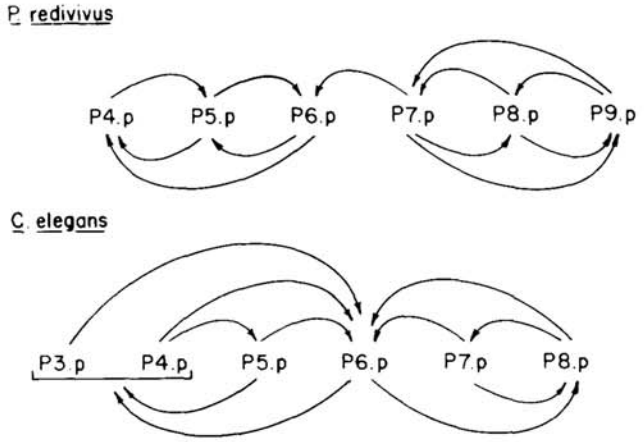


FIG. 21. Comparison of regulation of vulva development in the *P. redivivus* female and the *C. elegans* hermaphrodite. The fates of the Pn.p cells are as shown in Fig. 10. Arrows indicate changes in fate that have been induced by laser ablation of Pn.p cells (above) or gonadal cells (below). The fates of cells after gonadal cell ablation are determined by their division patterns. After gonad ablation in *C. elegans*, P(5–7).p undergo the P3.p, P4.p, and P5.p division patterns; for clarity, the complete set of arrows is not drawn; the bracket underneath P3.p and P4.p indicates that they have the same division pattern. In *P. redivivus*, the replacement of P5.p by P4.p has not been directly demonstrated; when P5/6R was ablated, P4 replaced the missing cell (see Table 2, experiment 13). See Tables 1 and 2 for *P. redivivus* data and Sulston and White (1980) and Kimble (1981a) for *C. elegans* data.

Cell–cell interactions are important in specifying the fates of certain cells in both species. For example, laser ablation experiments have revealed that the fates of cells involved in vulva development are determined by interactions both with other vulval precursor cells and with the gonad. Similarly, certain pairs of cells display a natural variability, assuming either of two distinct positions and acquiring either of two distinct fates; the absolute correlation of position and fate suggests that they are causally related.

TRANSFORMATIONS

Our earlier study of the differences between the gonadal cell lineages of *P. redivivus* and *C. elegans* led us to propose that the evolution of cell lineage involves four classes of cell lineage transformations (Sternberg and Horvitz, 1981). As discussed previously, some of these classes of transformations have been observed in

cell lineages of *C. elegans* mutants, which supports the hypothesis that they reflect simple changes in the genetic program. Similar transformations have also been observed after perturbation by laser ablation (Sulston and White, 1980; Kimble, 1981a,b) (see Results). Here we note selected examples of these transformations drawn from the differences between the nongonadal cell lineages of the two species.

(1) Change in the number of rounds of cell division. In the H1, H2 (Fig. 22), T, and P12 lineages in the *C. elegans* hermaphrodite and male and in the F, P10.p, and P11.p (Fig. 11) lineages of the *C. elegans* male there are divisions that do not occur in *P. redivivus*. In the H2 (Fig. 22), M (Fig. 23), and In lineages of *P. redivivus*, there are divisions that do not occur in *C. elegans*.

(2) Change in the fate of one cell to a fate associated with another cell. We consider the fate of a cell to be either its differentiated state or, in the case of a blast cell, its production of a certain number of cells of specific types by a particular pattern of cell divisions. P2.aap in *P. redivivus* females has the same fate as P(3–8).aap, whereas P2.aap in *C. elegans* hermaphrodites has the same fate as P1.aap and P(9–12).aap (Fig. 7). Similarly, P8.p participates in vulva development in *P. redivivus* females but not in *C. elegans* hermaphrodites, so that it seems to have "acquired" a fate otherwise associated with P(5–7).p. In some cases, the fate of a cell may remain constant, but its set of potential fates (as revealed by laser ablation) may be altered. For example, P9.p can participate in vulva formation in *P. redivivus* females but not in *C. elegans* hermaphrodites, so that it seems to have acquired a potential fate associated with at least P(4–8).p.

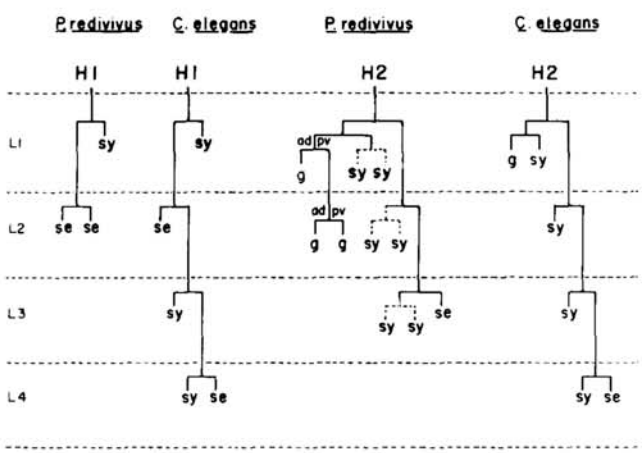


FIG. 22. Comparison of the *P. redivivus* and *C. elegans* H1 and H2 lineages. Dashed horizontal lines separate the larval periods. se, seam cells; sy, syncytial hypodermal nucleus; g, neuronal or glial cell. Dashed branches in the lineage tree indicate variable divisions. *C. elegans* lineages are adapted from Sulston and Horvitz (1977).

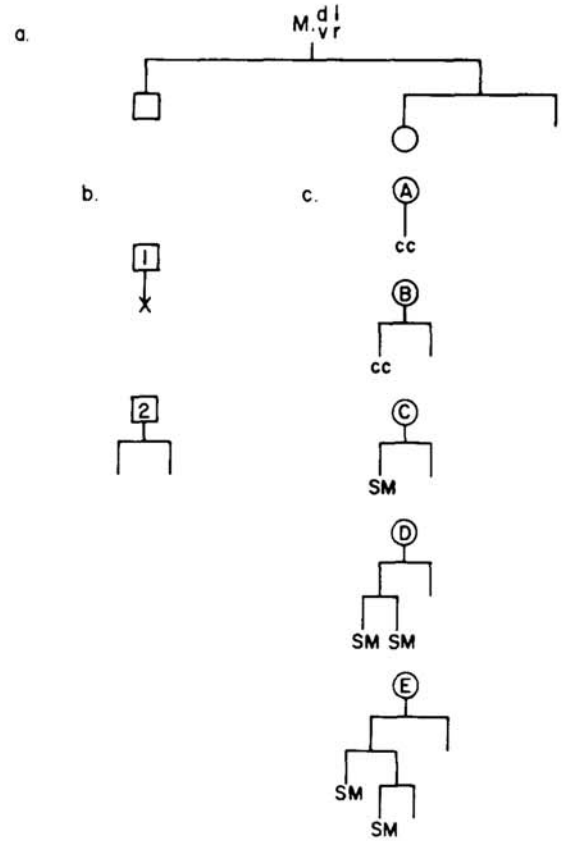


FIG. 23. Comparison of the $M._{vr}^{dl}$ lineages of *P. redivivus* and *C. elegans*. SM, sex myoblast or mesoblast, which divides further during the L3; cc, coelomocyte. Unlabeled cells are body muscles. The cell types shown here are those seen in the L2; no distinction is made among sex mesoblasts as to sex specificity or the types of progeny produced. (a) The invariant division pattern of $M._{vr}^{dl}$. (b) The portion of the M lineage that varies between species, but not between sexes or quadrants, is represented by a square: (1) *P. redivivus*; (2) *C. elegans*. (c) The portion of the M lineage that varies between species, between sexes, and among quadrants is represented by a circle. These lineages occur in (A) the dorsal quadrants of *C. elegans* hermaphrodites; (B) the dorsal quadrants of *P. redivivus* females; (C) the ventral quadrants of *P. redivivus* females and *C. elegans* hermaphrodites as well as the dorsal quadrants of *C. elegans* males; (D) the dorsal quadrants of *C. elegans* males; and (E) the dorsal and ventral quadrants of *P. redivivus* males. The dorsal $M.d_r^lpa$ and ventral $M.v_r^lpa$ cells appear to be homologous: they are lineally equivalent descendants of dorsal and ventral blast cells, respectively, and are the only M-cell descendants in the L1 that do not differentiate into body muscles (Sulston and Horvitz, 1977); furthermore, mutations at the *lin-12* locus, which result in transformations in cell fates among sets of homologous cells, also cause transformations between dorsal and ventral blast cells (P. Sternberg, I. Greenwald, C. Ferguson, and R. Horvitz, unpublished observations). The cell lineage transformations that might connect these $M._{vr}^{dl}pa$ lineages are as follows: A and B, suppression or addition of the $M.d_r^lpa$ division; B and C, a change in the fate of $M._{vr}^{dl}paa$ from SM to cc; C and D, symmetric division of $M._{vr}^{dl}paa$; D and E, asymmetric division of $M._{vr}^{dl}pap$.

(3) Polarity reversal. The anterior-most descendant of a blast cell acquires the fate of the posterior-most descendant, etc. B.ppap dies in *P. redivivus* males, but

B.ppaa dies in *C. elegans* males, consistent with the possibility that the B.ppa lineage (one division) may be of reversed polarity.

(4) "Altered segregation" of the potential to generate specific cell types. A cell acquires a developmental potential otherwise associated with its sister. As we noted previously (Sternberg and Horvitz, 1981), the left-right asymmetry in the fates of the sex mesoblast progeny in the *C. elegans* male sex mesoblast lineage (Sulston *et al.*, 1980) may result from an "altered segregation" between the left and right sides. Since the *P. redivivus* male sex mesoblast lineage appear to be bilaterally symmetrical, there is an apparent "altered segregation" between the species.

In some cases, different transformations could have indistinguishable consequences. For example, the K lineages of *P. redivivus* and *C. elegans* could differ either by a change in fate or by a change in the number and type of divisions. Similarly the B.ppa lineages of the two species could differ by a polarity reversal, as noted above, or by alterations in the fates of the two daughters. Although similar in their effects, such different transformations would presumably involve distinct underlying mechanisms.

Evolution of Cell Diversity

The evolution of cell diversity may involve cell duplication followed by the differential modification of some or all of the duplicated cells (Goodman, 1977; Chalfie *et al.*, 1981). Cells homologous by lineage history (i.e., lineally equivalent cells) seem likely to have arisen from the duplication of a specific precursor cell. Thus, the relationships among the fates of lineally equivalent cells may indicate the ways in which cell fates diverge during evolution.

In most cases, lineally equivalent cells are functionally and morphologically identical. Generally, even those lineally equivalent cells with different fates are nonetheless equivalent in that they share the same set of developmental potentials. In some of these cases, pairs of lineally equivalent cells may adopt either of two fates, with fate and position varying coordinately (e.g., B.alaa and B.araa in males of both species). In other cases, lineally equivalent cells with normally distinct fates have a number of developmental potentials, all but one of which are cryptic. For example, in *P. redivivus* females, P9.p always joins the hypodermal syncytium, but can participate in vulva formation after ablation of other Pn.p cells (Table 2; Fig. 21); similarly, cells that are normally vulval precursors join the hypodermal syncytium (as P9.p does normally) after ablation of the gonad.

We propose that the generation of lineally equivalent cells with multiple potentials may be an intermediate step in the evolution of cell diversity. Such multipotential cells might then express different potentials as a consequence of differences in their local environments; for example, intrinsically identical cells formed in different positions or at different times during development could well be exposed to distinct environmental cues (e.g., Lewis and Wolpert, 1976). Such cell extrinsic factors might affect the probability of the expression by each lineally equivalent cell of each of its set of alternative potentials. Continued evolution could lead first to the expression by each cell of only one of its potential fates and later to the loss of unexpressed potentials.

If this hypothesis were correct, members of sets of lineally equivalent but multipotential cells could become fixed with different fates in different species. Members of each pair of bilaterally symmetric Px/y cells (P1/2L and P1/2R, P3/4L and P3/4R, . . ., P11/12L and P11/12R) are lineally equivalent (J. Sulston, personal communication), and, in most cases, have distinct fates (Sulston and Horvitz, 1977). Some Px/y cells (P3/4L, P3/4R, P5/6L, and P5/6R) became Px or Py with approximately equal probabilities (Sulston and Horvitz, 1977) (see Results). In contrast, P11/12R always became P11 in *P. redivivus* and P12 in *C. elegans*; similarly, P11/12L became P12 in *P. redivivus* and P11 in *C. elegans*. Thus in a common ancestor of *P. redivivus* and *C. elegans*, P11/12L and P11/12R may have become P11 or P12 with equal probabilities, with the fixation of these fates evolving oppositely in the two species. Furthermore, members of some pairs of lineally equivalent, equipotential cells adopt different fates as if they are at an intermediate step of the evolutionary process proposed above. For example, in 14 out of 16 *C. elegans* hermaphrodites P1/2R became P1.

Lineage, Cell-Cell Interactions, and Morphogenesis

In some cases morphological differences between *P. redivivus* and *C. elegans* appear to reflect cell lineage differences. For example, the gonads of the *P. redivivus* female and *C. elegans* hermaphrodite differ by the lack of a posterior ovary in *P. redivivus*, and cell lineage differences are responsible (Sternberg and Horvitz, 1981). Similarly, the hypodermal portion of the *P. redivivus* and *C. elegans* male ventromedial preanal sensilla differ by the presence of an elongated sclerotic "hook" in *C. elegans*. The hook is formed by P10.papp. The lack of a hook in *P. redivivus* may result from the failure in this species of P10.pap to divide. In other cases differences in lineage have no apparent effect on form. For

example, although *P. redivivus* adults are twice the length of *C. elegans* adults, they have fewer body wall muscles and ventral hypodermal nuclei; however, *P. redivivus* does have approximately four times the number of lateral hypodermal nuclei.

Other morphological differences appear to be caused by differences in the morphogenesis of lineally equivalent cells. Such differences may reflect differences either in intrinsic developmental potential or in interactions with other cells. For example, the male tails of *P. redivivus* and *C. elegans* are strikingly different. The *C. elegans* male has a bursa (fan) with elongated caudal papillae (rays) embedded in it, and straight spicules. The *P. redivivus* male has a stubby tail with knob-like caudal papillae, and large curved spicules with hooked distal ends. The lineages (B, U, and F) that generate the cells of the spicules and gubernaculum are essentially identical. What causes the difference in final form? Sulston *et al.* (1980) have shown that proper morphogenesis of the spicules and gubernaculum in *C. elegans* requires the sex muscles derived from the M cell. There are 46 male sex muscles in *P. redivivus* and 41 in *C. elegans*, and the musculatures of the two species most likely differ. This difference in musculature could be at least partially responsible for the difference in spicule and gubernaculum shape.

Similarly, the Rn cell lineage that generates the neuronal and structural cells of the *C. elegans* caudal papillae (rays) is identical to the cell lineage that generates the compact nuclei of the *P. redivivus* knob-like caudal papillae. (The Rn cells are derived from V6 and T in both species and also from V5 in *C. elegans*.) The species-specific shapes of these structures may be determined by factors intrinsic to the cells involved; although both sets of Rn descendants are committed to form caudal papillae, they might have inherently distinct differentiated characteristics. Alternatively, the environment of the Rn cells during morphogenesis may be important.

The vulva of the *P. redivivus* female slants anteriorly and is anteroposteriorly asymmetric during its morphogenesis (Fig. 9). In contrast, the vulva of the *C. elegans* hermaphrodite is anteroposteriorly symmetric. (Both are bilaterally symmetric.) Although the vulval cell lineages differ between the species (Fig. 10), they both are anteroposteriorly symmetric around the anchor cell. Although this symmetry does not exclude differences in cell type on opposite sides of the vulva in *P. redivivus*, it does suggest that factors extrinsic to the vulva may affect its morphogenesis. For example, the gonad is known to influence vulva morphogenesis in both species (see Results) (Kimble, 1981a), and the anteroposterior asymmetry of the *P. redivivus* gonad could contribute to the asymmetry of the vulva.

SUBLINEAGES AND THE PROGRAMMING OF CELL LINEAGE

During the development of both *P. redivivus* and *C. elegans* certain distinct patterns of cell division that generate specific sets of progeny occur multiple times (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sternberg and Horvitz, 1981). Some of these patterns of division are expressed ectopically in mutants (Horvitz and Sulston, 1980; Sulston and Horvitz, 1981; Chalfie *et al.*, 1981; V. Ambros, W. Fixsen, C. Ferguson, I. Greenwald, P. Sternberg, and R. Horvitz, unpublished observations) or in wild-type *C. elegans* after physical perturbation by laser ablation (Sulston and White, 1980; Kimble, 1981a). Thus, these division patterns may be expressed as integral units. These observations have led to the suggestion that the developmental program specifying cell lineage may utilize a series of modular subprograms each of which encodes a multiply executed pattern of cell divisions (a "sublineage") (Chalfie *et al.*, 1981).

If indeed cell lineages are composed of separately programmed, modular sublineages, an understanding of development will require answering a number of questions concerning sublineages. For example, which multiply expressed cell division patterns are sublineages, i.e., are encoded as a single subroutine within the developmental program? What specifies when and where a particular sublineage is expressed? What defines the nature of each sublineage? Our studies of the cell lineages of *P. redivivus* offer insights into the role of sublineages in development in two ways. First, *P. redivivus* provides further examples of repeatedly expressed patterns of cell division. Second, the differences between species (as well as between sexes or between similar lineages within a species and sex) suggest that modifications of the program for cell lineage may involve either alterations within sublineages (by one of the transformations discussed above) or substitutions of one sublineage for another. A sublineage substitution, which is an example of the transformation we have called a "switch in fate," could profoundly alter a cell lineage in a single step. Similarly, a (single-step) transformation within a sublineage would coordinately affect multiple cell lineages.

The lineages of the lateral hypodermal precursors (Vn) provide a striking example of a repeatedly expressed pattern of cell division (Figs. 12 and 24). Each seam cell divides according to the same pattern of divisions to generate four syncytial hypodermal nuclei and two seam cells. Each resulting seam cell, except for those formed during the L4, divides similarly during the next larval period to generate an additional four syncytial hypodermal nuclei and two seam cells. This

characteristic seam cell division pattern occurs 70 times in the *P. redivivus* female and seems likely to be a sublineage. The lateral hypodermal lineages are examples of reiterative or "stem-cell" lineages in which a cell repeats the division pattern of one of its ancestors (see Chalfie *et al.*, 1981). The *P. redivivus* seam cell lineage (Fig. 24a) leads to both a great-grandparental and a parental reiteration as both se.apa and se.p reiterate the division pattern of the progenitor seam cell se. Any sublineage that produces cells that repeat that sublineage will result in a cell lineage reiteration.

Other repeatedly expressed patterns of cell division (i.e., possible sublineages) seen in *P. redivivus* correspond to those of *C. elegans*: the Rn lineages (Figs. 12 and 16) of the caudal papillae (repeated 14 times by V6 and T descendants in *P. redivivus* and 18 times by V5, V6, and T descendants in *C. elegans*); the T.pp lineage (expressed by T3 and T1.pp in *P. redivivus* and by both T.pp cells in *C. elegans*) of the lateral ectoderm (Fig. 17); the postdeirid lineages (derived from the two V5.pa cells); the Q lineages (Fig. 18) of the lateral ectoderm (repeated twice); the B.alap/B.arap and B.alpa/B.arpa lineages (Fig. 19) of the male proctodeum (repeated twice each); and the Pn.a and W lineages (Fig. 6) of the ventral cord (expressed 13 times, although with modifications).

The Vn cell lineages provide an example of an apparent interspecific sublineage substitution. Superficially, the Vn lineages of *P. redivivus* and *C. elegans* are very different. Yet for both species these lineages can be described essentially as follows: every seam cell divides at each larval molt, except the last, generating seam cells and syncytial hypodermal nuclei (Fig. 24). What differs between the species is the nature of the apparent sublineage expressed by each seam cell; the seam cell sublineage is considerably more extensive (Fig. 24) and variable (Fig. 15) in *P. redivivus*. Similarly in both species the differences in Vn lineages between the sexes appear to involve the utilization of different sublineages during the L4. For example, in the *P. redivivus* V6 lineages the caudal papilla (Rn) lineage is expressed in the male, and the lineage generating seam cells and syncytial hypodermal nuclei is expressed in the female (Fig. 12). Some differences between Vn lineages are constant among sexes and species. For example, in both sexes of both species the postdeirid lineage (generated by V5.pa) is substituted for a lineage generating seam cells and syncytial hypodermal nuclei (expressed by the other Vn.pa cells) during the L2 divisions of the V5 lineage. Thus, the programming of the Vn cell lineages most likely involves instructions specifying which of a number of alternative sublineages is expressed by each blast cell during each larval period.

The Pn lineages of *P. redivivus* and *C. elegans* (e.g., Fig. 6) may involve nested sublineages. As noted above, the similarity of the W and the Pn.a lineages suggests that the division pattern characteristic of these 13 cells is a sublineage. The Pn.a lineages can be considered to differ by the substitutions of distinct Pn.aap (Fig. 7) sublineages or fates within the Pn.a sublineage. Thus the programming of the Pn lineages may involve instructions at one level specifying the Pn.a (and Pn.p; see below) sublineages and instructions at a second level specifying which Pn.aap sublineage is utilized by each Pn.a cell. In addition, some of the Pn.a sublineages are slightly modified; e.g., P12.aaa does not divide in *P. redivivus*, and the differentiated characteristics of certain Pn.a descendants differ from those of their lineally

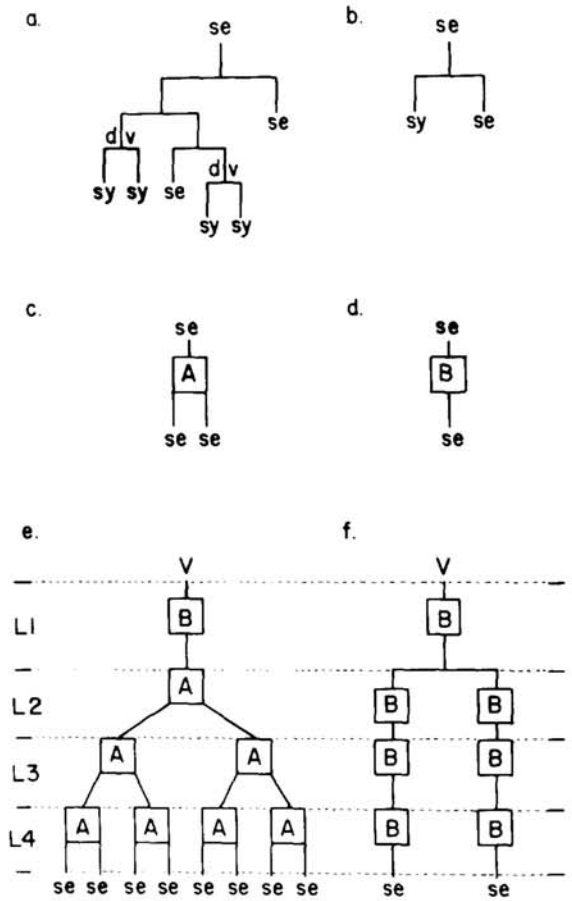


FIG. 24. Sublineages in the *P. redivivus* and *C. elegans* lateral hypodermal lineages (V1–V4). se, seam cell; sy, syncytial hypodermal nucleus. (a) *P. redivivus* sublineage. Each sublineage generates two seam cells and four syncytial hypodermal nuclei. (b) *C. elegans* sublineage. Each sublineage generates a single seam cell and a syncytial hypodermal nucleus. (c) Schematic representation of the *P. redivivus* sublineage. (d) Representation of the *C. elegans* sublineage. (e) Decomposition of the *P. redivivus* V(1–4) lineages (Fig. 12) into component sublineages. Note that a *C. elegans*-type sublineage is utilized during the L1. (f) Decomposition of the *C. elegans* V(1–4) lineages into component sublineages. The altered division pattern during the L2 could represent a distinct sublineage.

equivalent homologues (Sulston, 1976; Sulston and Horvitz, 1977).

A comparison of vulva development in the two species suggests that the instructions specifying the sublineages expressed by vulval precursor (Pn.p) cells may consist of several independent steps. One set of instructions may specify which cells are members of the vulval equivalence group, and hence which cells have the potential to express vulval sublineages. The vulval equivalence group consists of at least P(4-9).p in *P. redivivus* and P(3-8).p in *C. elegans* (Fig. 21). A second set of instructions may specify which cells within the equivalence group actually participate in vulva formation (Fig. 10). In *P. redivivus*, P(5-8).p participate in vulva formation, whereas in *C. elegans*, only P(5-7).p participate. A third set of instructions may specify which vulval sublineage each Pn.p cell expresses (i.e., whether each Pn.p cell adopts the primary or secondary fates, as defined by laser ablation experiments; see Results). In *P. redivivus* P6.p and P7.p have the primary fate, whereas in *C. elegans* only P6.p has the primary fate. A fourth set of instructions may specify the nature of each sublineage. The sublineages generated by cells with primary, secondary, and tertiary fates differ between *P. redivivus* and *C. elegans* (Fig. 10). For example, the P5.p vulval sublineage differs between the species even though in both species these cells have the secondary fate.

The mesodermal (M) lineages of *P. redivivus* and *C. elegans* also provide possible examples of sublineages and independent programming steps (Fig. 23). The fates of the four $M._{vr}^{dl}a$ cells seem to be determined coordinately: in *P. redivivus* all four $M._{vr}^{dl}a$ cells die and in *C. elegans* all four $M._{vr}^{dl}a$ cells generate two body muscle cells (Fig. 23b). Thus the four $M._{vr}^{dl}$ lineages may result from the expression of the same underlying sublineage and may differ by a coordinate change in the fate of $M._{vr}^{dl}a$. The fates of the four $M._{vr}^{dl}pa$ cells appear to involve sublineage modifications (Fig. 23c). Two sets of instructions may be responsible for defining $M._{vr}^{dl}pa$ cell fates: first, those causing the homologous cells $M.d_r^lpa$ and $M.v_r^lpa$ to differ (as they do in both species), and those specifying the particular fates these cells express (which differ between species).

IMPLICATIONS FOR DEVELOPMENTAL GENETICS

The different steps that appear to be involved in the programming of cell lineage suggest the existence of genes that control each of these steps. For example, as discussed above, which sublineage is expressed by each lateral hypodermal blast cell may be specified by spatial and temporal cues. Two genes in *C. elegans*, *lin-4* and *lin-14*, have been implicated in defining or implementing temporal information in the development of the lateral hypodermis: strains carrying the mutations *lin-4 (e912)* or *lin-14 (n355)* express the L1 lateral hypodermal lineages repeatedly, as if the specification of developmental timing has been altered (Chalfie *et al.*, 1981; V. Ambros and R. Horvitz, unpublished results). Similarly, in the development of the vulva the different fates of the Pn.p cells seem to depend on their positions (Sulston and White, 1980) (see Results). Mutations in the genes *lin-1*, *lin-2*, *lin-3*, *lin-7*, *lin-8*, and *lin-9* in *C. elegans* cause certain Pn.p cells to express fates normally associated with other Pn.p cells. These mutations appear likely to act by eliminating or altering the cell-cell interactions that have been implicated in the determination of the fates of the Pn.p cells (Horvitz and Sulston, 1980; Sulston and Horvitz, 1981).

Additional genes responsible for the programming (and evolutionary modification) of cell lineage may be identified by seeking other mutations with analogous effects on sublineages. Specifically, some such mutations should result in the inappropriate expression (in time, space, or possibly sex) of a sublineage. Others should result in the coordinate alteration of a sublineage wherever or whenever it is expressed. Cell lineage mutants with these phenotypes would be excellent candidates for defining genes that control development.

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The *Caenorhabditis elegans* Male: Postembryonic Development of Nongonadal Structures

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The nongonadal cells in the male nematode *Caenorhabditis elegans* have been followed through maturation by Nomarski microscopy. Many of the cells are incorporated into the copulatory apparatus, which includes the cloaca, copulatory spicules, sensilla, and musculature. This region has been reconstructed by serial section electron microscopy in order to identify the cell types that arise from known lineages. With the exception of certain bilaterally symmetrical pairs the cells have invariant fates. The development involves a variety of well-defined cell interactions, individual and collective cell movements, cell deaths mediated by designated killers, and the reorganisation of a muscle. The male structures overlie an almost unchanged hermaphrodite tail; their development is more complex than that of the hermaphrodite, and more liable to error.

INTRODUCTION

At hatching, the nematode *Caenorhabditis elegans* contains about 550 nongonadal nuclei; during postembryonic development the number increases to about 810 in the hermaphrodite and to about 970 in the male. The cell lineages leading to these increases were described by Sulston and Horvitz (1977), but at that time the fates of the male specific cells were largely unknown. We have now followed the nongonadal cellular development of the male up to maturity, and have reconstructed the posterior anatomy of the adult from electron micrographs; thus the migration, differentiation, and ultimate fate of each cell in the tail is now known. The development of the gonad has been elucidated by Kimble and Hirsh (1979), and its connection to the somatic structures will be described. We have mapped the nervous system only as far as is necessary to define each cell uniquely.

The development of the male is of interest both for its complexity and for its genetic accessibility. Since the principal sexual form of *C. elegans* is a self-fertilising hermaphrodite, functional males are not required for strain propagation, and male-specific mutants can be maintained without difficulty. Hodgkin (1974) has isolated a number of strains in which hermaphrodites are fertile but males are defective. In another class of mutant, transformer (Klass *et al.*, 1976; Hodgkin and Brenner, 1977), animals bearing two X chromosomes are male rather than hermaphrodite.

The study of such mutant strains is one approach to understanding the control of the developmental events described below. Another approach is selective cell ablation by means of a laser microbeam; experiments using this technique are described both in this and in the following paper.

MATERIALS AND METHODS

Caenorhabditis elegans, var Bristol, strain N2, was cultured as described by Brenner (1974).

The movements of cells were observed in living animals by Nomarski differential interference contrast microscopy. The animals were mounted on layers of 5% agar, as

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described by Sulston and Horvitz (1977) except that polyvinylpyrrolidone was not added to the S medium; a green interference filter was always inserted.

(1) Standard mount. The agar was trimmed around the cover slip, and the mount was simply flooded with immersion oil without the application of grease. Unfortunately, this convenient technique cannot be used with the immersion oil currently obtainable (Zeiss 518C), because of its toxicity to the nematodes. For this reason, we have continued to use the old type of oil, containing polychlorinated biphenyls (PCB). If the new type of oil is used, it must be kept out of contact with the agar. A layer of grease does not provide a sufficient barrier. One approach is to employ an 18 × 18 mm cover slip and to keep the oil away from the edges, which are sealed with grease or Voltalef oil (Judith Kimble, personal communication); another, applicable for periods of a few hours, is to use the quick mount, described below.

(2) Quick mount, used when the animal was to be retrieved after a brief examination. A 13-mm-diameter circular cover slip, coated with bacteria in the usual way, was placed over the nematodes; the agar was not trimmed, but covered with a 25-mm square of Saran Wrap having an 11-mm-diameter hole. Only a tiny drop of immersion oil was added, so that the plastic was not wetted by it. For recovery, the Saran Wrap was peeled off, and then the cover slip was raised, the animal being watched to ensure that it remained on the agar; if necessary, the cover slip was lowered and raised until it did so (extra fluid helped).

(3) Anaesthetic mount, used for laser surgery and for drawing with the camera lucida. The nematode was anaesthetised to the required extent in 0.5–1% 1-phenoxy-2-propanol and then mounted on agar containing 0.2% 1-phenoxy-2-propanol; by this procedure, anaesthesia is rapid (0.5–5 min, depending upon the age of the animal) yet controllable, and the animals do not deteriorate as fast as they do when mounted in more concentrated 1-phenoxy-2-propanol. The quick mount procedure was followed, except that no bacteria were applied to the cover slip.

(4) Invertible mount, used to follow certain cells through lethargi (the periods of inactivity during moults), when animals frequently turn over. It was particularly useful in tracking the muscle cells, which are obscured contralaterally by the intestine, through L3 lethargus.

To allow inversion, it is necessary to mount the animal in a very thin layer of aqueous medium; this in turn necessitates a plastic cover film, to permit gas exchange. The cover film was prepared in advance by coating a horizontal 38 x 76 mm microscope slide with 1.5 ml of 2% celloidin (Gurr, London) in amyl acetate. The slide was left for several days in a dust-free place, and the film was then cut into 10 x 10 mm squares; when required, a square was peeled off and the centre was lightly smeared with bacteria. In order to prepare the mount itself, a 22 x 50 mm coverslip was placed between two others raised 60 μm with Ofrex tape, all three being held onto a sheet of plate glass by films of water. A drop of hot 1% agarose was placed on the central coverslip and flattened by means of a siliconised slide supported on the outer coverslips. After cooling, the slide was slid away and the agarose was allowed to dry to about 40 μm (judged by experience; the final thickness of the mount was measured using the calibrated fine focus control). A very small drop of S medium containing the nematode was added, followed by the cover film. The edges of the agarose were allowed to dry completely, and then the mount was flooded with immersion oil. For convenience of handling, it was clamped in a metal frame (35 x 75 x 3 mm). Such mounts lasted for several hours before dehydration began to compress the nematode unduly; by that time the animal had moulted and was remounted in the normal way. PCB immer-

sion oil was used for invertible mounts, but, perhaps on account of the limited exposure period, the new oil can also be employed. Under PCB oil, the upper surface of the celloidin slowly becomes beaded with water droplets; these can be cleared by wiping very lightly with a shred of lens tissue.

Individual animals were always handled by pipette; 1.5 mm tubing was drawn in a flame, and cut to give a tip diameter of 0.2–0.3 mm. The pipette was attached to a mouth tube and kept partly filled with buffer during use. Provided that animals were not sucked into the wide part of the pipette, they never became trapped inside.

Drawings of anaesthetised animals were made with the help of a Zeiss camera lucida. Approximate depths of the nuclei in the animal were recorded by means of a colour code. Stereo pairs of the proctodeal nuclei were made by manual tracing, the spacing of the images of each nucleus being adjusted according to the colour code.

Developmental ages of animals are given in hours from the time of hatching at 20°C, according to the time scale of Sulston and Horvitz (1977).

Animals were prepared for electron microscopy in various ways. For bulk fixation, a male culture plate from which the bacteria had cleared was kept at 4°C overnight and then left for a few minutes in a covered dish containing some dry ice. The plate was inverted over 2% osmium tetroxide for 1–2 min, and the nematodes were washed off into 1% osmium tetroxide. After fixation for 1 hr, straight animals were selected and dehydrated and embedded as described by Ward *et al.* (1975). Series 4 and 5 (Table 1) were obtained in this way. For series 3 the animal was treated similarly, but was straightened manually after anaesthesia with carbon dioxide. For series 1 and 2 the animals were transferred directly from the Nomarski microscope to 1% osmium tetroxide, to ensure that development was arrested at a known stage.

In areas where the morphology and arrangement of cells is well defined (mesoderm, most of the proctodeum, part of the preanal ganglion), the assignment of cell fate did not require the electron microscopic reconstruction of animals of known lineage. This was helpful, because the lineage of only a limited number of cells can be watched in a single individual, and anatomical reconstruction is a time-consuming operation. To supply the missing information, two animals were analysed by electron microscopy after particular lineages had been followed (Table 1).

TABLE 1

ELECTRON MICROGRAPHIC SERIES

Series	Age of animal	Cells lineaged
1	42 hr	Left R3-R9; right R5-R9; dorsorectal ganglion
2	43 hr	—
3	Young adult	Preanal ganglion
4	Old adult	—
5	Old adult	—

A laser microbeam system developed by John White was used to kill individual cells, as described in the following paper.

The nomenclature used to describe cell lineages is the same as that employed by Sulston and Horvitz (1977). After a cell division, each daughter is given its parent's name followed by a letter representing its relative position: a, anterior; p, posterior; d, dorsal; v, ventral; l, left; r, right. Thus, Rn.aa is the anterior daughter of the anterior daughter of Rn. In the lineage charts, divisions are anterior (to the left)–posterior (to the right) unless otherwise designated.

Because many cell lineages are unknown, and some are ambiguous, it is desirable to name cells also in terms of their differentiated characters. The nomenclature for neurons was devised by John White. In his system, every cell is given a name consisting either of three upper case letters or of two upper case letters and a number; the second alternative is used only for groups of similar cells, each cell in a given group having the same letters but a different number. The

three-character name is unique except in the case of radially symmetric groups, of which the members appear to be identical: in such a group, every cell has the same three-character name, followed by L (left), R (right), D (dorsal), or V (ventral). Supporting cells in the nervous system, and some other structural cells, are named by a slight modification of the neuron system. In the figures, these names are distinguished from lineage designations by being printed in Gothic type.

For all the components of a given sensillum, the same initial pair of letters is used, i.e.—HO, hook sensillum; PC, postcloacal sensillum; PH, phasmid; Rn, ray number n; SP, spicule. The next one or two letters indicate the nature of the component, as follows—sh, sheath cell; so, socket cell; st, structural cell; A, B, C, D or V, neuron. For bilaterally paired sensilla, the name ends in L (left) or R (right). The correspondence between lineal names and systematic names is shown in the lineage charts and drawings.

RESULTS

Outline

The general layout of the external features and the copulatory apparatus is shown in Fig. 1. The nervous system and the mesoderm are shown separately in Figs. 11 and 25.

The first section of the results (Hypodermis) will deal with the overall shaping of the tail and the formation of the cuticular fan. The second (Proctodeum) will describe the internal copulatory apparatus: the union between the vas deferens and the alimentary tract, and the formation of the copulatory spicules. The third section covers the nervous system, including the sensilla shown in Fig. 1, and the fourth the mesoderm.

Hypodermis

Shaping of the tail. The adult male lacks the tapering tail characteristic of male larvae and hermaphrodites; during maturation, the tail cells withdraw anteriorly and, at the same time, lay down the cuticle of the fan. From the L2 stage onwards the tail of the male is increasingly swollen compared with that of the hermaphrodite, apparently on account of the additional cells which are generated within it. Before and during L4 lethargus, however, there are more rapid changes in shape, which seem to be due to movements of the hypodermis (Figs. 2–4). In mid-L4, the three hypodermal cells which occupy the posterior part of the tail begin to retreat anteriorly, together with their four nuclei. Some 5 hr before ecdysis, new cuticle can be seen around the retracted cytoplasm. The tips of the sensory rays, already embedded in the outer layer of the adult cuticle, are clearly

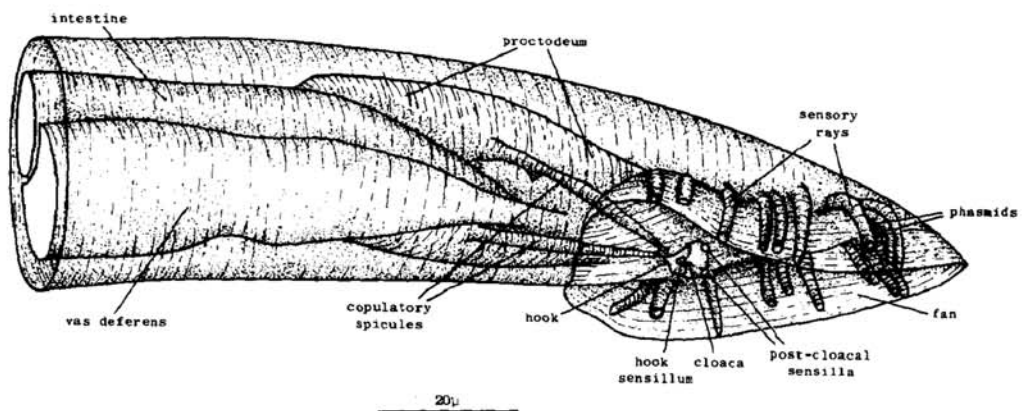

FIG. 1. Left subventral view of young adult tail, to show general anatomy.

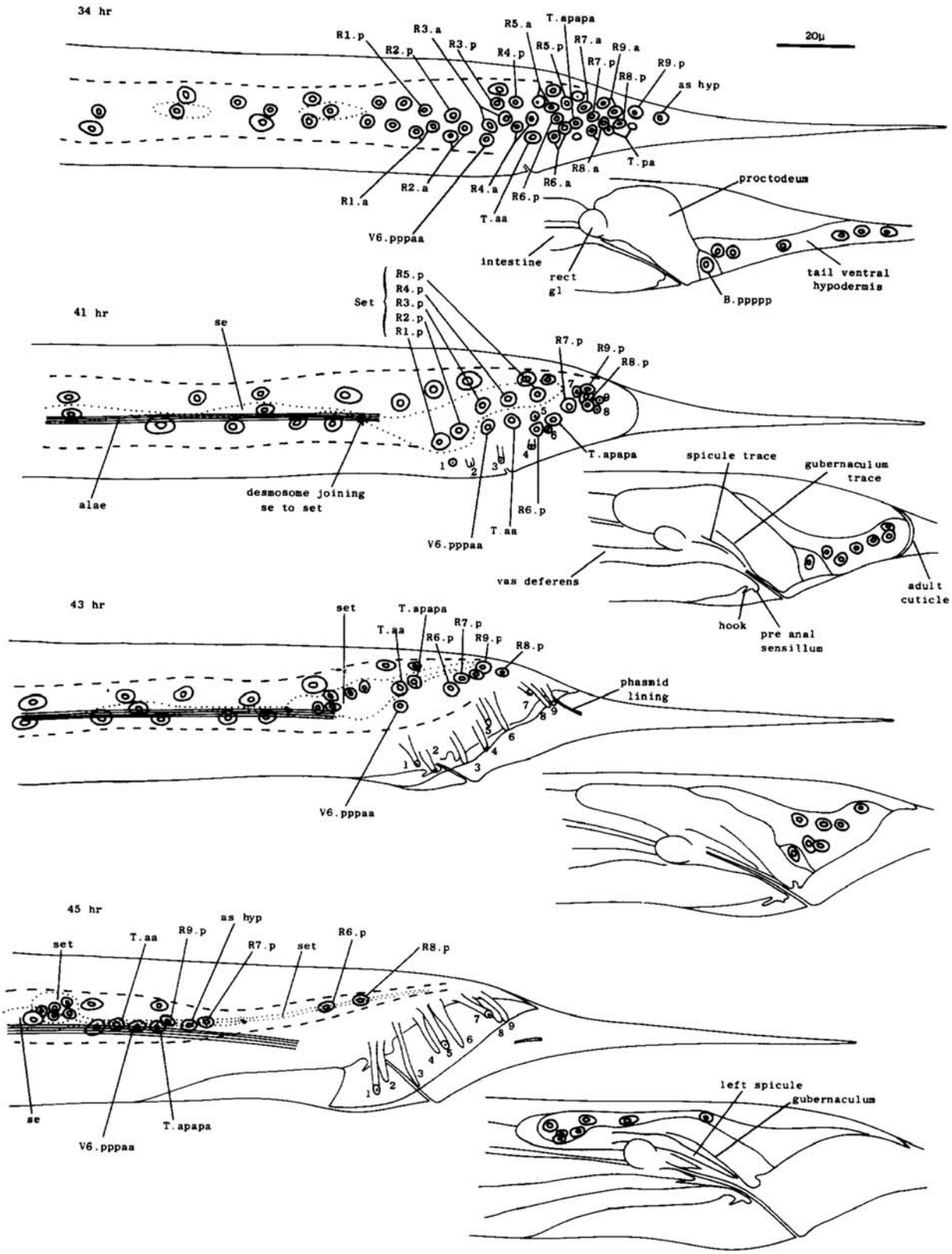
34 hr
R1.p
R2.p
R3.a
R3.p
R4.p
R5.a
T.apapa
R5.p
R7.a
R9.a
R7.p
R8.p
R9.p
as hyp
20μ
T.pa
R8.a
R6.a
R6.p
R1.a
R2.a
R4.a
T.aa
V6.pppaa
proctodeum
intestine
rect
gl
tail ventral
hypodermis
B.ppppp
41 hr
Set
R5.p
R4.p
R3.p
R2.p
R1.p
se
R7.p
R9.p
R8.p
T.apapa
alae
desmosome joining
se to set
R6.p
T.aa
V6.pppaa
spicule trace
gubernaculum
trace
vas deferens
adult
cuticle
hook
pre anal
sensillum
43 hr
set
T.apapa
T.aa
R6.p
R7.p
R9.p
R8.p
phasmid
lining
V6.pppaa
45 hr
as hyp
set
T.aa
R9.p
R7.p
set
R6.p
R8.p
se
V6.pppaa
T.apapa
left spicule
gubernaculum

FIG. 2

visible. The forward movement of the tail hypodermis continues, and now the lateral hypodermis, the ray cell bodies, and the dorsal parts of the proctodeum also begin to move anteriorly. Dorsally and ventrally the adult cuticle remains in close contact with the hypodermis, but laterally a wide gap opens between them; this gap is bridged by the rays, which remain attached to the outer layer of cuticle and are spun out behind the retreating cytoplasm. Next, the inner layer of the adult cuticle is laid down, and the lateral extensions of the outer layer collapse around the rays, forming the acellular webbing of the fan.

The preanal region is the last to reorganise; there is a general forward movement of the hypodermis, and spasmodic contractions of the newly formed diagonal muscles pull the ventral side away from the cuticle.

Tail seam. Seam cells are lateral hypodermal cells which do not fuse with the large hypodermal syncytium, divide repeatedly in larvae, generate alae, and possess large Golgi bodies during cuticle secretion. In the L4 the seam cells, having ceased to divide, fuse together into a continuous band on each side; after the L4 moult the seam appears to atrophy (John White, unpublished results; Sulston and Horvitz, 1977; Singh and Sulston, 1978).

In the male the three most posterior seam cells (V5, V6, and T) are diverted into the production of the rays (Fig. 17). However, in the late L4, the posterior daughters of the ray precursor cells R1, R2, R3, R4, and R5 fuse together and appear seam-like in their possession of large Golgi bodies (Fig. 3); we therefore call them the tail seam (set). The tail seam does not produce alae (Figs. 2 and 3), but it does shrink during L4 lethargus and leaves a narrow superficial band which forms a rearward extension of the body seam. The two seams never fuse, but remain attached to one another by a desmosome at their original point of contact (Fig. 5). Usually, all five nuclei of the tail seam are transported anteriorly in a bag of cytoplasm which remains connected via a narrow process to the posterior part (Fig. 2). However, in one animal electron microscopical reconstruction revealed that on each side one of the nuclei had remained posteriorly.

The function of the tail seam was investigated by ablation of its constituent cells soon after their birth in four animals. No other hypodermal cells replaced them, and the body seam did not grow posteriorly. The fan was somewhat attenuated, but electron microscopy revealed that the cuticle had collapsed to form it in the usual way. Thus, no essential role for the tail seam was identified.

It appears that the posterior daughters of R6, R7, R8, and R9 fuse with the large hypodermal syncytium. In series 1, fixed at a time when the nuclei are still identifiable

FIG. 2. Movement of hypodermal nuclei during tail maturation, left lateral view. The left-hand series of drawings shows the left lateral hypodermis and the right-hand series shows the tail ventral hypodermis. 34 hr: The hypodermal cells (Rn.p) derived from the ray precursor cells are not easily distinguished, other than by their characteristic positions, from the neuroblasts with which they are mingled. Dashed line shows edge of lateral hypodermis at surface of animal; dotted line shows edge of seam cells. 41 hr: The hypodermal cells of the ray groups now lie superficially to the neurons, which are not drawn. The outermost layer of adult cuticle has been laid down, complete with longitudinal alae and tips of the rays (numbered). The first traces of spicule and gubernacular cuticle can be seen. The body seam (se) and tail seam (set) are linked by a desmosome; alae are formed only by the former. At this stage, hypodermal nuclei can still be identified by position. The cells have retreated from the tapering tail. 43 hr: General forward movement, lengthening of the rays. L4 cuticle lining the phasmid channels is pulled out. Tail seam is shrinking and moving forwards. 45 hr: The animal is ready for ecdysis. Rays are fully extended and the fan has collapsed around them. The shrunken tail seam nuclei lie in a bag of cytoplasm at the anterior end of a long process; the body seam still runs back separately to the desmosome. The tail ventral hypodermis has largely withdrawn to lie dorsally to the proctodeum; as hyp, asymmetric hypodermal nucleus; rect gl, rectal gland.

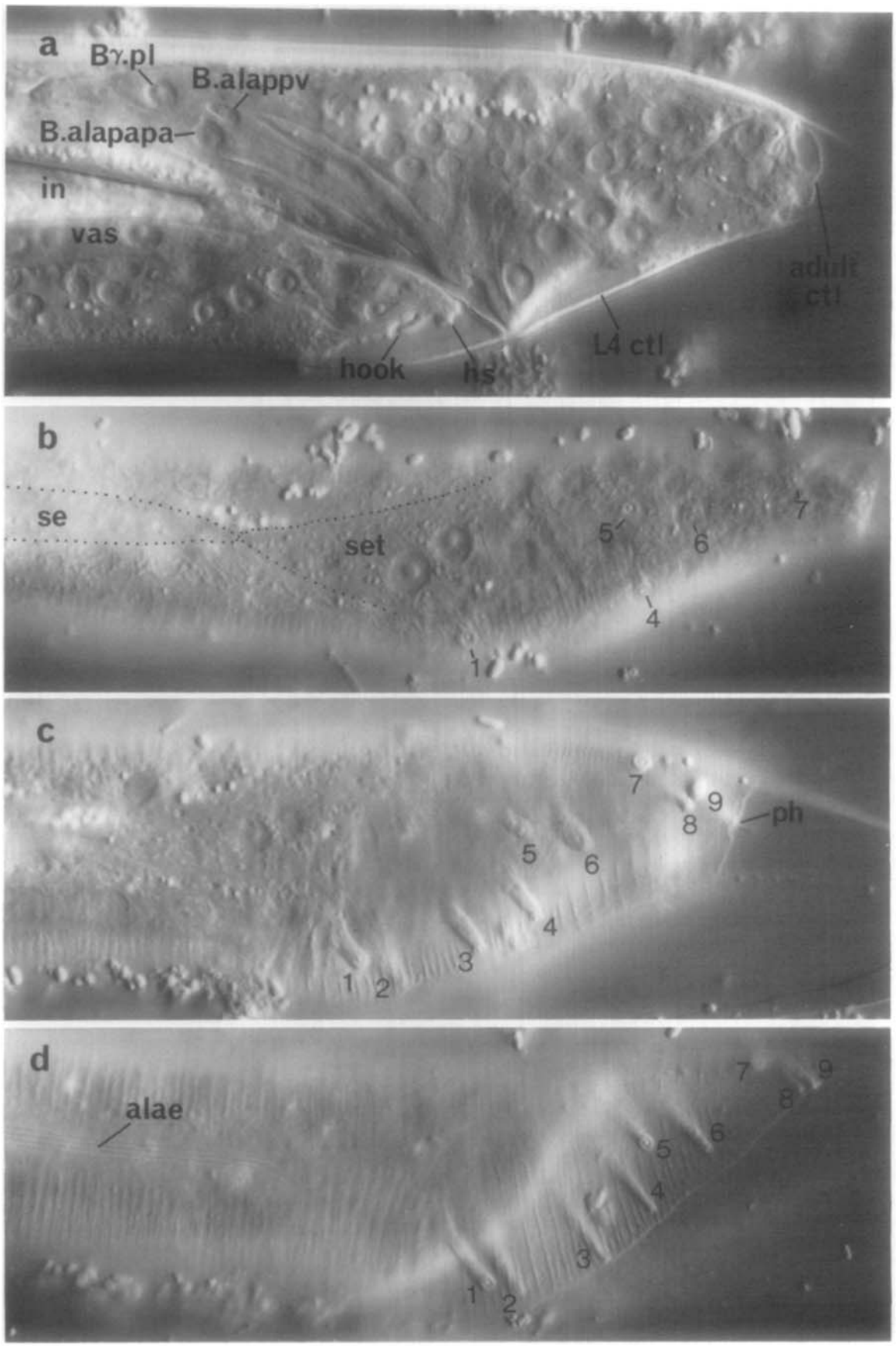
a
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FIG. 3

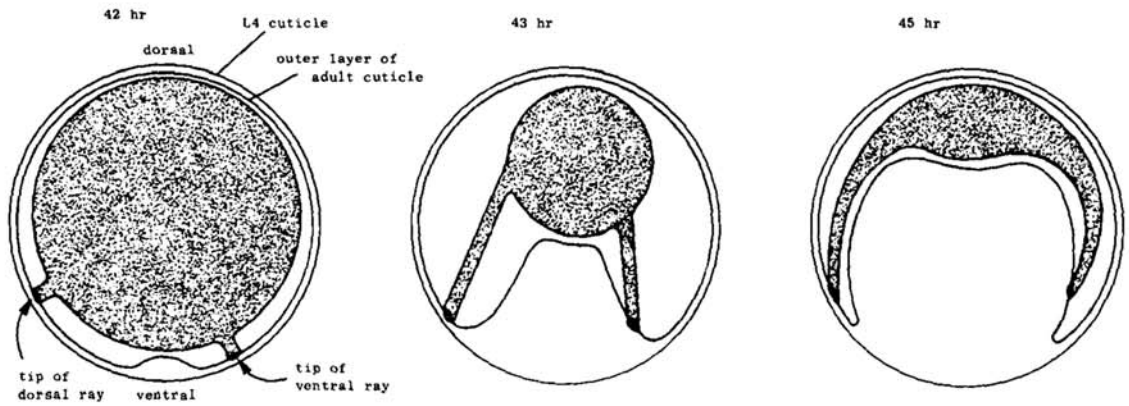

FIG. 4. Folding of outer layer of adult cuticle to form the fan. The hypothetical transverse section includes both a dorsal and a ventral ray (viz., rays whose tips lie on the dorsal or ventral surface, respectively, of the mature fan).

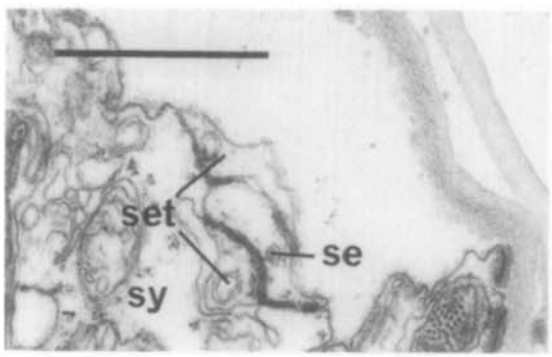

FIG. 5. Desmosome joining body seam (se) to tail seam (set). sy, large hypodermal syncytium. Electron micrograph of transverse section, series 4. Bar = 1 μm.

by their positions, only R8.p and R9.p are separate cells. In two adults examined by electron microscopy (series 4 and 5), all the posterior lateral hypodermal nuclei were found to lie either in the seams or in the large syncytium.

Proctodeum

The proctodeum is the junction between the alimentary and genital tracts, together with the copulatory spicules and the spicule channels. The blast cells involved in its formation are B, C, E, F, and K (Fig. 6). K is an asymmetric blast cell, which lies to the left of the rectum and divides once in the L1 (Sulston and Horvitz, 1977); its anterior daughter resembles the corresponding rectal hypodermal cell on the right, which does not divide. The latter was not named previously, and is now designated K′.

The arrangement of nuclei in and around the proctodeum at two stages is shown in Fig. 7. At the earlier time (34 hr), the divisions are already complete; then, in the L4, the cells move progressively to their final positions. Some remain near their birthplaces, while others migrate to new locations, but their behavior is essentially invariant and bilaterally symmetrical. During L4 lethargus there is little further migration of individual cells, but the entire proctodeum stretches anteriorly (see under Mesoderm) (Fig. 2). At the same time, the cell boundaries become irregular and easily confused in electron micrographs with membranous fragments in the cytoplasm; indeed, a number of the cells seem to degenerate after the L4 moult. For this reason series 1, from an animal fixed at 42 hr, was

FIG. 3. Maturation of the tail, Nomarski optics. Left-hand views. Bar = 20 μm. (a) 40 hr. Focus in midline. First signs of adult cuticle. ctl, cuticle; hs, hook sensillum; in, intestine. (b) 41 hr. Focus in left hypodermis. Tips of rays visible; note unique appearance of 6. Golgi activity in body seam (se) and tail seam (set), dotted outline. (c) 43 hr. Focus just below adult cuticle. Rays extending as cells retreat anteriorly. ph, phasmid. (d) 44 hr. Focus in adult cuticle. Rays full length, fan folding.

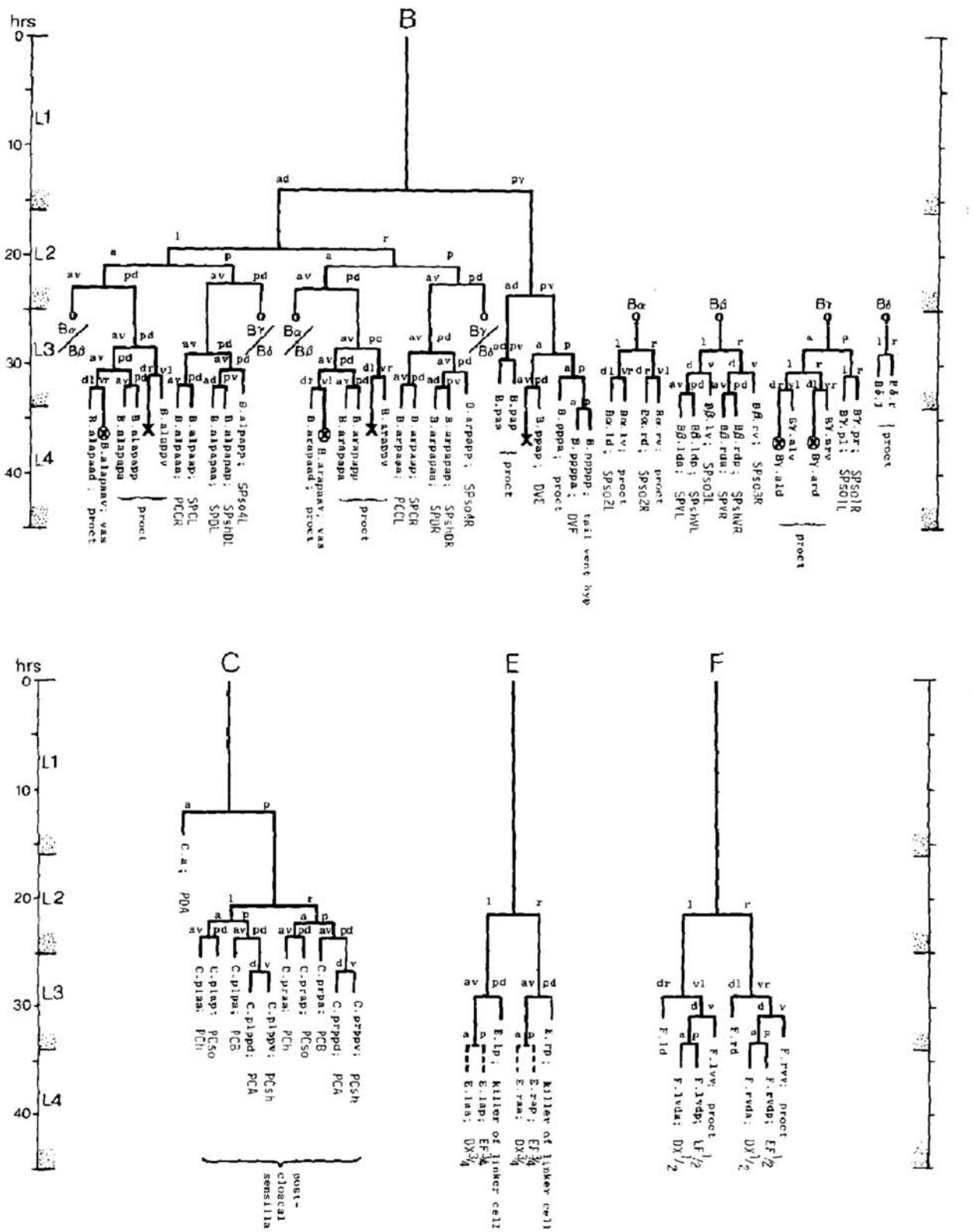

FIG. 6. Lineages of cells which divide only in the male. Circled crosses identify pairs of cells in which either the left-hand or the right-hand partner may die. proct, structural cells of proctodeum (Fig. 8); vas, participates in connection of vas to cloaca.

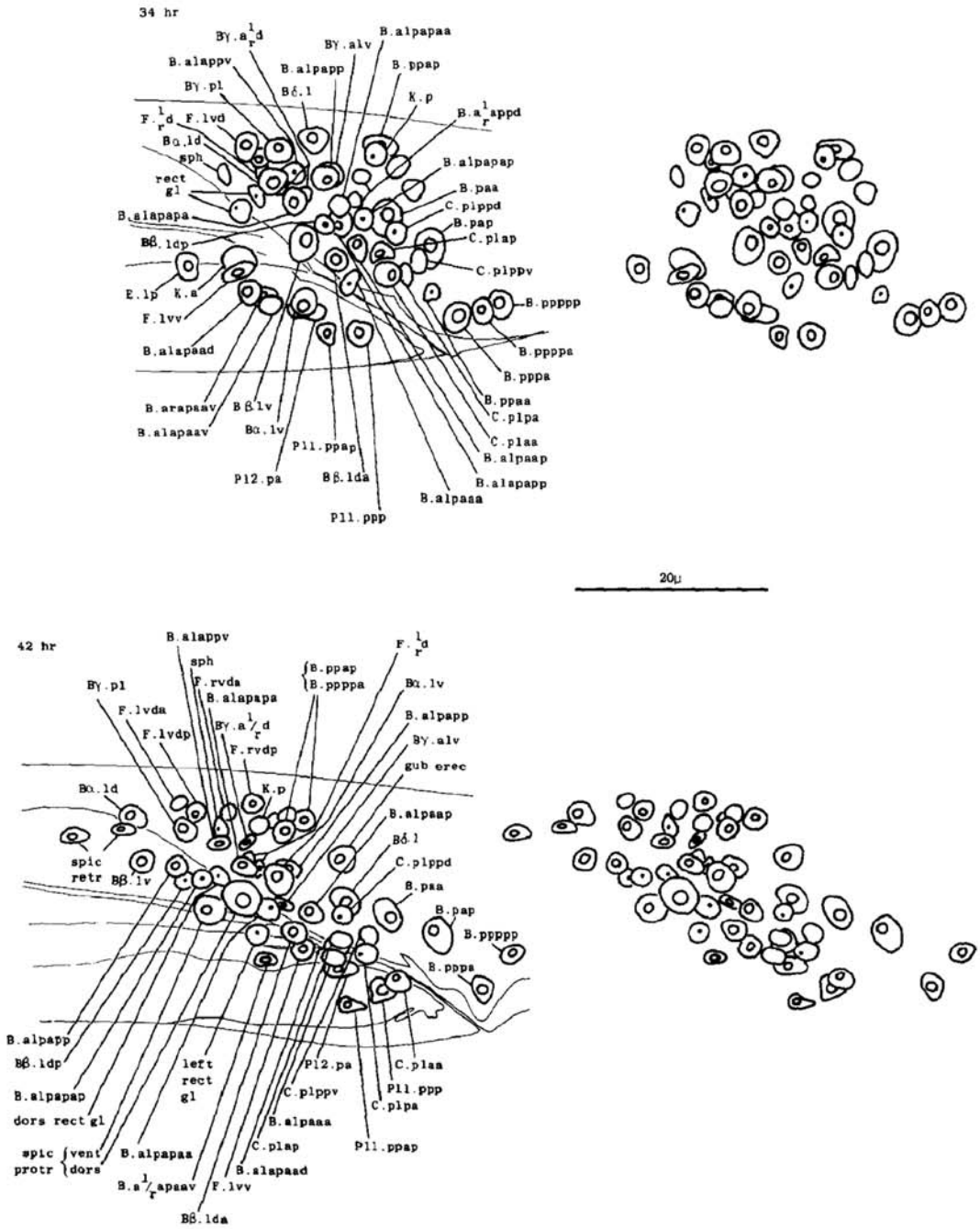

FIG. 7. Arrangement of nuclei in proctodeum, left cloacal ganglion, and dorsorectal ganglion in the L4 stage, left lateral view, stereo pairs. Only left and central nuclei are shown. These three regions are combined in a single drawing, because they were treated as a unit when cells were followed by light microscopy. The positions of the nuclei are largely invariant, especially at the later time, when they have stopped moving; at this time some of the muscle nuclei are also shown in their characteristic positions. (N.B. In Sulston and Horvitz, 1977, Fig. 21, 38 hr: cell pairs B.alpaaa/B.alpaap and Bβ.lda/Bβ.ldp were inadvertently reversed.)

used for the reconstruction of the proctodeum. Because of the invariant arrangement of nuclei, all the cells could be identified without difficulty (Fig. 8).

Asymmetric cell deaths. There are two asymmetric deaths in the B lineage (Fig. 6). They involve the symmetrical pairs of cells Bγ.ald/Bγ.ard and B.alapaav/B.arapaav (shown ⊗ in Fig. 6): in each pair, one member dies and the other survives. Before the

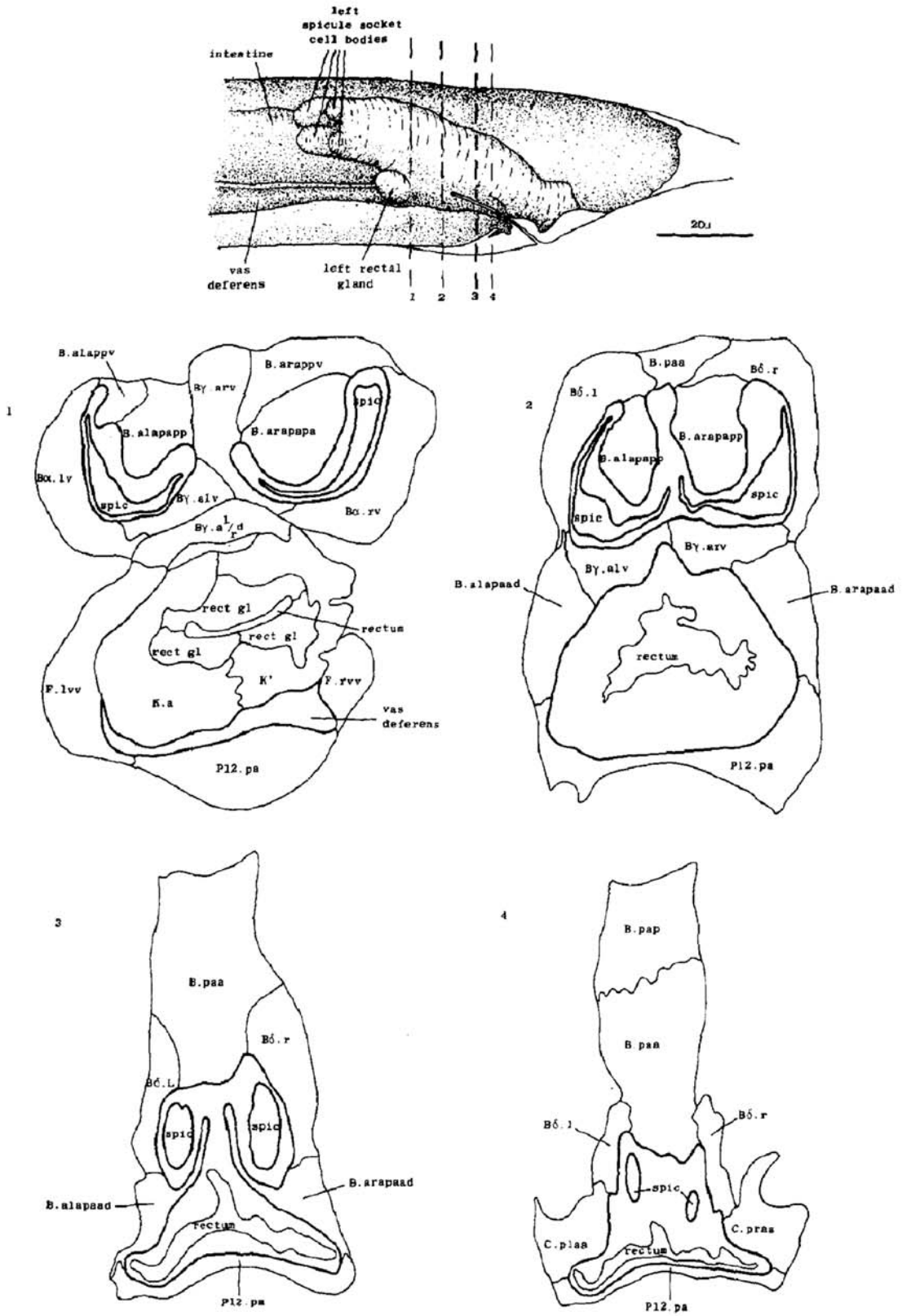

FIG. 8. Arrangement of cells in proctodeum at 42 hr (series 1). Cell outlines were traced from electron micrographs of transverse sections, at levels shown in left-hand perspective view at top. Outlines of the cloaca, spicules, and spicule channels are drawn boldly. At this stage the rectum is simply a tube of L4 cuticle. Scale refers to perspective drawing only.

deaths, the members of each pair lie close together in the midline of the animal; it is therefore likely that the two cells are potentially identical but have to compete for a unique fate.

Light microscopic observation, confirmed by electron micrographic reconstruction of one animal, indicates that B.alapaav or B.arapaav dies after being engulfed by P12.pa. After ablation of P12.pa, both B.alapaav and B.arapaav survive.

The dying member of the pair Bγ.ald/Bγ.ard is often closely associated with F.ld and F.rd; in series 1, F.ld and F.rd were found to be fused together and the dying cell lay within the resulting syncytium. However, ablation of F.ld and F.rd has not always prevented the death of $B\gamma.a^{l}/_{r}d$ and their role remains uncertain at present.

Spicules. The cuticle of each spicule is laid down around a bundle of processes derived from neurons and supporting cells (see under Nervous System) (Fig. 19). The U-shaped profile of the assembly seems to be determined in part by a row of structural cells which protrudes into the channel (Fig. 8).

Gubernaculum. A strip of thick, sclerotic cuticle is laid down in the roof of the proctodeum by the ventral edge of B.paa (with possible contributions from Bδ.l and Bδ.r). This structure is termed the gubernaculum (Chitwood and Chitwood, 1974) (Figs. 2 and 25c); presumably it serves to guide the tips of the spicules ventrally through the cloaca.

Vas deferens. During the L4 stage the gonad grows posteriorly along the ventral side of the body cavity. In the late L4, the most posterior cell of the gonad, the linker cell (Kimble and Hirsh, 1979), passes between E.lp and E.rp (or their fusion products: see under Preanal ganglion); it is engulfed by one of them and eventually dies (Figs. 9 and 10). Meanwhile, cells K.a and K′ grow anteriorly along the outside of the advancing gonad cells and establish contact with them, and $B.a^{l}/_{r}apaav$ meets the gonad ventrally. In the absence of E, K.a and K′ show no affinity for the gonad, and the connection is not made.

Nervous System

Ganglia. The nervous system of the tail

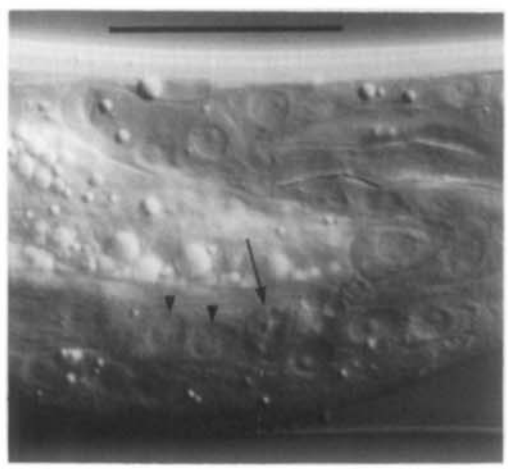

FIG. 10. Dying linker cell (arrowed) engulfed by left-hand killer cell whose nuclei E.lp and $E.^{l}/_{r}a$ are shown by arrowheads. Left-hand view. Bar = 20 μm.

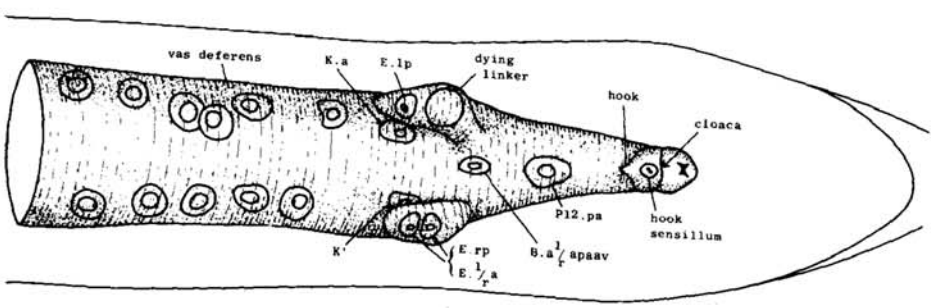

FIG. 9. Connection of vas deferens with cloaca, ventral view, mid L4 lethargus. The linker cell has been withdrawn by E.lp in this individual. Unlabelled nuclei are gonadal in origin. $B.a^{l}/_{r}apaav$ and P12.pa form the ventral side of the posterior vas; K.a and K′ form the lateral and dorsal sides.

can be divided into a series of ganglia (Fig. 11). As in the hermaphrodite, there are a pair of lumbar ganglia, a dorsorectal ganglion and a preanal ganglion; all of them contain more cell bodies in the male than they do in the hermaphrodite. In addition, the male has two groups of cell bodies, one on either side of the proctodeum, which we have named the cloacal ganglia. Most of the neuropil is found in the preanal ganglion, to which the other ganglia are connected by a series of commissures.

Sensilla. In describing the sensilla of the male tail, we shall employ the terminology which Ward *et al.* (1975) used for the sensilla of the head. The latter are present at hatching, and each of them comprises one or more neurons surrounded by two nonneuronal cells, the sheath and the socket. The sheath cell envelops the neurons, to which it is connected by desmosomes. The socket cell encircles the tips of the neurons; it is connected by desmosomes both to the hypodermis and to the sheath cell, but not to the neurons. In contrast, the tail sensilla of the male vary in the number of nonneuronal cells associated with them. They comprise a pair of phasmids (the only tail sensilla in the hermaphrodite), nine pairs of sensory rays, a pair of postcloacal sensilla, a pair of copulatory spicules, and a single sensillum in the sclerotic hook anterior to the cloaca. Their locations are shown in Fig. 1.

Lumbar ganglia. The lumbar ganglia (Figs. 11 and 12) contain the neurons and supporting cells of the phasmids and the rays. All the cells which are found in the hermaphrodite are also present in the male, and most of them appear to be morphologically similar in the two sexes. However, explicit lineage correlations have not been attempted for this group of cells in the male, and detailed consideration of them will be deferred to another publication.

The only neurons which do differ substantially in the male are the pair T.pppaa; they retain all the synapses which they possess in the hermaphrodite, and can thereby be recognised, but have many additional ones as well. The supporting cells of the phasmid also differ in the male, as described below.

Phasmids. At the L2 stage, the paired phasmids (Figs. 1 and 13) are identical in the male and the hermaphrodite, each having two neurons, a sheath cell, and two cells which jointly perform the "socket" function. One of these cells, PHso1, wraps around the tips of the neurons in the usual way; it is connected by desmosomes to the sheath and the second cell, PHso2, but not to the hypodermis. PHso2 is connected to PHso1 and the hypodermis, but not to the sheath.

Reconstruction of a 5-hr-old hermaphrodite revealed no discrete socket cell; instead, the phasmid opened to the outside through seam cell T. It is interesting that the latter meets the definition of a true

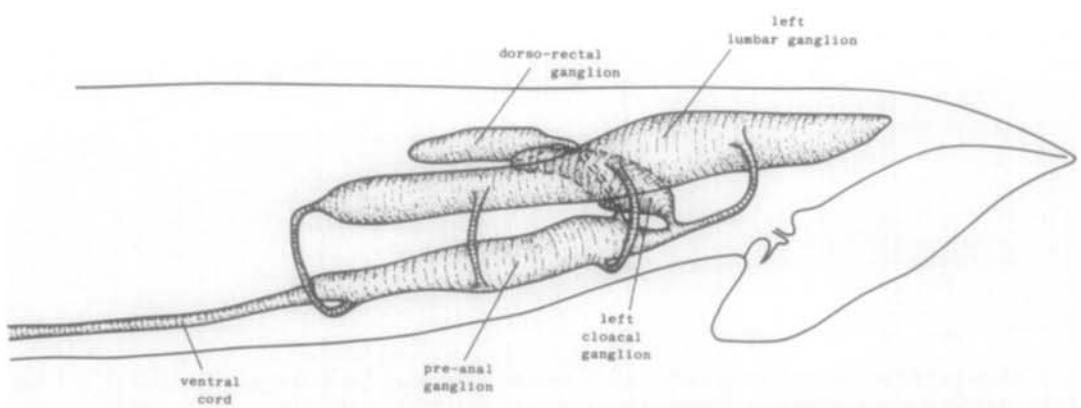

FIG. 11. Arrangement of left and central ganglia and principal nerve trunks: left-hand view.

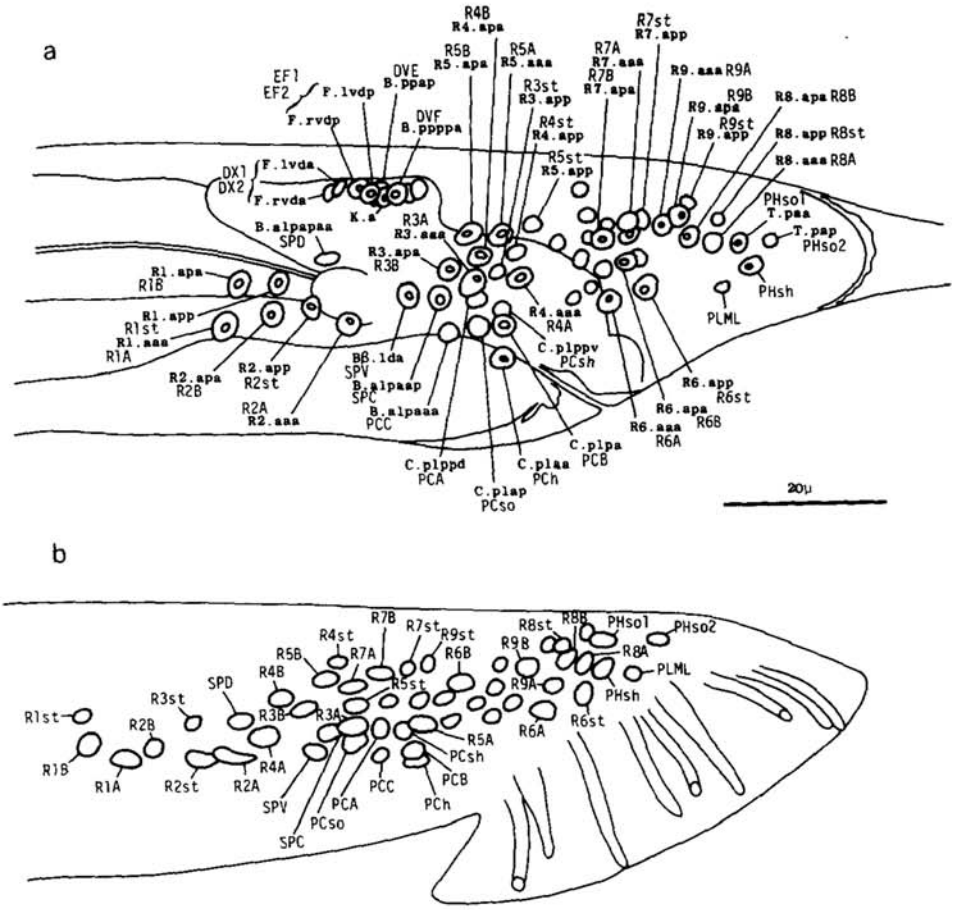

FIG. 12. (a) Typical arrangement of nuclei in left lumbar, left cloacal, and dorsorectal ganglion; left lateral view; late L4 (42 hr). Unmarked nuclei belong to juvenile or hermaphrodite groups. Dorsorectal ganglion is compact group of cells at dorsal edge of proctodeum. (b) Typical arrangement of nuclei in left lumbar and left cloacal ganglion; left lateral view; young adult. Positions in cloacal ganglion are essentially invariant at this stage; those in remainder of lumbar ganglion are variable.

socket cell, being attached by desmosomes both to the sheath and to the syncytial hypodermis. T is itself the ancestor of PHso1 and PHso2 (Fig. 17). Probably the phasmid has the same structure in the L1 male as it does in the hermaphrodite.

In adults, the phasmid of the male differs from that of the hermaphrodite. In the latter, PHso2 retains only a tenuous process that wraps around PHso1, and the latter is the principal socket cell. In the male, on the other hand, PHso2 is a true socket cell; PHso1 protrudes into the sheath, to which it is still attached by a desmosome, and in different animals it contains zero, one, or two basal bodies in its ensheathed part (Fig. 14). No neuronal characteristics of PHso1, other than the possession of basal bodies, have been observed.

Sensory rays. Each of the 18 rays comprises two neurons (RnA and RnB) and one structural cell (Rnst). The latter is attached both to the neurons and to the hypodermis by desmosomes; thus it appears to function as both sheath and socket cell to the ray neurons.

Except for ray 6, all the rays are very similar in structure. The two neurons can be distinguished on the basis of the ultrastructure of their endings, as illustrated in Fig. 15; in addition, the RnB neuron has a distinctive cell body, which is large and has dilated cisternae in the adult (Fig. 16). The tip of neuron B is surrounded by a small

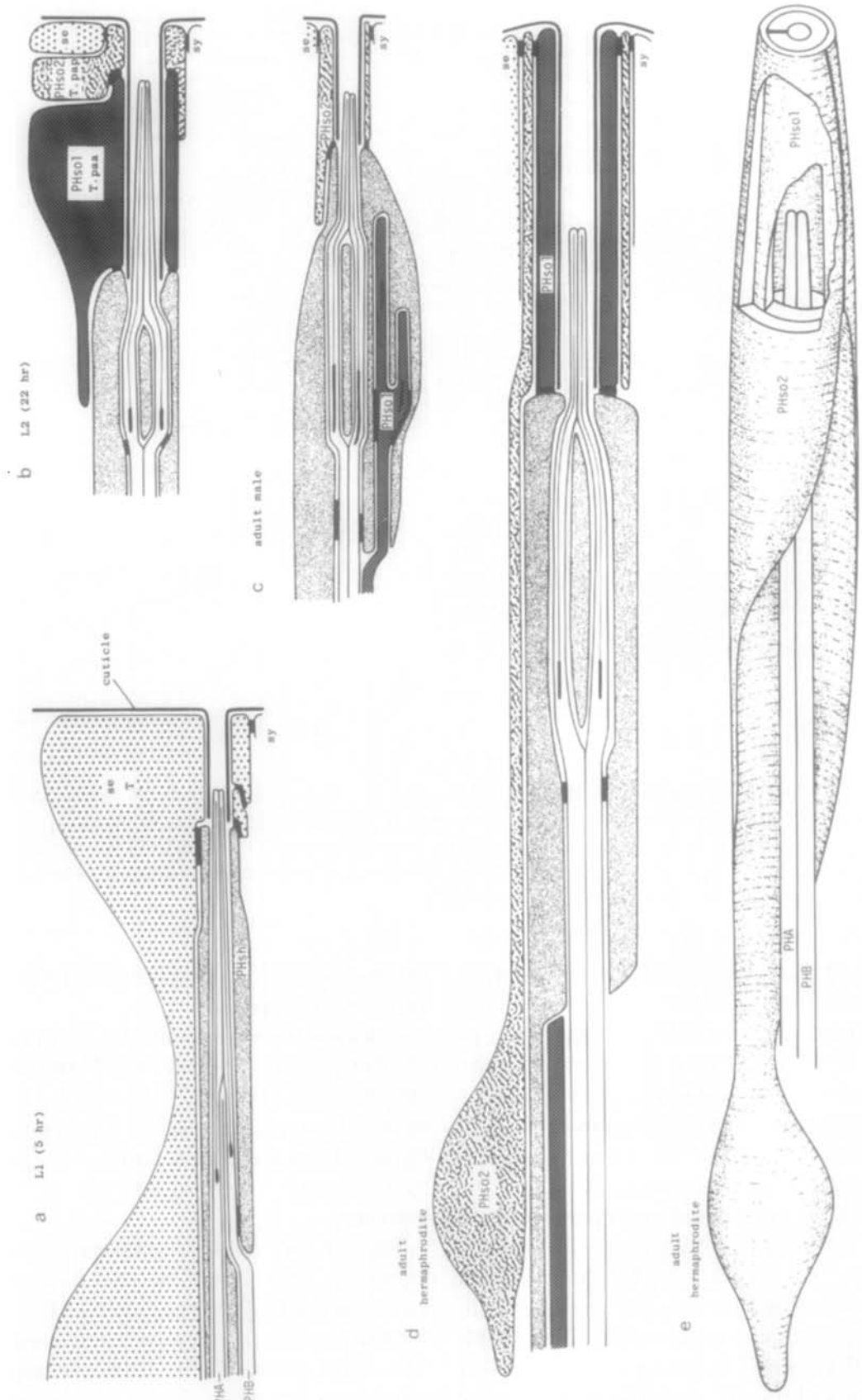
a L1 (5 hr)
b L2 (22 hr)
c adult male
d adult hermaphrodite
e adult hermaphrodite
cuticle
se
sy
T
PHsh
PHA
PHB
PHso1
PHso2
T.paa

FIG. 13

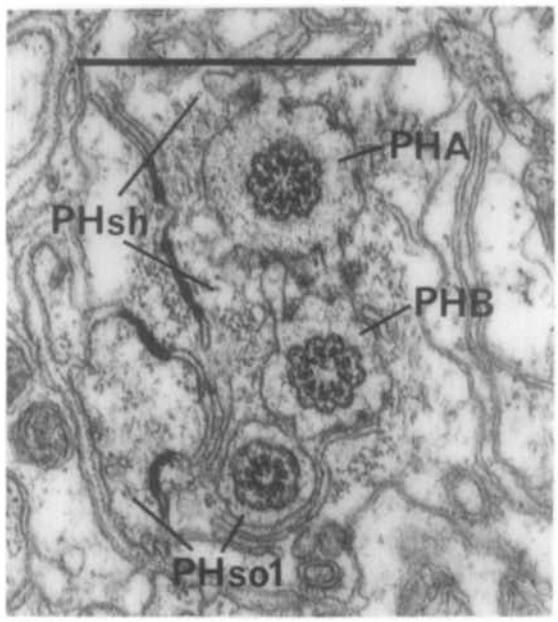

FIG. 14. Adult male phasmid, showing basal bodies in neurons (PHA, PHB) and socket cell (PHso1). Electron micrograph of transverse section, series 5. Bar = 1 μm.

cylinder of cuticle (Fig. 15, ts1). In ray 6, neuron A resembles the other RnA's; neuron B differs from other RnB's in not having a dark tip, in not obviously reaching the exterior, and in having a compact cell body. By Nomarski optics, the tip of ray 6 never displays the refractile ring and dot characteristic of the other rays (Fig. 3b and d).

The three cells which generate a given ray are formed from the anterior daughter of a single ray precursor cell Rn (Fig. 17). The posterior daughter, Rn.p, is a hypodermal cell which fuses either with the large hypodermal syncytium (sy) or with other ray hypodermal cells to form the tail seam (set); the fate of the hypodermal cell from a particular ray precursor cell is invariant.

In the animal chosen for series 1 most of the ray cells were followed until fixation at the beginning of L4 lethargus, when the developing rays could be seen (Fig. 3); all the rays appeared normal, except for left ray 3, which was missing. Subsequent electron microscopic reconstruction revealed that with this exception Rn.app became the structural cell. This assignment is consistent with the results of laser ablation experiments (Sulston and Horvitz, 1977) which showed that Rn.app is required for ray formation. The absence of left ray 3 was due to the failure of R3.app to form a process, although the neurons were completely differentiated. The loss of a ray is a common occurrence: in a random sample of adults, about half are defective in this way. Usually, the missing ray is 3, 8, or 9. Of the various nonlineaged animals which were reconstructed by electron microscopy, one lacked left ray 3 and another lacked right ray 9; in neither case could the corresponding structural cell be found, although the neurons were present and differentiated in the usual way. Perhaps the structural cells failed to form processes, like left R3.app in series 1, and were swept away by the movement of other cells during the L4 moult.

Cells Rn.aaa and Rn.apa were identifiable as neurons in series 1, but none of them had formed striated rootlets. However, by using the other criteria listed in Fig. 15, it was possible to distinguish between them in most cases; the results are shown in Table 2. It is very difficult to trace a reasonable number of ray cell bodies through L4 lethargus, on account of the rapid and extensive cell movements which take place. Therefore, we confirmed the assignments for Rn.aaa and Rn.apa by the following experiment.

The ray lineages of an animal were fol-

FIG. 13. Development of the phasmid; diagrammatic longitudinal sections. (a) 5 hr after hatching. Seam cell T has not completed cytokinesis of the first division. PHA, PHB, phasmid neurons containing basal bodies. (b) Late L2. Hermaphrodite and male phasmids are identical. T.paa wraps around the phasmidial channel; it is attached to itself, the sheath, and T.pap by desmosomes. T.pap is attached in turn to the seam (se) and syncytial (sy) hypodermis. (c) Adult male. The socket is PHso2 (T.pap). (d) Adult hermaphrodite. PHsol (T.paa) and PHso2 (T.pap) jointly perform the socket function, although PHso2 has become a thin sheet that encircles PHsol. (e) Adult hermaphrodite. The sheath and part of PHso2 have been removed to display PHso1.

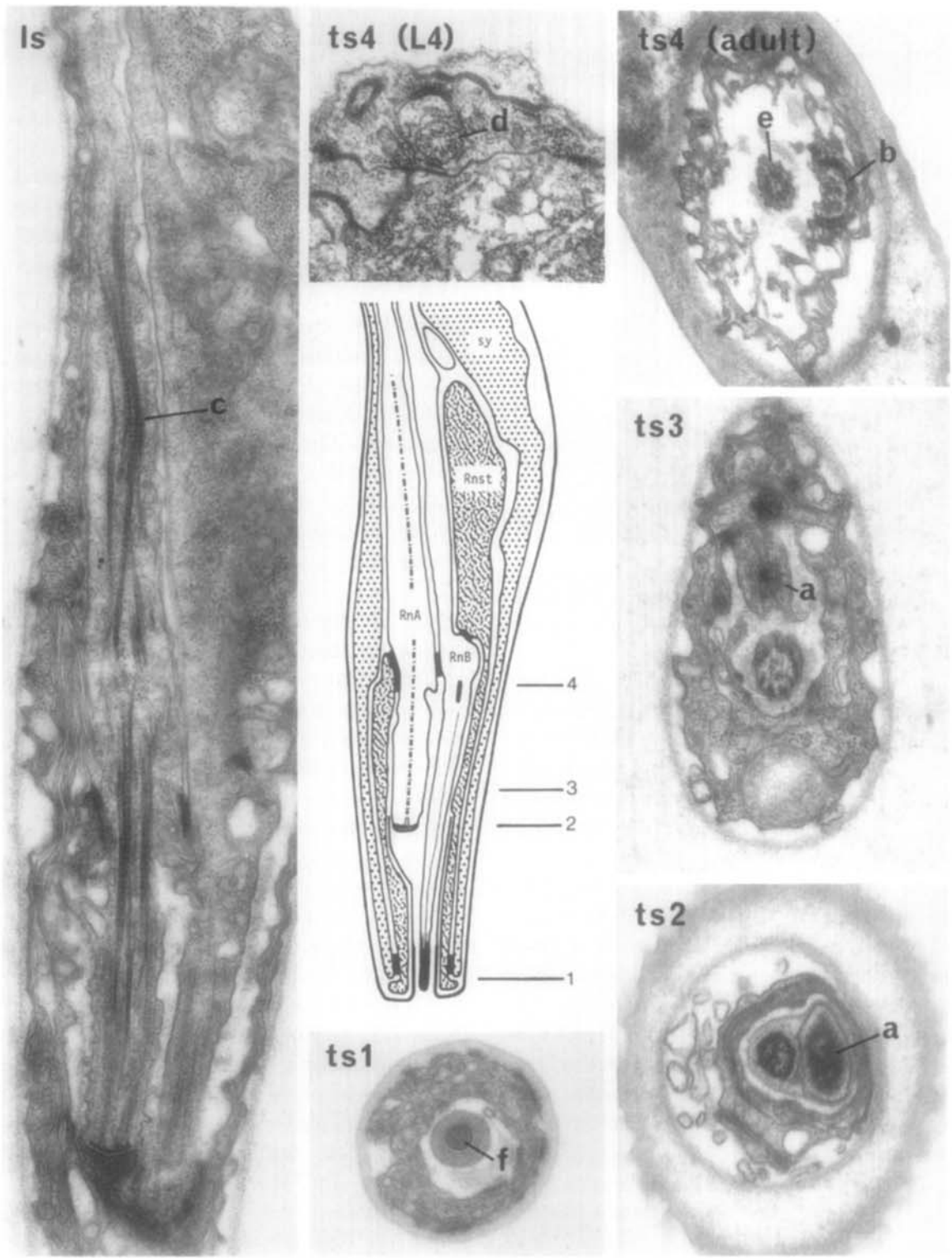

FIG. 15. The central drawing represents a longitudinal section through a sensory ray (other than ray 6). The electron micrographs (× 30,000) illustrate the diagnostic criteria (a–f) that were used to distinguish between the neurons (RnA and RnB). ls, longitudinal section; ts 1–4, transverse sections at levels 1–4; Rnst, ray structural cell. RnA has a long striated rootlet (c); it has no distinct basal body, but the broad tip contains darkly staining material which is arranged in a diffuse ring at slightly greater depth (a). RnB projects to the exterior, and has a narrow darkly staining tip (f); it contains a distinct basal body, which is particularly clear in young animals (d). RnA typically lies to one side of the channel, and forms desmosomes to the structural cell more distally than does RnB (b); RnB lies centrally in the channel (e).

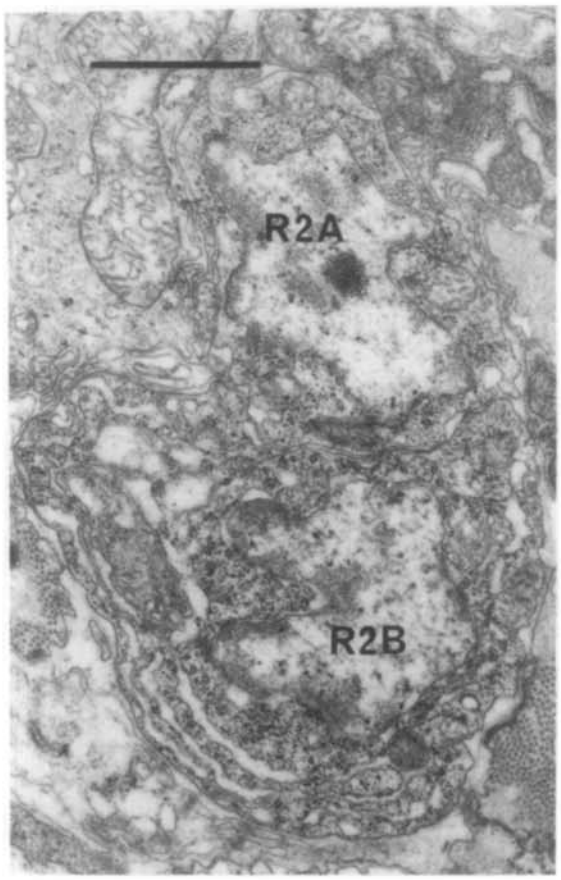

FIG. 16. Neuronal cell bodies of sensory ray 2, right side. R2B is larger than R2A, and the cytoplasm contains dilated cisternae. Electron micrograph of transverse section, series 4. Bar = 1 μm.

lowed until about 1 hr after the final divisions. All nine Rn.aaa cells were then ablated on the right side (by means of the laser microbeam), and all nine Rn.apa cells were ablated on the left; a minority of other cells were killed accidentally, but care was taken to ensure that none of the desired targets survived. Electron microscopical reconstruction of the resulting adult gave the results shown in Table 3. Five cells on the left formed striated rootlets, which are strong criteria for RnA neurons, while five on the right formed distinct basal bodies, indicative of RnB neurons. With the exception of one cell on the right there were no swollen cell bodies, but this may simply reflect reduced synthetic activity under the conditions of the experiment. We conclude that Rn.aaa cells develop into RnA neurons, and Rn.apa cells into RnB neurons.

Cloacal ganglia, postcloacal sensilla, and spicules. The cloacal ganglia contain the neurons and structural cells of the postcloacal sensilla and the neurons associated with the spicules. The correlation between lineage and cell type was carried out in two L4 lethargus animals (series 1 and 2) using the invariant position of the cell bodies as seen in the light microscope at this stage and electron microscopic reconstruction of the cell projections (Fig. 6).

With the exception of PCC (B.a^{1}/$_{r}$ paaa) the cells of the postcloacal sensillum (Fig. 18) are derived from blast cell C (Fig. 6). PCC and its sister SPC (see below) are morphologically similar in possessing a striated rootlet but lacking a basal body.

The cells of the spicule (Fig. 19) are derived from blast cell B (Fig. 6). In the adult the four socket cells become syncytial, as do the two sheaths. Associated with each spicule there is a motor neuron, (not shown in Fig. 19), which innervates the spicule protractor muscles. This neuron (SPC) also has an apparently sensory ending, containing a striated rootlet; the sensory ending is attached by half desmosomes to the muscle over the base of the spicule, and may be proprioceptive in function.

Dorsorectal ganglion. The dorsorectal ganglion of the male contains, in addition to cells found in the hermaphrodite, neurons from the B and F lineages (Figs. 6, 7, and 12). The positions of the cell bodies are variable and cannot be deduced without following the cell lineages in the light microscope. This was done for one animal (series 1).

The neurons derived from F, whose cell bodies lie in the dorsorectal ganglion, are very similar to those derived from E, whose cell bodies lie in the preanal ganglion (see below). For this reason the two groups will be described together.

F.$_{r}$^{1}vda and E.$_{r}$^{1}aa form a distinctive class, termed DX. They have small, darkly staining cell bodies that send processes along the dorsal edge of the proctodeum and

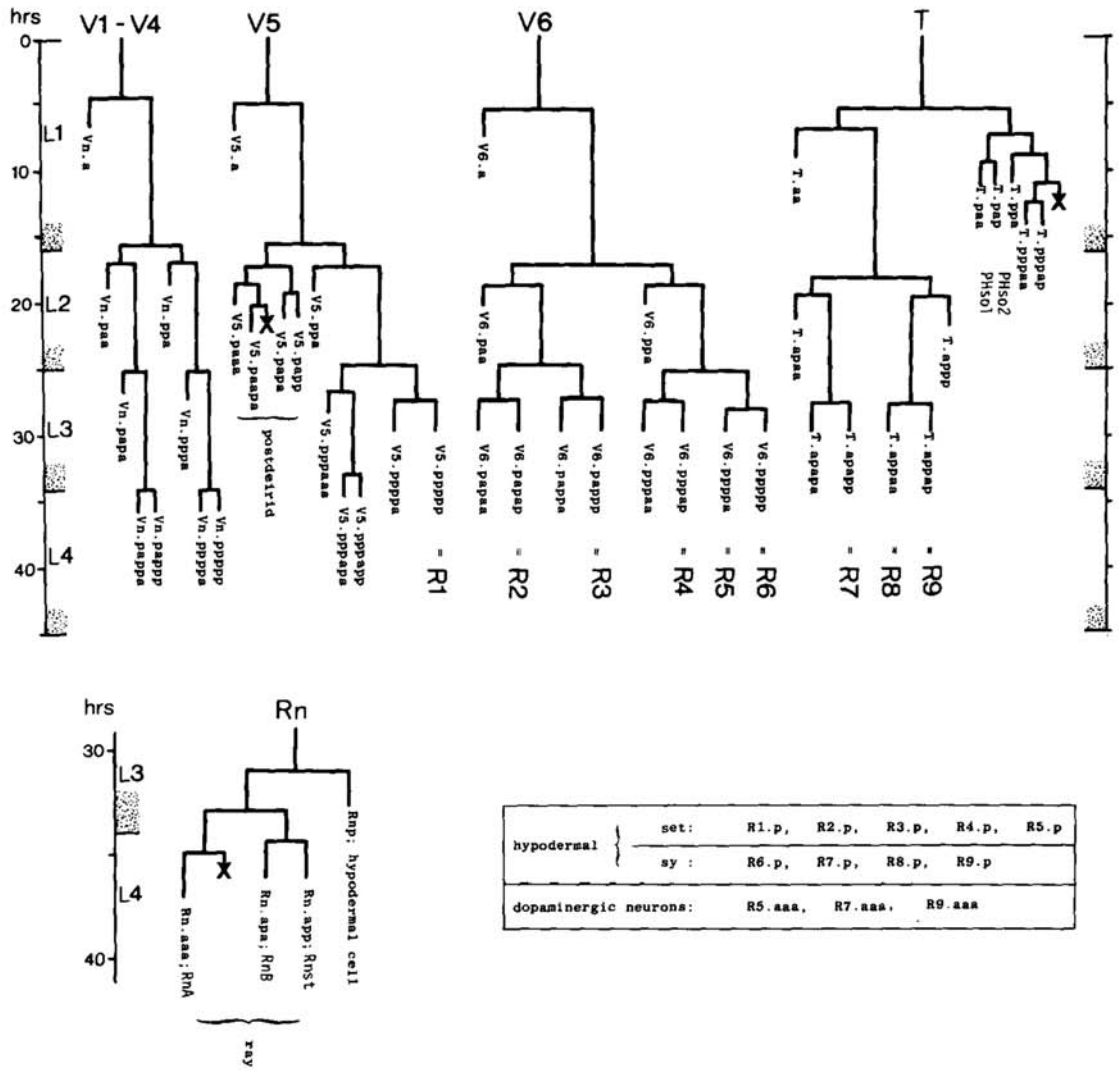

FIG. 17. Cell lineages of sensory rays and postdeirid on either side of the animal. In order to clarify the relationships among the ray cells, the ray precursor cells have been redesignated R1–R9.

through the basement membrane surrounding it to make electrical junctions with dorsal body muscles (Fig. 20); the processes sometimes also penetrate into the dorsal cord and run anteriorly. Neurons of no other class have been seen to penetrate basement membranes (White and Albertson, unpublished results).

$F._r{}^1$vdp and $E._r{}^1$ap form another class, termed EF. These large cells receive synaptic inputs from the rays in the preanal ganglion, and run together anteriorly at the dorsal left corner of the ventral cord; they may continue into the circumpharyngeal nerve ring. However, because they are only partially reconstructed, we cannot be as confident of their assignment to a single class as we are for their sister cells.

The similarity between E and F which is seen in the fates of their progeny extends to their lineages as well (Fig. 6).

Two neurons from the B lineage, B.ppap and B.ppppa, also have cell bodies in the dorsorectal ganglion. In series 1 a process from B.ppap enters the preanal ganglion on the right, while one from B.ppppa enters on the left. In series 4 two processes from the dorsorectal ganglion, tentatively identified as B.ppap and B.ppppa, both contain large vesicles. Provisionally the cell entering the

TABLE 2

CORRELATION BETWEEN ANCESTRY AND MORPHOLOGY OF RAY NEURONS IN SERIES 1 (42 hr)

Ancestry		Electron microscopic criteria[a]						Assignment
		RnA			RnB			
R9.aaa	left		b					R9A
	right							
R8.aaa	left	a	b					R8A
	right	a	b					R8A
R7.aaa	left	a	b					R7A
	right	a	b					R7A
R5.aaa	left	a	b					R5A
	right	a	b					R5A
R4.aaa	left							
	right		b					R4A
R9.apa	left							
	right							
R8.apa	left			d	e	f		R8B
	right			d	e	f		R8B
R7.apa	left			d	e	f		R7B
	right			d				R7B
R5.apa	left							
	right			d				R5B
R4.apa	left				e	f		R4B
	right			d		f		R4B

[a] Electron microscopic criteria are illustrated in Fig. 15. Striated rootlets (criterion c) have not developed at this age.

TABLE 3

CORRELATION BETWEEN ANCESTRY AND MORPHOLOGY OF RAY NEURONS IN YOUNG ADULT AFTER SELECTIVE CELL ABLATIONS[a]

Ancestry	Electron microscopic criteria						Assignment
	RnA			RnB			
Left							
R9.aaa		c					R9A
R6.aaa	a	c					R6A
R3.aaa		c					R3A
R2.aaa	a	c					R2A
R1.aaa		c					R1A
Right							
R9.apa				d			R9B
R8.apa				d			R8B
R7.apa				d			R7B
R4.apa				d		f	R4B
R3.apa				d			R3B

[a] See text.

preanal ganglion on the right has been called DVE (B.ppap) and that entering on the left DVF (B.ppppa).

Preanal ganglion. The preanal ganglion is the group of neurons and supporting cells which lie just anterior to the anus (or cloaca in the mature male); because the ganglion includes motor neurons of the classes found also in the ventral cord, its anterior boundary is somewhat arbitrary (Fig. 21).

At hatching, the ganglion comprises six neurons. During the L1 stage, divisions of P11 and P12 contribute motor neurons of the ventral cord types (Fig. 22); the male differs from the hermaphrodite in that P11.aap and P12.aap survive. The male also differs in the division of C (Fig. 6) whose posterior daughter forms the cells of the postcloacal sensilla (q.v.); in the hermaphrodite C does not divide, but differentiates into a neuron which resembles the anterior daughter of C in the male. The neuron is designated PDA in both sexes.

E is a cell which lies near the anus at hatching and is vestigial in the hermaphrodite. In the male E divides transversely and each of its daughters divides anteroposteriorly. At L3 lethargus either one, or occasionally both, of the anterior daughters migrates forwards into the preanal ganglion and there divides into a pair of neurons (described above under Dorsorectal ganglion). Anterior daughters which fail to move forwards do not divide, and usually fuse with one of the posterior daughters; one of the latter, whether fused or not, kills the gonadal linker cell (see above).

In the L3, P10.p moves posteriorly into the preanal ganglion. Both it and P11.p divide repeatedly and contribute neurons, supporting cells, and ventral hypodermal cells to the ganglion.

The morphology of the cells was studied initially in series 4. Cell assignments were made in series 3 (young adult stage) after the preanal ganglion cells had been traced from hatching. At early L4 lethargus most of the cells are sufficiently well defined in position and morphology to allow compar-

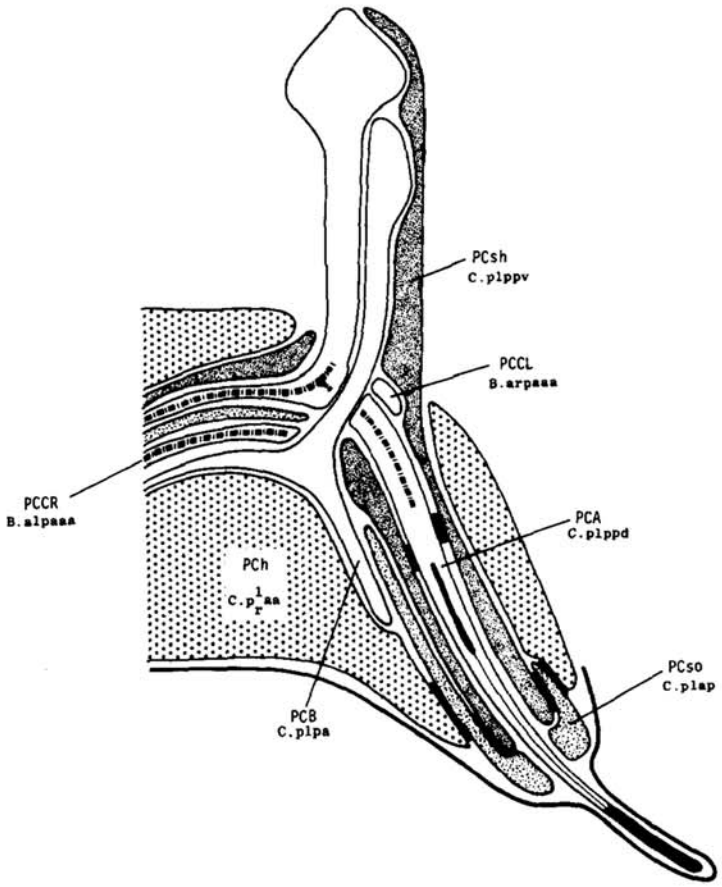

FIG. 18. Diagrammatic longitudinal section through the left postcloacal sensillum. The sensory neuron PCA has a short striated rootlet, a basal body, and a dense ending under the cuticle; its dorsal branch projects medially, sometimes crossing to the contralateral nerve cord and running anteriorly. PCB, which also has a dorsal branch, ends in the sheath cell (PCsh). The third neuron, PCC, originates contralaterally and terminates in PCsh; it has a long (7 μm) striated rootlet that ends at the midline, but lacks a basal body. The socket cell, PCso, is attached by desmosomes to PCsh and to the hypodermal cell PCh. The latter is the fusion product of C.plaa and C.praa.

ison between the lineage and fine structure of different individuals; on this basis, the assignments of these fixed cells were found to be identical in series 1. In addition, laser ablation experiments have shown that P10.papp, which is one of the mobile cells, invariably generates the hook (Fig. 1) (see following paper).

Some of the cells are elements of the hook sensillum (Fig. 23) and can be characterised by the morphology of their endings in it. However, many of the neurons can be defined uniquely only in terms of their processes and synaptic contacts (Fig. 24). Cells not shown in these figures are similar in the two sexes, and will be described in detail elsewhere.

Ventral cord. The ventral cord contains more cell bodies in the male than it does in the hermaphrodite, because of the increased survival of Pn.aap cells and the subsequent division of most of them (Fig. 22). The male cord has not yet been fully reconstructed, but we have preliminary information about the posterior half. The additional cell bodies can be recognised by their position to the left of the older ones, and by comparison of the ultrastructure of the male cord with that of the hermaphrodite (White *et al.*, 1976). In addition, those Pn.aap derivatives which lie in the preanal ganglion were positively identified in series 3.

The daughters of P7.aap and P8.aap are

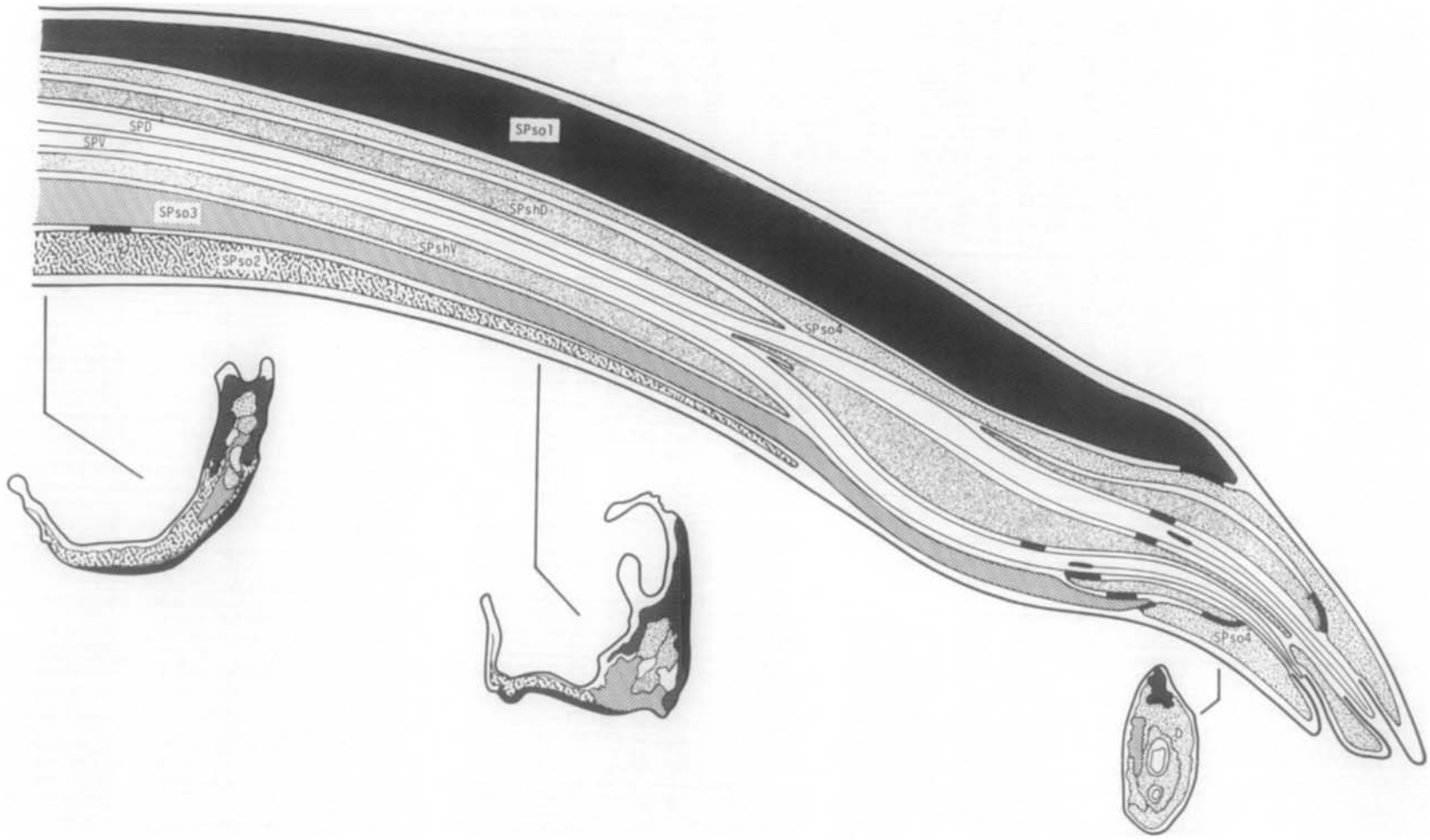

FIG. 19. Diagrammatic longitudinal section through spicule of a late L4 male (42 hr, series 1). Tracings of electron micrographs of transverse sections at three levels are included to show the detailed arrangement of the cells. The two ciliated neurons (SPD and SPV) open to the exterior. The two sheaths (SPshD and SPshV) have already begun to fuse at their tips.

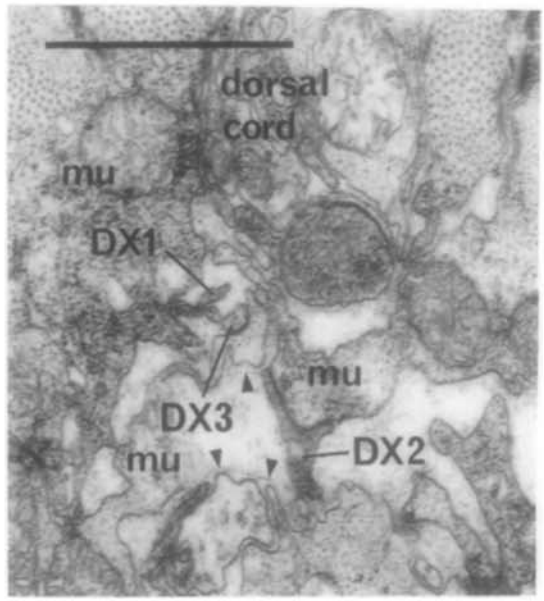

FIG. 20. Neurons of DX class passing through basement membranes (arrowheads) and forming electrical junctions with muscle cells (mu). Electron micrograph of transverse section, series 4. Bar = 1 μm.

all motor neurons. The posterior daughters of P9.aap, P10.aap, and P11.aap are interneurons with projections into the preanal ganglion. The anterior daughters of P9.aap, P10.aap, and P11.aap are neuron-like, but their processes are limited in extent and apparently devoid of synapses. P12.aap, which does not divide, is an interneuron of a different type.

The discontinuity in cell fate between P8.aap and P9.aap is interesting, because it parallels a change in the behaviour of the ventral hypodermal cells (see following paper). Furthermore, the hermaphrodite displays a discontinuity at the same point in the behaviour of ventral hypodermal cells and in the survival of Pn.aap cells (Sulston and Horvitz, 1977).

Mesoderm

The tail mesoderm is shown in Fig. 25. All the muscle cells found in this region of the hermaphrodite are also present in the male: namely, the sphincter, the anal depressor, the intestinal muscles (not drawn), and the body wall muscles. In addition, 41 male sex muscles and a coelomocyte are formed during the L4 stage (Figs. 26 and 27).

The following account is based on complete lineages of left muscle in two animals and right muscle in two different animals, together with numerous incomplete lineages.

The three pairs of sex mesoblasts migrate posteriorly during the L3 stage, and at about 34 hr begin to divide. In order to simplify description, the three mesoblasts on each side will be called SM1, SM2, and SM3 (Figs. 26 and 27). The divisions are complete by L3 ecdysis, but the migrations continue for several hours.

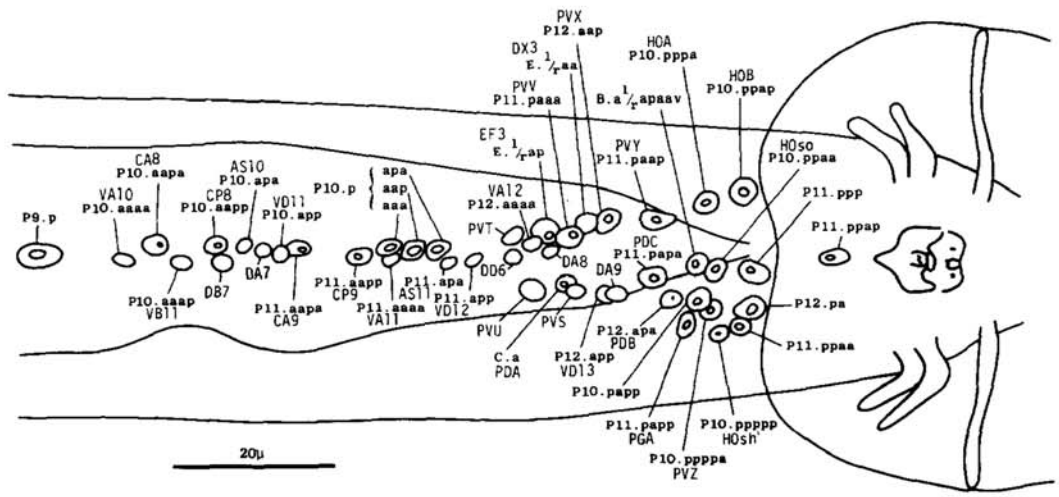

FIG. 21. Typical arrangement of nuclei in adult preanal ganglion and part of ventral cord. Ventral view. The preanal ganglion is considered to include P11.aapa and cells posterior to it.

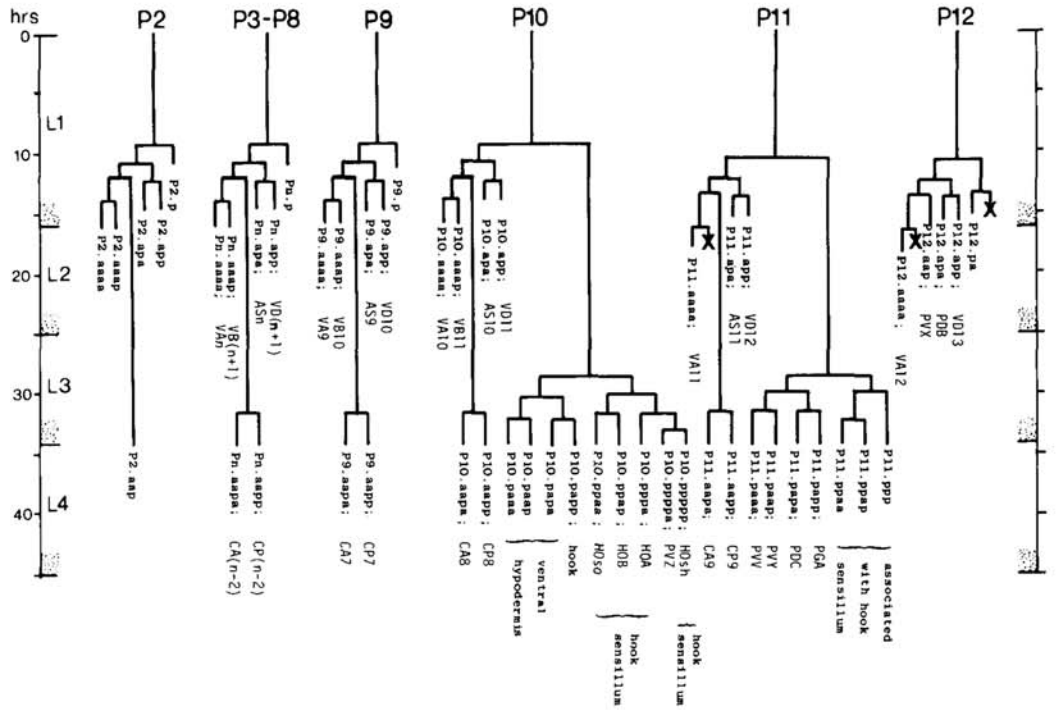

FIG. 22. Lineages of ventral cord precursor cells.

Symmetrical assignments. Most of the cells behave in the same way on both sides. SM2.apa and SM2.app move posteriorly past the cloaca, becoming the gubernacular retractor and anterior oblique muscles, respectively; SM1.aaa becomes the ventral spicule protractor. Some progeny of SM1 and SM2 become diagonal muscles, and do not have a rigidly defined arrangement.

Five of the progeny of SM3 become ventral longitudinal muscles, which move into particular positions next to the existing body muscle (Fig. 25). The sixth cell becomes the dorsal spicule protractor.

Asymmetrical assignments. At 31 hr (Fig. 27) three cells—SM2.pp, SM2.pa, and SM1.pp—lie close together dorsally. As the final round of divisions proceeds, SM2.pp and the daughters of SM1.pp and SM2.pa behave differently on the two sides.

On the left, the most posterior cell—SM2.pp—moves to the midline and then anteriorly, ultimately differentiating into the lone dorsal coelomocyte. The next cell—SM2.pap—migrates posteriorly and settles dorsal to the cloaca; it is followed and passed by SM2.paa, which continues to move over the posterior surface of the cloaca to a more ventral position. The two cells differentiate into the gubernacular erector and posterior oblique muscles, respectively. SM1.ppp becomes the dorsal spicule retractor, and SM1.ppa the ventral spicule retractor.

On the right, SM2.pp does not become a coelomocyte; instead, it migrates posteriorly and becomes the gubernacular erector. Similarly, each of the next three cells takes on the role which its anterior neighbour would play on the left: SM2.pap becomes the posterior oblique muscle, SM2.paa the dorsal spicule retractor, and SM1.ppp the ventral spicule retractor. The remaining cell—SM1.paa—becomes a diagonal muscle; thus the right side has one more diagonal muscle than the left side.

On both sides, but particularly on the right, the putative ventral spicule retractor makes a considerable ventral excursion before returning dorsally. This behaviour sug-

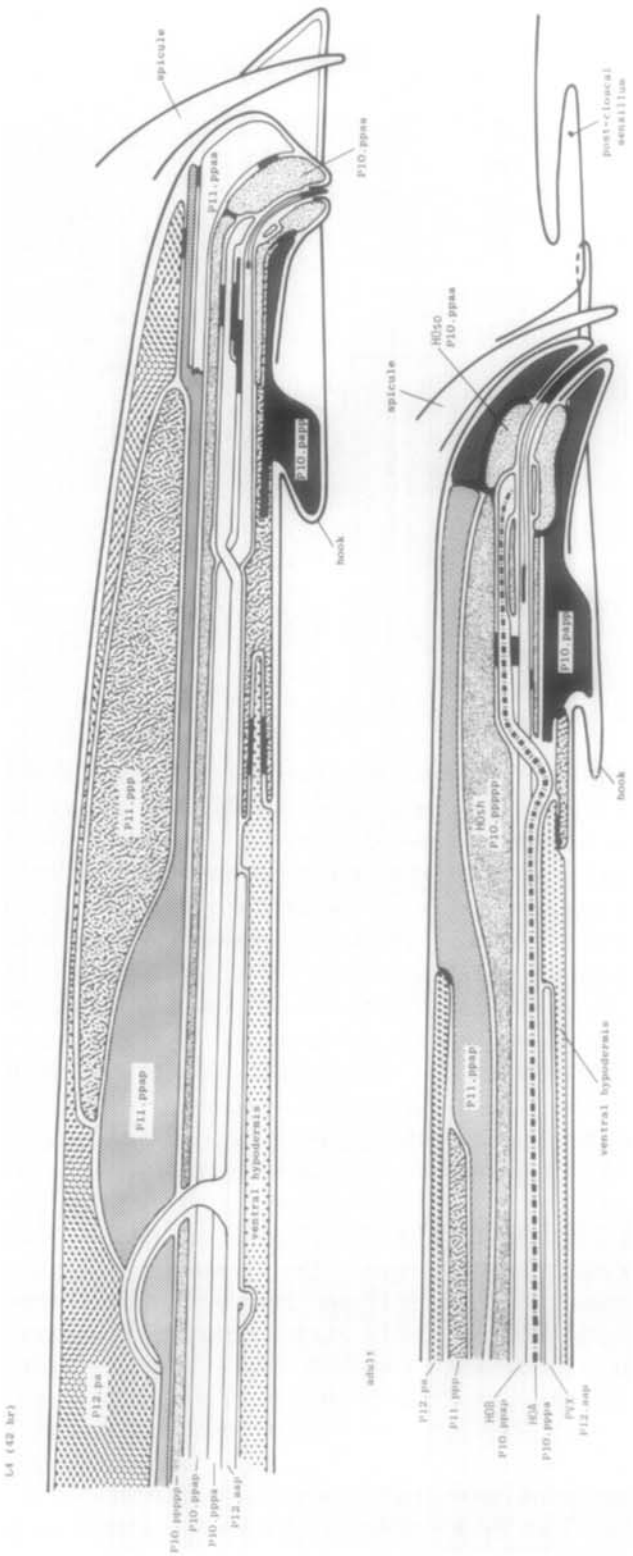

FIG. 23. Diagrammatic longitudinal section through the hook sensillum and associated cells. The two sensory neurons are similar to the ray neurons (Fig. 15), but they are supported by both a socket and a sheath cell. The posterior projection of P12.aap is associated with the neurons: the process runs on their ventral side, and then loops dorsally, passing between P12.pa and P11.ppap (shown in L4 only). The rearrangement of the structural cells in late L4 lethargus is part of the general reorganisation of the preanal ganglion. The cell bodies of P11.ppap and P11.ppp change places. The tips of P10.ppaa and P11.ppaa retreat from their initial hypodermal positions, the latter moving so far that it does not appear in the second figure.

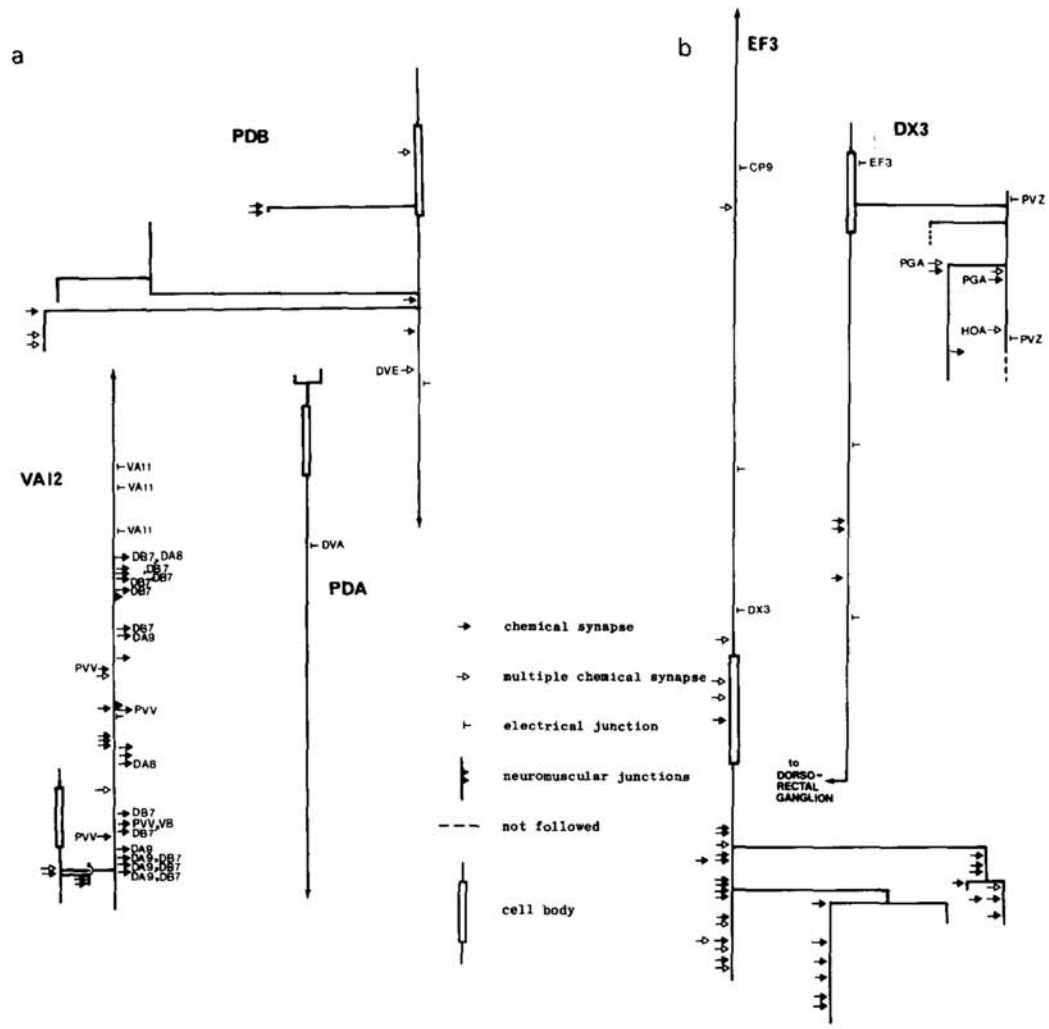

FIG. 24. Synaptic maps of neurons in the preanal ganglion. Top is anterior. (a) Cells present in males and hermaphrodites. PDB is modified in the adult male by becoming highly branched in the preanal ganglion. In both males and hermaphrodites its axon runs subventrally on the right to the posterior end of the animal and then runs anteriorly in the dorsal cord. PDA is a dorsal motor neuron and appears similar in the two sexes; however, in the hermaphrodite it is cell C, whilst in the male it is the anterior daughter of C. VA12 is also similar in the two sexes, although it is modified with respect to other VA neurons: few neuromuscular junctions are made, and chemical synapses to DAn and DB7 are frequent. (b) Derivatives of E. EF3 is a large cell that is postsynaptic and branched in the preanal ganglion. It runs anteriorly at the dorsal left edge of the ventral cord. DX3 has a small, darkly staining cell body; its posterior process characteristically runs over the cell body of PVZ and enters the dorsorectal ganglion from the left.

gests that it has some tendency to become a diagonal muscle, and indeed the loss of a spicule retractor in this way is a common occurrence after cell ablations (see following paper).

Morphogenesis. In addition to their role as muscles in the adult, some of these cells play a part in morphogenesis of the proctodeum. If the mesoblast (M) is destroyed in a young L1, the proctodeum develops normally until L4 lethargus but then fails to elongate anterodorsally. The result is a compressed structure containing crumpled spicules and gubernaculum. In series 1 the proctodeal structural cells B.alappv and B.arappv form specialised contacts with the dorsal spicule retractors, a finger from the structural cell being inserted into the muscle (Fig. 28); there are also darkenings of the cell membranes

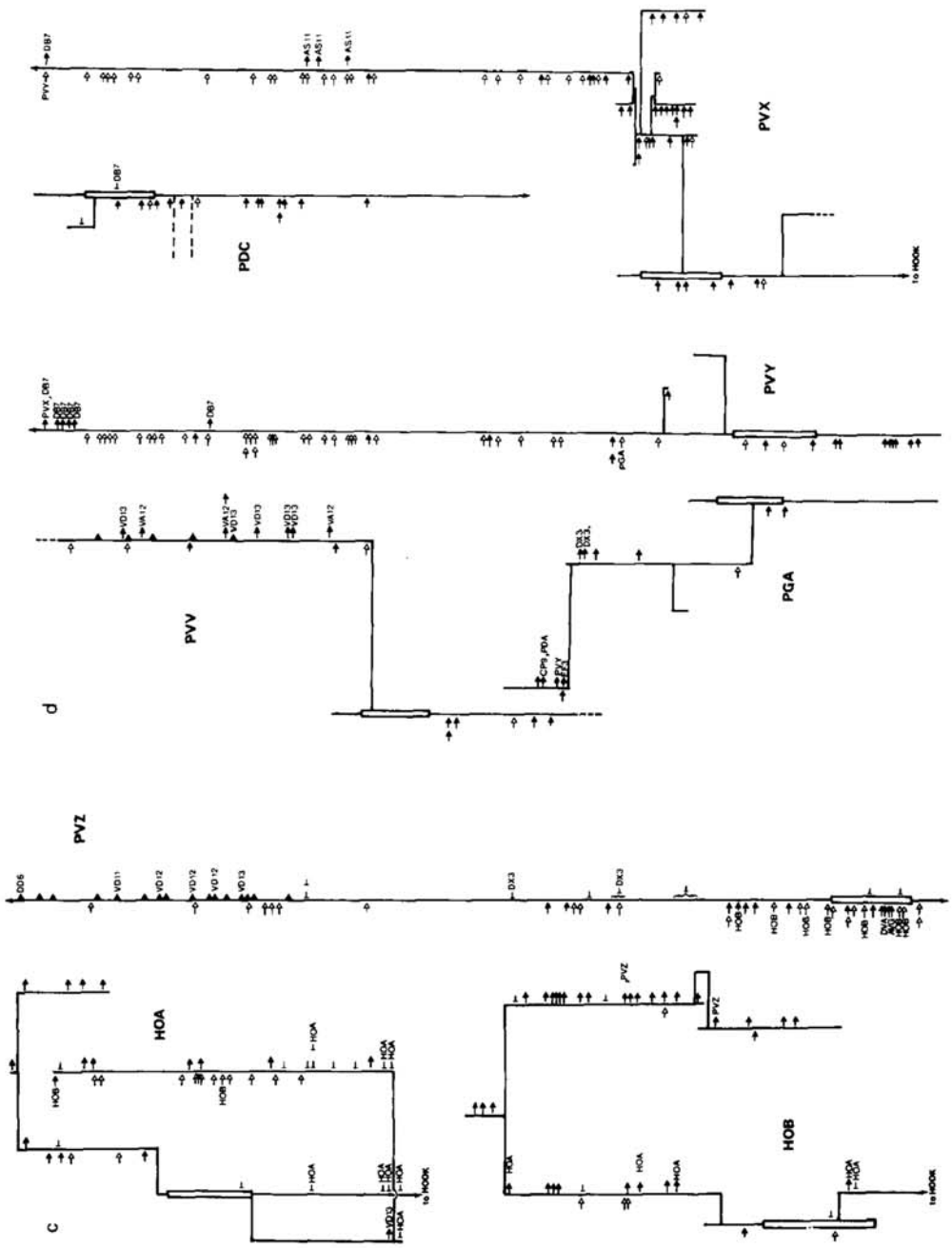

FIG. 24 (*continued*).

(c) Derivatives of P10. HOA and HOB are the neurons of the hook sensillum. HOB lies posterior to HOA and is easily distinguished in electron micrographs of adult males because of the dilated cisternae in its cell body (similar to RnB cells, Fig. 16). PVZ is a motor neuron whose axon runs anteriorly in the ventral cord dorsal to the VD motorneurons. (d) Derivatives of P11 and P12. PVV and PVY lie on the left, while PGA and PDC lie on the right. PDC sends a commissure to the dorsal cord. PVX resembles PVY in some respects. It is a large cell on the left of the preanal ganglion and sends a large empty-looking process anteriorly in the middle of the ventral cord. A posterior projection of PVX runs with HOA and HOB, but is not part of the hook sensillum (Fig. 23).

where B.a^1/$_r$appv contacts the dorsal spicule protractor. The anterior movement of the retractors, and the fact that B.a^1/$_r$appv is apparently dragged forward during L4 lethargus (Fig. 3a), squeezing between B.a^1/$_r$apapa and Bγ.p^1/$_r$, suggests that the

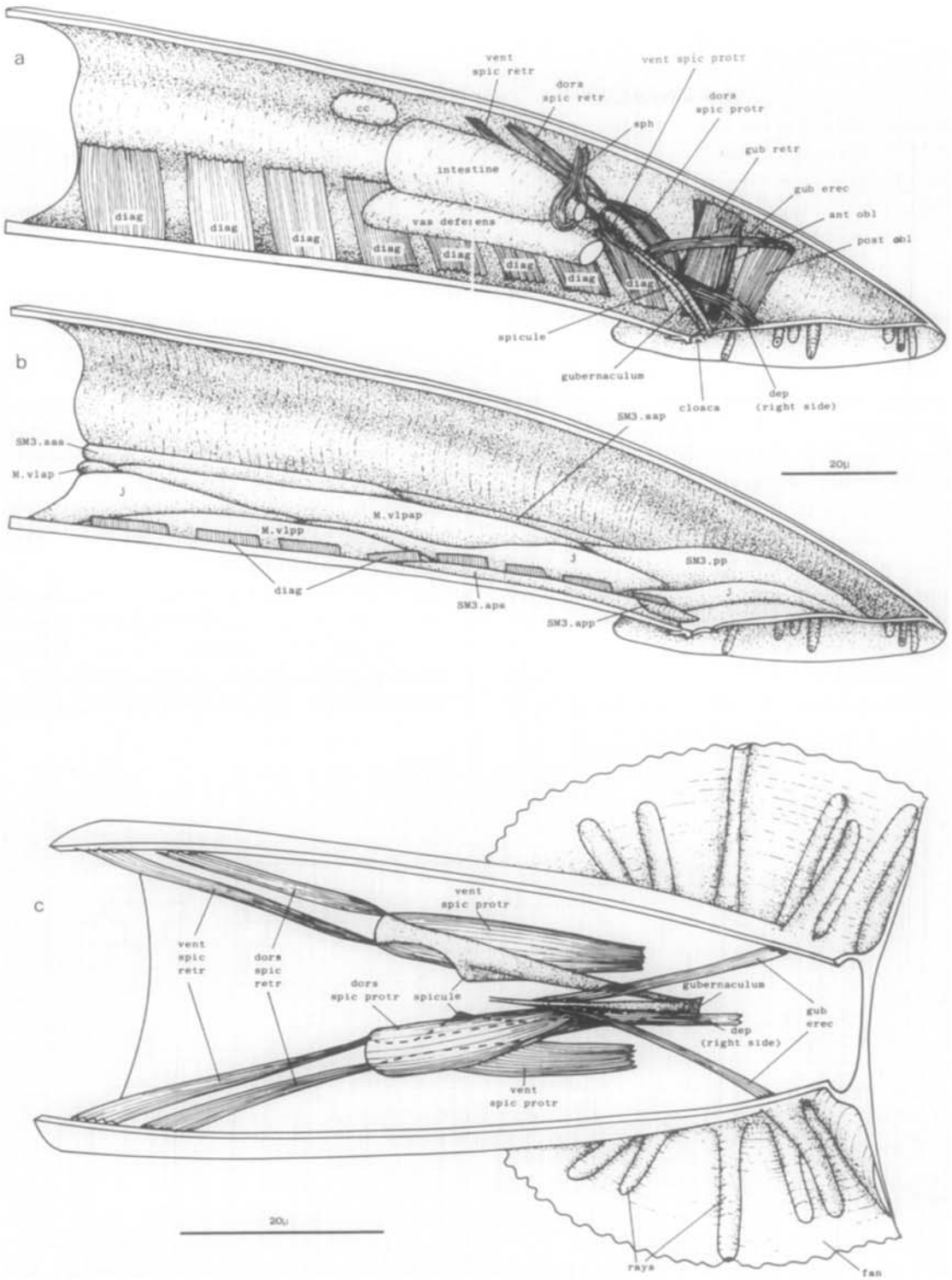

FIG. 25. Adult mesoderm. The drawings represent the contract nts, rather than the cell outlines, of the internal muscles. (a) and (b) Left-hand view of right half of ta. ernal muscles and coelomocyte; the intestinal muscle is omitted. (b) Ventral body wall muscles and ventral attachments of diagonals. SM3 derivatives intercalate with pre-existing body wall muscles; the remaining muscles are present in the hermaphrodite also, and their usual ancestry is indicated. j, juvenile (i.e., present at hatching). (c) Dorsal view of spicule muscles. Right-hand dorsal spicule protractor and arm of depressor (reorganised) have been removed to show spicule and ventral spicule protractor: posterior ends of ventral spicule protractors attach to ventral body wall. ant, anterior; post, posterior; dors, dorsal; vent, ventral; cc, coelomocyte; dep, anal depressor; diag, diagonal; erec, erector; gub, gubernacular; obl, oblique; protr, protractor; retr, retractor; sph, sphincter muscle; spic, spicule.

former contact is the more significant one. In confirmation of this, loss of both retractors on one side (by cell ablation) often results in a crumpled spicule; however, in some cases one of the spicule protractors seems to grow anteriorly and so compensate for the loss. If a single retractor is lost, the survivor can fork posteriorly and function as both. The retractors have few myofilaments at this stage, and are probably no longer independently mobile, because they have formed junctions with the body wall; their importance is probably that they couple the proctodeum to the anteriorly moving hypodermis.

Juvenile cells. Some of the juvenile muscles are modified during maturation. The most striking change is in the anal depressor, which is an H-shaped cell connecting the posterior edge of the anus to the dorsal body wall (Chitwood and Chitwood, 1974). In hermaphrodites, and in males up to L4 lethargus, the myofilaments run in the dorsoventral arms, and contraction of the depressor opens the anus in defaecation. During L4 lethargus in the male, the myofilaments are reoriented to run anteroposteriorly, so that the function of the muscle is entirely altered. The changes were studied in a series of electron microscopical reconstructions. In late L4, the anterior edges of the cell grew forward until they met the dorsal spicule protractors. In mid lethargus, the filaments became detached dorsally and shrank into the ventral half of the cell. They were next seen attached to the anterior edge, thus in a sense forming extensions of the dorsal spicule protractors; they were not seen to disappear at any stage. At the same time, the entire cell tilted forwards, in keeping with the general movement in the tail, but did not entirely lose its dorsal cytoplasmic attachments to the body wall.

The sphincter muscle becomes larger in

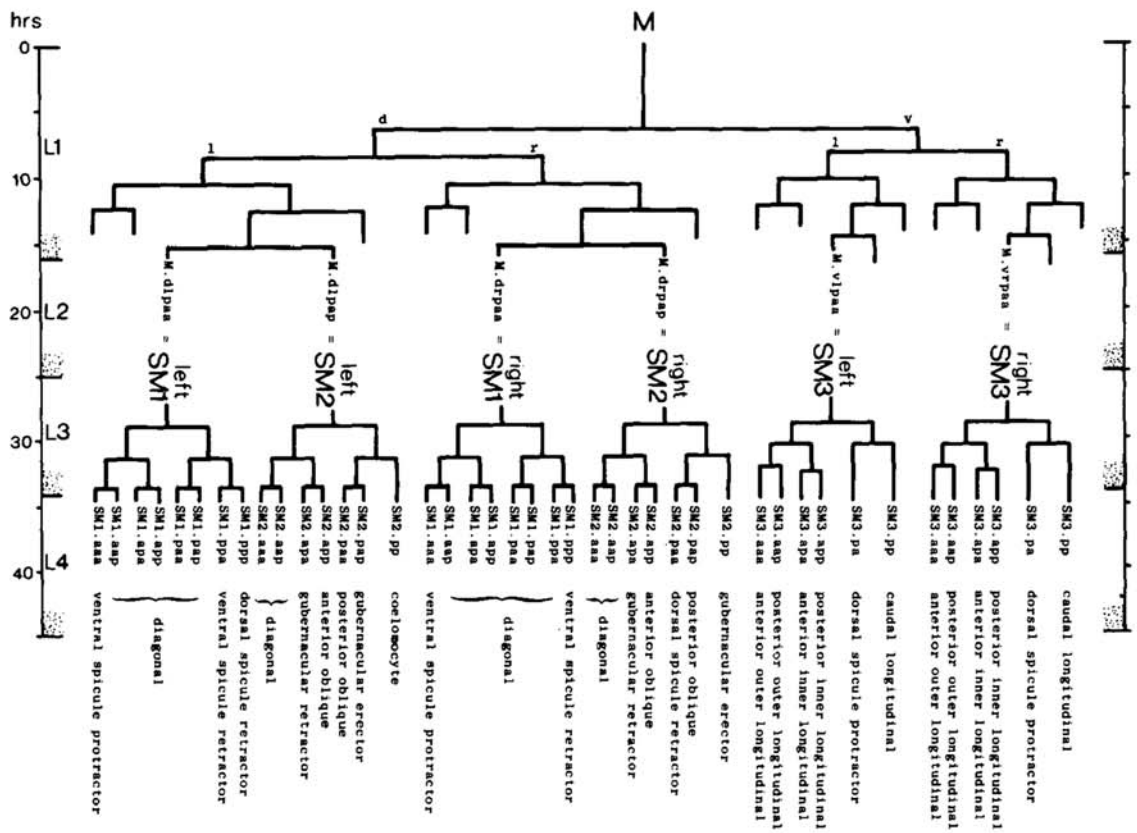

FIG. 26. Mesodermal lineages. The sex mesoblasts have been redesignated SM1–3 to facilitate description.

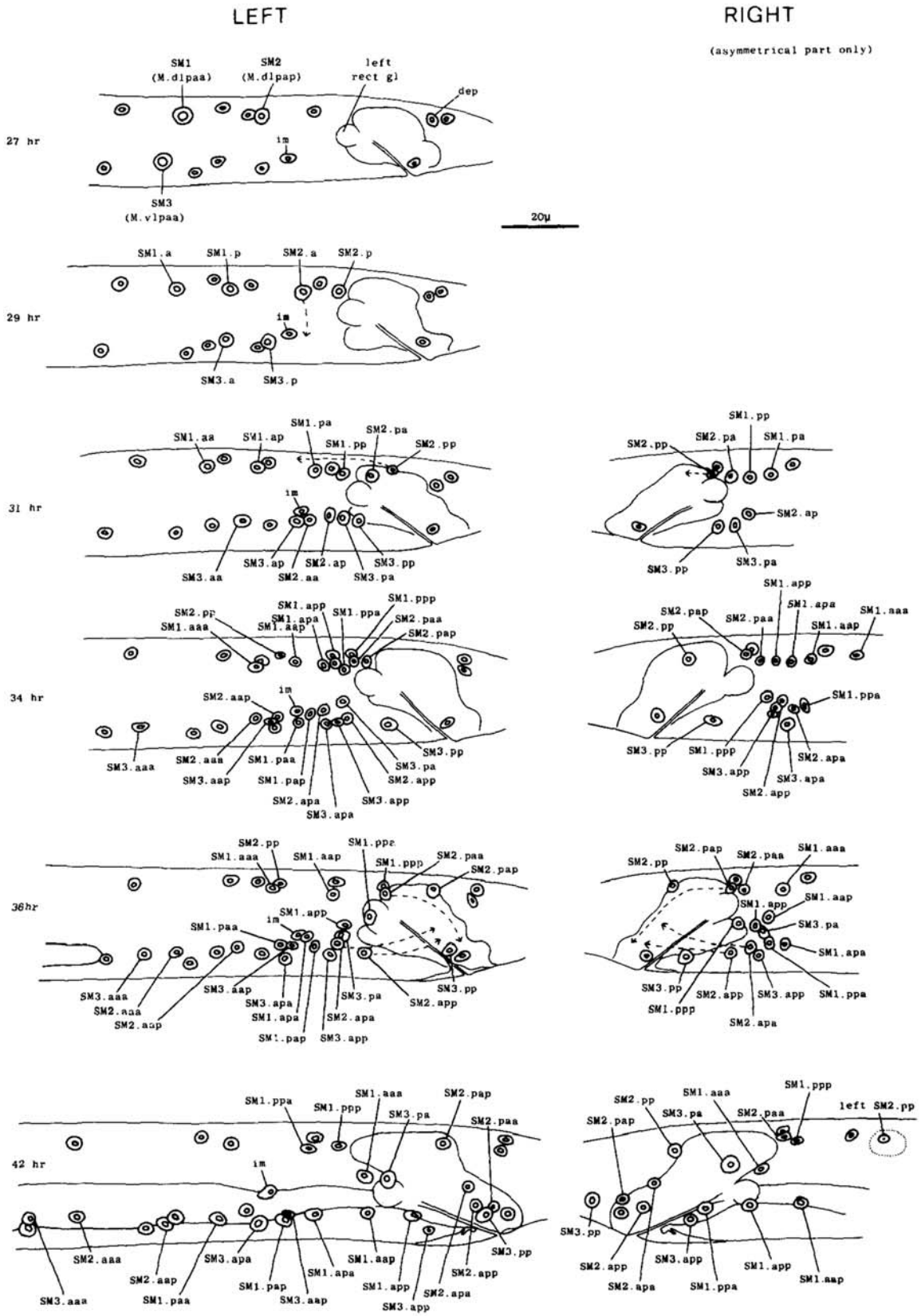

FIG. 27. Development of the mesoderm. The entire left-hand side is shown, but only the asymmetric region of the right. Note that the arrangement given previously (Sulston and Horvitz, 1977, Fig. 26) for the muscle nuclei at 35 hr has proved to be abnormal.

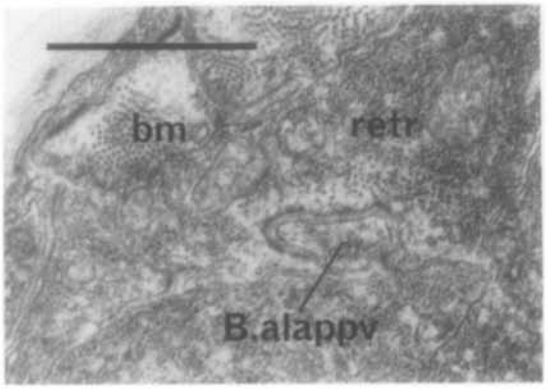

FIG. 28. Contact between dorsal spicule retractor muscle (retr) and proctodeal structural cell B.alappv at 42 hr. Note sparse myofilaments in retractor muscle at this time compared with body muscle (bm). Electron micrograph of transverse section, series 1. Bar = 1 μm.

the male. This corresponds to the function of the intestinorectal valve as the sole intestinal seal after cloacal development (in the hermaphrodite the principal seal is at the anus, and the sphincter contracts only during defaecation, possibly limiting outflow). In addition to the loop of myofilaments encircling the intestinorectal valve, the sphincter muscle develops filaments attaching this loop to the dorsal body wall. During ejaculation, the sphincter hypercontracts, thus not only clamping off the intestine but also drawing it away from the vas deferens so that sperm can pass freely. However, drug-induced hypercontraction of the sphincter does not in itself cause sperm release, and loss of the sphincter does not completely prevent sperm release.

DISCUSSION

Summary

We have described how the nongonadal cell lineages of the *Caenorhabditis elegans* male eventually lead to the secondary sexual apparatus of the adult. The cells were followed by using their nuclei as markers under Nomarski differential interference contrast optics, and late stages were reconstructed from electron micrographs of serial sections. In muscle and structural elements, the final positions of the cells are reproducible; in parts of the nervous system this is not the case, and the reconstruction had to be carried out on an animal of known lineage. The three most posterior hypodermal seam cells on each side (V5, V6, T) are responsible for generating the fan and the sensory rays (Figs. 1 and 17). Two ventral hypodermal cells, P10.p and P11.p, give rise to neurons and supporting cells in the preanal ganglion (Fig. 22). Of the juvenile cells which do not divide in the hermaphrodite, three (B, E, and F) form the union between the gonad and the alimentary tract, the copulatory spicules and associated neurons; the fourth (C) forms the postcloacal sensilla (Fig. 6). Three mesoblasts on each side (SM1, SM2, and SM3), which are formed from the juvenile mesoblast (M), produce the copulatory muscles and a coelomocyte (Fig. 26). Development culminates in a mass movement of cells which abruptly changes the overall shape of the tail (Fig. 2).

Cell Fate

Inspection of the cell assignments reveals some pattern in the correlation between cell lineage and cell fate. Thus, the sensory rays share a common terminal lineage; the repetitive formation of groups of neurons is reminiscent of the ventral cord lineage (Sulston and Horvitz, 1977). Elsewhere, there is some clustering of related cells into particular sensilla.

On the other hand, cells which appear quite similar in the adult are often drawn from disparate lineages. For example, the spicule socket cells, which are sufficiently alike to fuse together in the adult, come from seemingly nonhomologous branches of the B lineage in different parts of the proctodeum (cf. Sulston and Horvitz, 1977, Fig. 21). Similarly the dorsal and ventral spicule protractor muscles arise from diverse lineages at diverse locations. The fact that analogous cells can be well separated spatially at first, and only subsequently assemble at the same location, suggests that

their commitment is not an accident of position but rather is programmed into them at birth.

Sensilla

Inspection of Figs. 6 and 22 shows that lineages of sensilla can take a wider variety of forms than those proposed by Sulston and Horvitz (1977). Different tail sensilla contain from one to three supporting cells (in contrast to head sensilla, which invariably contain two). Furthermore, neurons and supporting cells do not necessarily occupy discrete branches of the lineage.

Nevertheless, it is worth searching for common features, because data presented previously (Sulston and Horvitz, 1977) and in the following paper indicate that the terminal branches of a number of lineages are determinate. One generalization that can be made is that sheath cells are more often sisters of neurons than are socket cells. Socket cells are sometimes formed later in development than the other components of sensilla, their function being at first performed by the seam cells from which they eventually arise; this is true of the phasmid and probably also of the deirid (John White, personal communication).

Comparison of the hook and ray sensilla reveals more specific similarities. The pair of neurons which innervate the hook sensillum (HOA and HOB) markedly resemble the pair (RnA and RnB, respectively) which innervate each ray (other than ray 6). It is therefore interesting that the lineage of the hook sensillum can be seen as a permutation of the ray lineage. On this view the programmed cell death in the ray lineage removes the potential ancestor of the sheath and motor neuron formed by the hook sensillum lineage; the ray structural cell would then be the homologue of the hook socket cell.

The cephalic sensilla may perhaps fit into the same pattern. Ward *et al.* (1975) showed that, in the male, each cephalic sensillum contains a pair of sensory endings: one lies beneath the cuticle, like the solitary, dopaminergic neuron found in the hermaphrodite, whilst the other penetrates to the exterior. The male has four swollen cell bodies, which are absent from the hermaphrodite, near the nerve ring (Sulston and Horvitz, 1977); these "cephalic companions" are thought to be the cell bodies of the four male-specific cephalic processes. Their morphology in the electron microscope is similar to that of RnB and HOB. There is thus an analogy between the cephalic neuron and RnA on the one hand, and between the cephalic companion and RnB on the other. However, the cephalic neuron does lack the striated rootlet typical of RnA. At present the lineages of the cephalic sensilla, which are embryonic, are unknown.

Comparison with Hermaphrodite Development

With few exceptions, all the nongonadal cells of the hermaphrodite are found also in the male, and most of them have similar functions in two sexes.

The only significant absence is that of the vulva and its associated musculature. The blast cells which generate the vulva in the hermaphrodite (P5.p, P6.p, P7.p) do not divide in the male, in which they form part of the ventral hypodermis. The hermaphrodite sex myoblasts (M.vlpaa, Mvrpaa), on the other hand, are also male sex myoblasts (renamed SM3), but their division pattern and the functions of their progeny are different in the two sexes.

The remaining male blast cells divide less, or not at all, in the hermaphrodite, but nevertheless become differentiated cells with well-defined functions. In the male, one or more of the progeny of a blast cell may take on the function which their parent performs in the hermaphrodite. Thus, C becomes a neuron in the hermaphrodite and its anterior daughter becomes a similar neuron in the male; the mesoblast Mdlpap (SM2) becomes a coelomocyte in the hermaphrodite whilst M.dlpappp (SM2.pp)

does so in the male; B, E, F, and the various lateral and ventral blast cells are all hypodermal cells in the hermaphrodite and except for E each produces hypodermal progeny in the male as well.

For the most part, then, the secondary sexual apparatus of the male overlies the existing hermaphrodite anatomy. This contrasts with the development of the gonad (Kimble and Hirsh, 1979), in which the division patterns of the hermaphrodite and the male diverge at an early stage.

Changes in Cell Function during Development

A number of cells play more than one role in the course of the development of the male, as the following examples show:

(1) The anal depressor muscle acts to open the anus during defaecation of larvae. During the final moult its myofilaments turn through 90°, and the muscle then acts as an accessory to the spicule protractors.

(2) The spicule retractor muscles are required during L4 lethargus for morphogenesis of the proctodeum. This function may not be related to their subsequent muscular nature; probably they simply act as couplers at this point.

(3) The seam cells, which lay down the lateral cuticle at each moult, are also blast cells in both the hermaphrodite and the male. In addition, the most posterior seam cell (T) is the socket cell of the juvenile phasmid, and segregates this function to two of its daughters during its subsequent division.

(4) Cells B, C, E, F, and K are responsible for forming parts of the rectum in larvae. At the same time they divide repeatedly to yield both structural cells and neurons.

Another striking example of changing cell roles is the reversal in information flow along certain neurons of the ventral cord at the L2 stage, described by White *et al.* (1978).

Symmetry

In the postembryonic lineages of the hermaphrodite, the two daughters of a transverse division across the midline have equivalent fates (Sulston and Horvitz, 1977). The lineages of an individual male contain several exceptions to this rule, but when several animals are examined it is found that in all but one case the two cells have equal potential.

Thus, in both cases of asymmetric cell death ($B.a^1/_r apaav$ and $B\gamma.a^1/_r d$) either the left or the right cell can die (Fig. 6). The decision may be due to a position effect, the left and right cells being ordered at random as in the case of the $B\alpha/B\beta$ and $B\gamma/B\delta$ pairs (Sulston and Horvitz, 1977). Similarly, we now know that the left and right daughters of E both have the potential to give rise to neuroblasts. This is not to say that all possible outcomes are equally probable: indeed it would be surprising if that were the case, since the animal is inherently asymmetrical at the cellular level. At present, though, not enough individuals have been watched for the probabilities to be estimated.

In a similar way, each individual is asymmetric while the population as a whole is symmetric with regard to the gonadal lineages (Kimble and Hirsh, 1979). The possible exception to the rule, the development of the mesoderm, is intriguing and unexpected. In fact, because this development is difficult to follow, it seemed likely at first that the observations were in error. However, the cell assignments have proved to be perfectly reproducible on each side.

Cell Death

The gonadal linker cell is engulfed by E.lp or E.rp, and dies soon afterwards. That these events are causally related is shown by the ablation of blast cell E, after which operation the linker cell does not die. It is confirmed by the observation that the linker cell survives in gonads which fail to reach the proctodeum, due to genetic defects or to ablation of other gonadal cells (J. Kimble, personal communication). Thus E.lp and E.rp seem to be killer cells directed

against the linker. In the same way P12.pa appears to be responsible for engulfing and killing either B.alapaav or B.arapaav, and the fusion product of F.ld and F.rd may be involved in the death of Bγ.ald or Bγ.ard.

It is possible that all programmed cell deaths in the nematode involve killer cells. Alison Robertson (personal communication) has found that ventral cord cells which are programmed to die are surrounded by the syncytial hypodermis before cytokinesis is complete; since overt signs of cell death follow only after some 30 min or more, it is likely (though not proved) that the hypodermis is killing them.

Developmental Variability

The development of the male is more prone to variation than that of the hermaphrodite.

(1) A common error is the loss of one or more rays, the most vulnerable being 3, 8, and 9; this appears to be due to the failure of the structural cell to form a process. Frequently, in place of ray 3, a thin process can be seen by Nomarski microscopy, suggesting that at least one of the neurons has been laid down in the normal way.

(2) A less common error is the fusion of rays 8 and 9.

(3) The lineage of cell E is variable: either one, or both, or neither of its anterior daughters may move forward into the preanal ganglion and there divide into two neurons.

None of these variations, or errors, is known to influence mating behavior significantly, but they do show that the male copulatory apparatus is constructed in a less precise way than other parts of the nematode.

Moulding of the Cuticle

During the final moult, the adult cuticle is shaped into a membranous fan encircling the ventral surface of the tail. The animal achieves this by secreting a layer of cuticle at a time when the tail is expanded: subsequently, the tail shrinks, and the oversized cuticle collapses in a well-defined way to form the fan (Fig. 4). This stratagem of collapsing a single-layered surface into a double layer is analogous to the formation of an insect wing (Seligman *et al.*, 1975), except that the layer is acellular rather than cellular. The collapse might be caused either by withdrawal of fluid from the subcuticular space or by an active contraction of the middle layer of cuticle. Contraction of cuticle secreted by the body seam has been implicated in the maturation of dauer larvae (Singh and Sulston, 1978). With this in mind, the tail seam was ablated in four animals; however, although the fan cuticle was somewhat attenuated by the loss of hypodermis, it still folded correctly.

Another apparent example of the use of cells as temporary moulds for the cuticle is found in the formation of the spicules. In the late L4, the bundle of processes from the socket, sheath, and neuronal cells of each spicule grow along a U-shaped channel (Fig. 8) and sclerotic cuticle is laid down around the bundle. Subsequently, the cells protruding into the channel shrink, leaving the resulting U-shaped spicule free to move.

In the course of this work, we have benefited particularly from discussions with Martin Chalfie, Jonathan Hodgkin, Bob Horvitz, Judith Kimble, and John White. We are also grateful to Marilyn Anness for help with electron microscopical reconstruction, and to Judith Daniels and Annette Lenton for help with the figures. D.G.A. was a research fellow of the Muscular Dystrophy Association of America, Inc.

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The Embryonic Cell Lineage of the Nematode *Caenorhabditis elegans*

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The embryonic cell lineage of *Caenorhabditis elegans* has been traced from zygote to newly hatched larva, with the result that the entire cell lineage of this organism is now known. During embryogenesis 671 cells are generated; in the hermaphrodite 113 of these (in the male 111) undergo programmed death and the remainder either differentiate terminally or become postembryonic blast cells. The embryonic lineage is highly invariant, as are the fates of the cells to which it gives rise. In spite of the fixed relationship between cell ancestry and cell fate, the correlation between them lacks much obvious pattern. Thus, although most neurons arise from the embryonic ectoderm, some are produced by the mesoderm and a few are sisters to muscles; again, lineal boundaries do not necessarily coincide with functional boundaries. Nevertheless, cell ablation experiments (as well as previous cell isolation experiments) demonstrate substantial cell autonomy in at least some sections of embryogenesis. We conclude that the cell lineage itself, complex as it is, plays an important role in determining cell fate. We discuss the origin of the repeat units (partial segments) in the body wall, the generation of the various orders of symmetry, the analysis of the lineage in terms of sublineages, and evolutionary implications.

INTRODUCTION

This report marks the completion of a project begun over one hundred years ago—namely the determination of the entire cell lineage of a nematode. Nematode embryos were attractive to nineteenth century biologists because of their simplicity and the reproducibility of their development, and considerable progress was made in determining their lineages by the use of fixed specimens (reviewed by Chitwood and Chitwood, 1974). By the technique of Nomarski microscopy, which is nondestructive and yet provides high resolution, cells can now be followed in living larvae (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sternberg and Horvitz, 1981) and eggs (Deppe *et al.*, 1978; this paper). The use of living material lends a previously unattainable continuity and certainty to the observations, and has permitted the origin and fate of every cell in one nematode species to be determined. Thus, not only are the broad relationships between tissues now known unambiguously but also the detailed pattern of cell fates is clearly revealed.

Our current interest in the cell lineage of a particular nematode, *Caenorhabditis elegans*, has arisen as part of a larger research effort comprising genetic, anatomical, and biochemical approaches to the development of this animal. The lineage is of significance both for what it can tell us immediately about relationships between cells and also as a framework into which future observations can be fitted.

The main purpose of this article is to present the embryonic cell lineage. A brief description of morphogenesis is given, although this is not intended to be an exhaustive account of cell movements and interactions. A few cell ablation experiments are described; these results are limited in scope, but do give some indication of the developmental flexibility (or lack of it) in the system. In addition, a key to differentiated cell types is provided as an Appendix.

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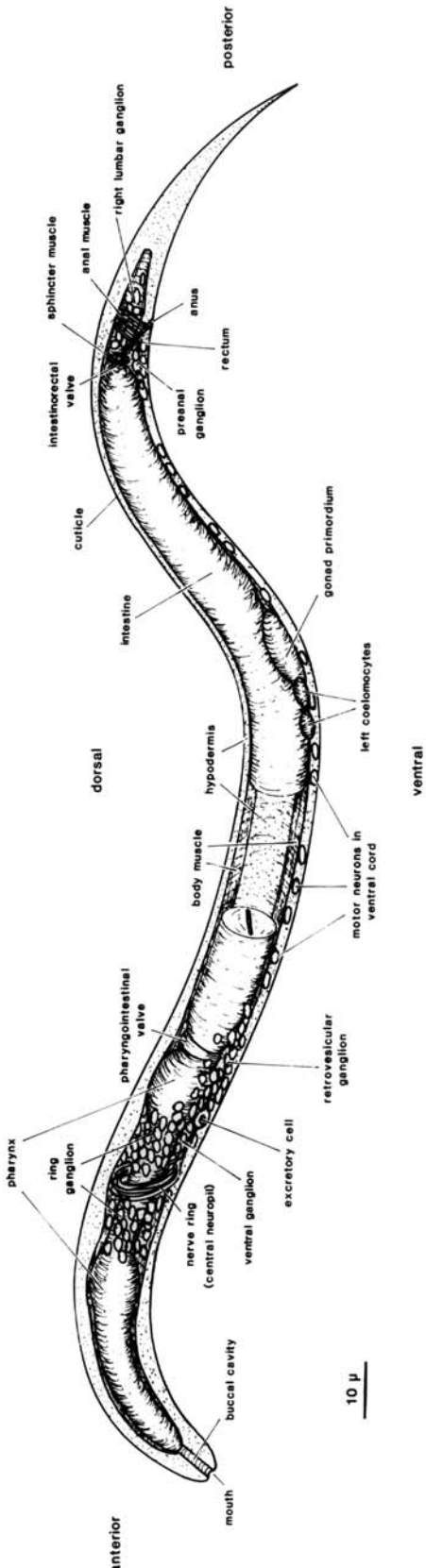


FIG. 1. General anatomy of newly hatched L1 larva, left lateral aspect, body wall cut away to midline. Part of the intestine is also cut away to show two of the four longitudinal bands of body muscle embedded in the hypodermis. Neuronal cell bodies are represented schematically. Most of the sensilla (amphids, cephalics, inner labials, outer labials, together with a number of accessory neurons) are grouped around the mouth (Ward *et al.*, 1975; also shown schematically in Fig. 18); elsewhere are the anterior deirids (paired, lateral, over rear bulb of pharynx), the phasmids (paired, lateral, posterior to lumbar ganglia), and the microtubule neurons (longitudinal lateral mechanoreceptors, one pair anterior and one pair posterior; Chalfie and Sulston, 1981). Body cavity is termed a pseudocoelom, since intestine is in direct contact with body wall. Principal changes during postembryonic development are: overall increase in size; growth and maturation of the gonad; formation of vulva (midventral) in hermaphrodite, and copulatory bursa (posterior) in male, both with associated musculature; cell division in hypodermis and ventral nervous system. Sensilla generated postembryonically are: postdeirids (paired, lateral, between gonad and anus) and ventral microtubule neurons in both sexes; numerous sensilla in the male copulatory bursa (Sulston *et al.*, 1980).

MATERIALS, METHODS, AND BACKGROUND INFORMATION

Culture

Caenorhabditis elegans (Bristol), strain N2, was maintained according to Brenner (1974). *Turbatrix aceti* and *Aphelencoides blastophthorus* were kindly given to us by David Hooper, Rothamsted Experimental Station, Harpenden, Herts, England; *Panagrellus redivivus* was obtained from Rothamsted in 1976, and is the strain studied by Sternberg and Horvitz (1981, 1982). *T. aceti* and *P. redivivus* were maintained in the same way as *C. elegans; A. blastophthorus* was grown on NGM plates infected with mixed fungi.

General Biology of C. elegans

C. elegans is a free-living nematode which has two sexual forms: a self-fertilising hermaphrodite and a male. Development begins in the egg, and continues through four larval stages (L1–L4) to the adult. A newly hatched L1 is depicted in Fig. 1.

The general anatomy of the newly hatched L1 larva has been described by Sulston and Horvitz (1977). As far as it goes this account is accurate except for the precise cell count in the head and the omission of the intestino-rectal valve (virL and virR, see Figs. 1 and 8c). Postembryonic cellular development has been described for the gonad by Kimble and Hirsh (1979), for the male tail by Sulston *et al.* (1980), and for other systems by Sulston and Horvitz (1977). Other references will be found in the tissue-by-tissue description below.

Light Microscopy

C. elegans eggs were transferred from plates which contained ample bacteria to water in a watch glass; alternatively, young eggs were obtained by cutting gravid hermaphrodites in water. With the help of a 50× dissecting microscope about 50 eggs of approximately the required age were selected; they were transferred to a layer of 5% agar, any that were in contact were moved apart with a fine hair, and they were viewed by Nomarski optics (Sulston and Horvitz, 1977; Sulston *et al.*, 1980). A single egg which happened to be at the required stage and in an appropriate orientation was chosen for observation. Representative specimens are shown in Fig. 2.

T. aceti eggs normally develop within the parent; they are both mechanically and osmotically fragile, and were therefore mounted on 1% agar in isotonic medium. Agar was dissolved in boiling water to a concentration of 2%, cooled to about 50°C, and mixed with an equal volume of a solution containing 180 m*M* NaCl, 8 m*M* KCl, 36 m*M* $CaCl_2$, 36 m*M* $MgSO_4$, 10 m*M* Hepes, pH 7.2 (cf

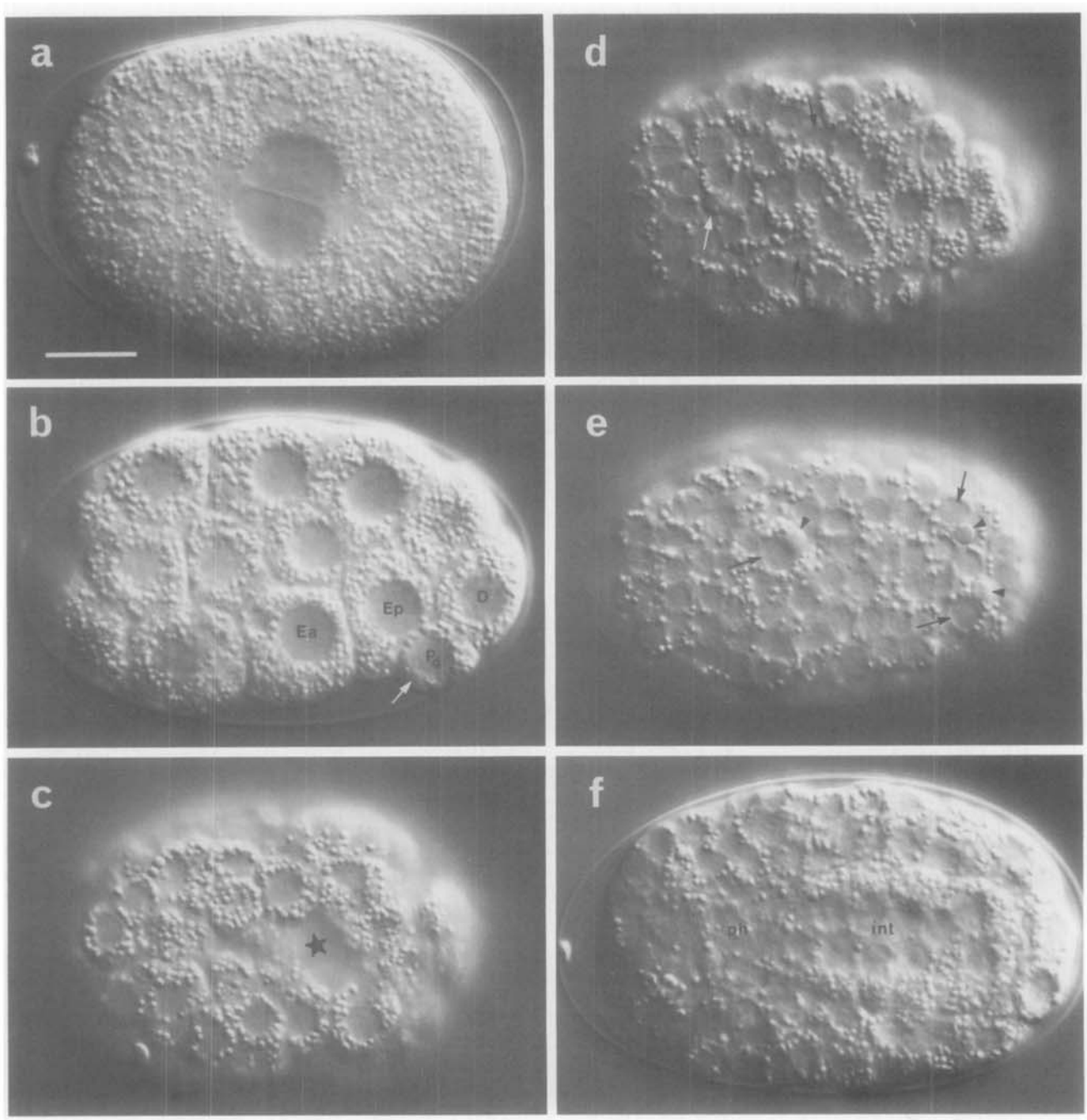


FIG. 2. Photomicrographs of embryos. (a–h) Nomarski optics, Microflash, anterior to left. Bar = 10 μm. A few landmark features are marked on the photographs, but no attempt has been made to label all the cells; for reliable identifications the line drawings should be used, preferably in conjunction with at least some tracing of the lineage. (a) Just before first cleavage. Midplane. Male and female pronuclei are apposed; first polar body visible beneath eggshell at anterior end (where it typically but not invariably resides). (b) Beginning of gastrulation. Left lateral aspect, midplane; cf. Fig 5. Ea and Ep are moving dorsally, into the interior. P_4 is recognisable by its small size, by the germinal plasm or nuage (arrowed; Strome and Wood, 1982; Krieg *et al.*, 1978) around its nucleus, and by the distinctness of its nuclear membrane. (c) Late gastrulation (ca. 210 min). Ventral aspect, superficial plane; cf. Fig. 6. The cleft through which the MS cells have just entered is starred. (d) ca. 280 min. Dorsal aspect, superficial plane; cf. Fig. 7a. Small neuroblasts anteriorly; larger hypodermal cells, loaded with granules, posteriorly. Furrows can be seen between hypodermal cells (cf. Fig. 10). White arrow, dying ABarpaaapp; black arrows, ADEshL and ADEshR. (e) ca. 260 min. Ventral aspect, superficial plane; cf. Fig. 7b. Small neuroblasts over entire surface. Dying cells (arrowheads) are engulfed by their sisters (arrowed): ABplpappaa, ABplpppapa, ABprpppapa. (f) ca. 280 min. Dorsal aspect, midplane. int, cylinder of intestinal cells, nine nuclei in this focal plane, cytoplasm heavily loaded with granules; ph, cylinder of pharyngeal cells, less distinctive, contain few granules. (g) First movement (ca. 430 min). Left lateral aspect, midplane; cf. Fig. 8c. int, intestine; ph, pharynx; white arrowhead, anterior sensory depression; black arrowhead, rectum; arrows, dorsal hypodermal ridge, heavily loaded with granules. (h) Threefold, rolling. Only the anterior two-thirds of the embryo is in focus. White arrowhead points to mouth, linked by pharynx (ph) to intestine (int). Arrows

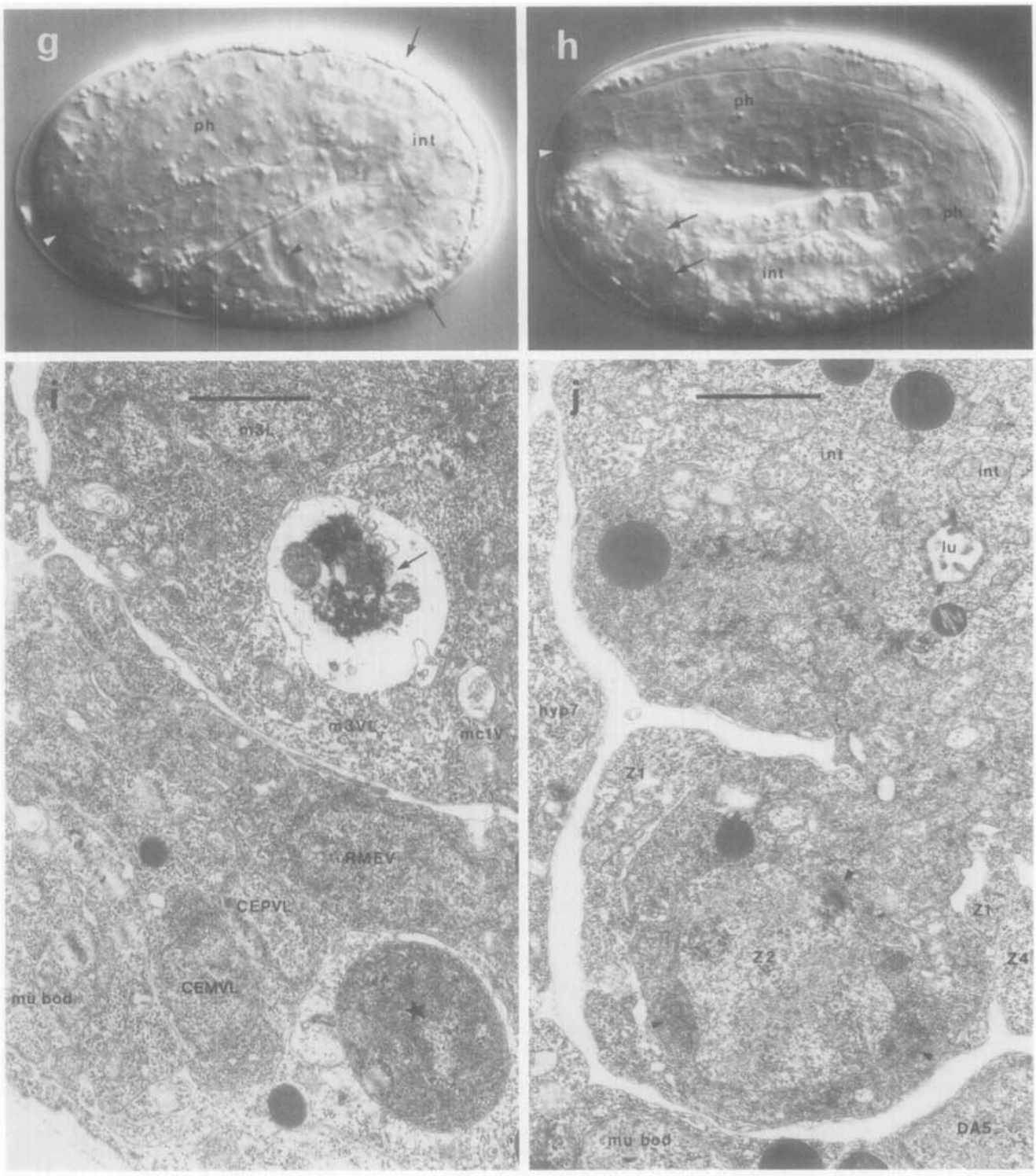


FIG. 2—*Continued.*

point to germ cells. (i) ca. 430 min; electron micrograph of transverse section to show phagocytosed cells. Recent death (starred) lies within a ventral hypodermal cell; older death (arrowed) lies within a pharyngeal muscle. Pharyngeal lumen seen at upper right, surrounded by desmosomes between muscles and marginal cells. Outer surface of embryo, seen at lower left, is covered by a tenuous membrane in addition to the hypodermal basement membrane. Bar = 1 μm. (j) ca. 470 min; electron micrograph of transverse section, to show protrusion of lobes from germ cell (Z2) into two intestinal cells (int). Germ cells are united via lobes, but Z3 is not visible in this section. Germinal plasm or nuage (arrowheads; cf. Fig. 2b) visible around Z2 nucleus. Lumen (lu) of intestine is sealed by desmosomes; its wall carries microvilli. Bar = 1 μm.

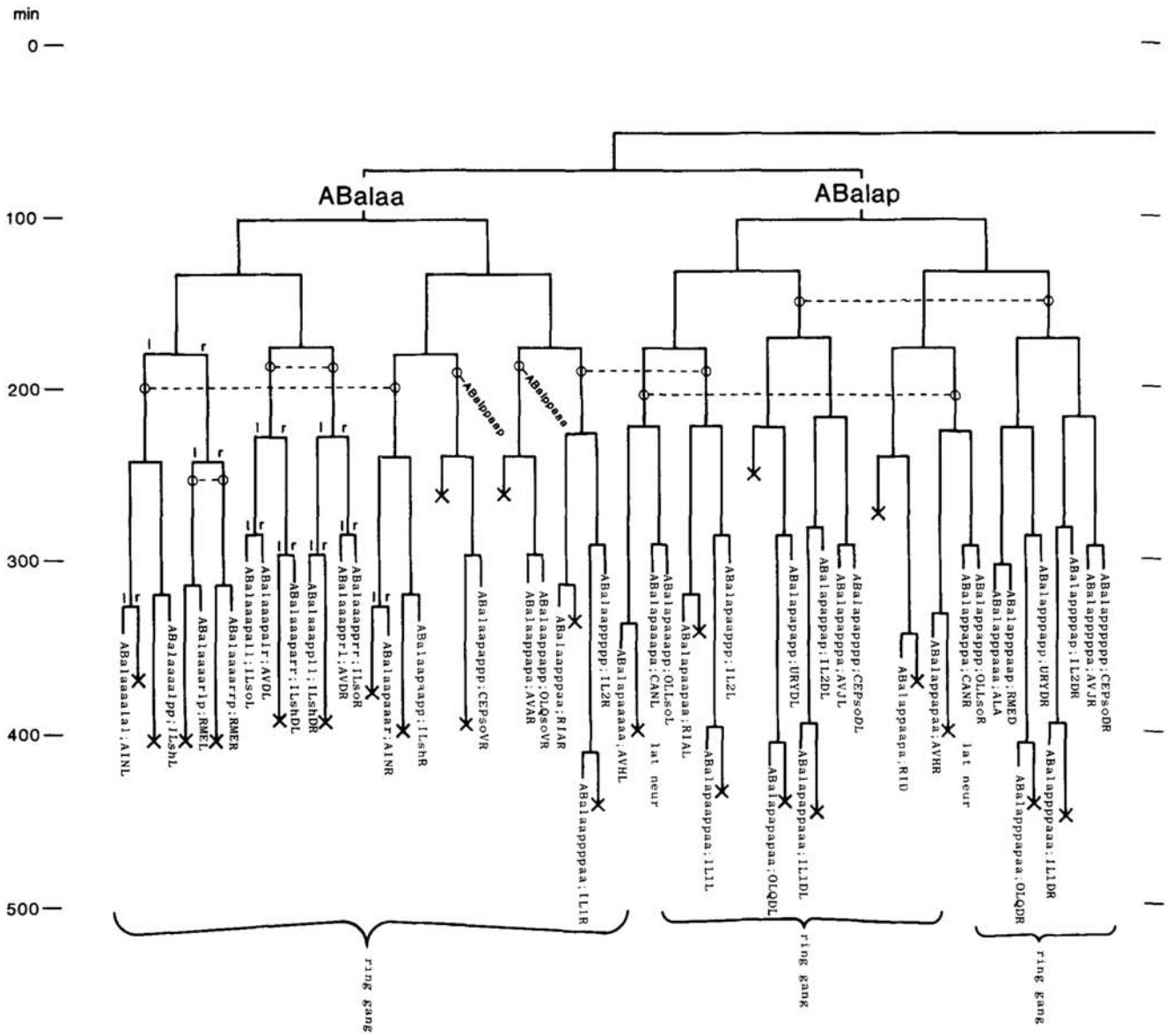


FIG. 3. The embryonic cell lineage of *C. elegans*. All interconnecting lines between the separate panels have been drawn, so that the pages can be copied, trimmed, and pasted together to give a complete chart. Vertical axis represents time at 20°C, from 0 min at first cleavage to 800 min at hatching. Many of the observations were made on eggs which were developing at slightly different rates (due to temperature variation and the effect of prolonged illumination); these primary results were normalised, by means of certain prominent cell divisions, to the course of events in eggs which were kept at 20°C and viewed infrequently. The precise times of individual events were not our primary concern, and should not be taken too seriously; the likely error varies from ±10% at the beginning of the lineage to ±2% at 400 min. Horizontal axis represents the direction of cell division. The majority of divisions have a marked anterior–posterior bias, and are shown with anterior to the left and posterior to the right, without any label. Only when this would lead to ambiguity in naming the daughters is an alternative direction indicated (l, left; r, right; d, dorsal; v, ventral); thus, our system is taxonomic rather than fully descriptive. The natural variation seen suggests that the precise direction of cell divisions is unimportant, at least in later development. Note that the daughters of a left–right division are not necessarily bilaterally symmetrical: for example, all the cells derived from ABalaaapa lie on the left of the animal and the right-hand daughters lie nearer the midline. Each terminal branch of the embryonic lineage is labelled either with X (indicating cell death; the position of the X on the time axis indicates the time of maximum refractility) or with a lineage name followed by a functional name. Large arrowheads denote cells which divide postembryonically, and small arrowheads denote nuclei which divide postembryonically, Symbols ○ and --- link precursors which give rise to bilaterally symmetrical groups of cells, the symbol ~ being included for cases of imperfect symmetry (see: Sublineages; Symmetry and Asymmetry). cord, ventral cord; gang, ganglion; lumb, lumbar; d-r, dorsorectal; p-a, preanal, r-v, retrovesicular; lat neur, isolated neuron lying laterally.

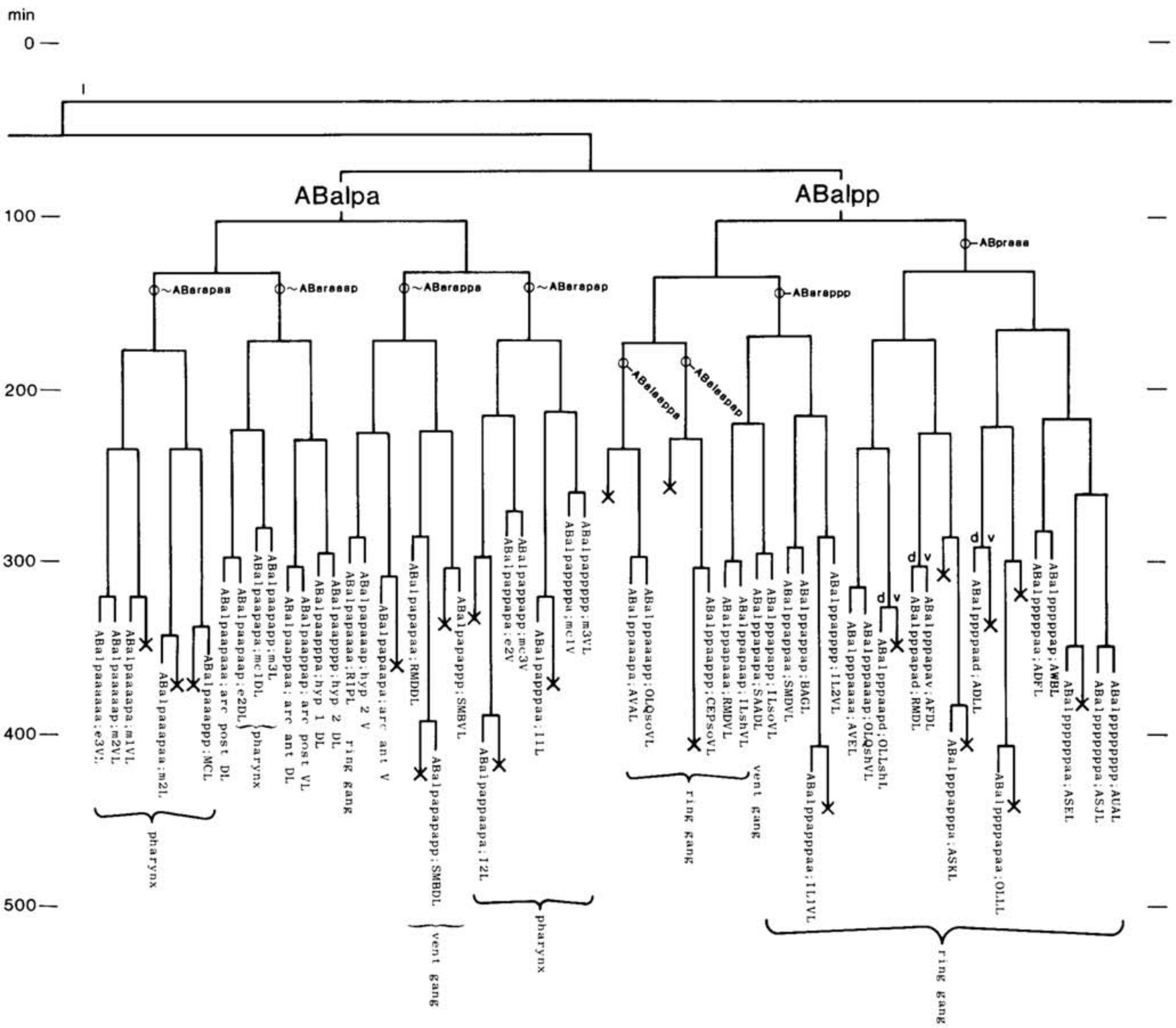


FIG. 3—*Continued.*

Laufer *et al.*, 1980). A single gravid adult was cut in half in water, and the extruded eggs were immediately pipetted onto a prepared agar layer. Even with these precautions, development continued for only about 7 hr, representing one-quarter of embryogenesis in *T. aceti.* However, the successive cell patterns were reproducible (and also similar to those of *C. elegans*), so the lineage could be traced unambiguously as described below (Strategy of Observation).

P. redivivus eggs were mounted like those of *T. aceti,* except that the gravid adults were placed directly on the agar layer and cut in half with scissors; the eggs were gently moved away from their parents with a fine hair.

A. blastophthorus eggs were teased out from agar/fungal blocks into water, and mounted like those of *C. elegans.*

Electron Microscopy

The egg shell excludes the usual fixatives and embedding media, and must be made permeable in some way before conventional methods can be applied.

The eggs examined in the course of the present work were first treated with NaOCl (2% available chlorine, 5–10 min); they were then fixed with OsO_4 (1%, 1 hr) and embedded and sectioned in the usual way (Ward *et al.*, 1975). In an improved version of this method the

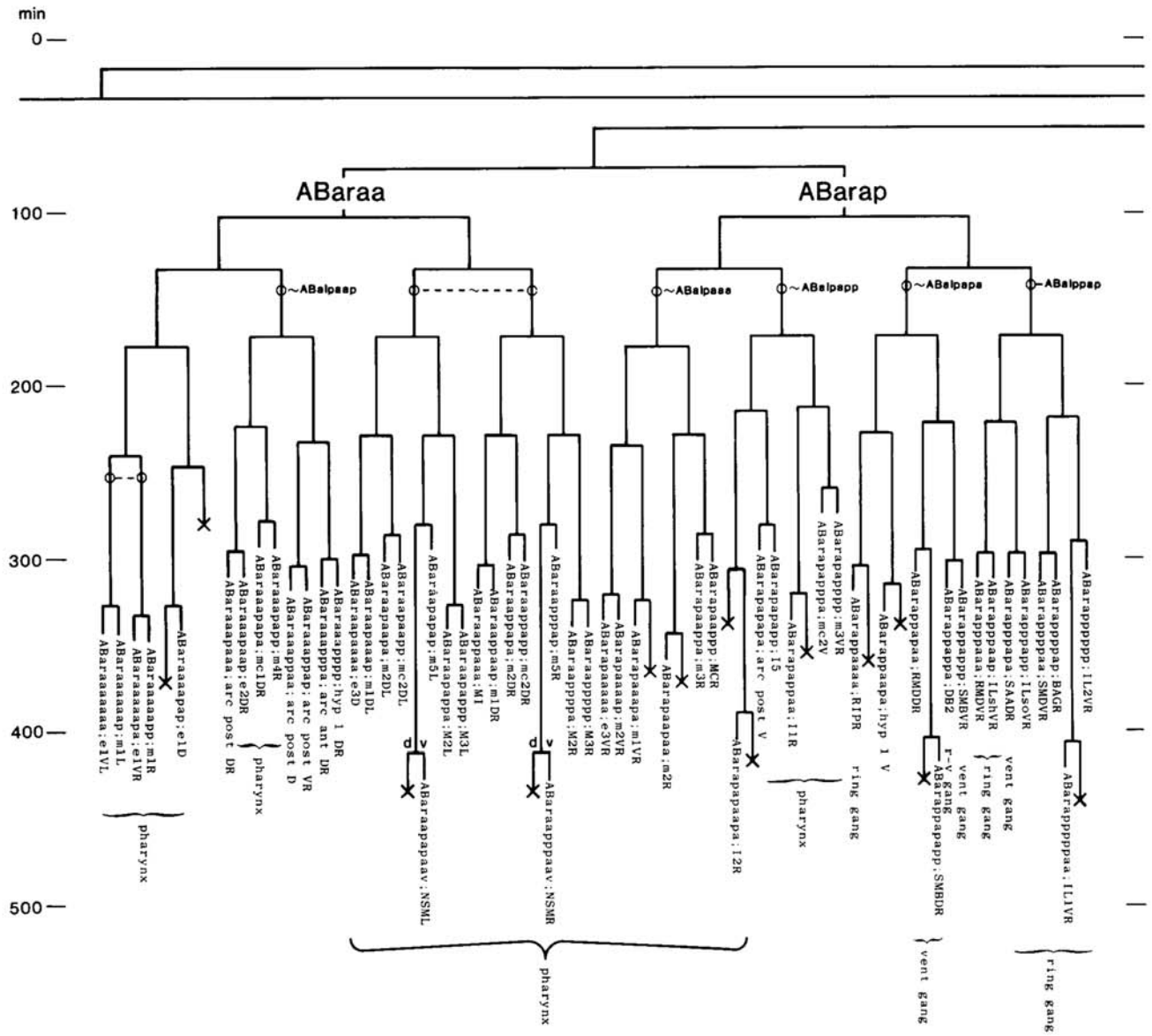


FIG. 3—*Continued.*

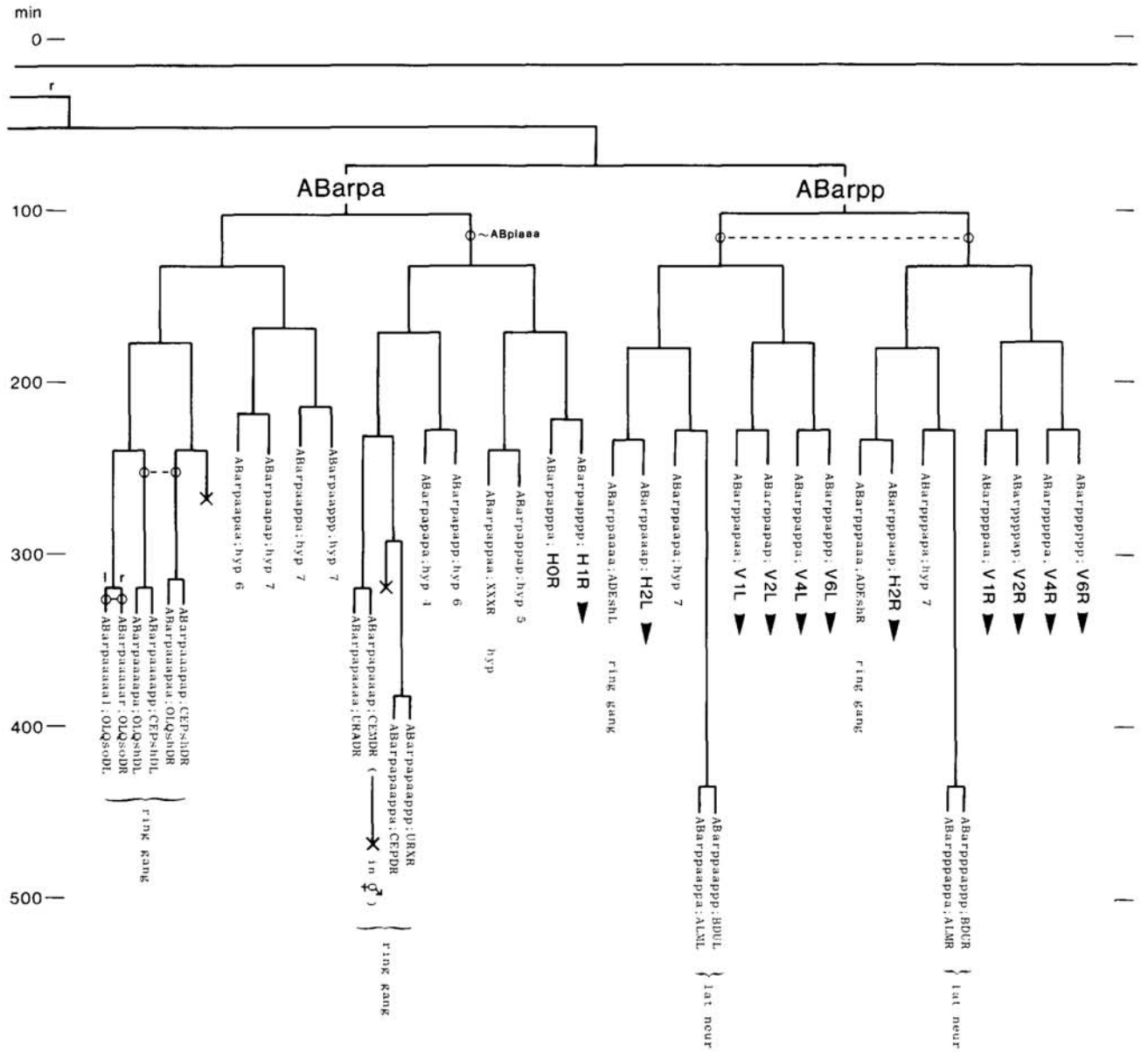
egg is treated with chitinase or 1-phenoxy-2-propanol after the NaOCl, and then prefixed with glutaraldehyde (Albertson and Thomson, 1982).

Permeabilisation with hypochlorite is always rather erratic; a better method for dealing with an individual egg is to puncture the shell with a laser microbeam (Schierenberg and Cole, *in* von Ehrenstein *et al.*, 1981).

For reconstruction of the anterior sensilla, L4 larvae and adults were prepared as follows. The nematode was transferred to 3% glutaraldehyde in 0.1 *M* Hepes, pH 7.4, and immediately cut in the posterior half. After about 2 min a second cut was made in the anterior half; after 1 hr the head was washed three times with 0.1 *M* Hepes, pH 7.4, and then postfixed with 1% OsO_4 in the same buffer for 1 hr. The specimen was embedded as usual (Ward *et al.*, 1975), and about 200 serial sections were cut from the anterior tip.

Strategy of Observation

All of the embryonic lineage was followed by direct observation. This method currently gives the best resolution in space and time, but has the disadvantage that the number of cells which can be followed in a single individual is limited by the short-term memory of the observer. Events were recorded by sketching the nuclei, using a colour code to indicate depth. A camera lucida was used at first, but the effective resolution was re-



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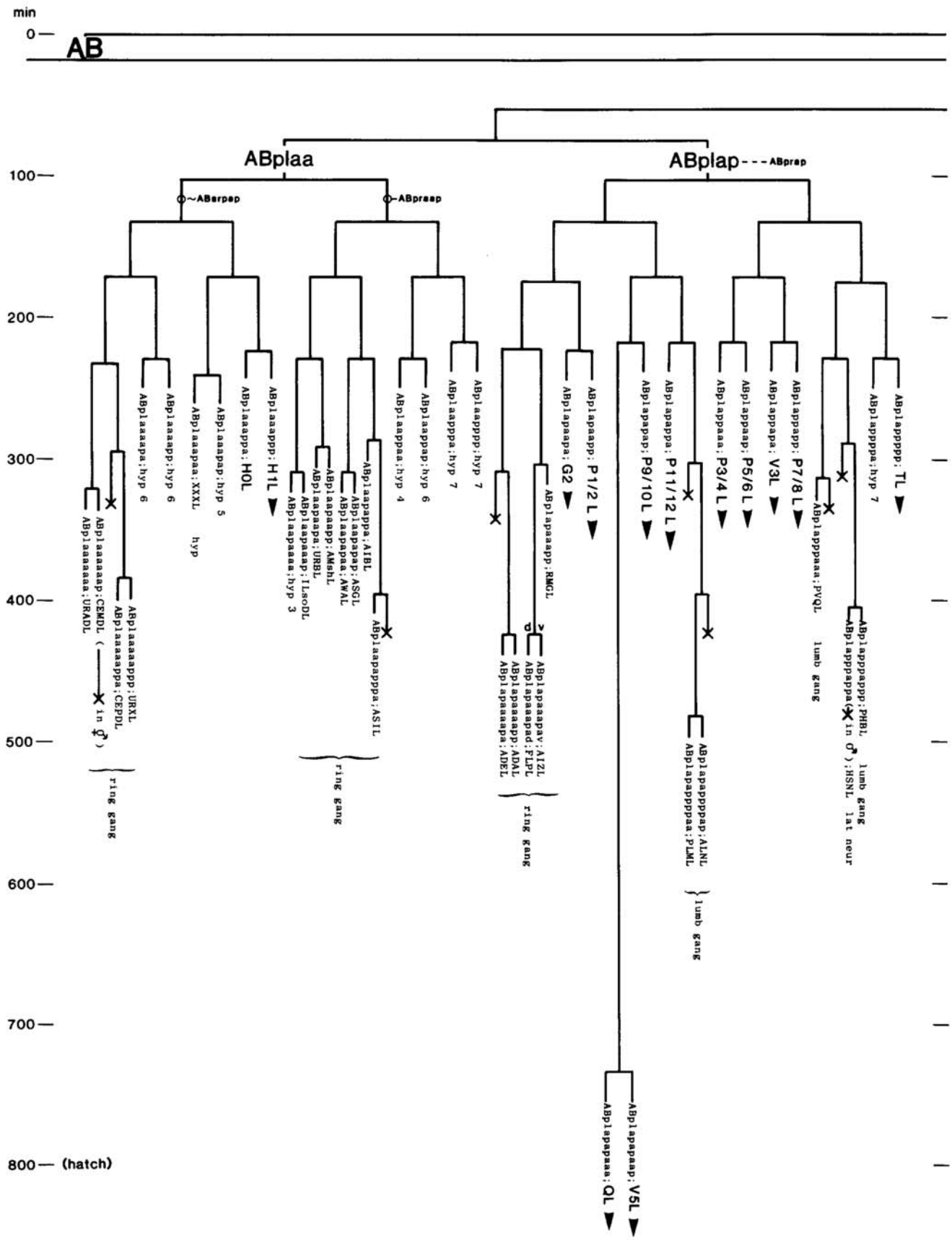
duced by this accessory, and the additional illumination required tended to damage the specimen. The best aid proved to be a pair of gossamer cross hairs in one eyepiece, under which the nucleus of interest could be located with the help of a gliding stage. The light was blocked whenever the specimen was not being viewed.

The earlier part of the lineage was also analysed using videotape recordings, and much of this work has already been described (Deppe *et al.*, 1978). The advantages of this technique are considerable: a permanent record is created, in which the cell lineage can be followed at leisure and in which long-range comparisons of cell movements and the timing of events can be made. However, it was not possible to trace the later cell divisions, particularly those taking place in the interior of the embryo, in this way. In order to resolve a small dividing nucleus it is necessary to be able to focus through it without jitter or excessive electronic noise, and at frequent intervals; this is not easily achieved with current equipment. Nevertheless, videotaping remains the technique of choice for studying early embryogenesis both in the wild type and in mutants and experimental animals.

Fortunately for the direct observer, it is unnecessary to follow the lineage from the beginning for each terminal cell. The developing egg displays a succession of



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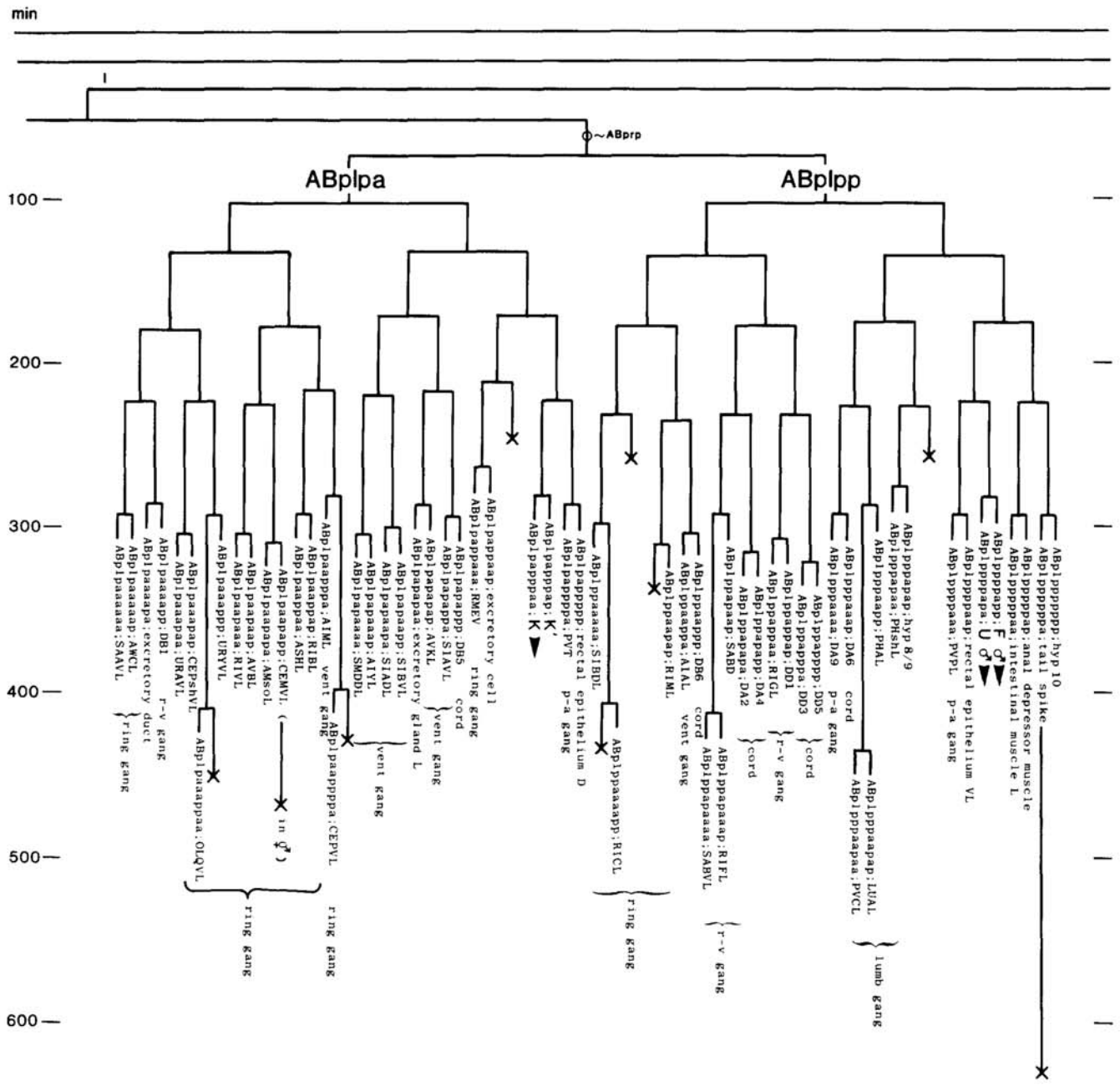
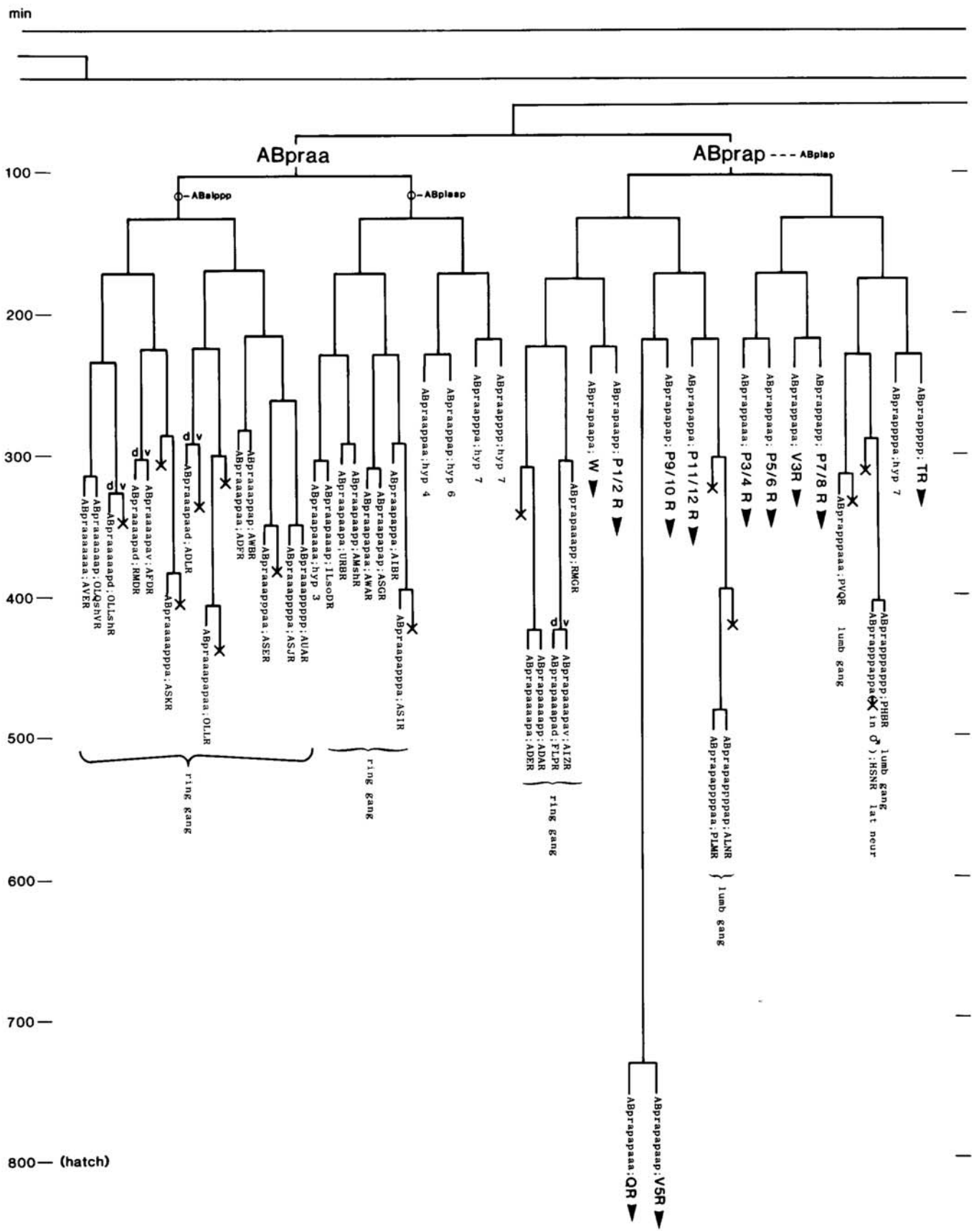


FIG. 3—*Continued.*

reproducible patterns, in which previously identified cells can be recognised. Some of the more useful ones are shown in Figs. 5–8. Although these diagrams were prepared with the aid of a camera lucida they are not intended to show the absolute positions of cells, which in any case vary appreciably from one individual to another; what is reproducible is the neighbourhood of each cell at a given time. The patterns change rapidly, but the behaviour of each cell is characteristic and provides an additional check on its identity. An inexperienced observer should be able to identify nuclei in the diagrams unambiguously by starting one division earlier and checking the arrangement of sister cells.

When placed on an agar layer under a coverslip, the embryo adopts a predictable, though age-dependent, orientation. At the four-cell stage it turns to display either the left or the right side; at gastrulation (100–150 min) it turns from left to dorsal or from right to ventral (these turns are only about 45°, because of the arrangement of blast cells); finally, at 350–400 min, the growing tail forces a return to a lateral aspect. No means of constraint compatible with good resolution was found



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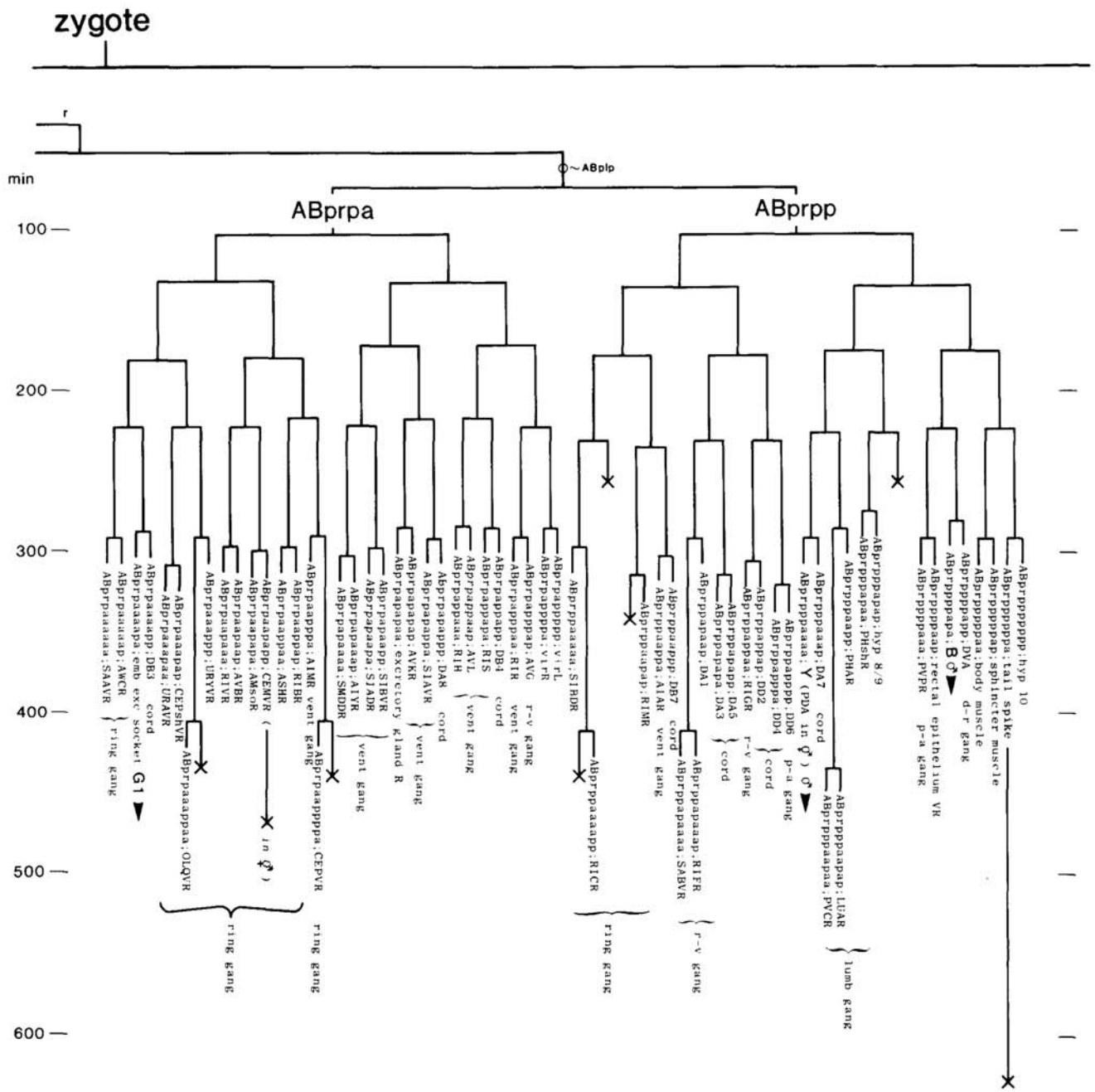


FIG. 3—*Continued.*

to prevent these turns, but they were controlled by selection of obliquely oriented embryos at appropriate times.

Cell deaths were recognised by a characteristic increase in refractility, followed by shrinkage and disappearance (Sulston and Horvitz, 1977; Robertson and Thomson, 1982).

The final step in our analysis was to identify the surviving cells in terms of the known larval and adult anatomy. As many cells as possible were identified at 430 min (by comparison with serial section reconstructions of animals at this stage), because thereafter observation is much more difficult on account of movement of the embryo. The most reliable assignments at this time are for cells which have already formed desmosomes or other structural connections (i.e., hypodermis, body muscles, sensory nervous system, alimentary tract) but some useful clues to the identity of other neurons can be gleaned from the initial outgrowth of their processes.

The cells which were not identifiable at this stage (mainly interneurons and motorneurons) were followed in small groups until the animal hatched. These obser-

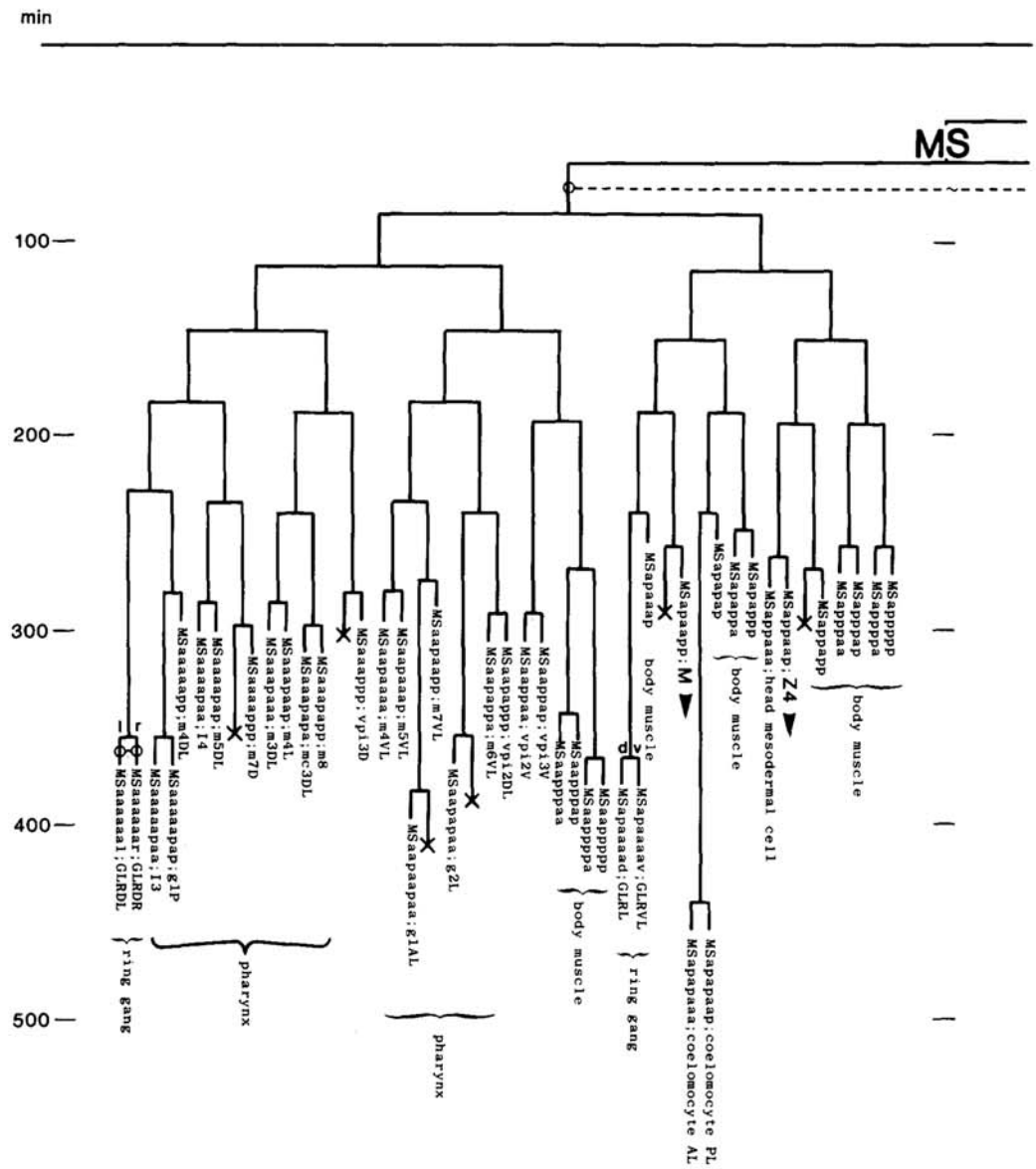


FIG. 3—*Continued.*

vations are tedious because from 450 min onwards the embryo rotates continually about its longitudinal axis, and it is necessary to train the eye to rapid pattern recognition for each cell group in turn. After hatching, some cells were identified from previously known L1 anatomy (J. G. White *et al.*, unpublished) and the remainder were traced into the adult. This is a relatively easy task because the larvae do not rotate and the patterns change only slowly, so that an entire ganglion can be followed in one individual.

Reliability

The lineage is based on a minimum of two direct observations for unique events, and one direct observation on each side for bilaterally symmetrical events. However, the great majority of events received considerably more confirmation than this. In addition, the earlier part of the lineage, extending to the terminal divisions in the intestine and the lateral hypodermis, was followed independently by videorecording.

Perhaps it is too much to hope that we have entirely avoided errors. It is worth emphasising, however, that any which have arisen are clerical in nature; there are no guesses, and the validity and reproducibility of a particular event can readily be checked by further observation.

Nomenclature

The system of lineage nomenclature is essentially that described by Sulston and Horvitz (1977). Certain key

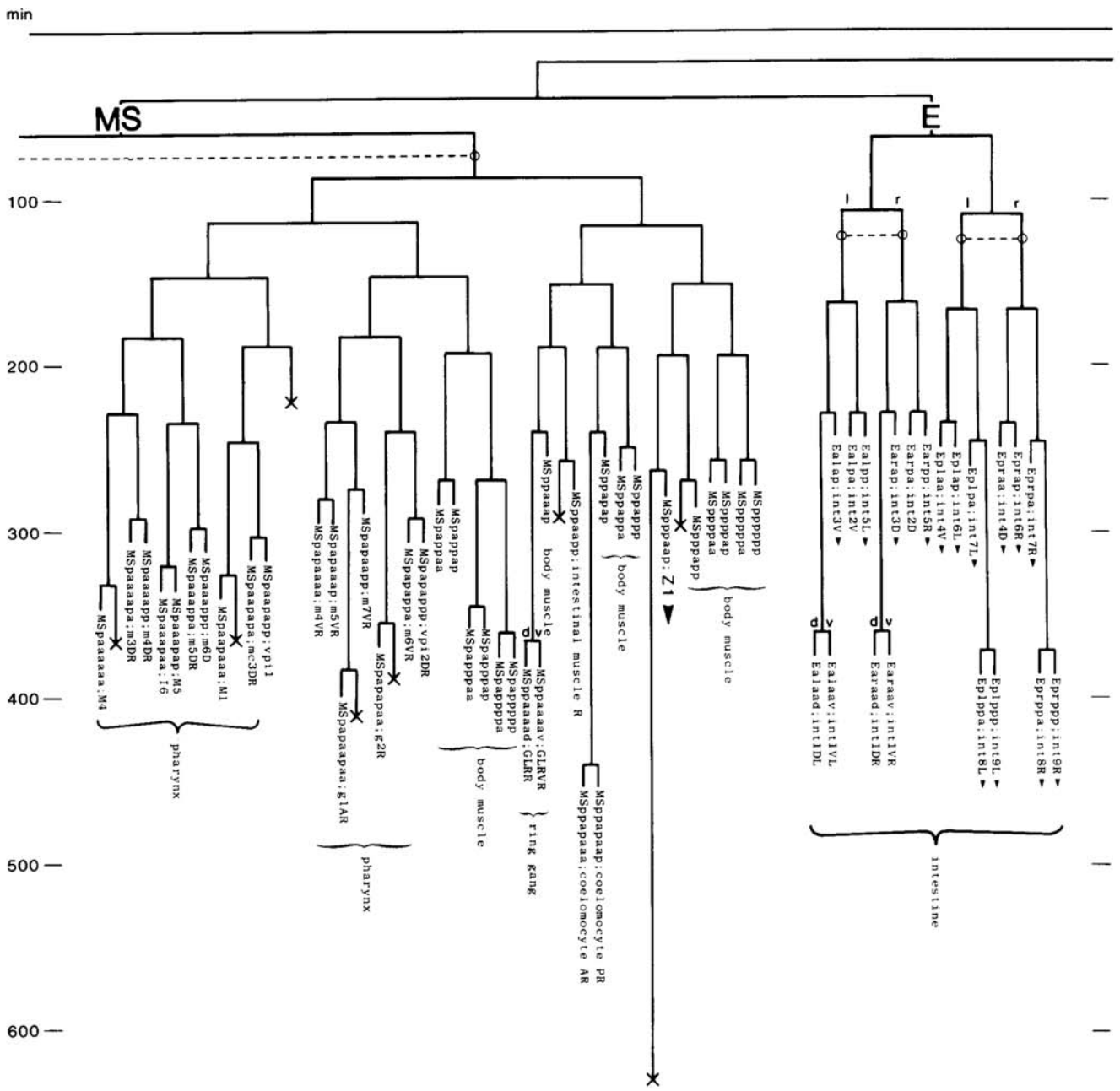


FIG. 3—*Continued.*

blast cells are given arbitrary names comprising uppercase letters and numbers; their progeny are named by adding lowercase letters indicating the approximate division axis according to an orthogonal coordinate system (a, anterior; p, posterior; l, left; r, right; d, dorsal; v, ventral); the next generation of cells is named by appending further letters in the same way; and so on. Existing blast cell names have been retained as far as possible, but certain changes are desirable to avoid confusion whilst conforming to our system: MS was formerly MSt, U was E, Y was C, W was "P0.a," QL was Q1, and QR was Q2. It should be noted that AB and B are entirely separate names, as are P_0–P_4 and P1–P12. A pair of cells may be designated by the use of internal parentheses, e.g., MS($^{a}_{p}$)pa means MSapa and MSppa. Pairs of identical postembryonic cells, lying on the left and right sides of the animal, have previously been given identical names; they are now distinguished by the addition of symmetry operators, as defined in the next paragraph. Sometimes developmental stages are named

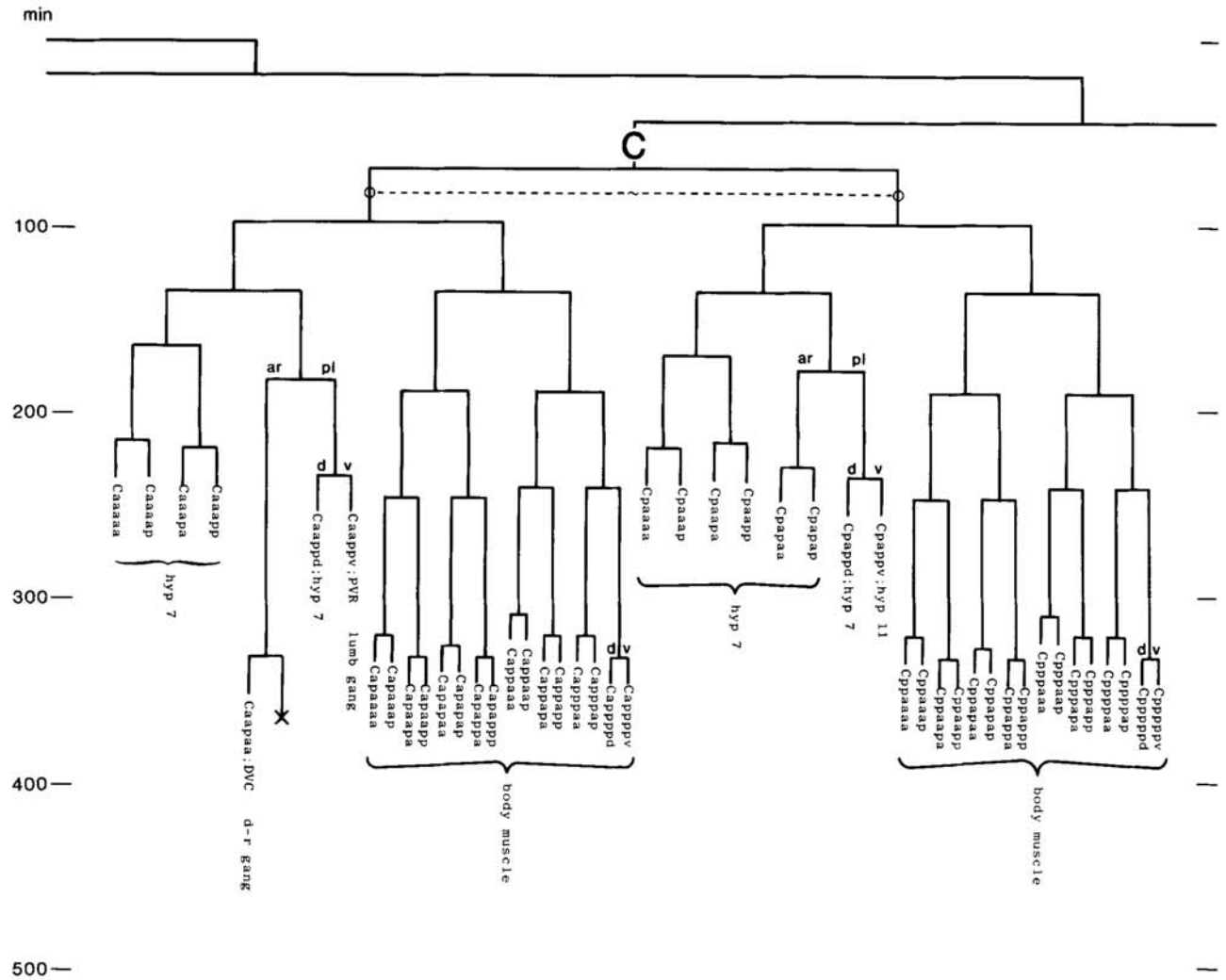


FIG. 3—*Continued.*

after the number of progeny generated by a particular founder cell; e.g., MS^8 means that MS has divided into eight cells.

Functional names are listed alphabetically in the Appendix, and follow a variety of systems:

Neurons and supporting cells other than those in the pharynx: White *et al.*, (in preparation). This system is largely self-explanatory (see Appendix), but note that it uses symmetry operators as suffixes to distinguish cells which differ only in position (A, anterior; P, posterior; D, dorsal; V, ventral; L, left; R, right).

Pharynx: Albertson and Thomson (1976), with the addition of symmetry operators.

Hypodermis: See Figs. 12–14; symmetry operators are used for the arcades and hypodermal rings 1 and 2.

Muscle: see Fig. 15.

Gonad: Kimble and Hirsh (1979).

Intestine: see Fig. 8c.

In descriptions of cell division the terms "equal" and "unequal" refer to the relative sizes of the daughters; "symmetrical" means that the daughters not only are equal in size but also are (or subsequently become) disposed symmetrically across the midline; "equational" means that the daughters are supposedly of equal developmental potential.

RESULTS AND COMMENTS

GENERAL DESCRIPTION

Invariance

The development of the embryo has proved to be essentially invariant (Fig. 3). There seems to be no naturally occurring indeterminacy like that found postembryonically in the gonad (Kimble and Hirsh, 1979), the ventral hypodermis, and the male tail (Sulston and Horvitz, 1977).

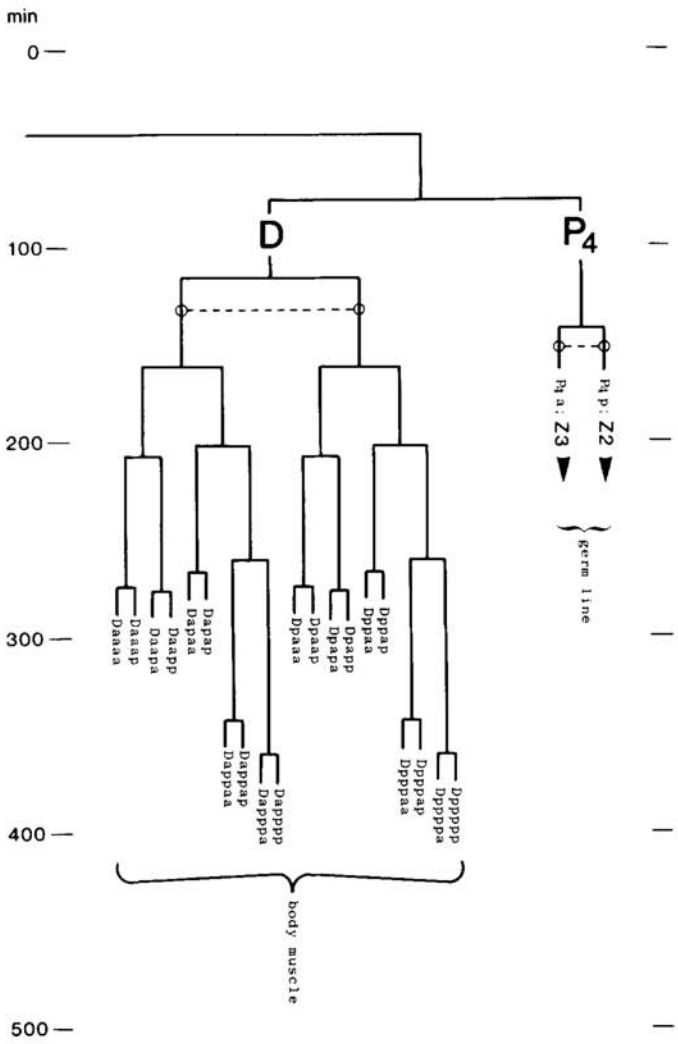


FIG. 3—*Continued.*

Cell Divisions and Cell Movement

The majority of cell divisions take place during the first half of embryogenesis. During the second half, the embryo changes greatly in external appearance: it elongates more than threefold, moves actively, synthesises cuticle, and initiates pharyngeal pumping before breaking out of the egg. This does not mean, however, that in the first half of embryogenesis there is no differentiation. On the contrary, by 430 min (the stage shown in Fig. 8), gastrulation and organogenesis are complete and the majority of the desmosomes seen in the L1 larva have already been made; subsequent events involve principally stretching and functional maturation of cells.

The Founder Cells

The fertilised egg cleaves into a larger anterior and a smaller posterior daughter. The latter (P_1) is a stem-like cell which continues to cleave unequally for a further three rounds of division (Fig. 9); it is then named P_4, and is the ancestor of the germ line. The anterior daughters AB, C, and D are called founder cells, and divide approximately equally with characteristic periods (Chitwood and Chitwood, 1974; Deppe *et al.*, 1978); the anterior daughter EMS divides unequally into the founder cells MS and E, which then proceed to divide equally. At one time the various founder cells were thought to give rise to discrete tissue types and were named accordingly; as will be seen later, it is actually not possible to classify all of them in a precise way and so this terminology will not be used here. We use the term "founder cell" (Schierenberg and Cassada, 1982) in preference to the classical "stem cell," which nowadays has a rather different connotation; an alternative expression is "embryonic blast cell" (Laufer *et al.*, 1980).

In addition to dividing with a characteristic period, the clone of cells derived from each founder cell behaves in a characteristic way, as follows:

AB. Starting from the anterior part of the egg, the cells spread over the entire surface except for the posterior dorsal region. Some of them lie inside the head temporarily at an early stage. Towards the end of gastrulation the AB pharyngeal precursors enter the interior through the ventral side of the head, and, later still, four muscles and the rectal cells sink into the ventral surface of the tail.

MS. Divides to eight cells on the ventral surface. In the course of the next two rounds of division the pharyngeal precursors sink inwards and form two rows in the head; meanwhile, the body muscle, coelomocyte, and somatic gonad precursors insinuate themselves between the intestine and the surface layer of AB cells.

E. Generates only intestine. Divides ventrally into two cells, which are the first to enter the interior in the course of gastrulation. Further division leads to a cylinder of cells with distinctively granular cytoplasm lying along the body axis.

C. Starting from the posterior dorsal side of the embryo, the cells spread anteriorly and posteriorly. The most posterior ones are body muscle precursors, which travel round to the ventral side and enter the interior immediately after D. Most of the remaining cells are employed in forming the dorsal hypodermis over the posterior two-thirds of the body; their nuclei migrate contralaterally (see Migrations).

D. Generates only body muscle. Enters the interior after division to four cells, at the same time as $MS(^{a}_{p})p$.

P_4. Generates only germ line. Enters the interior after E, and divides into two cells which extend lobes into the intestine (Fig. 17).

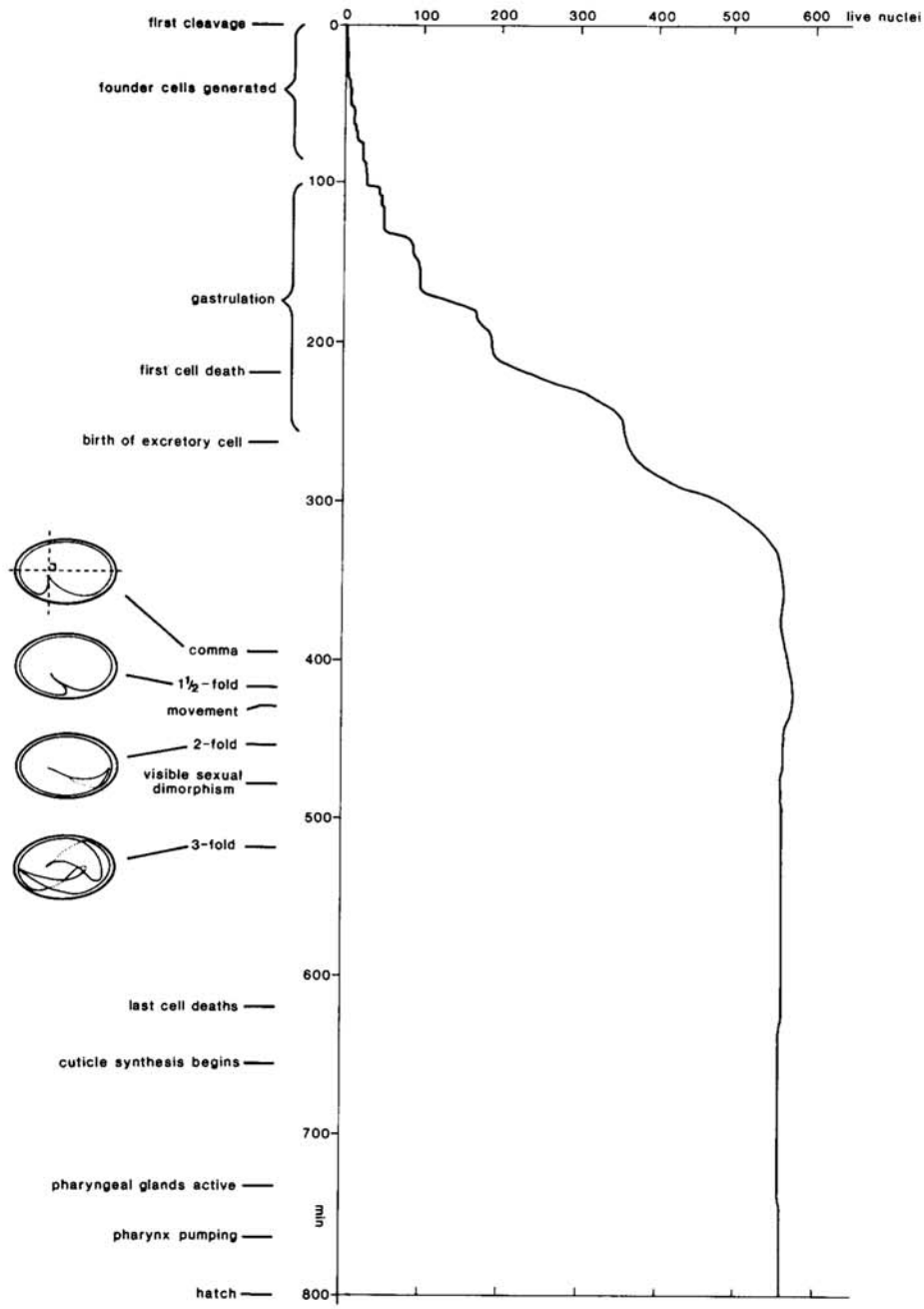


FIG. 4. Marker events and count of living nuclei during embryogenesis. Fertilisation is normally at −50 min. Note that the comma stage is defined in a precise way, as the moment at which the ventral surface of the tail lies perpendicular to the long axis of the egg.

Gastrulation

At 100 min after first cleavage, when the egg comprises 28 cells, gastrulation begins (Fig. 5). The first cells to enter the interior are Ea and Ep, which constitute the endoderm; they sink inwards from the ventral side, near the posterior end of the embryo. Next, at 120–200 min, are P_4 and the progeny of MS. The entry zone widens and lengthens, spreading first posteriorly as most of the remaining myoblasts (derived from C and D) enter (180–230 min), and then anteriorly as the AB-derived part of the pharynx enters (210–250 min). The ventral cleft closes from posterior (230 min) to anterior (290 min). As gastrulation proceeds, the clone of E cells and the precursors of the pharynx form a central cylinder, while the body myoblasts insinuate themselves between this cylinder and the outer layer of cells. Although most of the myoblasts enter the body

cavity during gastrulation, two do not. These are ABp($\frac{l}{r}$)pppppa, which do not sink inwards until the time of their terminal divisions at 290 min.

Later Cell Movements

At 250 min the ventral side is occupied largely by neuroblasts. During the following hour the latter undergo their last major round of division, and their progeny become covered by a sheet of hypodermis which grows circumferentially from a row of lateral cells on each side. Although there is a sufficient latero-ventral movement of cells to fill the gap left by the entry of the pharyngeal precursors, there is not a general flow of neural tissue through the ventral cleft. Dorsal and lateral neuroblasts sink directly inwards and their progeny become covered by adjacent hypodermal cells.

Migrations

As in postembryonic development, many cells move short distances past their neighbours but only a few embark upon long-range migrations. It seems that the lineage not only generates cells of the correct types but also places them, for the most part, in appropriate positions. The way in which this may have come about is suggested later (see Conclusion).

The cells that migrate furthest are: the postembryonic mesoblast M and its contralateral homologue mu int R (Fig. 16); the somatic gonad precursors Z1 and Z4 (Fig. 16); CANL and CANR, which move from the anterior end to a point midway along the body (Figs. 7a, 8a, 14); HSNL and HSNR, which move from the posterior end to a point midway along the body (Figs. 8a, 14); ALML and ALMR, which move posteriorly from the anterior end of the intestine (Figs. 8a, 14). All these migrations are longitudinal. The most extensive circumferential migration is that of the head mesodermal cell (hmc) and its contralateral homologue, but many of the body muscles also move circumferentially as they assemble into rows.

The group of cells which forms most of the dorsal hypodermis exhibits nuclear migration as opposed to cellular migration (see Fig. 10). These cells are born subdorsally, in two longitudinal rows; a cytoplasmic process grows circumferentially from each cell across the dorsal midline, and after a time the nucleus migrates along this process until it lies on the opposite side of the embryo. This type of migration is analogous to that seen postembryonically in the P cells (Sulston and Horvitz, 1977), and it is therefore interesting that mutations at two loci interfere with both processes (Sulston and Horvitz, 1981).

Programmed Cell Death

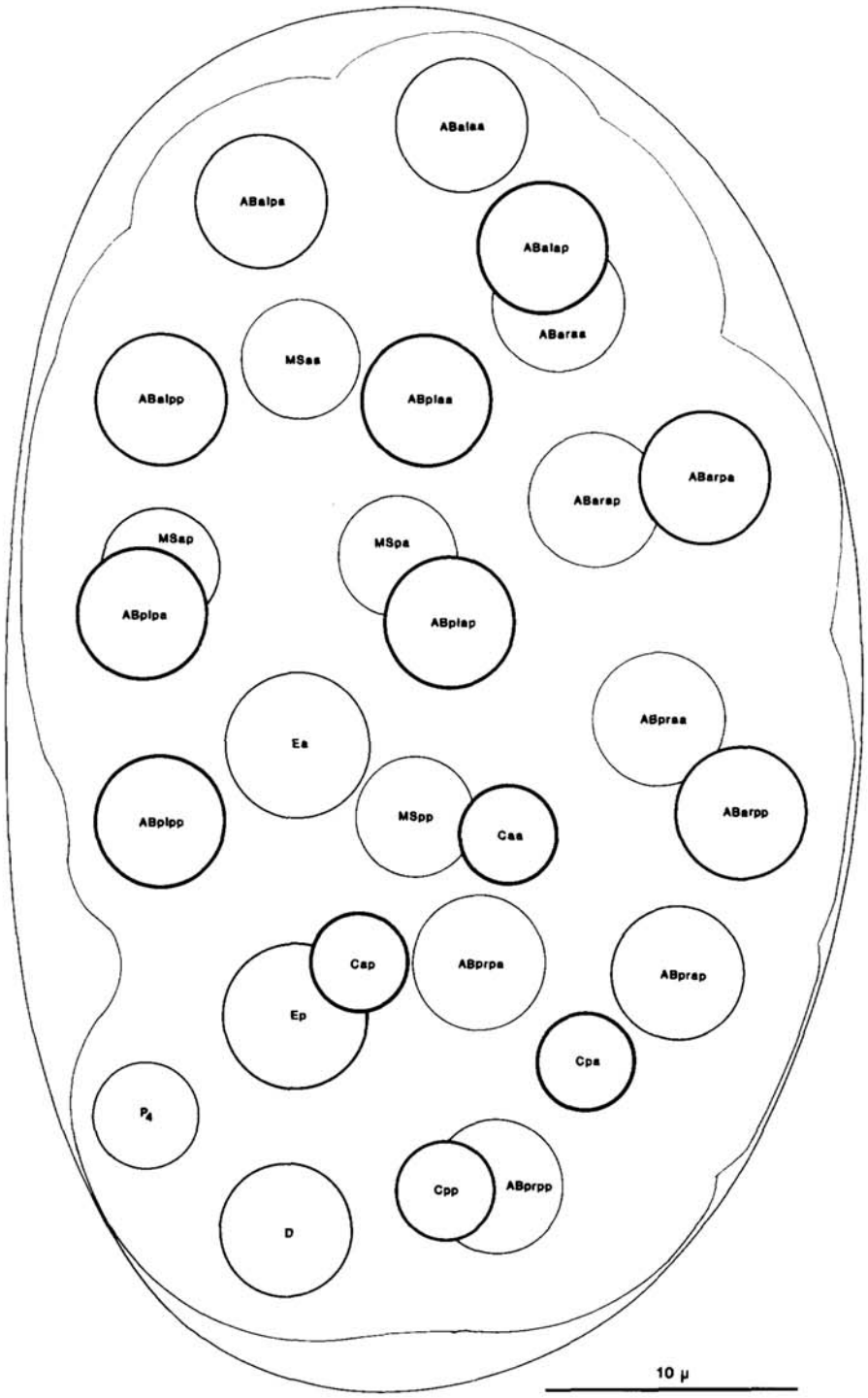
In the course of the lineage, one in six of all cells produced subsequently dies; their identity and the approximate times of their deaths are predictable. The mode of death is similar to that seen previously in the postembryonic lineages (Sulston and Horvitz, 1977; Robertson and Thomson, 1982). In some cases death occurs several hours after birth, so that it is possible for the cells to function in some manner before being discarded. A good example is the pair of tail spike cells, which fuse together, form a slender bundle of filaments in the tip of the tail, and then die. At the other extreme are the majority of programmed deaths, which occur 20 to 30 min after birth; these cells are born with very little cytoplasm, and die without differentiating in any obvious way. The limited sexual dimorphism seen in the embryo is a consequence of differential cell death (see Nervous System).

All the dying cells are promptly phagocytosed by their neighbours (cf. Robertson and Thomson, 1982). During the first wave of deaths, when the surviving cells are relatively large and rounded, this process can be followed by light microscopy; the engulfing cell is almost always the sister of the dying cell at this time (Fig. 2e). Later on, cell boundaries cannot usually be resolved by light microscopy but electron micrographs show that all dying cells (identified by their high electron density) lie within other cells (Fig. 2i); the principal phagocytes now seem to be the hypodermis (both seams and syncytia) and the pharyngeal muscles, though one death (possibly MSpaapp, which tends to be delayed) was found in the anterior intestine.

Other Nematode Species

Previous research showed convincingly that the pattern of early cleavage was uniform in the nematodes examined (all of which belong to the class Secernentea (Chitwood and Chitwood, 1974)). However, authors working on different species disagreed about the tissues to which certain founder cells gave rise; these disagreements may have been due either to the difficulties of interpreting observations on fixed specimens or to genuine differences between the species.

The most interesting discrepancy is in the origin of the somatic gonad, which was reported to arise from the founder cell P_4 in the nematodes *Turbatrix aceti* (Pai, 1927), *Ascaris megalocephala* (Boveri, 1892; but not sustained in later reports), and *Bradynema rigidum* (zur Strassen, 1959). Given the consistency of the early cleavage pattern of nematode embryos, a switch in the origin of such a vital tissue would be surprising indeed. Other discrepancies are in the fates of C progeny (said to be only five, and exclusively ectodermal, in *T. aceti*



FIGS. 5–8. Drawings of embryos. Circles and ovals represent nuclei, traced by means of a camera lucida, the thickness of the lines being inversely related to depth; outlines of the egg, embryo, and internal structures are traced with thin lines (regardless of depth). Anterior is towards the top of the page. Dying nuclei are stippled.

FIG. 5. Embryo, 100 min, left dorsal aspect; all nuclei included; cf. Fig. 2b. This stage has already been well characterised (Krieg *et al.*, 1978), and the observer quickly learns to recognise all the nuclei; it is a useful starting point both for lineages and for ablation experiments. An embryo in the orientation shown will present a dorsal aspect until it turns at 350 min; an embryo with the MS cells uppermost will present a ventral aspect. The intestinal precursors are entering the interior, leaving a characteristic depression on the ventral side.

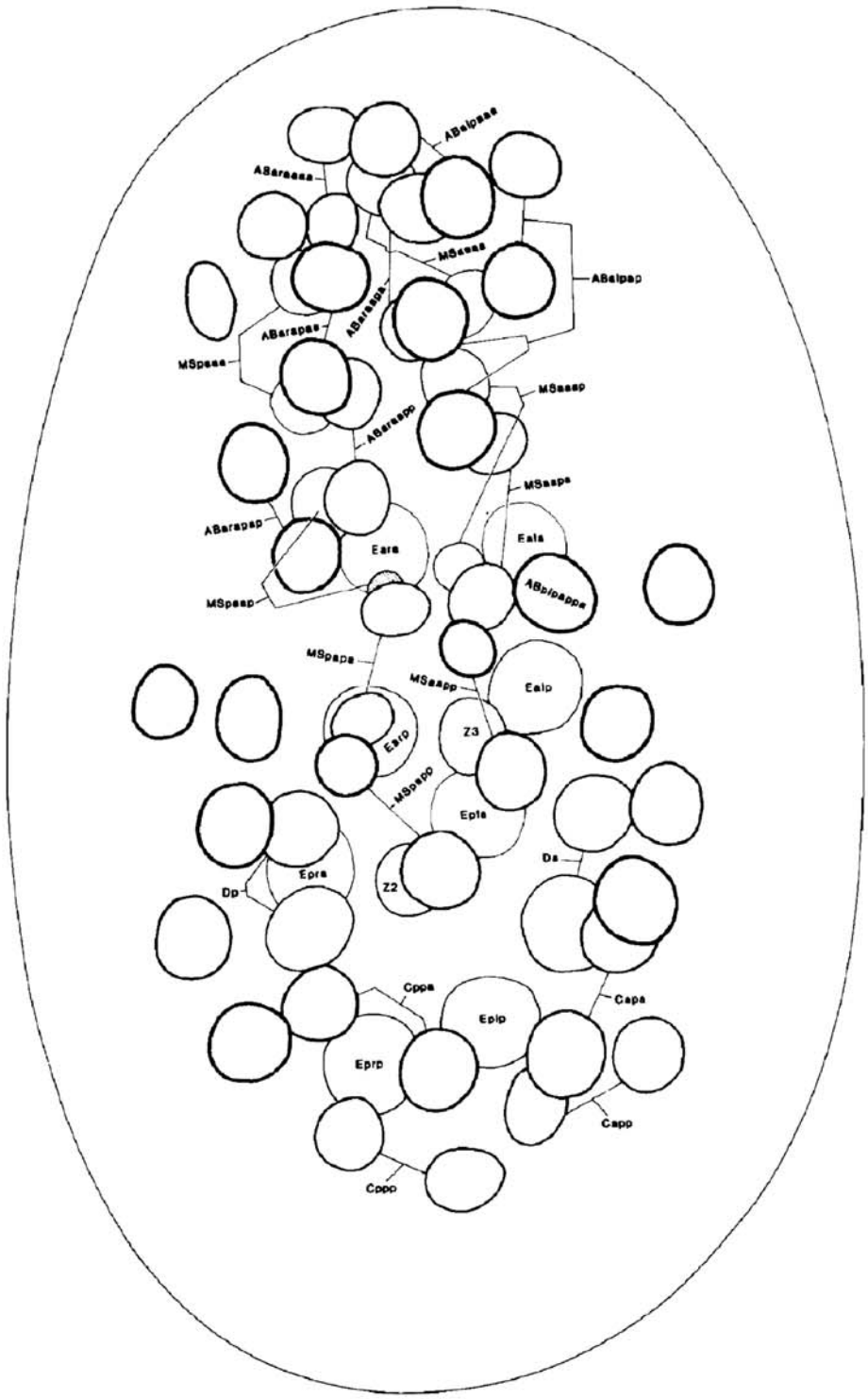


FIG. 6. Embryo, 200 min, ventral aspect, late gastrulation. The orientation of the egg can be recognised by the prominent ventral cleft; cf. Fig. 2c. All progeny of MS, E, D, and P_4 are shown, together with AB-derived pharyngeal precursors and C-derived myoblasts; other AB progeny (unlabelled) are included for perspective. MSaa and MSpa have each formed a chain of eight cells, of which all but the posterior two have entered the interior. The derivatives of ABaraap have also entered, and the remainder of the AB pharyngeal precursors will soon follow. Posteriorly, the mesodermal precursors derived from C and D are also entering the cleft. The germ line and the intestine can be seen lying more dorsally. The division of ABplpappa (earlier than its neighbours and very unequal) is a useful time point. Embryos of this age, but viewed from the dorsal side, were used as starting points for lineages of the dorsal pharynx and dorsal body muscle.

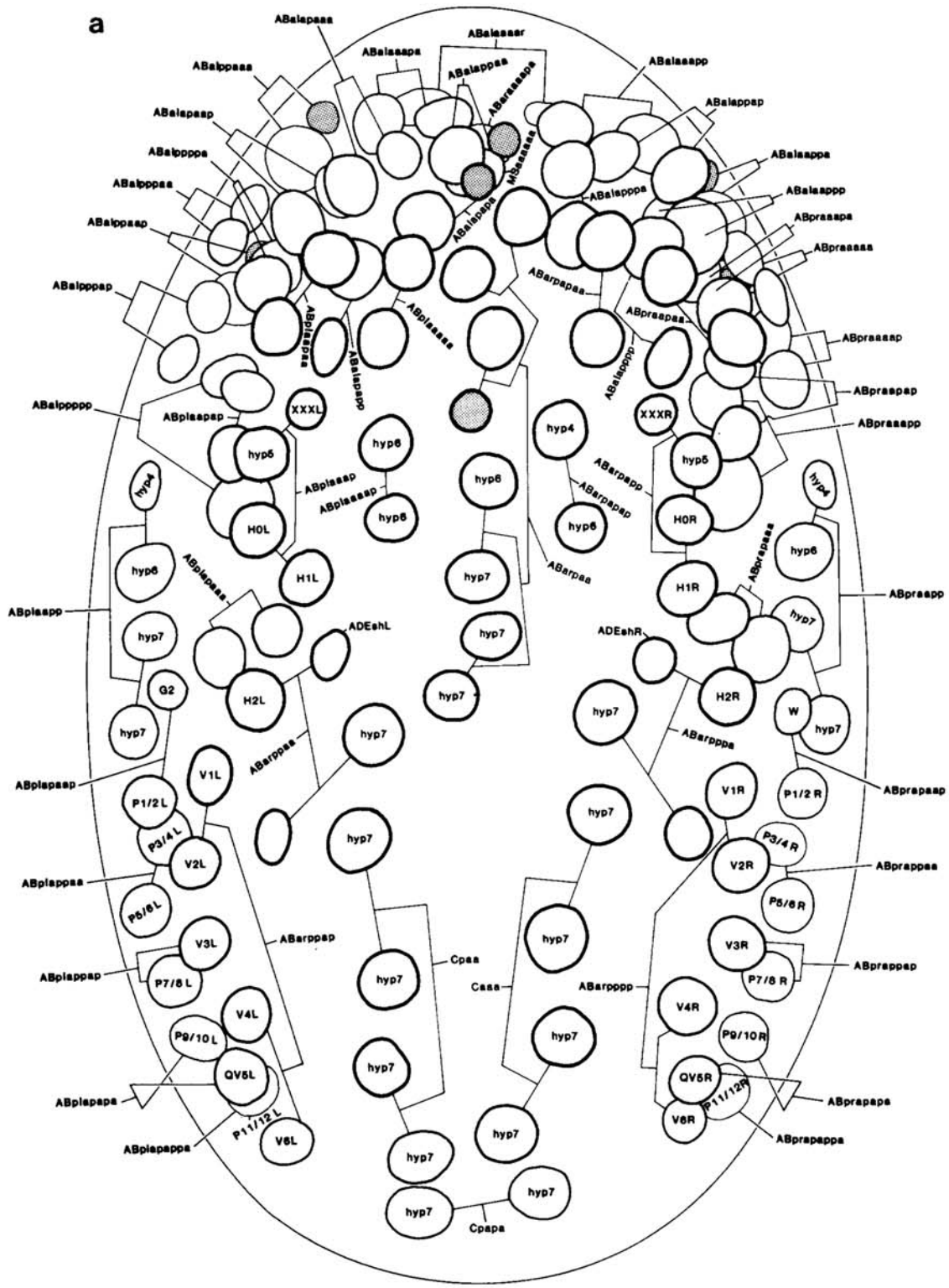


FIG. 7a. Embryo, 260 min, dorsal aspect, superficial nuclei; cf. Fig. 2d. Landmarks: nuclei of hyp4–hyp7, cell deaths; time points: division of various neuroblasts. The dorsal hypodermal cells have very granular cytoplasm and form prominent transverse ridges. In Fig. 7 some licence has been allowed in depicting cell deaths, because of their importance in pattern recognition; in fact, they do not all become refractile simultaneously.

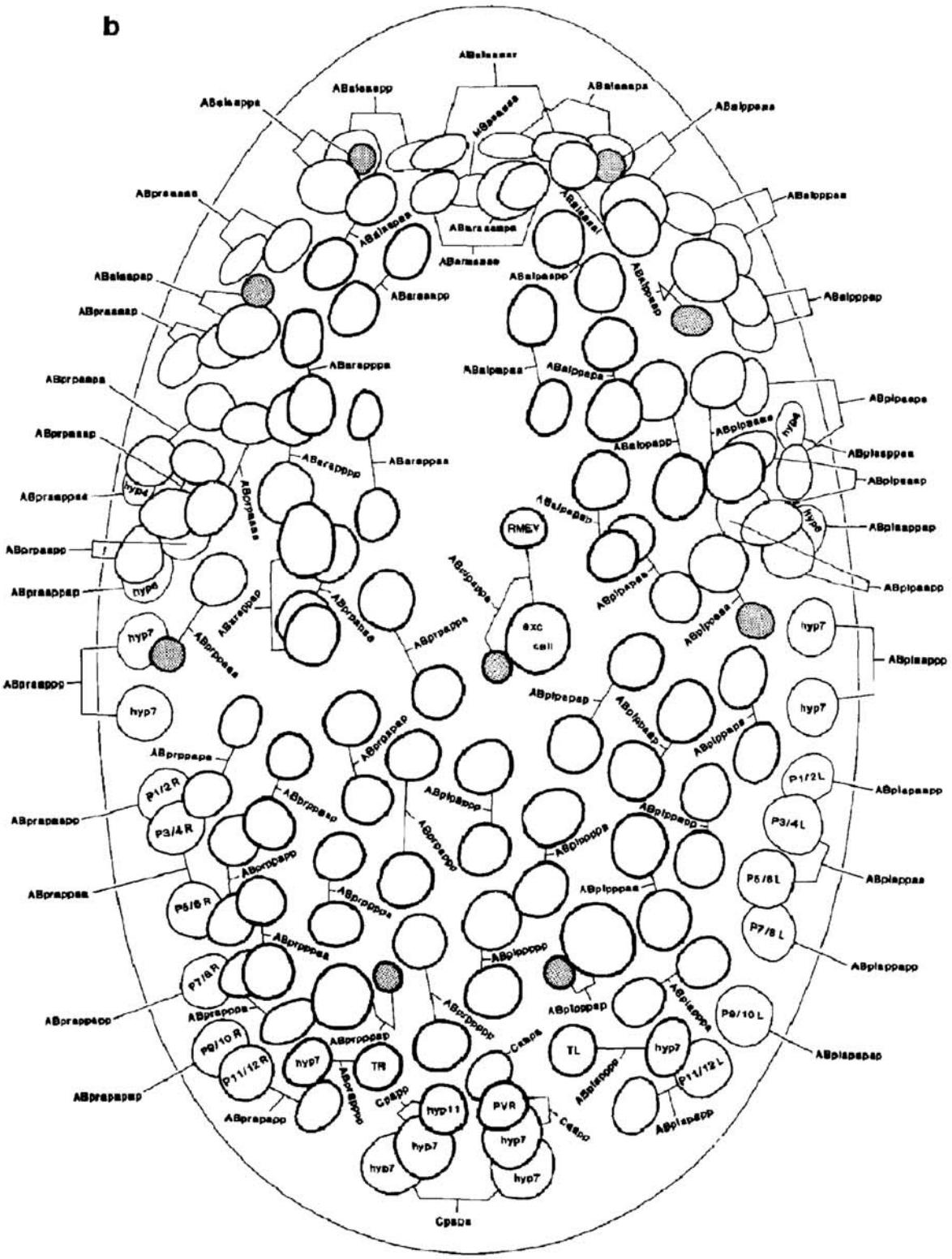


FIG. 7b. Embryo, 270 min, ventral aspect, superficial nuclei; cf. Fig. 2e. Landmarks: excretory cell, cell deaths; time point: division of mother of excretory cell. The gap anterior to the excretory cell contains pharyngeal and buccal precursors which are entering the interior.

(Pai, 1927)) and D progeny (reported to form the rectum, by several authors; reviewed by Chitwood and Chitwood (1974)). However, the latter assignment seems to be hypothetical, since it rests solely on the observation that the D cells enter the interior of the embryo. In order to go some way towards resolving these uncertainties, we have followed a few lineages in other nematode species.

The results for *T. aceti* are shown in Fig. 11. We find that this nematode is identical with *C. elegans* in the following respects: the origin of the somatic gonad and the germ line (cell assignments based on morphology at hatching); the origin and behaviour of "M" and its contralateral homologue (followed to the equivalent of 320 min, cf. Fig. 16); the behaviour of the progeny of C and D (followed explicitly to the equivalent of 230 min, by which time the mesoblasts are distinctive). *T. aceti* differs from *C. elegans* within the endoderm lineage, and it will not be surprising if it also differs in details of the MS and AB lineages.

We have also followed the origin of the founder cells and certain later lineages in the embryo of *Panagrellus redivivus*. This nematode is of interest because Sternberg and Horvitz (1981, 1982) have shown that its postembryonic lineage is quite similar to that of *C. elegans*, and that the newly hatched animal contains the same set of blast cells with one addition. We find that this extra blast cell, known as T3, has the embryonic ancestry Caappa; it is therefore homologous with the neuron PVR of *C. elegans*. On the basis of Nomarski microscopy, PVR is absent from *P. redivivus* (Sternberg, personal communication). *P. redivivus* proved to have the same endodermal lineage as *T. aceti*, to which it is closely related (Ritter, 1975); in one individual, however, division of Ea($^{l}_{r}$)ap resulted in an intestine having 20 cells instead of the usual 18.

zur Strassen (1959) based his conclusion—that in *B. rigidum* P_4 yields the somatic gonad—upon the observations that P_4 divides in the embryo and that the newly hatched larva contains only one germ cell. Although we were not able to obtain *B. rigidum*, we investigated another member of the order Tylenchida, *Aphelencoides blastophthorus*, which similarly contains a single germ cell at hatching. We find that P_4 does not divide in the embryo of *A. blastophthorus*, and becomes the solitary larval germ cell. Comparison with the detailed drawings provided by zur Strassen (1892) shows that the early cleavages of the two nematodes are very similar and reveals the reason for the discrepancy: he had inadvertently reversed anterior and posterior, so that the dividing cell which he took to be P_4 was in fact one of the AB group.

In conclusion, although differences of detail have been seen, the general pattern of cell fates shown in Fig. 9 is correct for the two rhabditids *C. elegans* and *T. aceti*, and may well be conserved throughout the class Secernentea.

TISSUE DESCRIPTION

Hypodermis

The hypodermis is a sheet of cells which forms the outer surface of the nematode and secretes the cuticle. Its postembryonic development has been described by Sulston and Horvitz (1977), and other aspects have been discussed by White (1974) and by Singh and Sulston (1978), but no comprehensive account has previously been given.

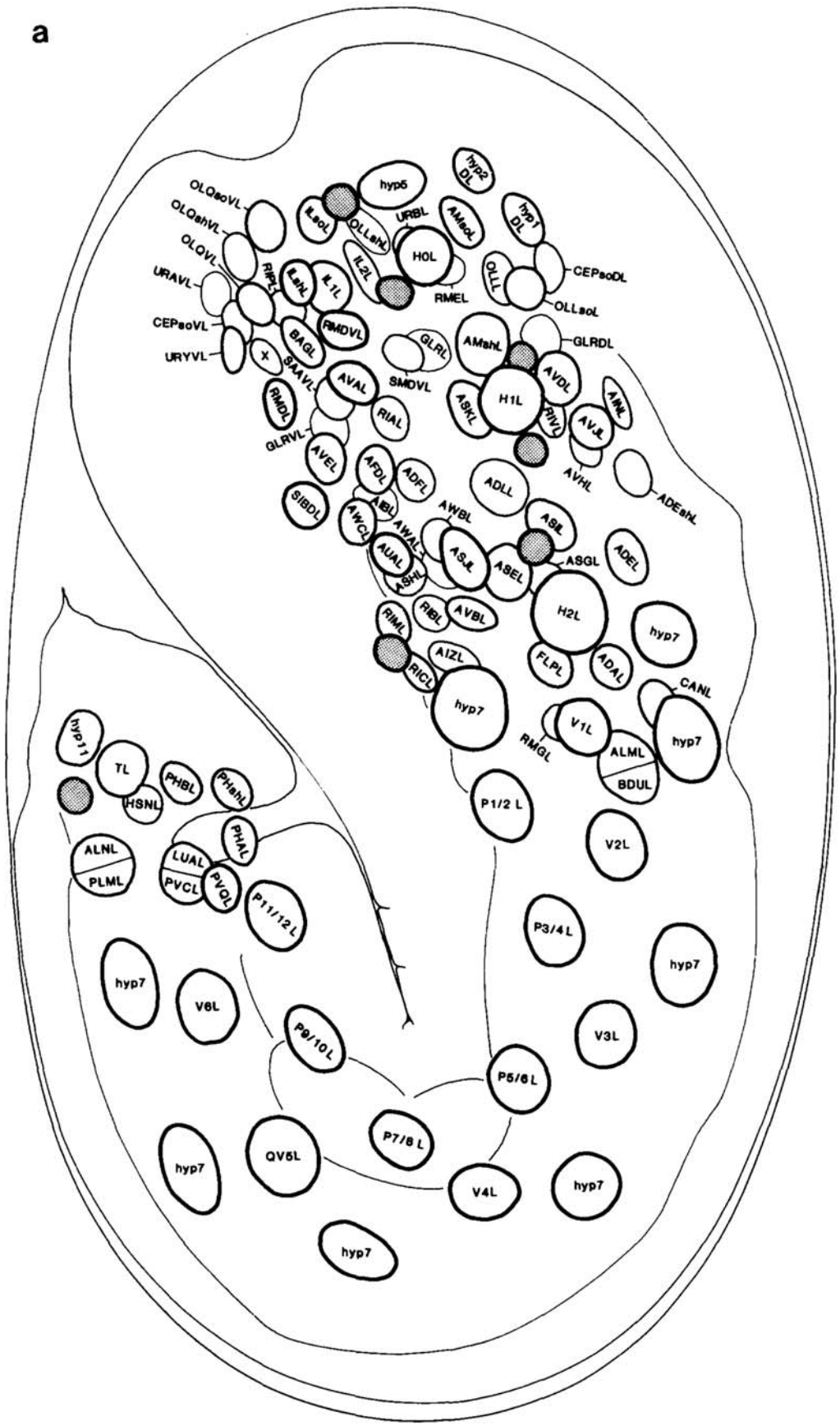
In the newly hatched L1 the anterior part of the hypodermis consists of a series of cylindrical syncytia linked together by desmosomes. These cylinders are numbered hyp1 to hyp7 from the mouth posteriorly (Fig. 12). The anterior and posterior arcades, which are historical names for the specialised hypodermis which lines the mouth (Chitwood and Chitwood, 1974), follow the

FIG. 8. This figure illustrates the arrangement of all left and central nuclei at 430 min after first cleavage; the right-hand side is a mirror image of the left, except where otherwise indicated. The three parts roughly represent three planes of focus (from superficial to central), but there is considerable overlap between them. The anterior sensory depression (not a mouth opening—see text) is at the top and the lengthening tail, terminating in the spike, curves round to the left. On the ventral side of the tail the rectal opening has appeared, and on the ventral side of the head the excretory duct leads to the excretory pore. Since the major hypodermal migrations are complete at this stage, the ancestry of nuclei in hyp3-hyp11 can be inferred from Fig. 13.

FIG. 8a. Left lateral ectoderm. No midplane nuclei are included, but the outlines of the pharynx, intestine, and gonad are shown for reference. Nuclei which will soon divide are labelled with the names of both presumptive daughters. The parent of QL and V5L is named QV5L. The pattern on the right is identical, except that: the homologue of hyp11 is PVR; an extra hyp7 nucleus (ABarpaappp) lies dorsal to H2R; there is no hyp2 DR.

FIG. 8b. Left and central mesoderm (excluding pharynx). M, mu int R, hmc, and MSpppaaa lie in the midplane. Unlabelled nuclei are in body muscles. The pattern on the right is identical, except that ABprpppppaa and ABprpppppap become a body muscle and the sphincter muscle, respectively, and lie slightly more anteriorly than their left-hand homologues.

FIG. 8c. Intestine, gonad, left central pharynx, and ectoderm; cf. Fig. 2g. The pattern on the right is identical, except that: the homologue of G2 is W, of I6 is M1, of U is B, and of K is K'; an asymmetric neuron RIS (ABprpappapa) lies anterior to AVKR.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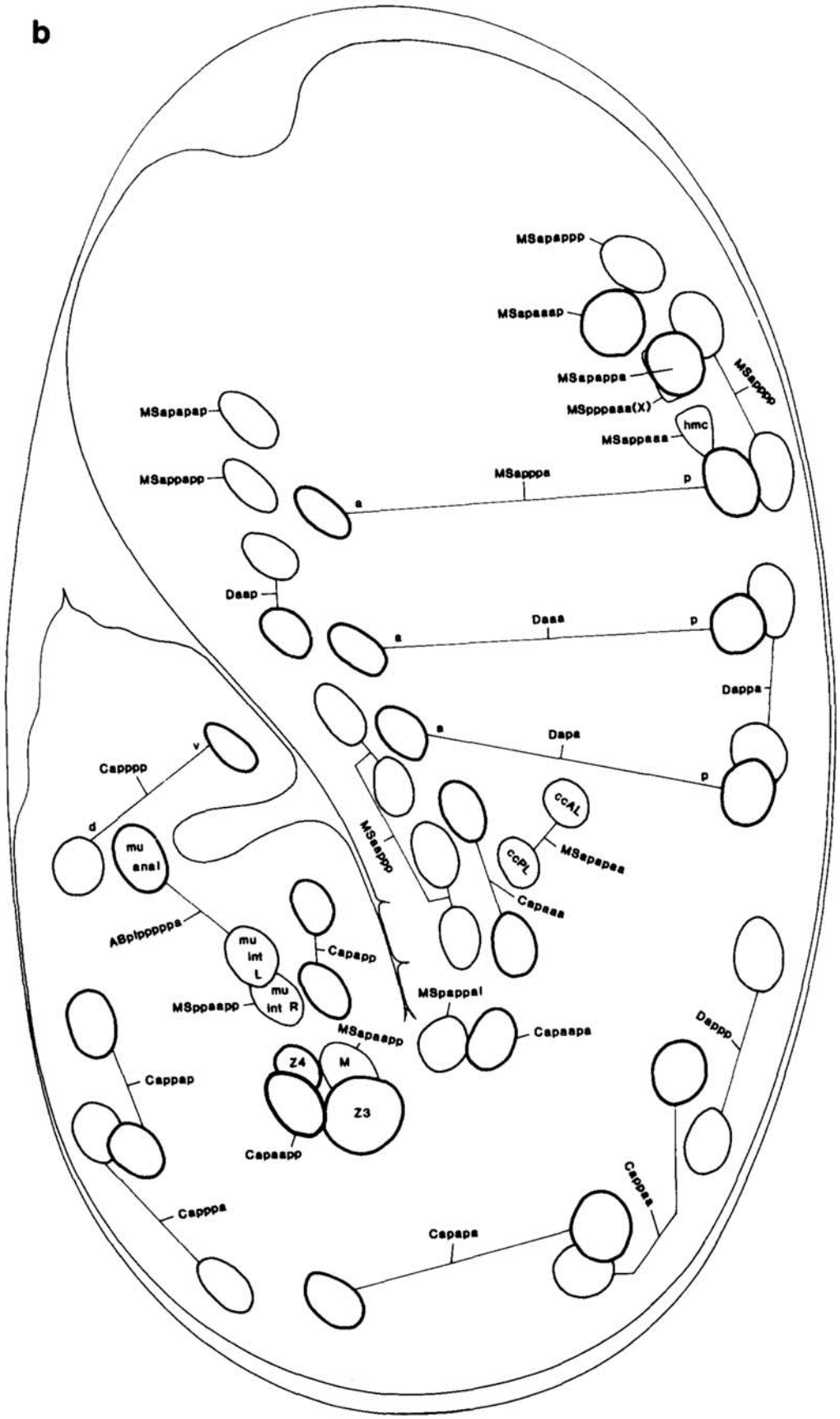
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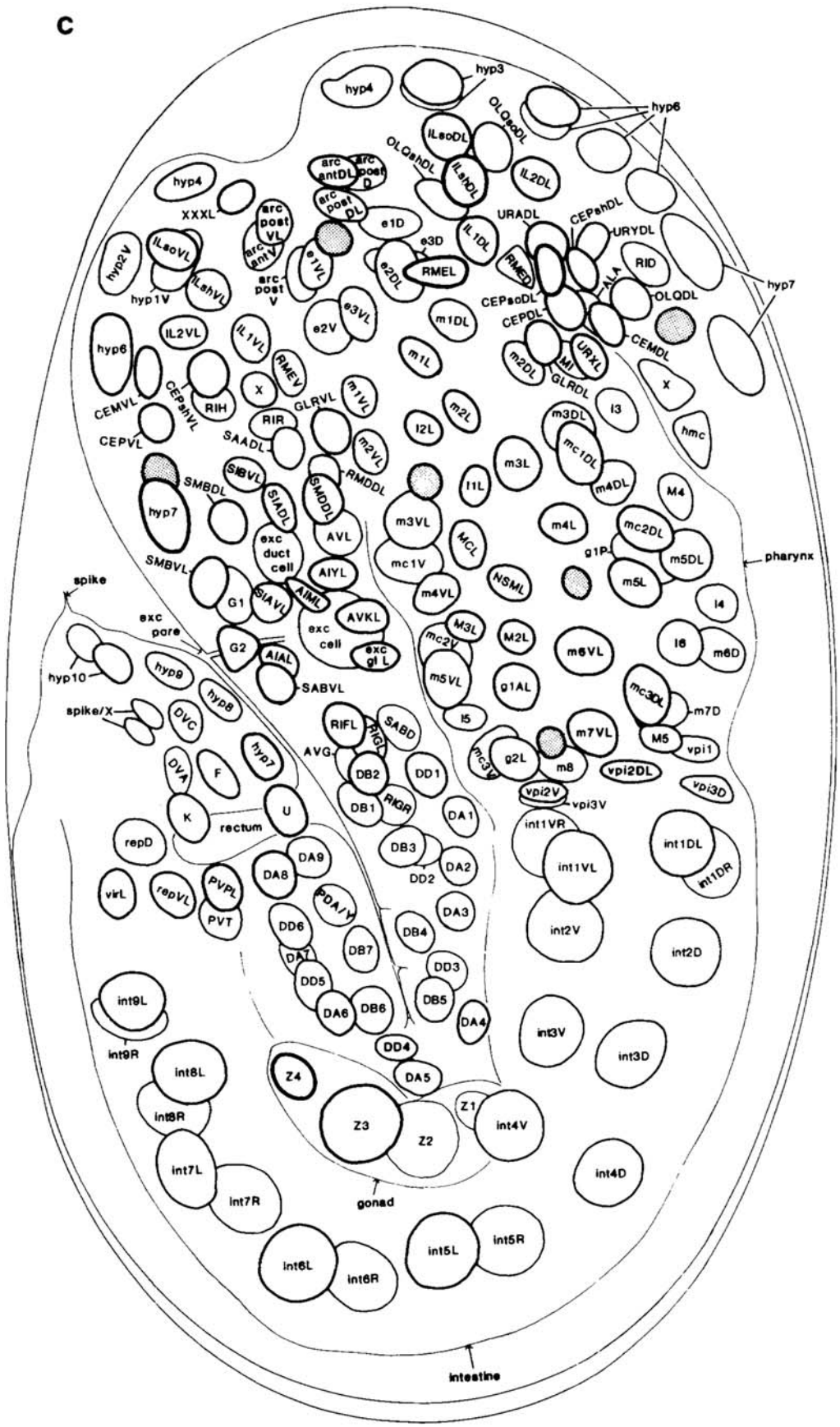
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GLRDL
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m4DL
M4
m3L
I1L
m4L
mc2DL
g1P
m5DL
pharynx
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I4
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MCL
mc1V
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NSML
M3L
M2L
m6VL
I6
m6D
mc2V
m5VL
g1AL
I5
mc3DL
m7D
m7VL
M5
vpi1
mc3V
g2L
m8
vpi2DL
vpi3D
vpi2V
vpi3V
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int1VL
int1DL
int1DR
int2V
int2D
int3V
int3D
int4V
int4D
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int7R
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int8R
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PDA/P
RIGL
RIGR
RIFL
SABD
AVG
intestine
hyp10
hyp9
hyp8
spike/X
DVC
DVA
F
K
U
rectum
repD
virL
repVL
PVPL
PVT
hyp7
spike
exc pore
G1
G2
SABVL
SIAVL
AIML
AIAL
AIYL
AVKL
exc cell
exc gl L
exc duct cell
AVL
SIADL
SMDDL
RMDDL
SAADL
SMBVL
SMBDL
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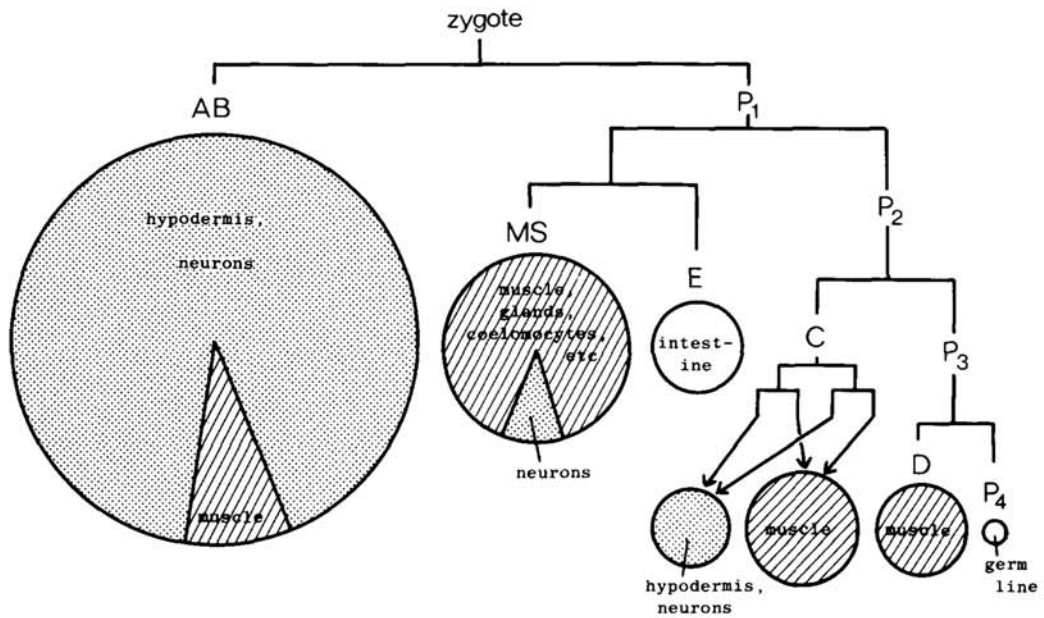


FIG. 9. Generation of the founder cells, and a summary of cell types derived from them. Areas of circles and sectors are proportional to number of cells. Stippling represents typically ectodermal tissue and striping typically mesodermal tissue.

same plan. hyp7 extends back on the dorsal side and encircles the body again at the anus; the midventral surface is occupied by the P cells (ventral cord blast cells). The tail is completed by three mononucleate cells (hyp8,9,11) and a binucleate cell (hyp10). All the hypodermal syncytia arise as mononucleate cells which subsequently fuse together.

On each side of the animal there is a longitudinal row of specialised hypodermal cells, called seam cells (H0–H2, V1–V6, T); they remain separate from the rest of the hypodermis, and are responsible for making the lateral cuticular ridges known as alae (Singh and Sulston, 1978). All except H0 are blast cells in the wild type, and even H0 has been seen to divide in certain mutants (E. Hedgecock, personal communication).

The location of the larger hypodermal nuclei in the L1 is shown in Figs. 13 and 14. During postembryonic development many more nuclei, generated by division of the seam cells and P cells, are added to hyp7, which comes to occupy most of the body surface. For this reason hyp7 has been called the large hypodermal syncytium.

Embryonically, the hypodermis is derived from the founder cells AB and C. The dorsal nuclei undergo a strange contralateral migration in midembryogenesis (see Migrations). The finely tapering spike of the tail is formed by a process which passes posteriorly through hyp10 and contains a bundle of filaments; the process is formed by a binucleate cell (ABp($^{l}_{r}$)ppppppa) which subsequently dies. The dorsal ridge of hyp7 seems to act as a storage organ during late embryogenesis; the concentration of refractile granules in it becomes more and more marked while that in the intestine diminishes.

The pattern of generation of the postembryonic blast cells (nearly all of which are hypodermal at hatching) is more intriguing than informative. Most of the seam cells are made from ABarpp, but V3 and V5 are closely related to the P cells. The special origin of V5 is not unexpected, in view of its unique postembryonic programme (Sulston and Horvitz, 1977); on the other hand, there is no known difference in behaviour between V3 and the other seam cells. By ancestry, the P cells fall neatly into the equivalence groups previously revealed by postembryonic laser ablation (Sulston and White, 1980); embryonic laser ablations are consistent with the hypothesis that the equivalence groups are determined cell autonomously, although these experiments are not conclusive (see Cell Interaction Experiments).

It is likely that the hypodermis is primarily responsible for the overall architecture of the animal, but the way in which it achieves this is largely unknown. One hint comes from ablation experiments in the head, which suggest that tension in the head hypodermis is necessary for elongation of the tail (see Cell Interaction Experiments). Certainly the cuticle is not involved in the shaping process, because the first sign of cuticle formation is at 600–650 min. At this time the seam cells acquire large Golgi bodies visible by Nomarski microscopy (cf Singh and Sulston, 1978), and cuticle can be seen at the mouth and in the rectum. The paired lateral alae characteristic of the L1 do not appear until just before hatching, and are apparently generated by cir-

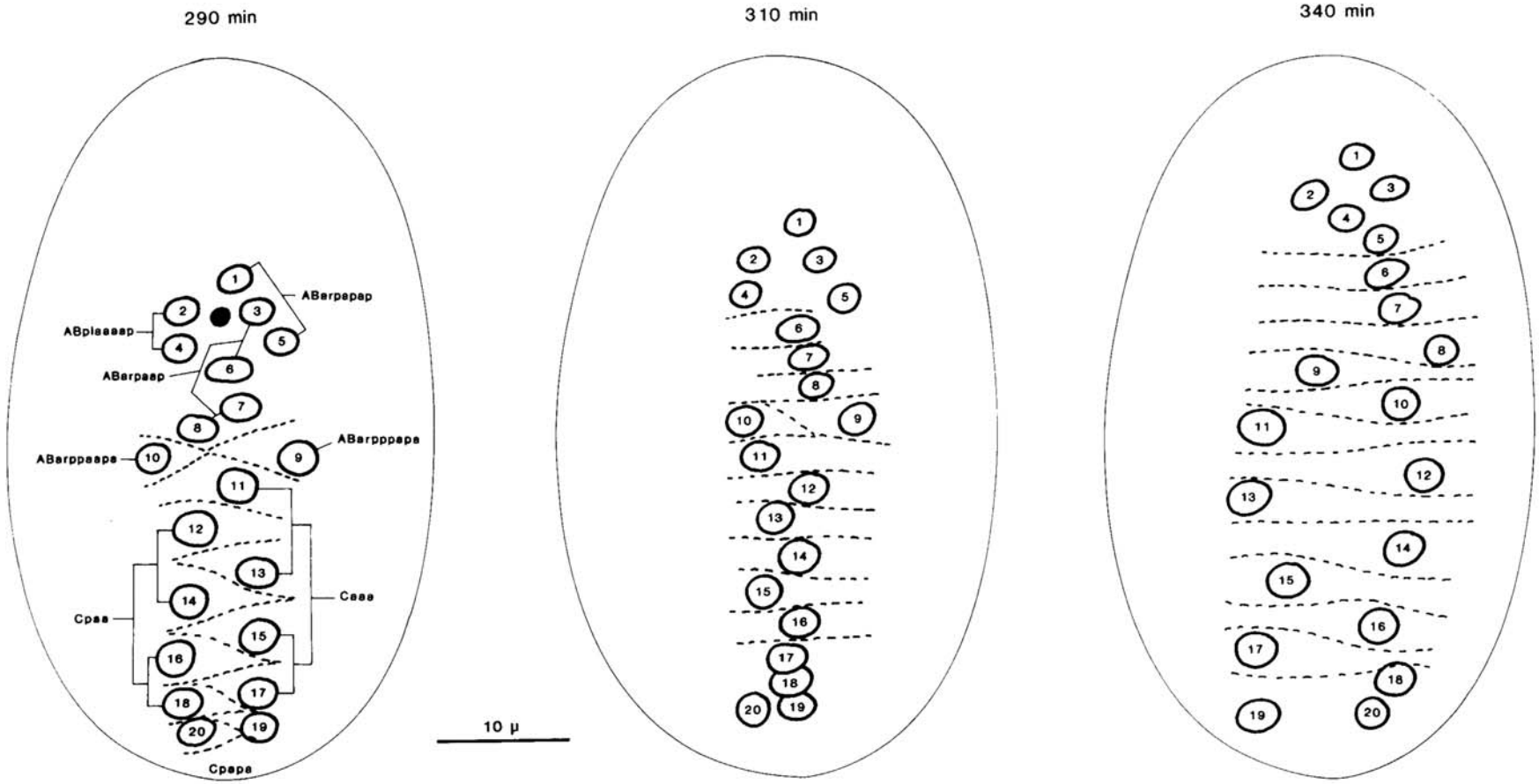


FIG. 10. Contralateral migration of dorsal hypodermal nuclei. Dorsal aspect. Cell boundaries visible by Nomarski microscopy (because of surface depression) are shown as dotted lines. Numbers are to provide continuity and have no other significance. Cpapp($^{a}_{p}$) and Caapp($^{a}_{p}$) (see Fig. 7b) countermigrate similarly, but are only visible from the ventral side at this stage.

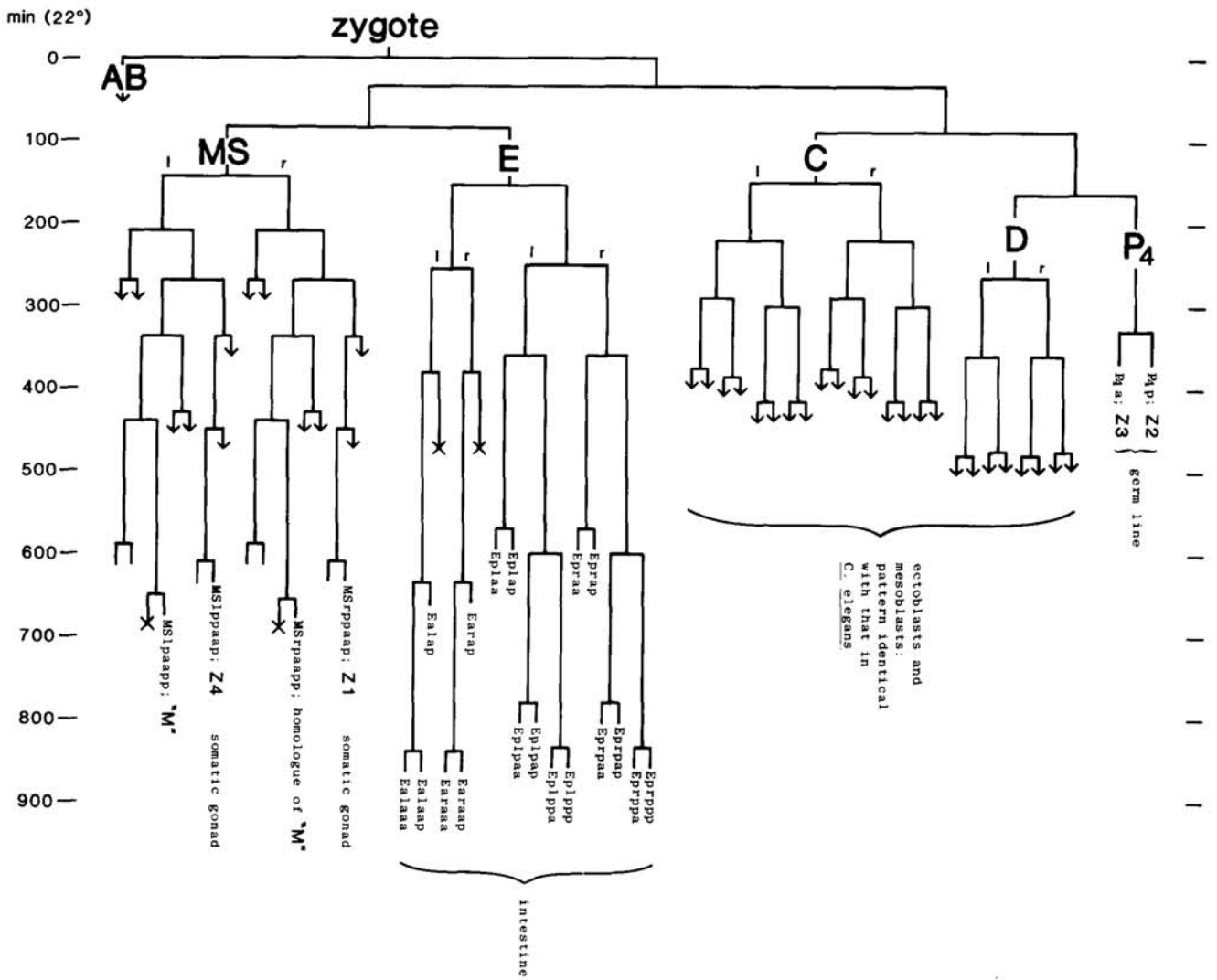


FIG. 11. Partial embryonic lineage of *Turbatrix aceti*. Conventions as for Fig. 3. Times (at 22°C) are approximate, for reasons given in text; the time scale is adjusted to facilitate comparison with Fig. 3. Arrows represent continued division, not followed.

cumferential contraction of the seam-specific cuticle (as is the case for dauer larva alae (Singh and Sulston, 1978)).

Excretory System

The excretory system has been described by Mounier (1981) and Nelson *et al.* (1983). It is derived from AB. Internally, it comprises four cells: the excretory cell, the duct cell, and two gland cells (which eventually become fused (Nelson *et al.*, 1983)). A fifth cell surrounds the excretory pore, and forms the interface between the excretory duct and the hypodermal syncytia: it is known as the excretory socket or pore cell. In the embryo, the socket cell is G1; after hatching, G1 becomes a neuroblast and the socket function is taken over by G2; finally, G2 divides into a neuroblast (G2.a) and the mature excretory socket (G2.p).

Regulative interaction is seen within the pairs excretory duct/G1 and G2/W (see Cell Interaction Experiments).

Nervous System

The complete adult nervous system has been described by White *et al.* (in preparation). Regions which were described previously are: the anterior nervous system (Ward *et al.*, 1975; Ware *et al.*, 1975); the ventral cord (White *et al.*, 1976); the pharynx (Albertson and Thomson, 1976); the male tail (Sulston *et al.*, 1980).

Of the 222 neurons present in the newly hatched L1 hermaphrodite, 2 arise from founder cell C, 6 from MS, and the rest from AB. All the supporting cells of the sensilla arise from AB.

The lineage patterns are complex, and will be discussed later. Most neurons are born fairly close to their

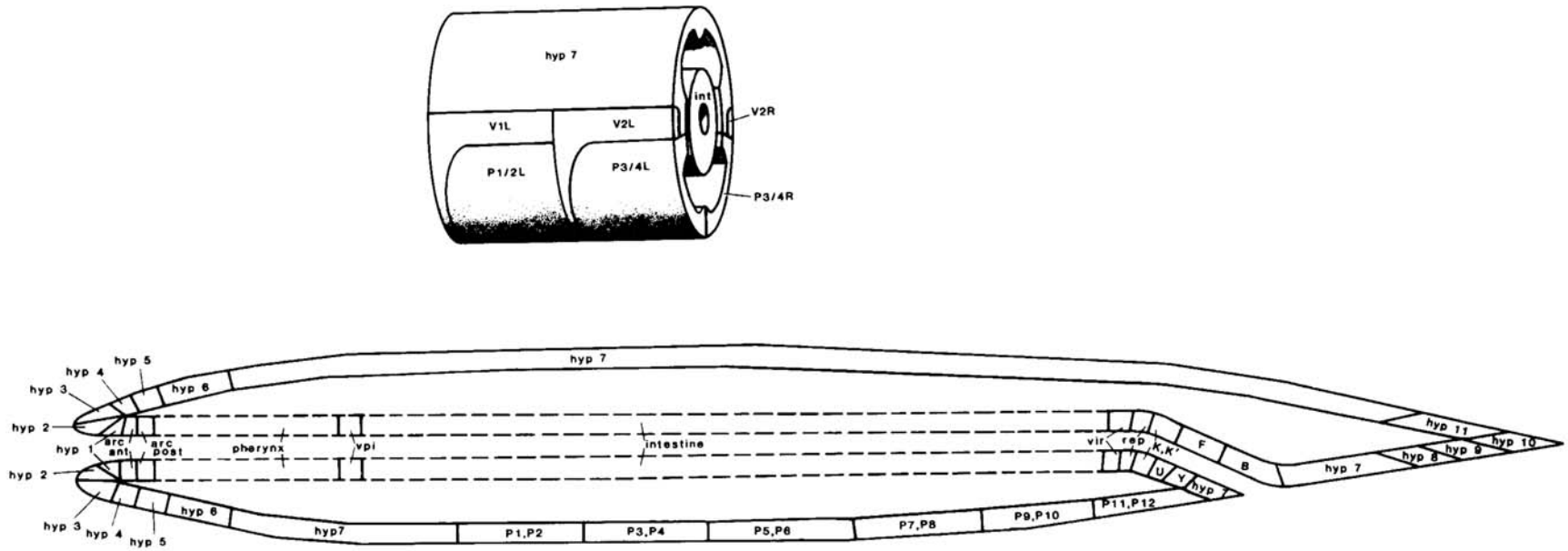


FIG. 12. Schematic longitudinal section to illustrate cells and syncytia forming surface coverings of newly hatched L1. Part of the digestive tract is dotted to indicate that boundaries are not shown; for details of this region see Albertson and Thomson (1976) (pharynx) and Fig. 17 (remainder). Inset (above) indicates three-dimensional arrangement in central region; the four longitudinal grooves between hypodermis and intestine are occupied by body muscle. Commas indicate two cells meeting in plane of drawing. During postembryonic development: hyp7 enlarges (by fusion) into the P-cell region; in the hermaphrodite a vulva is formed and Y becomes a neuron; in the male there are extensive changes in the tail (Sulston and Horvitz, 1977; Sulston *et al.*, 1980).

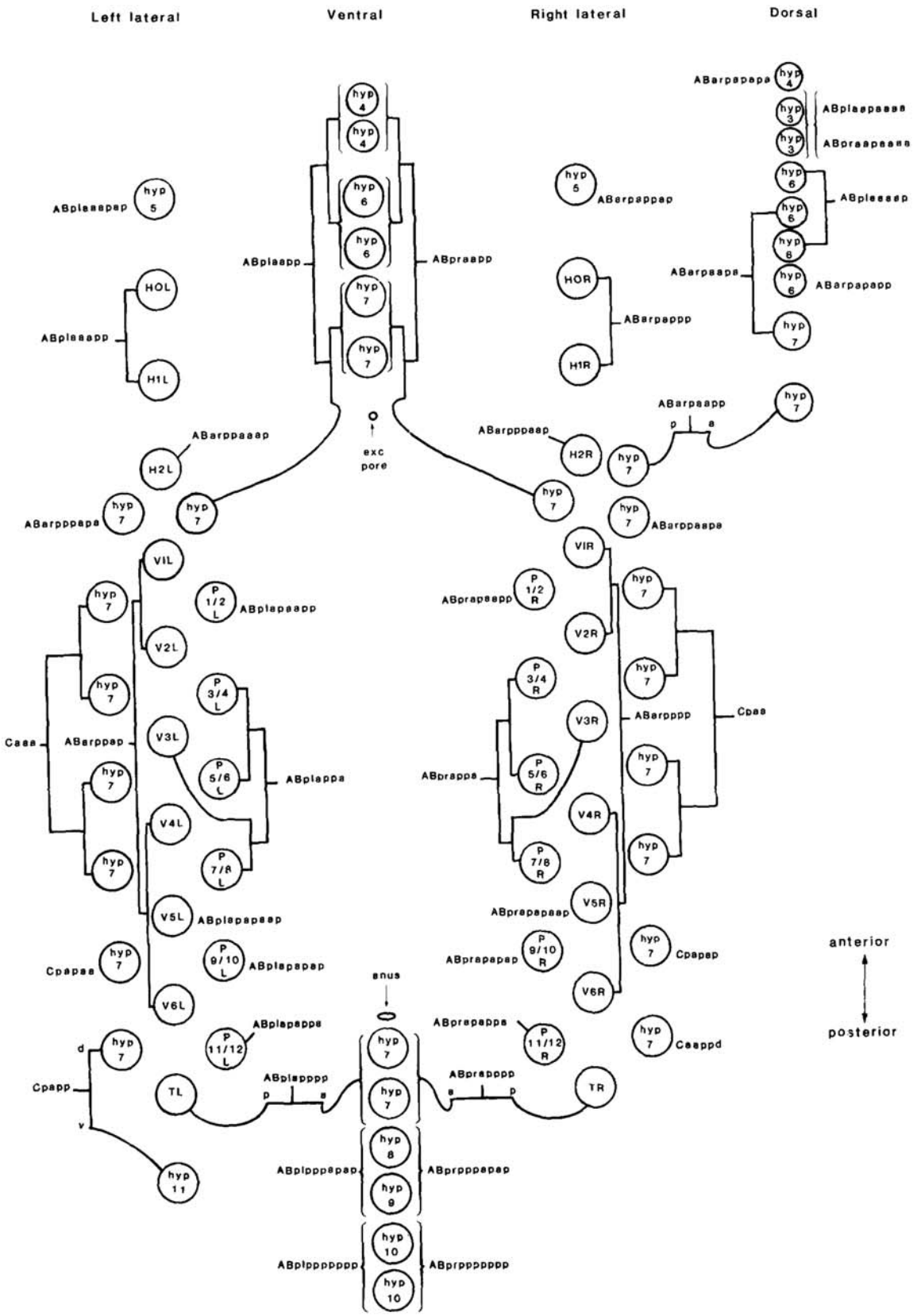


FIG. 13. Arrangement of larger hypodermal nuclei in newly hatched L1. Schematic cylindrical projection, viewed from within animal. Brackets indicate pairs of nuclei, of bilaterally symmetrical origin, whose anterior-posterior ordering is uncertain.

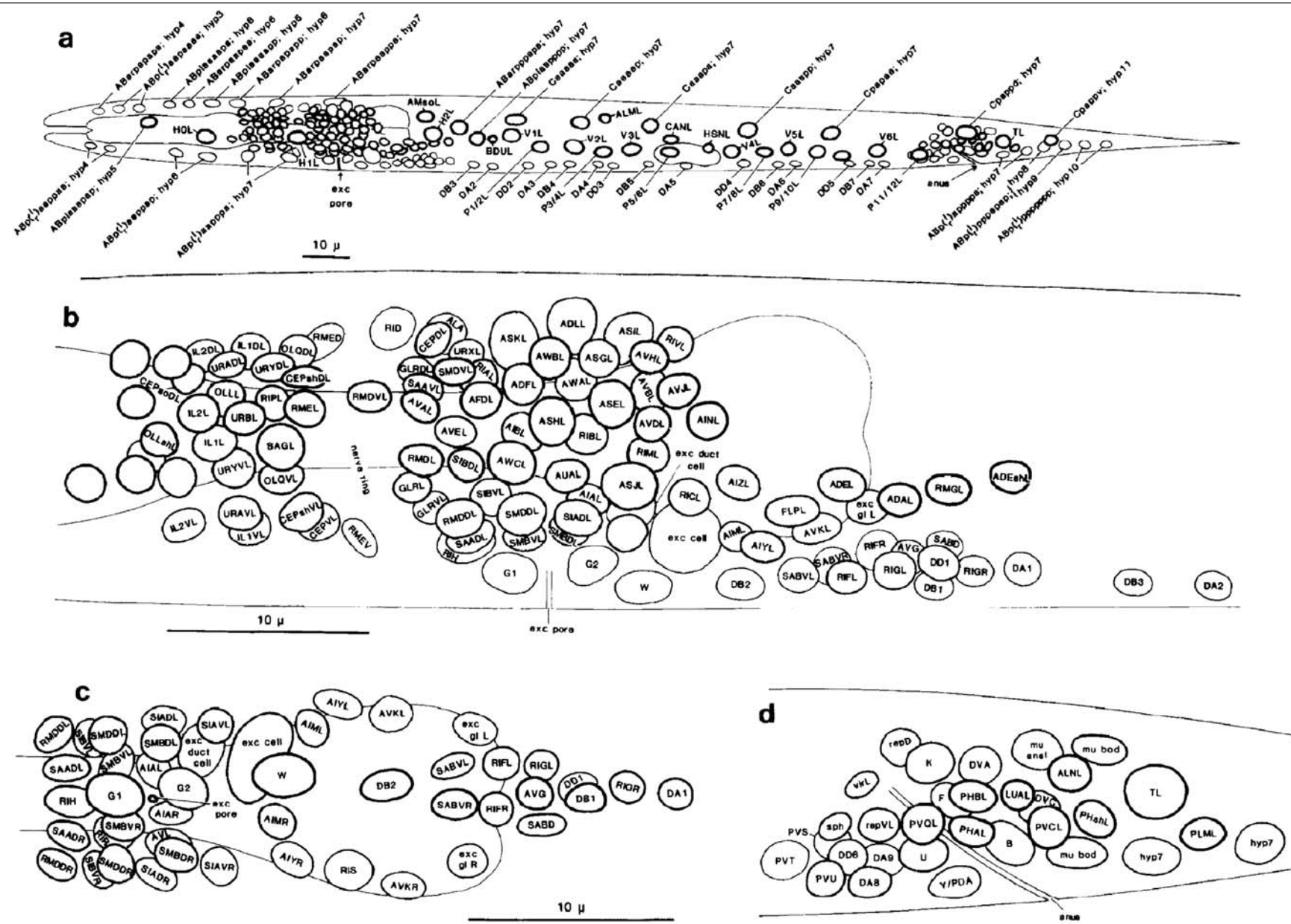


FIG. 14. Arrangement of neuronal and larger hypodermal nuclei in newly hatched L1; based on camera lucida drawings. (a) Entire animal: left lateral aspect. Pattern on the right is identical, except that: additional hyp7 nucleus (ABarpaappp) lies dorsal to H2R; homologue of QL is QR, of hyp11 is PVR. (b) Ring, ventral, and retrovesicular ganglia: left lateral aspect. Note that arrangement of ring ganglion cells around posterior bulb of pharynx is very variable at this stage. Anatomy anterior to the ring is not wholly known, since cells in this region were mostly identified by their processes in the embryo. (c) Ventral and retrovesicular ganglia: ventral aspect. (d) Preanal and left lumbar ganglia, rectal cells: left lateral aspect.

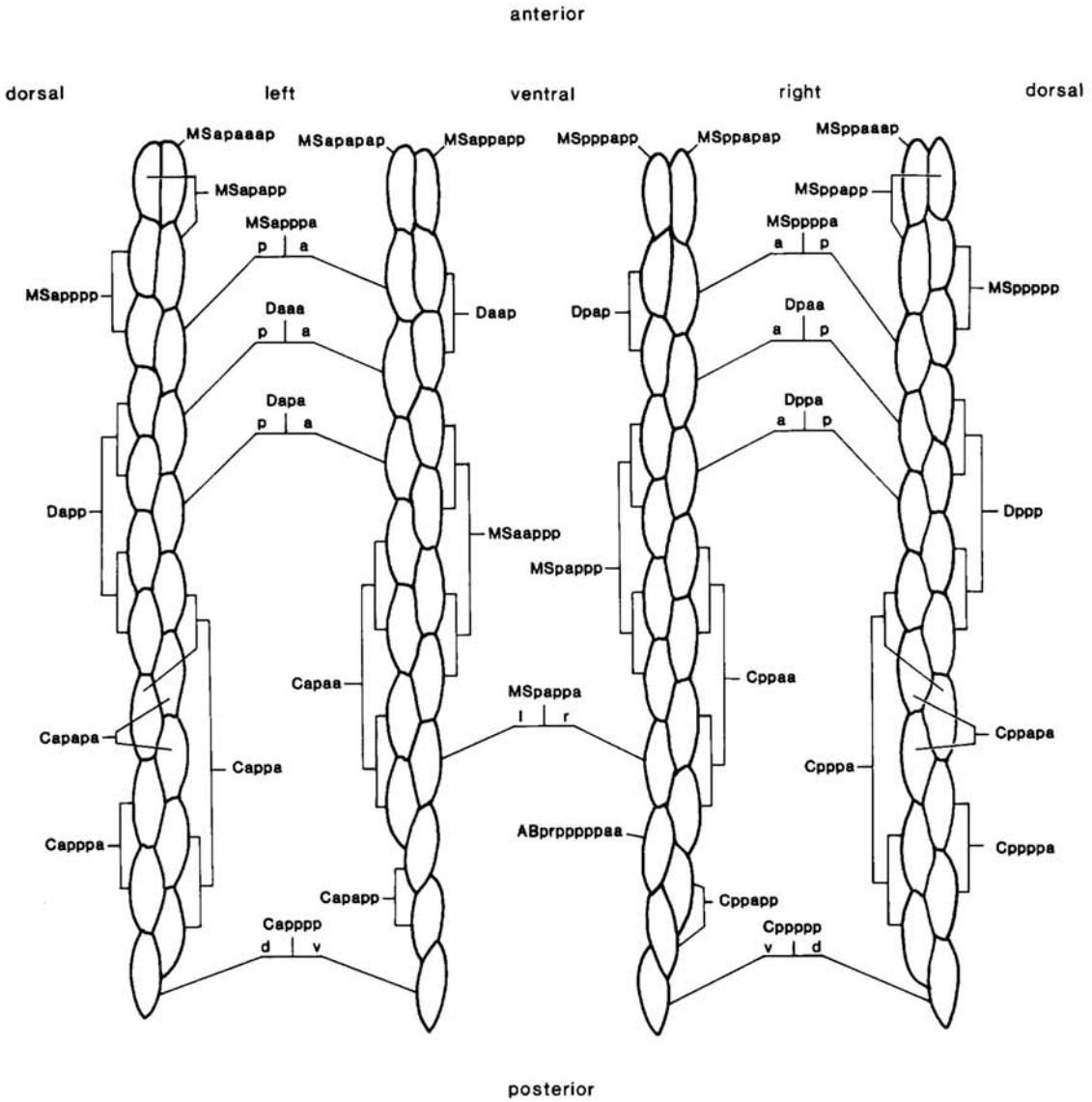


FIG. 15. Arrangement of body muscles at 430 min; schematic cylindrical projection, viewed from within animal. The shapes of the muscles are not intended to be realistic, but each cell is defined uniquely by its position in the pattern. Note that, with the exception of ABprpppppaa, the assignments are bilaterally symmetrical.

ultimate positions, though a few migrate long distances (see Migrations) and many migrate short distances relative to their neighbours. There are two occasions upon which mass movements of neuroblasts and neurons are noticeable. The first (230–290 min) leads to closure of the ventral cleft at the end of gastrulation. In the second (about 400 min) the anterior neurons move towards the tip of the head, and the rudiments of the sensilla are formed; the neurons then move posteriorly again, the sensory cell bodies laying down their dendritic processes as they go. At the same time, a depression appears in the tip of the head (Fig. 8c); this does not involve morphogenetic cell death, and is presumably a way of providing more surface area for the sensilla. The depression is not a primordial mouth, because it subsequently everts; the buccal cavity arises further inside, between the arcade cells.

After 430 min the tip of the head elongates and the pharynx grows forward through the mass of neurons surrounding the developing nerve ring; at the same time the head becomes thinner. The pattern of neurons changes rapidly at first but stabilises after about 2 hr. In late embryogenesis it is possible to recognise all the neurons in the ring ganglion by their positions; at hatching the arrangement of the most posterior ones changes in an unpredictable way, perhaps as a result of pharyngeal movements.

At about 470 min sexual dimorphism becomes visible

for the first time: in the hermaphrodite the cephalic companions (CEM) die, whilst in the male the hermaphrodite-specific neurons (HSN) die. It appears that these decisions are not made at the time that the cells are born, because all six behave at first in the same way in both sexes. In the hermaphrodite the CEMs have time to grow into the cephalic sensilla, where they form desmosomes with the sheaths and the cephalic neurons; in the male the HSNs migrate anteriorly at the same rate as they do in the hermaphrodite.

In postembryonic development a periodically repeated sublineage generates five classes of motorneurons in the ventral cord (Sulston and Horvitz, 1977; White *et al.*, 1976). In the embryo, however, there is no such repeated sublineage to produce the three classes of juvenile motorneurons (DA, DB, and DD) which are interspersed along the ventral cord (Fig. 14). All that can be said is that classes DA (together with SAB, the analogue of DA in the retrovesicular ganglion) and DD are each generated semiclonally, whilst DB neurons have a variety of unique origins and are not closely related to one another.

Mesoderm (Excluding the Pharynx)

The anatomy of the larval mesoderm has been described by Sulston and Horvitz (1977).

Of the 81 body muscles present in the L1, 80 are generated in a symmetrical fashion by MS, C, and D; the remaining one is generated by AB. A schematic cylindrical projection of their arrangement is shown in Fig. 15. The pattern of overlaps between the spindle-shaped cells is already apparent at 430 min, and allows unambiguous assignment at this stage.

The unique AB body muscle is one of a group of four muscles generated preanally by AB. The two mother cells of this group (ABp($\frac{l}{r}$)pppppa) remain on the outside of the embryo until their division at 295 min. The other three members of the group become the anal muscle, the sphincter muscle, and one of the two intestinal muscles.

The postembryonic mesoblast (M) is born on the left, next to the pharynx. It migrates posteriorly, following a distinctive path between the two germ line cells (Fig. 16); it remains on the midline for some time, but then gradually shifts to the right-hand side of the intestine. The contralateral homologue of M migrates in a similar way, preceding M along the midline between the germ cells, but then differentiates into the second intestinal muscle.

The head mesodermal cell (hmc) is one of a pair of homologues (sisters to the somatic gonad cells) which migrate to the dorsal midline. There the two cells align

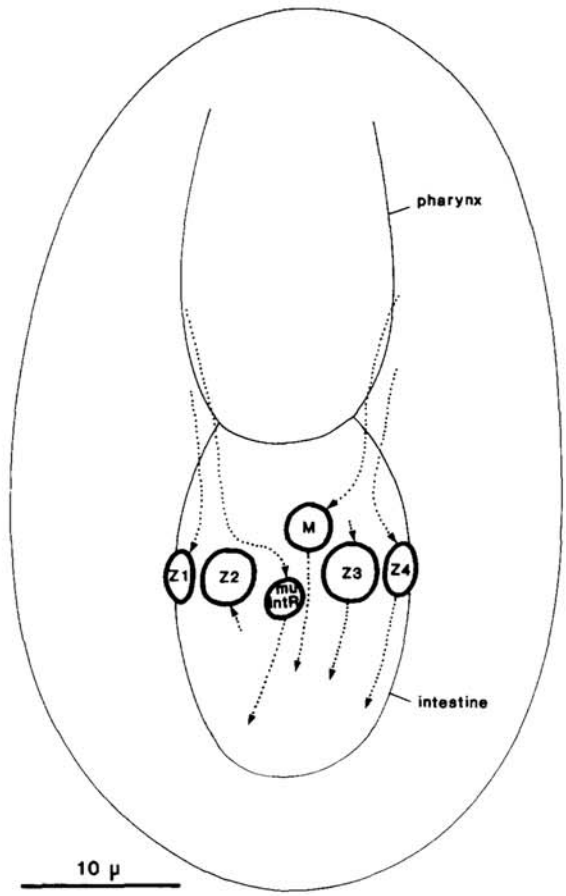


FIG. 16. Migration of M, its contralateral homologue (mu int R) and the cells of the gonad from 250 to 400 min. Ventral aspect. Nuclei are drawn at 320 min.

themselves anterior-posteriorly and appear very similar until late embryogenesis, when the anterior one dies.

The four coelomocytes are generated symmetrically. Their reproducible and sexually specific arrangement arises as a result of later movements, the reasons for which are not understood.

Alimentary Tract

The alimentary tract is a single tube which comprises the following components: (mouth), buccal cavity, pharynx, pharyngo-intestinal valve, intestine, intestino-rectal valve, rectum, (anus). Part of it is shown schematically in Fig. 17.

Buccal cavity, pharynx, and pharyngo-intestinal valve. The pharynx is a pump which ingests bacteria and crushes them; it is a complex organ, comprising muscles, structural cells, neurons, and glands. Its anatomy in the adult has been described by Albertson and Thomson (1976); their account needs amendment only in that

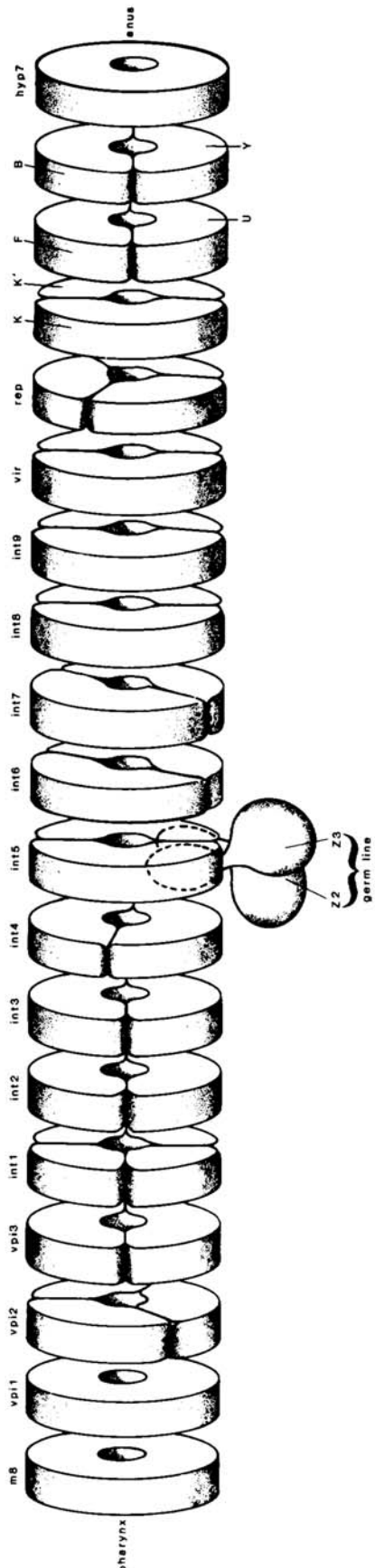


FIG. 17. Part of alimentary tract, 430 min, schematic. Except for absence of germ line lobes, arrangement is similar in L1. int, rep, vpi1, and vpi2 bear microvilli on their inner surfaces.

the m2 muscles are binucleate. The buccal cavity, which is formed by the arcade cells and the anterior end of the pharynx, has been described by Wright and Thomson (1981).

All the mechanical elements of these tissues (arcade, epidermal cells, muscles, marginal cells, valve) can be recognised in the 430-min embryo by the pattern of desmosomes which they form. The neurons and glands of the pharynx were followed until they settled into the mature pattern of nuclei which persists essentially unchanged from late embryogenesis to the adult. A series of cell fusions, which take place either before or soon after hatching, yield the multinucleate cells seen in the adult. In muscle class m1, all six cells fuse together; in each of the muscle classes m2, m3, m4, and m5, the six cells fuse in pairs—DL with DR, L with VL, and R with VR; in gland class g1, AR fuses with P.

The arcade, pharynx, and pharyngo-intestinal valve are generated by two granddaughters of MS and three great-great-granddaughters of AB. These precursors, however, do not yield exclusively these tissues. At the anterior end, there are no obvious lineal boundaries between the future hypodermis, arcade, and pharynx, in spite of the specialisations which become apparent later. Conversely, there is no functional boundary between pharyngeal components derived from MS and those derived from AB. For example, apparently identical cells arise respectively from MS and AB in muscle rings m3, m4, and m5 (see Appendix), and indeed three MS muscle cells go so far as to fuse with seemingly identical AB partners (m4VR with m4R, m5VL with m5L, m5VR with m5R).

Descendants of the precursors enter the body cavity from the ventral side during late gastrulation. First to enter are the MS cells (120–200 min), next are the ABaraap cells (210 min), and last are the remaining AB cells (220–250 min). At first the dividing cells form a cylinder anterior to the intestine; gradually a distinct boundary appears at the surface of the developing pharynx; then, at about 400 min, it is compressed posteriorly and becomes almost spherical, but subsequently it gradually elongates, first anteriorly and then posteriorly. The transient compression coincides with a flux of anterior sensory neurons towards the tip of the head (see Nervous System) but the causal relationship between these events is unknown.

The three g1 gland cells migrate in a reproducible way. Their movements approximately follow the subsequent course of their secretory processes, and may be responsible for laying down the latter. The cell bodies of the anterior muscles and epidermis also move substantially in late embryogenesis (compare Fig. 8c with Albertson and Thomson (1976)).

The structural elements of the mature pharynx have

an exact threefold rotational axis of symmetry, yet there is no trace of a threefold axis in their lineages; rather, the lineages show approximate bilateral symmetry and the third symmetry element arises by piecemeal recruitment of cells.

After 430 min (Fig. 8c) the pharynx continues to elongate, and within an hour the two bulbs and the isthmus are apparent. A refractile thread gradually appears along the axis of the pharynx and protrudes from the mouth. At 600–650 min the formation of L1 cuticle begins. The straight-sided cylinder of the buccal cavity appears, still plugged by the tip of the thread, and the pharyngeal lumen becomes outlined. One hour before hatching, the g1 glands become active, just as they do before ecdysis (Singh and Sulston, 1978). Half an hour later the pharynx begins to pump spasmodically, the mouth plug falls away, and the refractile thread is broken up and discharged into the intestine.

Intestine. The intestine comprises a chain of paired cells (Fig. 17). At hatching they are mononucleate, but subsequently most of them become binucleate by nuclear division (Sulston and Horvitz, 1977). Occasionally an extra cell is found in a newly hatched larva, presumably as a result of an extra division in the E lineage (cf. Other Nematode Species: *P. redivivus*). The anterior ring of four cells (int1) is specialised in having shorter microvilli than the rest of the intestine.

The intestine is derived exclusively from founder cell E, which gives rise to no other tissue. The daughters of E are the first cells to enter the body cavity during gastrulation (90 min). By 300 min they have formed two rows of eight cells, one on the left and one on the right. The anterior pair divide dorso-ventrally to yield int1, which attaches to the pharyngo-intestinal valve. Before attaching to int1, the rest of the anterior intestine undergoes a 90° left-handed twist, so providing half the total twist noted by Sulston and Horvitz (1977); the remainder seems to be due to packing of the posterior nuclei, because no twist is seen in the attachment of the intestine to the intestino-rectal valve (Fig. 17).

Intestino-rectal valve and rectum. The cells which form these structures are shown schematically in Fig. 17. Some of them have been given new names: rectal epithelium was formerly rectal gland, U was E, and Y was C. K is a blast cell; its contralateral homologue K′ is a blast cell in the *C. elegans* mutant *lin-4* (Chalfie *et al.*, 1981) and also in *Panagrellus redivivus* (Sternberg and Horvitz, 1982). F, U, B, and Y are blast cells in the male. All these cells underlie cuticle in the L1. During late larval development of the hermaphrodite Y withdraws from the hypodermis, without division, and becomes a neuron.

There is some similarity between the intestino-rectal valve and the pharyngo-intestinal valve: in both, the intestine attaches to a ring of two cells which do not bear microvilli, which attaches in turn to a ring of three cells which do bear microvilli. Only the intestino-rectal valve is a true valve, in that it can be closed actively by means of a sphincter muscle which surrounds it.

Gonad

At hatching, the gonad comprises two germ line cells (Z2 and Z3) and two somatic cells (Z1 and Z4). Its subsequent development has been described by Kimble and Hirsh (1979), and its structure in the adult by Hirsh *et al.* (1976) and by Klass *et al.* (1976).

The germ cells and somatic cells have separate embryonic origins; the former are the daughters of founder cell P_4, and the latter arise by identical lineages from MSa and MSp. After their birth, the somatic cells migrate posteriorly and attach to the germ cells. At this stage the gonad is oriented transversely across the animal (Fig. 16), but, probably as an inevitable consequence of the elongation and narrowing of the embryo, it gradually adopts the oblique position shown in Fig. 8c. The homologous origin of the somatic cells is concordant with their equivalent behaviour in postembryonic laser ablation studies (Kimble, 1981).

Electron micrographs of a 470-min embryo show that the germ cells are united and protrude large lobes into two intestinal cells (Figs. 2j, 17); after hatching, the protrusions are absent. Perhaps the germ cells are nursed by the intestine until their attachment to the somatic cells, for it is known that the latter are essential for their survival and division in larvae (Kimble and White, 1981).

Cell Interaction Experiments

This section describes some investigations using the technique of cell ablation by means of a laser microbeam (Sulston and White, 1980). Although the number of experimental animals is small, the invariance of the wild-type lineages ensures that any abnormalities observed are highly significant.

Ablation is more difficult in eggs than in larvae. Small cells can be killed satisfactorily (although it is difficult to avoid damage to their neighbours), but attempts to kill the large cells present early in embryogenesis frequently cause death of the entire embryo. Damage to these young eggs can be minimised by mounting them on 1% agar in an isotonic medium (see Light Microscopy: *T. aceti*), since heavy pulses seem sometimes to cause the shell to leak transiently. In this way the nucleus of any given cell can be destroyed, or at least prevented from dividing; although the cytoplasm inevitably persists, it is often displaced from its usual position. The technique used is to pulse the target cell

repeatedly at an energy level sufficient to produce refractile debris in its nucleus; whenever the cell appears dangerously weakened (low refractility of the cytoplasm, excessive Brownian motion) it is rested for a minute or two before pulsing is continued. After a successful operation the cell, heavily loaded with refractile debris, appears largely to have lost contact with its neighbours and remains visible as an undivided blob.

Early Ablations

After ablation of P_1, AB continued to cleave and in due course generated a large number of cell deaths. There was no recognisable organogenesis or morphogenesis, and no particular cells could be identified by light microscopy, though nuclei characteristic of hypodermal and neural tissue were visible.

In a series of animals, the two daughters and four granddaughters of AB were ablated in turn. The remaining cells divided in a superficially normal way, and intestinal, dorsal hypodermal, and germ line cells became recognisable. The arrangement of tissues, however, was defective, and all these embryos arrested at the comma stage (see Fig. 4).

Ablation of P_4 led to the production of larvae with no germ line. The somatic gonads of these animals grow more or less normally but are devoid of gametes: P_4 is not replaced by any other cell.

Although these results are superficially consistent with cell autonomous development, they are inconclusive because the presence of the dying cells may inhibit potential replacements (see Discussion).

Body Muscle from C and D

The following cells were ablated in a series of animals: Da, Dp, Capa, Capp, Cppa, Cppp. In each case, the resultant larva lacked approximately the quota of muscles which the dead precursor would normally have made (accurate muscle counts, especially in damaged animals, are difficult). The missing cells left a gap, and the remaining muscles did not spread out much to fill it. The experiments are subject to the caveat given for the early ablations.

MS Lineage

A number of experiments were carried out at the MS^4 to MS^{16} stages. The great advantage of this group of cells is that they are gastrulating at the time of ablation; in favourable cases, the target cell can be damaged sufficiently to cause it to detach from its neighbours and to remain at the surface of the embryo for some time, although eventually it, too, becomes enclosed within the body cavity. This circumstance should permit the remaining cells to interact regulatively, if they are capable of doing so.

Various derivatives of $MS(^a_p)p$ were ablated at MS^4 or MS^8, and the following cells were scored in the L1 and later larval stages: postembryonic mesoblast (M), right intestinal muscle (homologue of M), coelomocytes, somatic gonad (Z1, Z4: regarded as equivalent), head mesodermal cell (difficult), body muscle complement (approximate). In all cases the survivors appeared to generate those cells, and only those cells, which they would have produced in an intact embryo.

This result is particularly striking in the case of M and the right intestinal muscle, which are homologous and migrate along the midline in contact with one another (Fig. 16). M is a large cell with a characteristic postembryonic lineage; it is therefore easy to score, and the result is very clear-cut (five animals). This experiment and the following one are important counterexamples to the regulative interaction shown by two pairs of AB cells (see below).

The head mesodermal cell (hmc) is more difficult to score, so the finding in this case was confirmed by watching for the death of its homologue. When MSapp or MSappa was ablated (three animals) the death was seen and hmc was absent from the larva; when MSppp or MSpppa was ablated (three animals) the death was not seen and hmc was present in the larva. Therefore, in spite of the similar embryonic appearance and position of hmc and its homologue, the latter is programmed to die even in the absence of the former.

Extension of these experiments to $MS(^a_p)$ leads to pharyngeal damage, as predicted from the lineage. Not surprisingly, these animals feed poorly or not at all. Detailed analysis, which would require electron microscopical reconstruction, has not been undertaken.

AB Lineage

In the first two sections below, experiments which produced viable animals are described. In (a) the resulting animals were scored solely by light microscopy; in (b) the ultrastructure of the anterior sensilla was determined by electron microscopy. A third section (c) lists some experiments in which the animals died.

(a) The following cells were ablated at the AB^{32} stage: ABplapa (three animals), ABplaaa (one animal), ABarppa (one animal). The resulting larvae were scored, by Nomarski microscopy, for the postembryonic blast cells and certain neurons; although the blast cells were sometimes displaced in experimental animals they could still be accurately identified by their division patterns. With one exception (see below), the only blast cells

missing were those normally generated by the ablated precursor. Furthermore, the surviving blast cells behaved normally during postembryonic development, even to the extent of respecting the equivalence group boundaries (Sulston and White, 1980) in the usual way.

Because of the presence of the dying AB cells on the surface of the embryos these results do not unambiguously demonstrate cell autonomy, though they are consistent with it. The best evidence is that from ABplapa, in that the progeny of this precursor would normally move to two separated regions in the embryo, and it is unlikely that a dying blob of cytoplasm could make appropriate contacts in both places.

The exception was that, after the ablation of ABplapa, W was missing and G2 was present. In these animals ABprapaapa, which normally becomes W, presumably became G2 in place of the missing ABplapaapa. This example of regulative interaction was confirmed by the direct ablation of ABplapaapa (one animal) and ABprapaapa (two animals) at 260 min, before the cells moved ventrally: all three animals subsequently formed G2 and lacked W.

A second example of regulation was revealed by the ablation of ABplpaaaap (two animals) and ABprpaaaap (one animal): all three animals subsequently formed the excretory duct (normally ABplpaaaapa) and lacked G1 (normally ABprpaaaapa).

It appears that G2/W and duct/G1 are a forward extension of the P-cell regulative system. The latter is a series of six pairs of lateral cells (Fig. 13), which move to the ventral midline during postembryonic development. The members of each pair are equivalent, but in certain cases they compete for a particular "primary" fate (Sulston and White, 1980). The presumptive G2/W and duct/G1 move to the ventral midline and compete for a primary fate in the same way but at an earlier time.

(b) The following cells were ablated at the AB^{32} stage: ABalaaa, ABalaap, ABalapa, ABalapp, ABalppa, ABalppp, ABpraaa, ABprpaa. The pattern of cell deaths was scored at 270 min, certain neurons (RMED, ALA, RID, CAN) and the postembryonic blast cells were scored in the young L1, and finally the anterior sensilla of the L4 larva or adult were reconstructed by serial section electron microscopy. The results concerning the anterior sensilla are collected in Fig. 18.

In each case, those components which would normally have been generated by the ablated precursor were not seen in the resulting animal, indicating that no replacement of one cell by another had occurred (Fig. 18). This result strongly suggests that the selection of asymmetrical precursors for symmetrical roles in the head (see Discussion: Sublineages) is a cell autonomous process, because, according to the lineage, the time of selection is mostly later than the time of ablation: in this interval the dying cell acquires a position and a shape very different from that normally occupied by its progeny.

In most cases there were a few unpredicted deficiencies in the sensilla. These can be accounted for in various ways: the difficulty of recognising supporting cells in the absence of their neurons, displacement of unsupported neurons beyond the zone of reconstruction (in several cases such neurons were found lying beneath the hypodermis and well posterior of their usual positions), and perhaps failure of expression as a result of displacement. Since these missing cells are not numerous, they do not seriously weaken our conclusion.

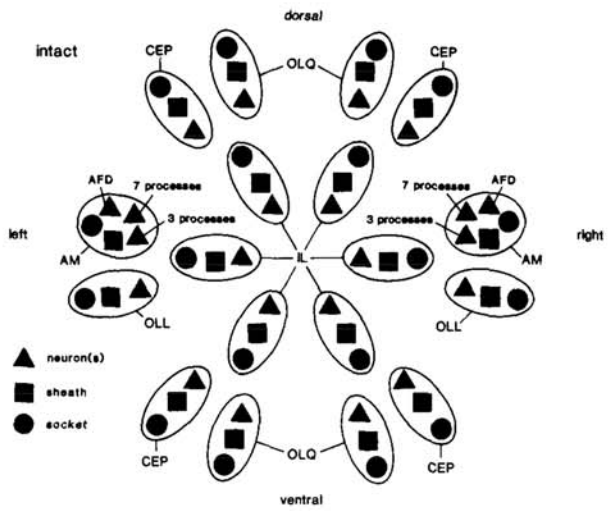
Although replacement regulation at the cellular level was not seen, these experiments provided some interesting examples of anomalous assembly of sensilla (see arrows in Fig. 18). One clear conclusion is that socket cells are not restricted to association with neurons of a particular type.

(c) A number of ablations in the AB lineage repeatedly yielded animals that failed to hatch. Many of the precursors for which this was true (ABplaaa (two animals), ABarpap (five animals), ABpraap (two animals), ABplapp (seven animals), ABprapp (one animal) are normally responsible for generating large patches of hypodermis. After ablation of any one of them, overall morphogenesis proceeded as usual until the start of elongation at 400–450 min, and then the cells inside the embryo oozed out through the missing area. Elongation ceased when this happened, suggesting that it is driven by circumferential contraction in the anterior part of the embryo.

Ablation of ABplpap (three animals) and ABprpap (three animals) also caused problems. Of these animals, only one of the former hatched, apparently because the dying precursor interferes with closure of the ventral cleft. However, before the embryos burst, it was possible to see that the excretory cell was made only when ABplpap was present. The one animal that hatched lacked both the excretory cell and the rectum, as predicted, and failed to grow.

Other Late Ablations

Various other cells were ablated at 250–300 min. The object of these experiments was primarily to deduce the function of the cells or of their progeny (e.g., interneurons of the ventral cord, rectal cells, postembryonic blast cells). No further replacement regulation was revealed, but many cells remain untested. Three examples of mosaic assembly of the nematode are of interest: after ablation of ABplpapppa (parent of K and K′, Fig. 17) the rectum was blocked; after ablation of ABprpapppp



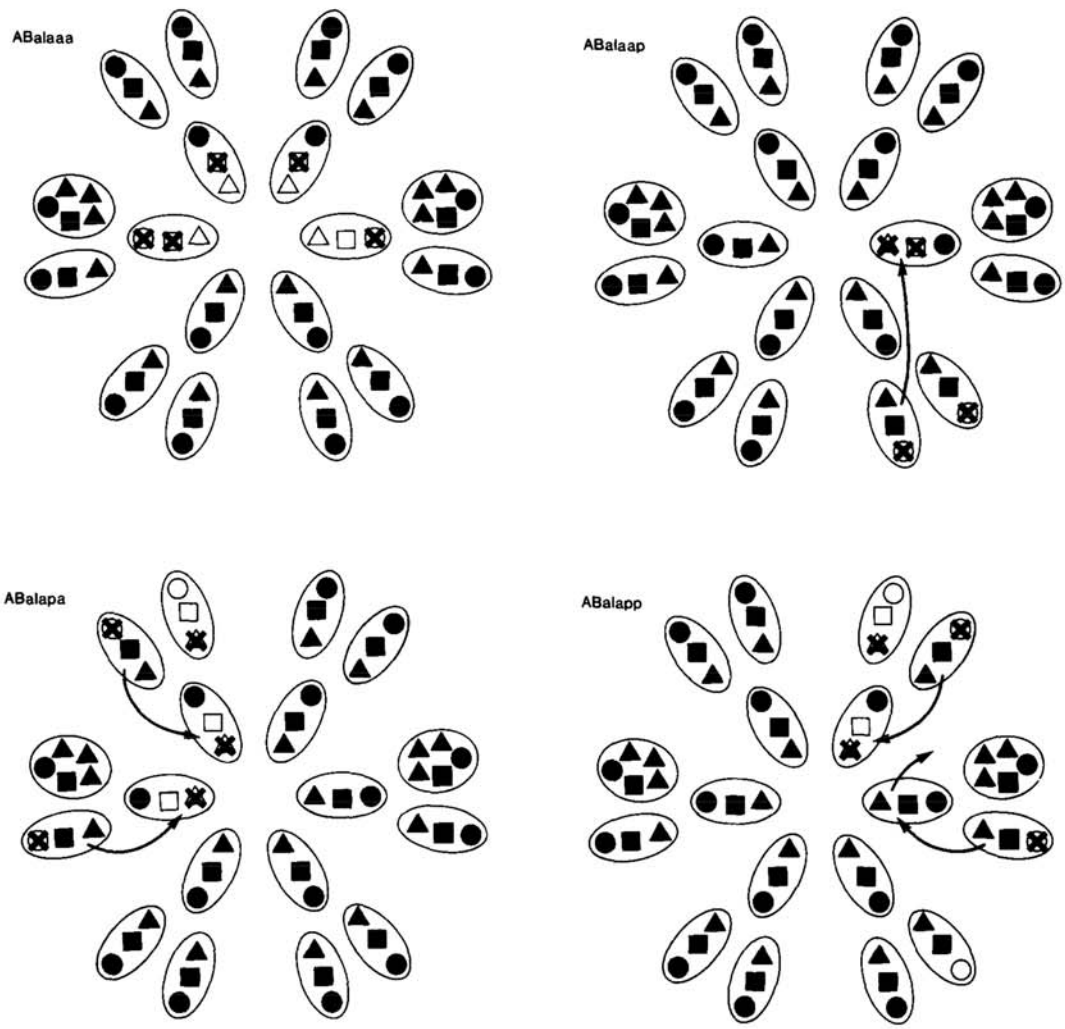


FIG. 18. Laser ablations of precursors of anterior sensilla. *Intact.* Anterior sensilla are represented by ovals, enclosing symbols representing neurons and supporting cells (see Ward *et al.*, 1975, for realistic representation). In inner labials (IL), triangle represents two neurons. In amphids (AM), "wing" cells (AWA, AWB, AWC) are not scored; remaining amphid neurons are split into three groups: AFD ("finger" cell, scorable), a group of five cells sending seven ciliated processes, and a group of three cells sending three ciliated processes into the amphid channel (the two neurons with double processes—ADF and ADL—are included in the seven process group, but otherwise the designations

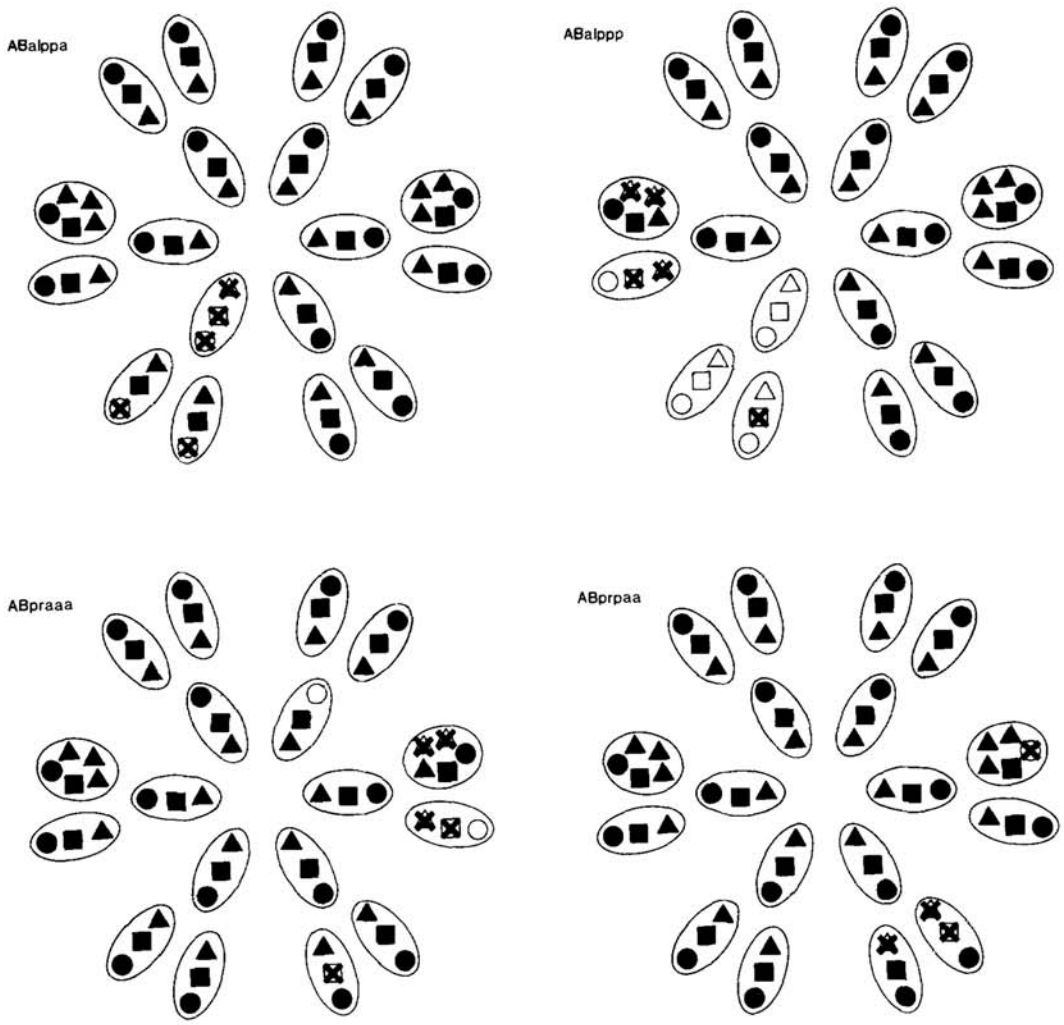


FIG. 18—*Continued.*

(parent of the intestino-rectal valve cells, Fig. 17) the posterior end of the intestine was not connected to the rectum; after ablation of ABalpaappp and ABalpapaaa (parents of hyp2) the buccal cavity was formed in the normal way at the anterior end of the pharynx, but was not connected to the mouth.

Summary

Two cases of regulative interaction were found. Both occur in late embryogenesis and involve the confrontation of similar cells at the ventral midline, a situation which is already familiar from postembryonic studies. In other respects the results are consistent with cell autonomous development, the evidence for autonomy being strong for the MS and late AB lineages. Interactions before the 50-cell stage are difficult to analyse by laser ablation.

DISCUSSION

In this paper we have described the cell lineage that gives rise to the newly hatched larva of *Caenorhabditis*

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are purely numerical). *Ablations.* Ablated precursor is shown at top left of each figure. Empty symbols indicate that the designated cell was not found; stippled symbols indicate uncertainty; X's indicate the cells which the ablated precursor would normally have produced. Note that, without exception, X's cover empty symbols. In some animals neurons became associated with inappropriate sockets; since sockets tended to remain in their usual positions, these changes are indicated by arrows leading from the neurons (and their sheaths: see below) to the sockets. Criteria used to identify particular cell types included: striated rootlets in IL1 and OLQ neurons; square linked array of four filled microtubules in OLQ neurons; dark deposit and supernumerary microtubules at tip of CEP neurons; prominent lips generated by IL sockets. In the region reconstructed there are no criteria for distinguishing between types of sheath; where a neuron is transferred to a different socket, so that the sheath designation is ambiguous, the neuron is arbitrarily assumed to move with its own sheath.

elegans. Since postembryonic development has already been followed (see Background Information for references), our knowledge of the cell lineage of this organism is now complete. The embryonic cell lineage is drawn out in Fig. 3, a complete list of cells is given in the Appendix, and a summary of cell types appears in Table 1.

The main value of this information is as a basis for more detailed studies of development, though certain features of it seem of more general interest and are discussed below. There is, however, little point in thinking about the details of the cell lineage unless it has some function beyond mere cell proliferation; we shall therefore ask at the outset the question: To what extent are the fates of cells constrained by their ancestry?

Invariance, Cell Autonomy, and Cell Interaction

The embryonic cell lineage is essentially invariant. The patterns of division, programmed death, and terminal differentiation are constant from one individual to another, and no great differences are seen in timing. At any given moment in development, each blastomere not only has a predictable future but also has a reproducible position and a defined group of neighbours.

Although invariance is consistent with cell autono-

TABLE 1
NUCLEAR COUNTS

	L1								
	AB	MS	E	C	D	P_4	Total	Blast	Adult total
Hypodermis (excluding rectum)	72			13			85	35	213^{211}
Body nervous system									
Neurons	200^{202}			2			202^{204}		282^{361}
Supporting cells	40						40		50^{86}
Ring glia		6					6		6
Body mesoderm									
Muscle	1	28		32	20		81		111^{136}
Coelomocytes		4					4		6^{5}
Excretory system	4						4		4
hmc		1					1		1
Mesoblast (M)		1					1	1	
Alimentary tract									
Arcade	9						9		9
Pharynx									
Structural	16	2					18		18
Neurons	14	6					20		20
Muscles	19	18					37		37
Glands		5					5		5
Valve p/i		6					6		6
Intestine			20				20	14^{+}	34
Valve i/r	2						2		2
Muscle	3	1					4		4
Rectum	9						9	1^{5}	8^{31}
Gonad									
Somatic		2					2	2	143^{55}
Germ line						2	2	2	indef
Total survivors	389^{391}	80	20	47	20	2	558^{560}	55^{59}	959^{1031} (+ germ)
Deaths	98^{96}	14		1			113^{111}		131^{148}
Total produced	487	94	20	48	20	2	671		1090^{1179} (+ germ)

Note. Cell count is less than nuclear count because of cell fusions and postembryonic divisions in the intestine (+) which are of nuclei only. Main entries are for hermaphrodite; male counts, where different, are shown as superscripts. Postembryonic blast cells (column 8) are included in L1 totals (column 7). Hypodermis includes XXX's, Q's, and hermaphrodite vulva. Rectum becomes cloaca in adult male. Sex muscles and body muscles are placed in a single category. Rectal cell Y becomes a neuron during postembryonic development of hermaphrodite. indef: indefinite.

mous development, it is not in itself diagnostic, because it might be the result of either the absence or the reproducibility of cell-cell interactions; in order to distinguish between these possibilities it is necessary to perturb the system experimentally. Two approaches have been fruitful.

Blastomere isolation. The first approach is the isolation of early blastomeres in a supportive medium and the observation of their subsequent development. Using this method, Laufer *et al.* (1980) demonstrated that the unique characters of the founder cells (see General Description) are internally determined and do not depend upon interaction with neighbouring cells. They found that the progeny of isolated blastomeres do not undergo normal morphogenesis (for which, therefore, cell interactions are required), but did not examine later cell lineages in detail; it is thus an open question whether determination to follow a particular division pattern is dependent upon extrinsic factors or not.

However, an isolated daughter of AB can give rise to a group of cells in which unequal divisions and subsequent deaths are seen (J.E.S., preliminary results), so at least some differentiation can take place within an isolated founder cell lineage. The absence of morphogenesis in such cell groups means that there are few parameters for the identification of cell types: we do not know whether cells are undetermined or are simply failing to express their determined characteristics. The same difficulty arises with embryos in which early blastomeres have been ablated.

Cell ablation. The second approach is to destroy particular cells and to observe the behaviour of the remainder. Numerous experiments of this kind have been carried out in the past, usually by ultraviolet irradiation but also by crushing or ligation (for bibliography, see Nigon, 1965; Seck 1938). The first method has the advantage of precision, and is reasonably effective in preventing the nucleus of a cell from dividing, but leaves the cytoplasm intact and potentially able to interact with other cells. All these researchers (with the exception of Pai (1927), whose data were subsequently discredited, e.g., Seck (1938)) found that the founder cells were generated and maintained their individual characters regardless of the fates of their neighbours.

More recently, a laser microbeam apparatus has been built by J.G.W.; this instrument is effective in killing, and causing the disappearance of, entire cells, and has provided useful information about cell-cell interaction in developing larvae (Sulston and White, 1980; Kimble, 1981). Its application to eggs, however, is problematical because, except at relatively late stages, cells cannot be killed outright without causing death of the organism. Nevertheless, earlier blastomeres can be prevented from dividing and displaced; their relationships with other cells are thereby presumably altered, and the presence of an abnormally positioned large blob of cytoplasm probably distorts the relationships between the surviving cells as well. In this way a reasonable test for cell-cell interaction can be applied from about the 50-cell stage onwards. In a series of experiments covering this period, no examples of cell-cell interaction affecting embryonic lineage, and only two examples of interaction affecting fate, were found. As explained above (Cell Interaction Experiments: AB lineage), the latter two cases can properly be regarded as part of a postembryonic regulative episode in which pairs of homologous cells confront one another at the midline.

It is worth emphasising two areas in which regulative interaction has not been seen. One is the mesoderm, in which the behaviour of early blastomeres allows cells to be ablated relatively cleanly, and in which a variety of differentiated cell types can readily be scored by light microscopy. The absence of detectable regulation in either lineage or fate is therefore striking, particularly in the case of two cell pairs which confront one another at the midline (in the same way as the AB pairs just mentioned). The other area is the anterior sensory system, which is rich in pairs of precursors that are analogous but not homologous (see Symmetry and Asymmetry).

Another technique for killing individual cells is treatment with psoralen followed by irradiation with an ultraviolet microbeam. In this way James Priess (personal communication) has shown that removal of E or Ea precludes normal morphogenesis of the head, and that certain late divisions and deaths are dependent upon the presence of unrelated cells. The source and specificity of the implied inductive influences remain to be discovered.

The possibilities for regulation of cell shape seem to be rather limited. For this reason, ablation of a blastomere which generates a substantial patch of hypodermis usually leads to a burst embryo. Individual muscles and postembryonic blast cells lie in approximately their usual positions even when a number of their fellows have been lost. At the ultrastructural level, however, anomalies can be caused in the assembly of neural components (see Fig. 18).

Early divisions of the founder cells. None of the experiments so far described have shed much light on this intermediate period. The initial divisions of MS, C, D, and P_4 are more or less equational in terms of their subsequent lineages; indeed, although these divisions are approximately anterior-posterior, they give rise ultimately to left-right symmetrical groups of cells. (This pattern is more apparent in *Turbatrix* and *Ascaris*, because the initial divisions of MS, C, and D are left-right.) AB and E are different, in that they do not gen-

erate elements of left–right symmetry until their second rounds of division. Nevertheless, their initial divisions may be equational. In particular, the first anaphase of AB is transverse across the midline, and only at telophase do its daughters skew into an anterior–posterior configuration; this progression is seen more clearly in *Ascaris*, in which the transverse arrangement persists for much longer.

Classical attempts to investigate the cell autonomy of the early AB lineage in *Ascaris*, by the study of squashed eggs and giant eggs, led only to a mass of circumstantial data and heated controversy (see Seck, 1938). Blastomere isolation experiments would seem to offer the best hope of settling the matter, though this approach runs into further difficulties, as explained above. For the present, the bases for the difference between ABa/ABp, as well as those for the more subtle distinctions betweeen MSa/MSp, Ea/Ep, and Ca/Cp, remain unknown.

Summary. In our limited survey, no replacement of any embryonic precursor by another was observed, but the postembryonic behaviour of two pairs of cells was found to be decided by regulative interaction in midembryogenesis. There is evidence for inductive effects upon morphogenesis, cell divisions, and cell deaths during the second half of embryogenesis. The period beween the generation of the founder cells and the 50-cell stage has not yet been satisfactorily investigated.

Embryonic Germ Layers and Cell Fate

In their literal sense, the terms ectoderm, mesoderm, and endoderm refer to the three distinct layers of cells formed at gastrulation in triploblastic embryos—the so-called germ layers. By extension, the same terms are applied, in mature animals, to the groups of tissues typically derived from the germ layers. To avoid confusion, we shall qualify the terms with "embryonic" when using them in the former sense.

It has long been known that the correlation between germ layer and tissue type is not perfect. In vertebrate embryos, for example, part of the embryonic ectoderm, known as the neural crest, contributes cells to structures (such as muscle and cartilage) which are more typically derived from embryonic mesoderm (reviewed by le Douarin, 1980). Is this also true for the nematode?

The ancestry of various cell types in *C. elegans* is summarised in Fig. 9. From the behaviour of their progeny at gastrulation the founder cells can be classified as follows:—embryonic endoderm: E; embryonic mesoderm: MS, D, part of C; embryonic ectoderm: AB, remainder of C (though the progeny of AB which contribute to the pharynx might also be classified as embryonic mesoderm). Thus neurons can be derived from embryonic mesoderm and muscles from embryonic ectoderm.

The mingling in ancestry is most extensive in the pharynx. For example, there are three cases of neurons which are sisters to muscles (strictly, myo-epithelial cells), showing that divergence between these two fates can occur as late as the terminal division. Conversely, a consideration of symmetry and patterns of cell fusion in muscle rings m3, m4, and m5 (see Alimentary Tract and Appendix) indicates that a particular cell type may be generated by more than one developmental pathway.

The pharynx may perhaps be regarded as atypical, in that it is a remarkably self-contained organ with its own nervous system and highly specialised myo-epithelial cells. The very late derivation of four muscle cells from the AB lineage is a more straightforward example. The parents of these cells remain on the outside of the embryo until 290 min. Two of the muscles are associated with the rectum and are unique, but the other two are identical with cells derived from MS: one is an intestinal muscle and the other is a body muscle.

The conclusion is that, although there is a broad mapping of particular cell types onto particular blast cells, absolute distinctions are not laid down at an early stage. One reasonable explanation for this observation is that the broad categories correspond to a primitive ancestral condition, and that in the course of evolution their boundaries have occasionally been transgressed; such an event may well be improbable, in that it would involve reprogramming a partially committed cell, but may nevertheless occasionally be selected.

Lineal Boundaries and Functional Boundaries

Two identifiable tissues are generated as single exclusive clones; i.e. in each case one precursor generates all the cells of the tissue and no others. These tissues are the intestine (founder cell E) and the germ line (founder cell P_4).

Such coincidence of lineal boundaries with functional boundaries is the exception rather than the rule. Examples of partial clonal derivation can be recognised: some precursors generate only, but not all, cells of a given type (e.g., D, Cap, and MSpppp all yield exclusive clones of body muscle); other precursors generate all, but not only, cells of a given type (e.g., MS yields all the coelomocytes and the entire somatic gonad). However, throughout most of the lineage cell types are intermingled even at the terminal divisions.

As a result of this heterogeneity of ancestry with respect to cell fate, many lineal boundaries cross the various somatic structures in an apparently meaningless way. For example, the neurons and supporting cells of a given embryonic sensillum never arise as an ex-

clusive clone, and the boundary between AB and MS meanders through binucleate cells.

It is interesting that exactly the same conclusion is reached with regard to polyclones in the epidermis of insects (Garcia-Bellido *et al.*, 1973; Lawrence, 1981). Here, the reproducible lineal boundaries surround the progeny not of single cells but of groups of cells called compartments; the cell lineages within a given compartment are indeterminate. Like the clonal boundaries in the nematode, compartment boundaries do not necessarily circumscribe recognisable functional units and yet are reproducibly positioned from one individual to another.

Segments

Nematodes are not usually considered to be segmented animals. However, the lateral hypodermis of the newly hatched L1 larva is divided into periodically repeated units (Fig. 13). The repeat unit is most obvious in the middle of the body, where it comprises a ventral cord blast cell (P), a lateral hypodermal blast cell (V), and a syncytial hypodermal nucleus (hyp7); the head and the tail can plausibly be regarded as containing additional degenerate units. How do these units arise?

Inspection of the embryonic lineage reveals no corresponding periodic repeat. The three types of cell are generated semiclonally (Fig. 13) and the repeat units are formed by reproducible but piecemeal recruitment. Presumably the regularity of the structure depends upon cell affinity and perhaps upon packing constraints in the embryo. There appears to be no correlation between the repetition in the hypodermis and that in the endoderm, muscle, or juvenile nervous system. During the postembryonic expansion of the ventral nervous system (Sulston and Horvitz, 1977), the regularly repeated array of neurons derived from the P cells is simply superimposed upon the different, and less regular, repeat pattern of juvenile neurons.

This mode of limited "segmentation" partially parallels the true segmentation of the leech, which also develops by an invariant cell lineage (Stent and Weisblat, 1982). The leech embryo has five stem cells (four ectodermal and one mesodermal) on either side; every segment is founded by a group of cells comprising one or two daughters from each of the stem cells. Consequently, as in the nematode, a given cell in the mature animal is more closely related to its homologues in other segments than it is to most of the cells in its own segment.

Sublineages

We shall use the term "sublineage" as an abbreviation for the more descriptive, but cumbersome, phrase "intrinsically determined sublineage"—namely, a fragment of the lineage which is thought to be generated by a programme within its precursor cell. At present there is no direct evidence for such inherent programming, and so strictly the concept is hypothetical; nevertheless, in certain cases a variety of circumstantial evidence can be adduced to support it. The notion has been discussed elsewhere (Chalfie *et al.*, 1981; Sternberg and Horvitz, 1982) and need not be further elaborated now, but it is convenient to begin by listing two of the available criteria for postulating the existence of a sublineage: (1) the generation of the same lineage, giving rise to the same set of cell fates, from a series of precursors of diverse origin and position; (2) evidence for cell autonomy within the lineage, obtained from laser ablation experiments or the study of mutants.

By the first criterion, two sublineages can be found in the head of the embryo (Fig. 19). The sixfold inner labial sublineage generates the neurons of the six identical inner labial sensilla. The outer labial sublineage can be regarded as either sixfold or (four + two)fold; this ambiguity is of interest in the light of the morphological difference between the OLQ and OLL outer labials (Fig. 19 legend; White *et al.*, in preparation).

In postembryonic development, parts of the lineage which are simply bilaterally symmetrical do not usually qualify as sublineages by the first criterion above, because their precursors are of symmetrical origin. However, in the case of the embryo it is possible to take the analysis a step further.

In the lineage chart (Fig. 3), pairs of precursors which give rise to similar or identical division patterns and groups of cells on the left and right sides are indicated. In the posterior part of the animal, bilateral symmetry arises at an early stage, as a result of equational divisions of precursors, and the groups are large. In the anterior part, however, symmetry arises late, in a piecemeal fashion, and the groups are small; for example, identical lineages arise from ABalaappp and

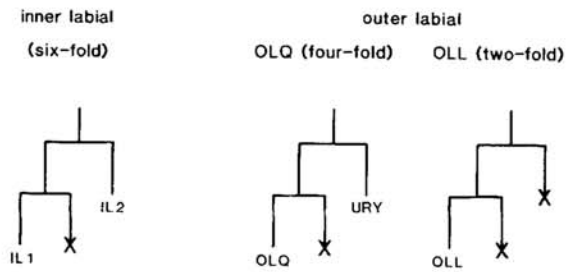

FIG. 19. Labial sublineages; see text. OLL resembles OLQ in its radial position (Ward *et al.*, 1975) but differs in having neither a square array of four microtubules nor a striated rootlet. OLL sublineage is related equally closely to OLQ and inner labial sublineages.

ABalapaap, which are not themselves of identical origin. There are therefore grounds for supposing that these symmetrical pieces of lineage are sublineages, particularly since their precursors are frequently not disposed symmetrically and do not have identical neighbours on the left and the right.

The latter observations suggest further that the precursors are determined, not by interaction with their neighbours, but by their ancestry. Evidence in favour of this conjecture was obtained from a series of laser ablation experiments, in which all tested precursors behaved autonomously. Some implications of this finding are discussed below (Symmetry and Asymmetry).

The assignment of sublineages is often a matter for subjective judgment, in that two sections of the lineage may be alike in some respects and discrepant in others. Thus, ABalpaaa and ABarapaa give rise to sublineages identical except for the fate of one cell, and it is reasonable to suppose that identical programmes are being used by the two precursors; the fate of the one anomalous cell may be determined either by position or by additional intrinsic factors. A more extreme example is provided by the pair ABalapap and ABalappp; in this case a dividing cell in one version of the sublineage is replaced by a cell death in the other, but there are still enough common features to suggest a shared programme.

Programmed Cell Death and Sexual Dimorphism

The large number of programmed cell deaths, and their reproducibility, is evident from the lineage. The most likely reason for the occurrence of most of them is that, because of the existence of sublineages, unneeded cells are frequently generated along with needed ones (Sulston and Horvitz, 1977; Horvitz *et al.*, 1982).

Direct support for this point of view is provided by the sexually specific deaths seen in the embryonic lineage. In the hermaphrodite the putative CEM cells die but the HSN cells survive, whilst in the male the situation is reversed; before the deaths, however, each cell begins to differentiate in the same way regardless of sex. Rather than generate two different patterns of cell division, the nematode uses the same sublineages in both sexes and kills those cells that are not required. Sexually specific deaths are also seen in hermaphrodite larvae, but in other cases no surviving homologues of the dead cells are available for study. However, by means of mutants in which cell death does not occur (recently isolated by H. Ellis, personal communication; Horvitz *et al.*, 1982), it should now be possible to exhume all of them.

There are few obvious restrictions upon the patterns of cell deaths. They are found in ectoderm, mesoderm, and (in *Turbatrix* and *Panagrellus*) endoderm. Several precursors give rise to two cell deaths in successive divisions, but none give rise to two in a single division. Presumably there is some selective pressure on the organism to dispense with programmed deaths, which must represent a waste of time and materials. Logically, sister deaths could be readily eliminated (by programming the death of their parent) whereas single deaths might require more extensive rearrangement of control elements. Sternberg and Horvitz (1981) have reported sister deaths in the *Panagrellus* gonad, but suggest, on account of their variability, that they are of recent evolutionary origin.

Rotational Symmetry

In addition to bilateral symmetry (see Sublineages) parts of the nematode display two-, three-, four-, and sixfold rotational axes of symmetry. The embryonic lineage, on the other hand, is in part bilaterally symmetrical and in part asymmetrical; the ways in which it generates the more elaborate symmetries of the mature nematode will now be discussed.

Twofold At 300 min the gonad is a bilaterally symmetrical structure consisting of two identical subunits, each comprising two cells of different types (Fig. 16). It subsequently turns to lie obliquely and acquires a twofold rotational axis of symmetry. This behaviour can be understood if, during late embryogenesis, the two subunits interact specifically with one another but not with nongonadal tissues, the oblique position being a consequence of packing. The autonomy of the gonad was pointed out by Kimble and Hirsh (1979) who found that, apart from the initial events in the male, postembryonic gonadogenesis is reproducibly oriented with regard to the axes of the gonad rather than to those of the body.

Threefold The muscles and structural cells of the pharynx have a precise threefold rotational axis of symmetry, whilst the nervous system of the pharynx is bilaterally symmetrical. Broadly speaking, two of the three identical sets of mechanical elements are generated by bilaterally symmetrical lineages, and the third set is assembled by piecemeal recruitment. The threefold symmetrical arrays of the arcade, hyp1, hyp4, and parts of the valves are produced in a similar fashion. This plan of development contrasts with that found for the vas deferens (formed in the larval male), in which structures having a threefold rotational axis of symmetry are generated by three equivalent precursor cells which give rise to identical sublineages (Kimble and Hirsh, 1979; Kimble, 1981).

Four- and sixfold At the tip of the head the various classes of sensilla are arranged in rings with bilateral

(amphid), fourfold (cephalic), and sixfold symmetry (inner labial); the outer labial sensilla can be regarded as having either sixfold or (four + two)fold symmetry. Various authors have speculated that one of the higher modes of symmetry may represent the fundamental plan of the nematode head (discussed by Ward *et al.*, 1975). If this were the case, the plan should be detectable as a corresponding pattern of precursor cells. However, apart from the sublineages giving rise to the labial neurons (see Sublineages), no mode of symmetry other than bilateral is detectable in the embryonic lineage. Even the origin of the labial neurons does not support the idea that sixfold symmetry is fundamental, because the sublineages for the inner and outer labial sensilla are generated by unrelated precursors and do not include the supporting cells.

Symmetry and Asymmetry

It is clear from the events already discussed that, at numerous points in the lineage, symmetry is broken. This is obviously a necessary step on the pathways to unique cells and threefold symmetrical structures, but it can also occur in the generation of bilaterally symmetrical structures (see Sublineages).

More than one symmetry change can occur during the ancestry of a single cell. An extreme example is provided by a sequence from the complete (embryonic and postembryonic) lineage of the male; in it, symmetry is generated three times and broken twice, as follows. Cells B and U are produced by identical right and left lineages; they break symmetry and arrange themselves respectively posterior and anterior to the anus; during larval development, the anterior daughter of B divides symmetrically and gives rise to identical lineages on the left and right sides; two pairs of its progeny break symmetry again and arrange themselves anterior-posteriorly; finally, all four of these progeny generate symmetrical groups of cells on the two sides of the animal (Sulston *et al.*, 1980).

In addition to symmetry changes involving precursors, which are indicated in Fig. 3, certain new symmetries appear at the level of terminal differentiation (Fig. 20). These "phase shifts" in otherwise symmetrical patterns of fate assignments are reminiscent of those seen in the postembryonic development of male-specific muscle (Sulston *et al.*, 1980; Sulston and White, 1980). They may have arisen as a result of selective pressure to produce either unique cells or threefold groups, and various hypothetical schemes can be constructed to explain their evolution. Sternberg and Horvitz (1981) have termed such events "altered segregations," to express the observation that two cells may acquire the same developmental potential by different routes.

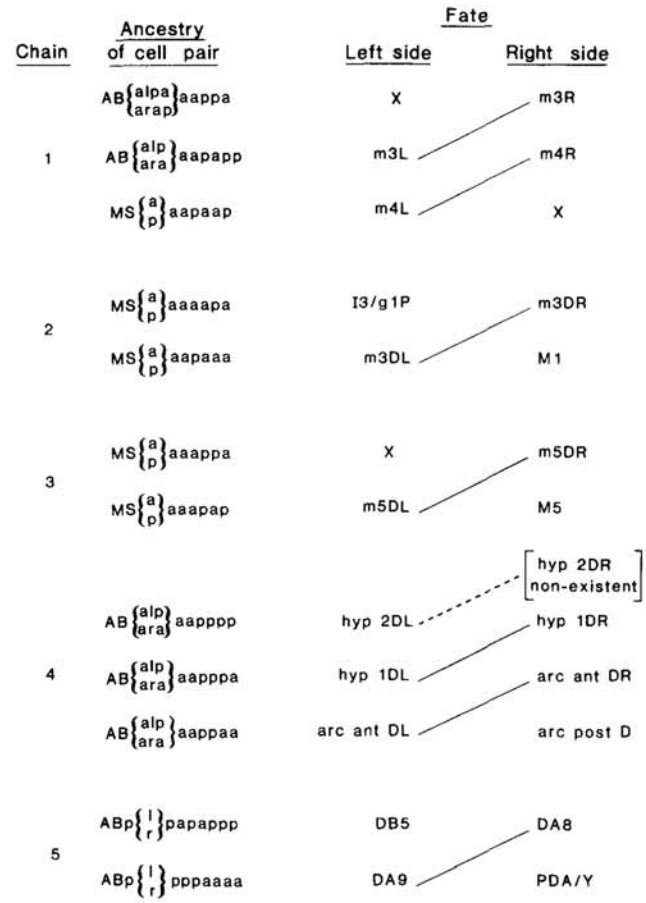

FIG. 20. Phase shifts in patterns of fates of symmetrical cell pairs. Members of each pair are judged to be homologous partly by ancestry and partly by the assignments of their relatives. Chain 4 goes some way to account for the strange form of hyp2, a cylindrical syncytium which arises by the fusion of a ventral cell and a dorsal left cell; the corresponding dorsal right cell, anticipated on grounds of symmetry, is absent—perhaps sacrificed in the past for some greater goal. Chain 5 is unimpressive, but is included because apart from DB5 and DA8 pairs of homologous neurons always belong to the same functional class; it is thus remarkable that ABprpppaaaa, rather than ABprpapappp, becomes PDA/Y. In chain 1 the unique cell which may once have justified the phase shift has itself been eliminated: a striking illustration of the accretion of logical debris to the lineage.

How are the symmetry decisions taken? Laser ablation experiments show that two cases of symmetry breakage (G2/W and duct/G1, see Cell Interaction Experiments) involve competitive interaction between the homologous partners, which confront one another at the midline. In all other cases tested the behaviour of the cells seems to be independent of their neighbours and of their exact position. We are therefore forced to conclude that the various symmetries arise during development largely from a series of piecemeal decisions, each individually selected in the course of evolution. The precise bilateral symmetry of the head, for example, is not determined by a single global influence (such as a

symmetrical morphogenetic field) but is the end result of many small decisions. Bilateral symmetry is evidently a favourable asset for an animal, and is selected even at the level of individual neurons and sensilla.

Conclusion

Perhaps the most striking findings are firstly the complexity and secondly the cell autonomy of the lineage. As regards the former, many of the guesses that have been made in the past about the ultimate fates of embryonic cells are correct in general but wrong in detail: the assignment of cell function follows certain broad rules to which there are numerous exceptions. As regards the latter, there are two examples of specific interaction between cells affecting their fates, and there is evidence for induction late in embryogenesis; doubtless other instances of cell-to-cell signalling remain to be discovered, but much of the detailed control of division pattern and fate, including such niceties as the generation of precise bilateral symmetry, seems to be vested in autonomous programmes within individual cells.

With hindsight, we can rationalise both this complexity and this rigidity. The nematode belongs to an ancient phylum, and its cell lineage is a piece of frozen evolution. In the course of time, new cell types were generated from precursors selected not so much for their intrinsic properties as for the accident of their position in the embryo. Perhaps the mutations responsible for these novel developmental events acted directly upon the intrinsic behaviour of the precursors, but equally they may have operated via extrinsic regulative mechanisms. In the latter case, however, cell-cell interactions that were initially necessary for developmental decisions may have been gradually supplanted by autonomous programmes that were fast, economical, and reliable, the loss of flexibility being outweighed by the gain in efficiency. On this view, the perverse assignments, the cell deaths, the long-range migrations—all the features which could, it seems, be eliminated from a more efficient design—are so many developmental fossils. These are the places to look for clues both to the course of evolution and to the mechanisms by which the lineage is controlled today.

APPENDIX

PARTS LIST: *Caenorhabditis elegans* (Bristol), NEWLY HATCHED LARVA

This index was prepared by condensing a list of all cells in the adult animal, then adding comments and references. A complete listing is available on request to MRC, Cambridge. References: (1) Ward *et al.*, 1975; (2) Ware *et al.*, 1975; (3) White *et al.*, 1976; (4) Albertson and Thomson, 1976; (5) Singh and Sulston, 1978; (6) Kimble and Hirsh, 1979; (7) Sulston *et al.*, 1975; (8) Chalfie and Sulston, 1981; (9) Sulston and Horvitz, 1977; (10) Sulston *et al.*, 1980; (11) White *et al.*, 1978; (12) Horvitz *et al.*, 1982; (13) Wright and Thomson, 1981; (14) Gossett *et al.*, 1982; (15) Nelson *et al.*, 1983.

AB	P_0 a	Embryonic founder cell	
ADAL ADAR	AB plapaaaapp AB prapaaaapp	Ring interneuron	
ADEL ADER	AB plapaaaapa AB prapaaaapa	Anterior deirid, sensory neuron, receptor in lateral alae, contains dopamine	7
ADEshL ADEshR	AB arppaaaa AB arpppaaa	Anterior deirid sheath	
ADFL ADFR	AB alpppppaa AB praaappaa	Amphid neuron, dual ciliated sensory endings, probably chemosensory, enters ring via commissure from ventral ganglion	1, 2
ADLL ADLR	AB alppppaad AB praaapaad	Amphid neuron, dual ciliated sensory endings, probably chemosensory, projects directly to ring	1, 2
AFDL AFDR	AB alpppapav AB praaaapav	Amphid finger cell, neuron associated with amphid sheath	1, 2
AIAL AIAR	AB plppaappa AB prppaappa	Amphid interneuron	
AIBL AIBR	AB plaapappa AB praapappa	Amphid interneuron	
AIML AIMR	AB plpaapppa AB prpaapppa	Ring interneuron	
AINL AINR	AB alaaaalal AB alaapaaar	Ring interneuron	

AIYL AIYR	AB plpapaaap AB prpapaaap	Amphid interneuron	
AIZL AIZR	AB plapaaapav AB prapaaapav	Amphid interneuron	
ALA	AB alapppaaa	Neuron, sends processes laterally and along dorsal cord	
ALML ALMR	AB arppaappa AB arpppappa	Anterior lateral microtubule cell, touch receptor neuron	8
ALNL ALNR	AB plapappppap AB prapappppap	Neuron associated with ALM	8
AMshL AMshR	AB plaapaapp AB praapaapp	Amphid sheath	
AMsoL AMsoR	AB plpaapapa AB prpaapapa	Amphid socket	
ASEL ASER ASGL ASGR ASHL ASHR ASIL ASIR ASJL ASJR ASKL ASKR	AB alpppppppaa AB praaapppaa AB plaapapap AB praapapap AB plpaappaa AB prpaappaa AB plaapapppa AB praapapppa AB alpppppppa AB praaappppa AB alpppapppa AB praaaapppa	Amphid neurons, single ciliated endings, probably chemosensory; project into ring via commissure from ventral ganglion, make diverse synaptic connections in ring neuropile	1, 2
AUAL AUAR	AB alpppppppp AB praaappppp	Neuron, process runs with amphid neuron processes but lacks ciliated ending	
AVAL AVAR	AB alppaaapa AB alaappapa	Ventral cord interneuron, synapses onto VA, DA, and AS motor neurons; formerly called alpha	3
AVBL AVBR	AB plpaapaap AB prpaapaap	Ventral cord interneuron, synapses onto VB and DB motor neurons; formerly called beta	3
AVDL AVDR	AB alaaapalr AB alaaapprl	Ventral cord interneuron, synapses onto VA, DA, and AS motor neurons; formerly called delta	3
AVEL AVER	AB alpppaaaa AB praaaaaaa	Ventral cord interneuron, like AVD but outputs restricted to anterior cord	
AVG	AB prpapppap	Ventral cord interneuron, few synapses	
AVHL AVHR	AB alapaaaaa AB alappapaa	Neuron, mainly presynaptic in ring and postsynaptic in ventral cord	
AVJL AVJR	AB alapapppa AB alappppppa	Neuron, mainly postsynaptic in ventral cord and presynaptic in ring	
AVKL AVKR	AB plpapapap AB prpapapap	Ring and ventral cord interneuron	
AVL	AB prpappaap	Ring and ventral cord interneuron, few synapses	
AWAL AWAR AWBL AWBR AWCL AWCR	AB plaapapaa AB praapapaa AB alpppppap AB praaappap AB plpaaaaap AB prpaaaaap	Amphid wing cells, neurons having ciliated sheet-like sensory endings closely associated with amphid sheath	1, 2
B	AB prpppapa	Rectal cell, postembryonic blast cell in male	9, 10
BAGL BAGR	AB alppappap AB arappppap	Neuron, ciliated ending in head, no supporting cells, associated with ILso	1, 2
BDUL BDUR	AB arppaappp AB arpppappp	Neuron, process runs along excretory canal and into ring, unique darkly staining synaptic vesicles	

C	P_0 ppa	Embryonic founder cell	
CANL CANR	AB alapaaapa AB alappappa	Process runs along excretory canal, no synapses seen, essential for survival	
CEMDL CEMDR CEMVL CEMVR	AB plaaaaaap AB arpapaaap AB plpaapapp AB prpaapapp	Male specific cephalic neurons (die in hermaphrodite embryo), open to outside, possibly mediate male chemotaxis towards hermaphrodites	10
CEPDL CEPDR CEPVL CEPVR	AB plaaaaappa AB arpapaappa AB plpaappppa AB prpaappppa	Cephalic neurons, contain dopamine	1, 2, 7
CEPshDL CEPshDR CEPshVL CEPshVR	AB arpaaaapp AB arpaaapap AB plpaaapap AB prpaaapap	Cephalic sheath, sheet-like processes envelop neuropile of ring and part of ventral ganglion	1, 2
CEPsoDL CEPsoDR CEPsoVL CEPsoVR	AB alapapppp AB alappppppp AB alppaappp AB alaapappp	Cephalic socket	1, 2
D	P_0 pppa	Embryonic founder cell	
DA1 DA2 DA3 DA4 DA5 DA6 DA7 DA8 DA9	AB prppapaap AB plppapapa AB prppapapa AB plppapapp AB prppapapp AB plpppaaap AB prpppaaap AB prpapappp AB plpppaaaa	Ventral cord motor neurons, innervate dorsal muscles	3
DB1/3 DB2 DB3/1 DB4 DB5 DB6 DB7	AB plpaaaapp AB arappappa AB prpaaaapp AB prpappapp AB plpapappp AB plppaappp AB prppaappp	Ventral cord motor neurons, innervate dorsal muscles	3
DD1 DD2 DD3 DD4 DD5 DD6	AB plppappap AB prppappap AB plppapppa AB prppapppa AB plppappppp AB prppappppp	Ventral cord motor neurons, probably reciprocal inhibitors, change their pattern of motor synapses during postembryonic development	3, 11
DVA DVC	AB prpppppapp C aapaa	Ring interneurons, cell bodies in dorsorectal ganglion	
E	P_0 pap	Embryonic founder cell	
F	AB plpppppapp	Rectal cell, postembryonic blast cell in male	9, 10
FLPL FLPR	AB plapaaapad AB prapaaapad	Neuron, ciliated ending in head, no supporting cells, associated with ILso	1, 2
GLRDL GLRDR GLRL GLRR GLRVL GLRVR	MS aaaaaal MS aaaaaar MS apaaaad MS ppaaaad MS apaaaav MS ppaaaav	Set of six cells that form a thin cylindrical sheet between pharynx and ring neuropile; no chemical synapses, but gap junctions with muscle arms and RME motor neurons	
G1 G2	AB prpaaaapa AB plapaapa	Postembryonic blast cells; excretory socket is G1 in embryo, G2 in L1, G2.p later	9, 15

H0L	AB plaaappa	Seam hypodermal cell	5, 9
H0R	AB arpapppa		
H1L	AB plaaappp	Seam hypodermal cell, postembryonic blast cell	
H1R	AB arpapppp		
H2L	AB arppaaap	Seam hypodermal cell, postembryonic blast cell, functions as deirid socket in L1	
H2R	AB arpppaap		
HSNL	AB plapppappa	Hermaphrodite-specific motor neurons (die in male embryo), innervate vulval muscles	9
HSNR	AB prapppappa		
I1L	AB alpapppaa	Anterior sensory, input from RIP	4
I1R	AB arapappaa		
I2L	AB alpappaapa	Anterior sensory	
I2R	AB arapapaapa	Anterior sensory	
I3	MS aaaaapaa	Anterior sensory	
I4	MS aaaapaa		
I5	AB arapapapp	Posterior sensory	
I6	MS paaapaa	Posterior sensory	
IL1DL	AB alapappaaa	Inner labial neuron, ciliated ending with striated rootlet, synapses directly onto muscle cells	1, 2
IL1DR	AB alappppaaa		
IL1L	AB alapaappaa		
IL1R	AB alaappppaa		
IL1VL	AB alppapppaa		
IL1VR	AB arappppppaa		
IL2DL	AB alapappap	Inner labial neuron, ciliated ending without striated rootlet, open to outside	1, 2
IL2DR	AB alappppap		
IL2L	AB alapaappp		
IL2R	AB alaappppp		
IL2VL	AB alppappppp		
IL2VR	AB arappppppp		
ILshDL	AB alaaaparr	Inner labial sheath	1, 2
ILshDR	AB alaaappll		
ILshL	AB alaaaalpp		
ILshR	AB alaapaapp		
ILshVL	AB alppapaap		
ILshVR	AB arapppaap		
ILsoDL	AB plaapaaap	Inner labial socket	1, 2
ILsoDR	AB praapaaap		
ILsoL	AB alaaapall		
ILsoR	AB alaaapprr		
ILsoVL	AB alppapapp		
ILsoVR	AB arapppapp		
K	AB plpapppaa	Rectal cell, postembryonic blast cell	9
K′	AB plpapppap	Rectal cell	
LUAL	AB plpppaapap	Interneuron, short process in posterior end of ventral cord	
LUAR	AB prpppaapap		
M	MS apaapp	Postembryonic mesoblast	9
M1	MS paapaaa	Pharyngeal motor neurons	4
M2L	AB araapappa		
M2R	AB araappppa		
M3L	AB araapappp	Pharyngeal sensory-motor neuron	
M3R	AB araapppppp		
M4	MS paaaaaa	Pharyngeal motor neurons	
M5	MS paaapap		
MCL	AB alpaaappp	Pharyngeal neurons that synapse onto the marginal cells	4
MCR	AB arapaappp		
MI	AB araappaaa	Pharyngeal motor neuron/interneuron	4
MS	P_0 paa	Embryonic founder cell	

NSML NSMR	AB araapapaav AB araapppaav	Pharyngeal neurosecretory motor neuron, probably contains serotonin	4, 12
OLLL OLLR	AB alpppapaa AB praaapapaa	Lateral outer labial neuron, ciliated ending without striated rootlet	1, 2
OLLshL OLLshR	AB alpppaapd AB praaaaapd	Lateral outer labial sheath	1, 2
OLLsoL OLLsoR	AB alapaaapp AB alappappp	Lateral outer labial socket	1, 2
OLQDL OLQDR OLQVL OLQVR	AB alapapapaa AB alapppapaa AB plpaaappaa AB prpaaappaa	Quadrant outer labial neuron, ciliated ending with striated rootlet	1, 2
OLQshDL OLQshDR OLQshVL OLQshVR	AB arpaaaapa AB arpaaapaa AB alpppaaap AB praaaaaap	Quadrant outer labial sheath	1, 2
OLQsoDL OLQsoDR OLQsoVL OLQsoVR	AB arpaaaaal AB arpaaaaar AB alppaaapp AB alaappapp	Quadrant outer labial socket	1, 2
P_4	P_0 pppp	Embryonic founder cell: germ line	
P1/2L P1/2R P3/4L P3/4R P5/6L P5/6R P7/8L P7/8R P9/10L P9/10R P11/12L P11/12R	AB plapaapp AB prapaapp AB plappaaa AB prappaaa AB plappaap AB prappaap AB plappapp AB prappapp AB plapapap AB prapapap AB plapappa AB prapappa	Postembryonic blast cells for ventral cord neurons, ventral hypodermis, vulva, male preanal ganglion; form ventral hypodermis in L1	9, 10
PDA	AB prpppaaaa	Motor neuron, process in dorsal cord; same cell as Y (qv) in hermaphrodite, as Y.a in male	9
PHAL PHAR PHBL PHBR	AB plpppaapp AB prpppaapp AB plapppappp AB prapppappp	Phasmid neurons, ciliated endings, probably chemosensory, similar to amphids but situated in tail	10
PHshL PHshR	AB plpppapaa AB prpppapaa	Phasmid sheath	10
PLML PLMR	AB plapappppaa AB prapappppaa	Posterior lateral microtubule cell, touch receptor neuron	8
PVCL PVCR	AB plpppaapaa AB prpppaapaa	Ventral cord interneuron, cell body in lumbar ganglion, synapses onto VB and DB motor neurons; formerly called delta	3
PVPL PVPR	AB plpppppaaa AB prpppppaaa	Interneuron, cell body in lumbar ganglion, projects along ventral cord to ring	
PVQL PVQR	AB plapppaaa AB prapppaaa	Interneuron, projects along ventral cord to ring	
PVR	C aappa	Interneuron, projects along ventral cord to ring	
PVT	AB plpappppa	Interneuron, projects along ventral cord, has several branches in ring	
QL QR	AB plapapaaa AB prapapaaa	Postembryonic neuroblast, migrates anteriorly Postembryonic neuroblast, migrates posteriorly	9
RIAL RIAR	AB alapaapaa AB alaapppaa	Ring interneuron, many synapses	

RIBL RIBR	AB plpaappap AB prpaappap	Ring interneuron	
RICL RICR	AB plppaaaapp AB prppaaaapp	Ring interneuron	
RID	AB alappaapa	Ring interneuron, projects along dorsal cord	
RIFL RIFR	AB plppapaaap AB prppapaaap	Ring interneuron	
RIGL RIGR	AB plppappaa AB prppappaa	Ring interneuron	
RIH	AB prpappaaa	Ring interneuron	
RIML RIMR	AB plppaapap AB prppaapap	Ring interneuron	
RIPL RIPR	AB alpapaaaa AB arappaaaa	Ring/pharynx interneuron, only direct connection between pharynx and ring	
RIR	AB prpapppaa	Ring interneuron	
RIS	AB prpappapa	Ring interneuron	
RIVL RIVR	AB plpaapaaa AB prpaapaaa	Ring interneuron	
RMDDL RMDDR RMDL RMDR RMDVL RMDVR	AB alpapapaa AB arappapaa AB alpppapad AB praaaapad AB alppapaaa AB arapppaaa	Ring motor neuron/interneuron, many synapses	
RMED RMEL RMER RMEV	AB alapppaap AB alaaaarlp AB alaaaarrp AB plpappaaa	Ring motor neuron	
RMGL RMGR	AB plapaaapp AB prapaaapp	Ring interneuron	
SAADL SAADR SAAVL SAAVR	AB alppapapa AB arapppapa AB plpaaaaaa AB prpaaaaaa	Ring interneuron, anteriorly projecting process that runs sublaterally	
SABD SABVL SABVR	AB plppapaap AB plppapaaaa AB prppapaaaa	Ring interneuron, anteriorly projecting process that runs sublaterally, synapses to anterior body muscles in L1	
SIADL SIADR SIAVL SIAVR SIBDL SIBDR SIBVL SIBVR	AB plpapaapa AB prpapaapa AB plpapappa AB prpapappa AB plppaaaaa AB prppaaaaa AB plpapaapp AB prpapaapp	Receives a few synapses in the ring, has a posteriorly directed process that runs sublaterally	
SMBDL SMBDR SMBVL SMBVR SMDDL SMDDR SMDVL SMDVR	AB alpapapapp AB arappapapp AB alpapappp AB arappappp AB plpapaaaa AB prpapaaaa AB alppappaa AB arappppaa	Ring motor neuron/interneuron, has a posteriorly directed process that runs sublaterally	
TL TR	AB plappppp AB prappppp	Tail seam hypodermal cell, postembryonic blast cell, functions as phasmid socket in L1	5, 9, 10
U	AB plpppapa	Rectal cell, postembryonic blast cell in male	9

URADL	AB plaaaaaaa		
URADR	AB arpapaaaa	Ring motor neuron, nonciliated ending in head, associated with OLQ in embryo	
URAVL	AB plpaaapaa		
URAVR	AB prpaaapaa		
URBL	AB plaapaapa	Neuron, presynaptic in ring, nonciliated ending in head, associated with OLL in embryo	
URBR	AB praapaapa		
URXL	AB plaaaaappp	Ring interneuron, nonciliated ending in head, associated with CEPD in embryo	
URXR	AB arpapaappp		
URYDL	AB alapapapp		
URYDR	AB alapppapp	Neuron, presynaptic in ring, nonciliated ending in head, associated with OLQ in embryo	
URYVL	AB plpaaappp		
URYVR	AB prpaaappp		
V1L	AB arppapaa		
V1R	AB arppppaa		
V2L	AB arppapap		
V2R	AB arppppap		
V3L	AB plappapa		
V3R	AB prappapa	Seam hypodermal cell, postembryonic blast cell	5, 9
V4L	AB arppappa		
V4R	AB arpppppa		
V5L	AB plapapaap		
V5R	AB prapapaap		
V6L	AB arppappp		
V6R	AB arpppppp		
W	AB prapaapa	Postembryonic neuroblast, analogous to Pn.a	9
XXXL	AB plaaapaa	Embryonic head hypodermal cell; no obvious function later	
XXXR	AB arpappaa		
Y	AB prpppaaaa	Rectal cell at hatching, becomes PDA in hermaphrodite, postembryonic blast cell in male	9
Z1	MS pppaap	Somatic gonad precursor cell	6
Z2	P_4 p	Germ line precursor cell	6
Z3	P_4 a	Germ line precursor cell	
Z4	MS appaap	Somatic gonad precursor cell	6
arc ant	AB X3	Interface between pharynx and hypodermis, form anterior part of buccal cavity	13
arc post	AB X3		
ccAL	MS apapaaa		
ccAR	MS ppapaaa	Embryonic coelomocytes	9
ccPL	MS apapaap		
ccPR	MS ppapaap		
e1D	AB araaaapap		
e1VL	AB araaaaaaa		
e1VR	AB araaaaapa		
e2DL	AB alpaapaap		
e2DR	AB araaapaap	Pharyngeal epithelial cells	4
e2V	AB alpappapa		
e3D	AB araapaaaa		
e3VL	AB alpaaaaaa		
e3VR	AB arapaaaaa		
exc cell	AB plpappaap	Large H-shaped excretory cell	15
exc duct	AB plpaaaapa	Excretory duct	15
exc gl L	AB plpapapaa	Excretory glands, fused, send processes to ring, open into excretory duct	15
exc gl R	AB prpapapaa		
g1AL	MS aapaapaa		
g1AR	MS papaapaa		
g1P	MS aaaaapap	Pharyngeal gland cells	4
g2L	MS aapapaa		
g2R	MS papapaa		

hmc	MS appaaa	Head mesodermal cell, function unknown	9
hyp1	AB X3		
hyp2	AB X2		
hyp3	AB X2		
hyp4	AB X3	Cylindrical hypodermal syncytia	
hyp5	AB X2		
hyp6	AB X6		
hyp7	AB X11		
hyp7	C X12		
hyp8/9	AB plpppapap		
hyp8/9	AB prpppapap	Tail ventral hypodermis	
hyp10	AB X2		
hyp11	C pappa	Tail dorsal hypodermis	
int	E X20	Intestinal cells	
m1DL	AB araapaaap		
m1DR	AB araappaap		
m1L	AB araaaaaap		
m1R	AB araaaaapp		
m1VL	AB alpaaaapa		
m1VR	AB arapaaapa		
m2DL	AB araapaapa		
m2DR	AB araappapa		
m2L	AB alpaaapaa		
m2R	AB arapaapaa		
m2VL	AB alpaaaaap		
m2VR	AB arapaaaap		
m3DL	MS aaapaaa		
m3DR	MS paaaapa		
m3L	AB alpaapapp		
m3R	AB arapaappa		
m3VL	AB alpappppp		
m3VR	AB arapappppp		
m4DL	MS aaaaapp	Pharyngeal muscle cells	4
m4DR	MS paaaapp		
m4L	MS aaapaap		
m4R	AB araaapapp		
m4VL	MS aapaaaa		
m4VR	MS papaaaa		
m5DL	MS aaaapap		
m5DR	MS paaappa		
m5L	AB araapapap		
m5R	AB araapppap		
m5VL	MS aapaaap		
m5VR	MS papaaap		
m6D	MS paaappp		
m6VL	MS aapappa		
m6VR	MS papappa		
m7D	MS aaaappp		
m7VL	MS aapaapp		
m7VR	MS papaapp		
m8	MS aaapapp		
mc1DL	AB alpaapapa		
mc1DR	AB araaapapa		
mc1V	AB alpappppa		
mc2DL	AB araapaapp		
mc2DR	AB araappapp	Pharyngeal marginal cells	4
mc2V	AB arapappppa		
mc3DL	MS aaapapa		
mc3DR	MS paapapa		
mc3V	AB alpappapp		
mu anal	AB plpppppap	Anal depressor muscle	9, 10

mu bod	AB prpppppaa	Embryonic body wall muscles	9, 14
mu bod	C X32		
mu bod	D X20		
mu bod	MS X28		
mu int L	AB plpppppaa	Intestinal muscles, attach to intestine and body wall anterior to anus	9
mu int R	MS ppaapp		
mu sph	AB prpppppap	Sphincter muscle of intestino-rectal valve	9, 10
rect D	AB plpappppp	Rectal epithelial cells, adjacent to intestino-rectal valve, have microvilli	9
rect VL	AB plpppaap		
rect VR	AB prpppaap		
spike/X	AB plpppppa	Used during embryogenesis to make tail spike, then die	
spike/X	AB prpppppa		
virL	AB prpappppp	Intestino-rectal valve	
virR	AB prpappppa		
vpi1	MS paapapp	Pharyngo-intestinal valve	
vpi2DL	MS aapappp		
vpi2DR	MS papappp		
vpi2V	MS aappaa		
vpi3D	MS aaappp		
vpi3V	MS aappap		

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The Postembryonic Cell Lineages of the Hermaphrodite and Male Gonads in *Caenorhabditis elegans*

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The ancestry of the cells in the hermaphrodite and male gonadal somatic structures of *C. elegans* has been traced from the two gonadal somatic progenitor cells (Z1 and Z4) that are present in the newly hatched larvae of both sexes. The lineages of Z1 and Z4 are essentially invariant. In hermaphrodites, they give rise to a symmetrical group of structures consisting of 143 cells, and in males, they give rise to an asymmetrical group of structures consisting of 56 cells. The male gonad can be distinguished from the hermaphrodite gonad soon after the first division of Z1 and Z4. However, the development of Z1 and Z4 in hermaphrodites shares several features in common with their development in males suggesting that the two programs are controlled by similar mechanisms. In the hermaphrodite lineage, a variability in the positions of two cells is correlated with a variability in the lineages of four cells. This variability suggests that cell–cell interaction may play a more significant role in organisms that develop by invariant lineages than has hitherto been considered. None of the somatic structures (e.g., uterus, spermatheca, vas deferens) develops as a clone of a single cell. Instead, cells that arise early in the Z1–Z4 lineage generally contribute descendants to more than one structure, and individual structures consist of descendants of more than one lineage.

INTRODUCTION

How the developmental history of a cell influences its capacity to differentiate persists as one of the central questions in developmental biology. Cell lineages pose this fundamental question in a framework that permits an analysis of specific developmental events. Classically, cell lineage studies were used to explore the organization of the zygote and the basis of cell determination during embryogenesis. From these studies came the idea that many eggs are mosaics of cytoplasmic determinants that are distributed to specific cells by an invariant pattern of cell divisions (see Wilson, 1925).

The nematode *Caenorhabditis elegans* is an excellent organism for lineage studies because it has a limited number of cells and is genetically well defined (Brenner, 1974). Since *C. elegans* is transparent, individual cells can be distinguished microscopically and followed in living worms. The genetic control of lineage patterns can be studied using mutations that alter known lineages. Such mutations also provide a means of perturbing the normal pattern to investigate how changes in cell divisions or positions affect subsequent development. A similar method of approach to the genetic control of cell lineages was used by Lees and Waddington (1942) using the bristle lineage in *Drosophila* as a model system. However, they were required to infer developmental events from fixed specimens.

Considerable work has already been done on lineages in *C. elegans*. A description of the postembryonic nongonadal lineages has been completed (Sulston and Horvitz, 1977), and work on the embryonic lineages is in progress (Deppe *et al.*, 1978). Laser

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ablation experiments have demonstrated that cells are rigidly determined in at least some lineages (Sulston and Horvitz, 1977). Analysis of a lineage mutant in *C. elegans* has shown that nerve precursor cells blocked in division are still capable of exhibiting the differentiated phenotypes of their descendants (Albertson *et al.*, 1978).

Previous studies in our laboratory have included a morphological description of the developing gonads in hermaphrodites and males (Hirsh *et al.*, 1976; Klass *et al.*, 1976), and the isolation of temperature-sensitive gonadogenesis-defective mutants (Hirsh and Vanderslice, 1976). This paper presents the postembryonic gonadal lineages of *C. elegans*. The lineages of the germ line progenitor cells are variable from worm to worm, and are discussed only briefly here. Although clonal information may be relevant to the generation of oocytes and sperm, other methods, such as laser ablation of precursor cells, will be necessary to elucidate the germ line lineages. By contrast, the lineages which generate the somatic structures of the gonad proceed according to an essentially invariant pattern.

The lineages that give rise to the somatic structures of the gonad are of particular interest because they display a complete program of development from only two cells, which are present when the worm hatches, to multiple cell types that are organized into discrete organs in the adult gonad. Although the embryonic ancestry of the two somatic progenitor cells is not known in *C. elegans*, Pai (1927) reported that the equivalent two cells of another nematode, *Turbatrix aceti*, arose from an embryonic stem cell, S5, as sisters. Since work on the embryonic lineage of *C. elegans* is in progress (Deppe *et al.*, 1978), the complete lineage of these structures should be known. Another interesting aspect of this lineage is that the two cells follow one developmental pattern in the hermaphrodite and another in the male, so that a comparison of homologous programs is possible.

MATERIALS AND METHODS

Nematode strain. Wild-type *C. elegans* var. Bristol, designated N2, is from the University of Colorado, Boulder, stock. The nematodes were handled as described by Brenner (1974) and Hirsh *et al.* (1976). Worms for lineage studies were kept at either 16 or 20°C until they were picked for observation.

Technique to obtain cell lineages. We used the technique of Sulston and Horvitz (1977) for mounting live worms for observation over long periods of time. Briefly, a worm is placed on a thin agar pad on a microscope slide. A slurry of *E. coli* is applied in a small disk to a coverslip. The coverslip is placed so that the worm is trapped between the agar surface and the coverslip in a thin layer of S medium (0.1 *M* NaCl and 0.05 *M* KH_2PO_4, pH 6.0). The worm is able to crawl on the agar surface and feed on the bacteria supplied. The edge of the coverslip is sealed with Vaseline or immersion oil so that the preparation does not dry out.

Our observations were made using a Zeiss Universal microscope equipped with Plan 100 objective and Nomarski differential interference contrast optics. One limit of this technique is that cell boundaries are indistinct. Thus, the bulk of the lineage data reflects the behavior of cell nuclei. Drawings were made as frequently as possible during periods of active migration and rapid division, and as frequently as necessary (every 0.5 to 2 hr) when nuclei were less active. The complete lineages were compiled from observations of over 50 hermaphrodites and 20 males. Photomicrographs were taken with a Zeiss microflash illuminator.

Nomenclature. We have used the nomenclature of Sulston and Horvitz (1977) for naming cells. A cell's daughters are each named by adding to the name of the mother cell a single lower case letter describing the position of each daughter relative to the other just after division (a = anterior, p = posterior, d = dorsal, v = ventral, l = left,

r = right). Thus, if the cleavage plane of Z1 is oriented perpendicular to the anterior–posterior axis, the resulting anterior daughter is identified as Z1.a and the posterior daughter as Z1.p. If a division occurs obliquely, the resulting daughters are identified by adding a single letter signifying one axis only.

Lineage charts. The lineage trees presented here follow the rules formulated by Sulston and Horvitz (1977) as far as possible. A division is depicted by a branch point in which the left branch represents an anterior, dorsal, or left daughter and the right branch represents a posterior, ventral, or right daughter. Branches are marked by the same letters used in naming cells except that combinations of letters are used to describe oblique divisions. In the case of simple anterior–posterior divisions, the letters are omitted. In each lineage chart, the vertical coordinate represents time. Since the lineages are obtained at varying temperatures (19–23°C), the times divisions take place are normalized to 20°C. The times at which individual molts occur have been used as time standards for this correction. The horizontal coordinate of the lineage chart represents the anterior–posterior axis with anterior to the left for the entire hermaphrodite lineage and the first half of the male lineage. In the male lineage chart, the horizontal coordinate system in the last half of the divisions represents the distal–proximal axis with proximal to the left. The proximal end of the gonad is the opening at the cloaca; the distal is the farthest from the opening. Anterior, then, is to the left in the chart until the gonad reflexes, and it is to the right for the rest of the divisions. This is necessary, because in males, unlike hermaphrodites, divisions occur in the bend of the gonad. Since these divisions take place at variable points in the bend, the positions of the daughters vary with respect to the coordinates of the worm. However, the divisions are invariant when viewed with respect to the distal–proximal axis of the gonad.

Some variability is observed in the times at which divisions occur and in the orientation of cleavage planes. For example, in some worms Z1 divides first, in others Z4 divides first and, in a rare worm, they divide at the same time. Since they almost always divide within an hour of each other, they are represented in the lineage chart as dividing at the same time. A similar limited variability is observed in the cleavage planes, and here too, an average is shown in the lineage charts.

One-micron sections. Adult hermaphrodites were cut posterior to the pharynx or anterior to the anus in a drop of 2% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.4). A good cut will release the anterior or posterior gonadal arm intact into the fixative, though it remains attached to the worm by the somatic structures. The dissected animals were fixed in 2% glutaraldehyde for 1 hr, postfixed in 1% OsO_4 for 1 hr, stained *en bloc* in 1% uranyl acetate for 1 hr, dehydrated in increasing concentrations of ethanol, transferred to propylene oxide, and embedded in Epon. Serial sections of 1-μm thickness stained with toluidine blue were examined by light microscopy.

RESULTS

Hirsh *et al.* (1976) and Klass *et al.* (1976) have described the major features of postembryonic gonadogenesis in hermaphrodites and males, respectively. Figure 1 summarizes these findings. In both sexes, gonadal development starts from a four-celled gonadal primordium present at hatching and proceeds through all four larval stages (L1, L2, L3, and L4). Hermaphrodite gonadogenesis is characterized by twofold rotational symmetry, whereas male gonadogenesis is asymmetrical. Both hermaphrodite and male gonads display a distal–proximal axis with proximal toward the vulva in hermaphrodites and toward the cloaca in the male. The maturation of gametes occurs from distal to proximal. The somatic structures are located most proximally in the

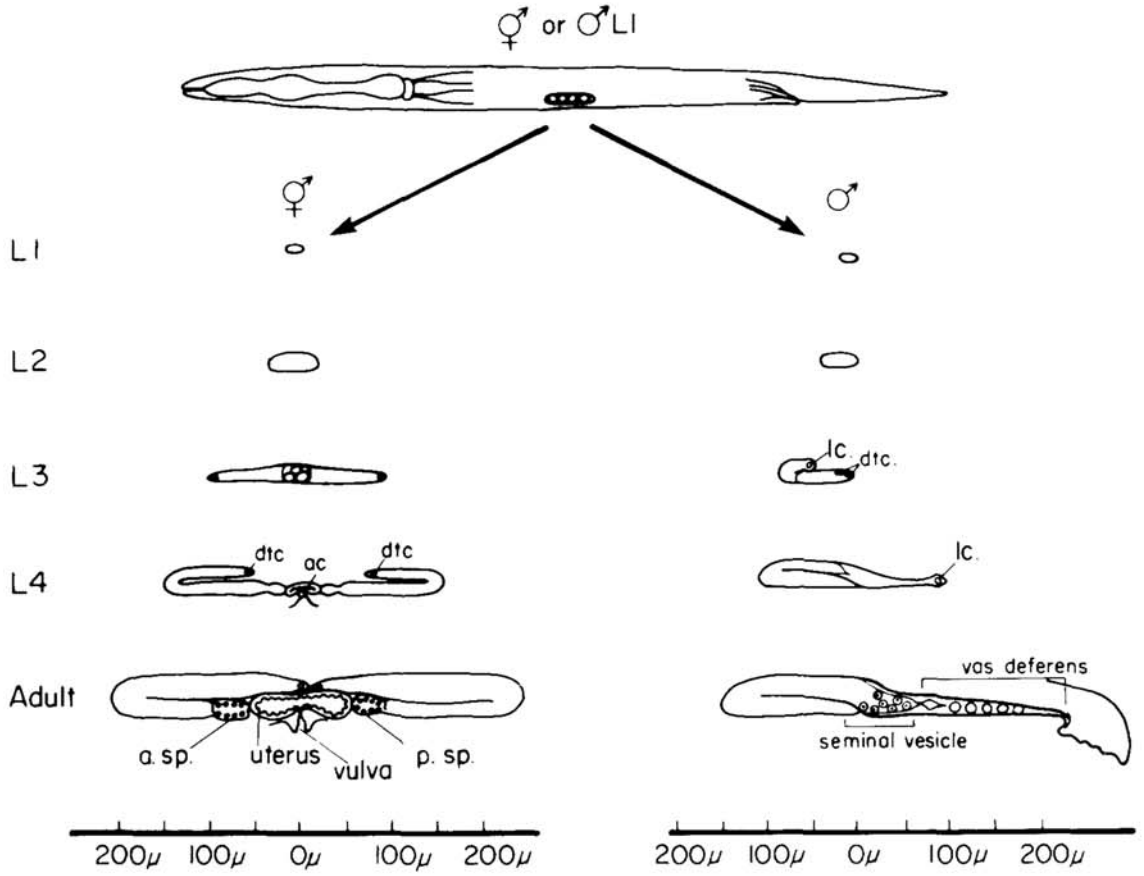

FIG. 1. Overview of gonad development in hermaphrodites and males. At the top, the four celled gonadal primordium is shown in its midventral position in the newly hatched worm. In hermaphrodites (left column), the developing gonad elongates anteriorly and posteriorly during L1, L2, and L3. The growing tips reflex around the time of the L3–L4 molt. In males (right column), the developing gonad initially grows only in an anterior direction, and reflexes at about the L2–L3 molt. The main somatic structures of the adult hermaphrodite and male are shown schematically in the bottom two drawings. dtc, distal tip cell; ac, anchor cell; lc, linker cell; a. sp., anterior spermatheca; p. sp., posterior spermatheca.

reproductive system.

The gonadal primordium in the hermaphrodite is indistinguishable morphologically from that of the male. In each sex, the four primordial cells are located midventrally along the anterior–posterior axis (Fig. 2A). These four cells are named Z1, Z2, Z3, and Z4 from anterior to posterior. Z1 and Z2 are located to the right of the worm's midsagittal plane, and Z3 and Z4 are to the left of that plane (Fig. 2B). Thus, the primordium exhibits twofold rotational symmetry. Although the hermaphrodite and male primordia are equivalent structures, newly hatched worms can be easily sexed according to three secondary sex characteristics recognized by Sulston and Horvitz (1977).

The organization of the somatic and germ line progenitor cells in the four-celled gonadal primordium of nematodes was revealed many years ago (reviewed in Chitwood and Chitwood, 1950). In both hermaphrodite and male worms, cells Z1 and Z4 give rise to the somatic structures of the gonad, and Z2 and Z3 give rise to the germ line cells. The primary focus of this paper is the Z1–Z4 somatic lineage. The cell lineages of Z2 and Z3 are variable in both sexes with respect to the planes of cell division and the times at which particular divisions occur. Z2–Z3 divisions occur continuously from L1 through adulthood. In hermaphrodites, the anterior and posterior gonadal arms each contain descendants of both Z2 and Z3. The plane of cell division can be

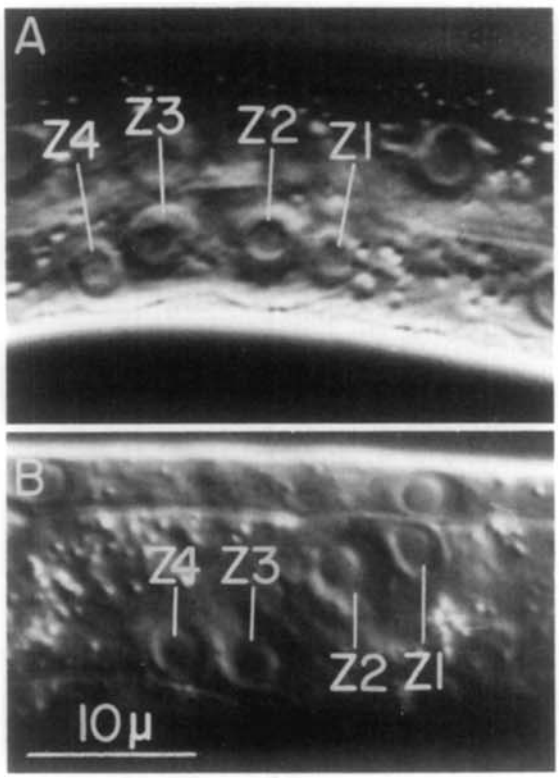

FIG. 2. Gonadal primordium in young L1 hermaphrodite; Nomarski optics. A. Lateral view. This worm is unusual in that Z1, Z2, Z3, and Z4 are all visible in the same focal plane. B. Ventral view.

left–right, dorsal–ventral, anterior–posterior, or oblique along any axis. A particular cell can divide relatively soon after its birth or it can be considerably delayed. No migrations have been observed among the germ line cells.

In contrast to the germ line cells, the somatic progenitor cells, Z1 and Z4, follow a nearly invariant pattern of cell divisions, migrations, and differentiation. The timing of events and the orientation of cleavages are essentially the same from worm to worm. The lineages of Z1 and Z4 are characterized by two periods of mitoses. The earlier period occurs during the first larval stage (L1) and the later one spans L3 and extends into L4.

Hermaphrodite Z1–Z4 Lineage (See Fig. 3)

Early Mitotic Period

Z1 and Z4 start dividing midway during the first larval stage (L1), and give rise to 12 cells by the L1–L2 molt, or shortly thereafter. After the first division, the anterior daughter of Z1, Z1.a, and the posterior daughter of Z4, Z4.p, occupy respectively the anterior and posterior tips of the primordium, and Z1.p and Z4.a lie ventral to the germ line cells (Fig. 4a). When Z1.a and Z4.p divide, Z1.aa and Z4.pp remain at the anterior and posterior tips of the gonad, and their siblings, Z1.ap and Z4.pa become positioned more dorsally in the primordium (Fig. 4b). Z1.p and Z4.a divide asymmetrically, and each gives rise to one larger daughter (Z1.pa and Z4.ap) and one small daughter (Z1.pp and Z4.aa) (Fig. 4b). Z1.pa, Z1.pp, Z4.aa, and Z4.ap, lying ventrally in the primordium, each divide to complete the early divisions (Fig. 4c). By the time of this final round of divisions, Z1.pa has moved to the left side, and Z4.ap has moved to the right side of the developing gonad. The daughters arising from these cells remain on the side to which the mother cell moved previously (Fig. 4d). Thus, four descendants of Z1 now lie on the opposite side that Z1 originally occupied and similarly for the descendants of Z4 (cf. Fig. 2 and Fig. 4d). Thus, the division pattern and cell movements carried out by Z1 are related by twofold rotational symmetry to the division pattern and cell movements of Z4.

Amitotic Period

The somatic cells of the gonad do not divide again until late L2 or early L3, but the germ line cells approximately quadruple in number during L2. Thus, the somatic cells become separated from each other by an increasing number of germ line cells. Z1.aa remains at the anterior tip and Z4.pp at the posterior tip. Since the growing tips are the future distal tips in hermaphrodites, Z1.aa and Z4.pp are called distal tip cells. These two cells appear to serve a leader function, preceding the extending gonadal arms throughout gonadogenesis (Fig. 5). They do not divide again and they remain at the gonadal tips even in the adult. During

L2, the gonadal primordium elongates from 25–30 to 50–70 μm.

The second half of L2 is characterized by a marked growth without cell division and a positional change of ten Z1 and Z4 descendants (Z1.aa and Z4.pp remain at the distal tips). Just before the L2–L3 molt, these ten cells, five Z1 descendants and five Z4 descendants, move to the center of the gonad and overlap each other. During this process, the germ line cells are completely displaced from the central region. This rearrangement of somatic cells forms the hermaphrodite somatic primordium (Fig. 5). This primordium represents a new association of all the cells that will divide further to generate the individual somatic structures of the hermaphrodite gonad.

The ten cells arrange themselves in one of two alternative configurations in the somatic primordium (Fig. 6). The positions of eight of the somatic primordial cells are invariant. However, two cells, Z1.ppp and Z4.aaa, assume one of two alternative positions. Late in L2, either Z1.ppp or Z4.aaa moves into the midsagittal plane on the ventral surface of the gonad. Whichever cell acquires this midsagittal position is fated to become the anchor cell—a small, round cell which divides no further, and seems to be involved in the formation of the vulva. If Z1.ppp moves in from the left side of the gonad, only four cells remain on the left, whereas five cells are present on the right. This is called the 5R configuration (Fig. 6a). Conversely, if Z4.aaa moves in from the right side, four remain on the right and five on the left. This is the 5L configuration (Fig. 6b). Individual worms that developed by passing through a 5R configuration were observed to give rise to 5L and 5R progeny. Similarly, progeny of 5L worms can develop through either the 5L or 5R pathway.

Late Mitotic Period

During L3 and L4, nine somatic cells (all the cells in the somatic primordium except the anchor cell) divide to generate 140 cells for a total of 143 cells in the L4, including the distal tip cells and the anchor cell. These cell divisions and subsequent differentiation give rise to five somatic structures: the anterior and posterior sheaths which encapsulate the germ line component of the gonad, the anterior and posterior spermathecae which store sperm from each gonadal arm, and a central uterus (Fig. 7).

(a) Sheath lineage. Two cells (Z1.ap and Z1.paa) in the somatic primordium each contribute five descendants to the anterior sheath, and two (Z4.pa and Z4.app) contribute five descendants to the posterior sheath (Fig. 8). Thus, each sheath consists of 10 cells. The sheath precursor cells also give rise to a major portion of the spermatheca.

The future sheath cells arise next to the developing spermatheca. They become flat elongated cells and spread over the surface of the germ line tube. They either migrate distally or are dragged along as the germ line arm elongates. Unless the nuclei stay along the visible edge of the gonadal arm, the sheath cells are very difficult to see. Therefore, 1-μm serial sections were cut from dissected gonads embedded in Epon, and stained with toluidine blue. The sheath cell nuclei, easily visible in cross section, were counted to make sure that no further division had occurred, and to ascertain the adult positions of these cells (Fig. 8).

(b) Spermathecal lineage. The anterior and posterior spermathecae each arise from four cells of the somatic primordium (Fig. 9). Two cells each contribute nine descendants, and two cells each contribute three descendants.

The spermathecae develop as spiral structures that join the developing uterus to the anterior or posterior sheath. Each spermatheca has a single left-handed twist in it along its longitudinal axis. These two structures are related by twofold rotational symmetry. As the spermathecal cells differentiate, the nuclei become small and irregular. The cytoplasm takes on a granular appearance and it becomes impossible to see the cytoplasmic boundaries. A lumen

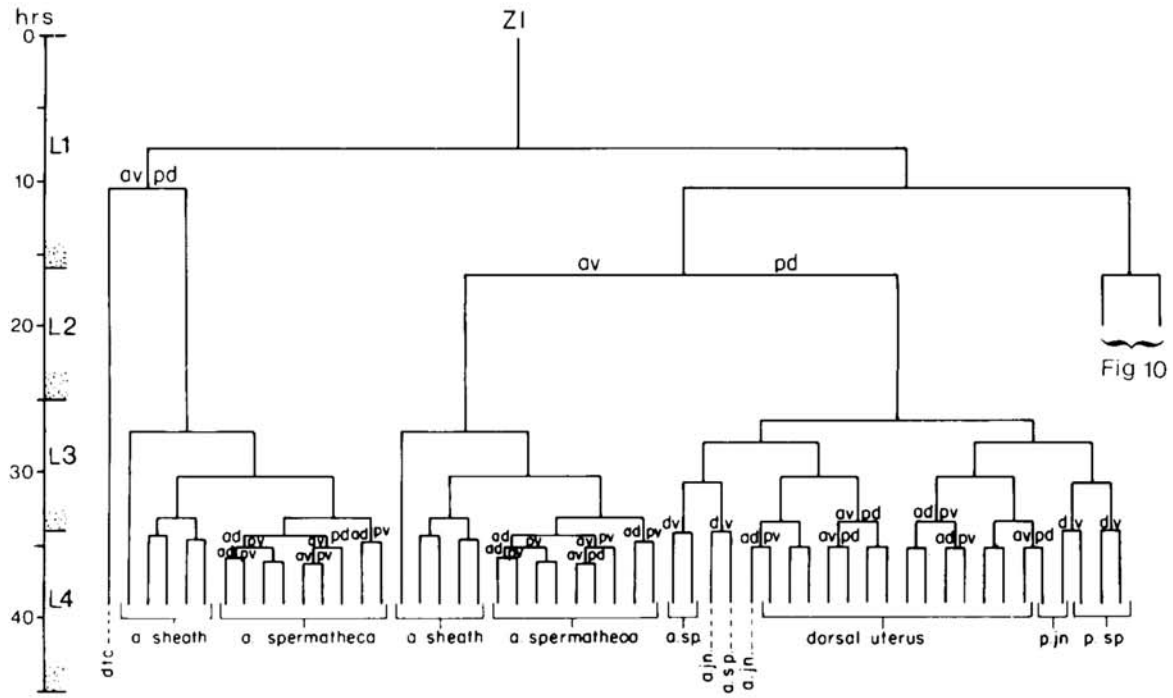

FIG. 3. Hermaphrodite lineage of gonadal somatic progenitor cells Z1 and Z4. Only the invariant lineages are shown here; the variant lineages are shown in Fig. 10. Divisions give rise to an anterior daughter and a posterior daughter unless otherwise indicated. Anterior is to the left. The fates of descendants are indicated either individually or in groups; a, anterior; p, posterior; dtc, distal tip cell; sp, spermatheca; jn, spermathecal–uterine junction.

forms as the last divisions occur in the developing spermatheca. The adult spermatheca is a flexible tube that is continually contorted by the muscular contractions of the proximal arm sheath and the uterus.

(c) Uterine lineage. The dorsal portion of the uterus arises invariantly from two cells in the somatic primordium (Fig. 3). The ventral uterus can arise according to either of two alternative lineages (Fig. 10). These two lineages correspond to the two alternative configurations of the somatic primordium. One lineage is followed if the cells assume the 5R configuration (Fig. 10a), whereas another is followed from the 5L configuration (Fig. 10b). These two lineages, expressed in different animals, are related by twofold rotational symmetry.

The difference in the ventral uterine lineage in 5L and 5R worms provides the only example of variability in the hermaphrodite lineage of Z1 and Z4. The 5R and 5L configurations differ only in the positions of two cells, but the lineages of four cells are affected. Figure 11 shows diagrammatically how the ancestry of cells in specific locations in the ventral uterus differs depending on whether the 5R or the 5L pathway is followed.

A uterine lumen begins to form during the last divisions of the uterine lineage. At this stage, the uterine cells are cuboidal with round nuclei and clear cytoplasm (Fig. 7). During the latter half of L4, the uterine cells become very thin. During this differentiation of the uterine epithelium, the uterine muscle cells, arising from a different lineage (Sulston and Horvitz, 1977), become attached to the uterus. The uterus begins to undergo massive distortion due to contractions, so the individual cells have not been traced through this period.

(d) Spermathecal–uterine junction lineage. Six cells contribute to the formation of the anterior, and six to the posterior, spermathecal–uterine junction. Their identities are indicated in Figs. 3 and 10. The adult junction consists of a junctional core that

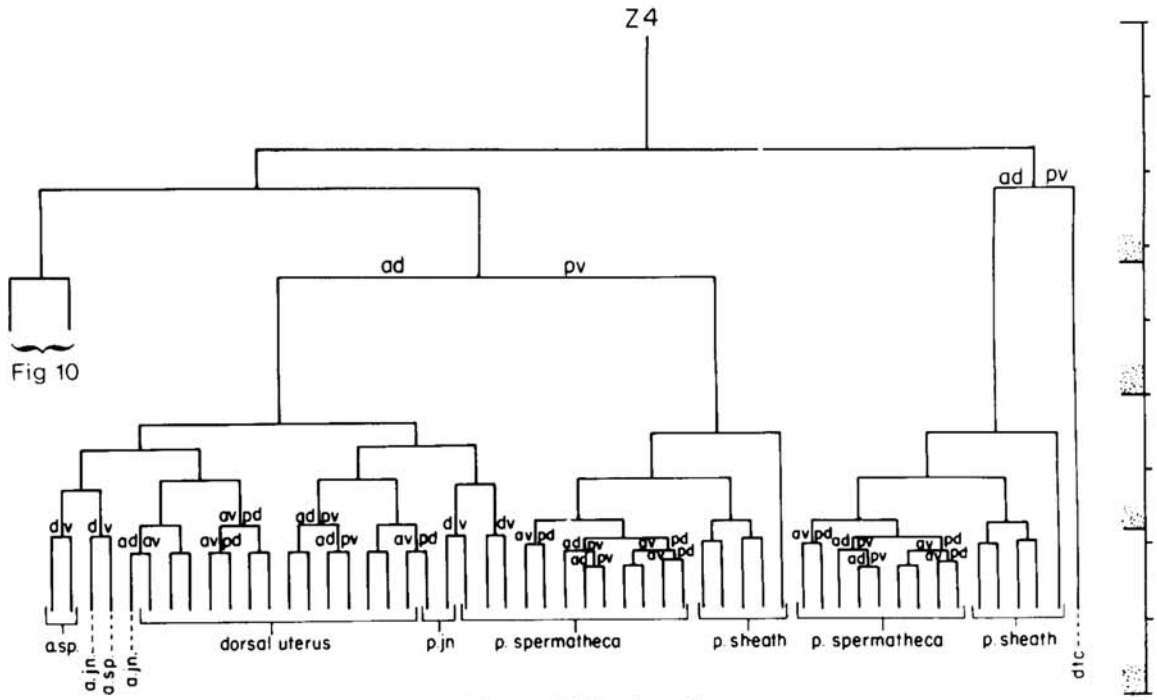

FIGURE 3 (*Continued*)

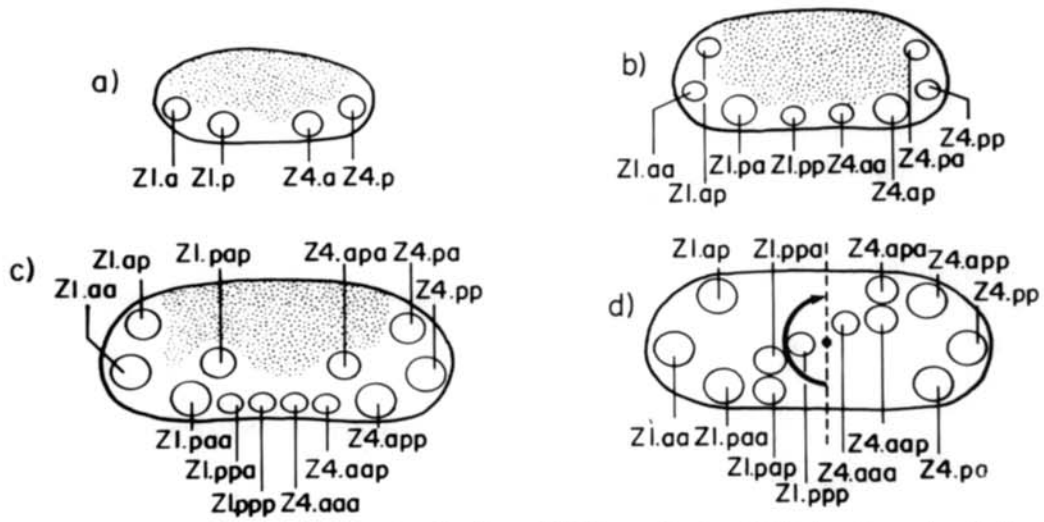

FIG. 4. Spatial arrangement of hermaphrodite Z1 and Z4 descendant nuclei during the early mitotic period. The space occupied by Z2 and Z3 descendants is depicted by stippling. (a), (b), and (c) give lateral views after each round of divisions; (d) is a dorsal view of the same stage as (c) and shows that the positions of Z1 descendants are related to the positions of Z4 descendants by twofold rotational symmetry. The axis of symmetry is depicted as a dot, and the rotational operation is indicated by an arrow.

is surrounded by the nuclei of four cells (Fig. 12).

Two cells combine to make the junctional core. Because these two cells arise in what otherwise would be a spermathecal lineage, their identities are shown in Fig. 9. During L4, these two cells enlarge to form a plug between the lumens of the uterus and the spermatheca. During the L4 to adult molt, they differentiate into the core of the junction. The nuclei protrude into the uterine lumen as the main bulk of their cytoplasm elongates and takes on the shape of the junction's core (Fig. 12). These two nuclei are present until the first fertilized egg squeezes through the junction into the

FIG. 5. Hermaphrodite developing gonad after formation of the somatic primordium; Nomarski optics. This focal plane shows the anterior arm (Z2 and Z3 descendants), the anterior distal tip cell (Z1.aa), and three cells in the somatic primordium: 1, Z4.apa; 2, Z4.aap; 3, Z4.aaa.

uterus. Their subsequent fates have not been determined.

The four other cells that contribute to the junction arise from what otherwise would be uterine lineages. These cells separate themselves from the developing uterus with which they had been associated and surround the junctional core to complete formation of the junction.

(e) Anchor cell. The anchor cell occupies a midventral position in the gonad during L3. The vulval precursor cells are located in the hypodermis underlying the gonad and are centered around the anchor cell. (See Sulston and Horvitz, 1977, for a more complete account of vulva formation.) As invagination of the developing vulva begins, the anchor cell assumes a position at its apex. During invagination, the anchor cell appears round and exhibits a prominent nucleolus and a granular cytoplasm. Once the invagination is complete, small vacuoles appear in the anchor cell cytoplasm ventral to the nucleus. The anchor cell then elongates, and its nucleus moves off center, leaving a cytoplasmic membrane spanning the vulval orifice. The anchor cell seems to be missing in the adult, but its fate is not known.

The Male Z1–Z4 Lineage (See Fig. 13)

Early Mitotic Period

In males, the early period of divisions generates 10 cells by early L2. As in hermaphrodites, Z1 and Z4 begin to divide about halfway through L1, and Z1.a and Z4.p occupy tip positions in the primordium, while Z1.p and Z4.a lie ventrally to the germ cells. Shortly after this first division, a fundamental difference in symmetry emerges between hermaphrodite and male developing gonads. The first indication of this difference is the anterior migration of Z4.a which disrupts the twofold rotational symmetry of the male primordium (Fig. 14a). As Z4.a pushes forward, Z1.p displaces Z1.a at the anterior end of the primordium. The position of Z1.a changes from anterior to Z1.p, to dorsal to Z1.p, and then to a more posterior dorsal position (Fig. 14b). Cells Z1.a and Z4.p do not divide again in the male. As the male gonad elongates, Z1.a moves posteriorly along the dorsal margin of the primordium, until it lies just anterior to Z4.p at the posterior or distal tip of the gonad. These two cells become the male distal tip cells. In one animal, Z1.a did

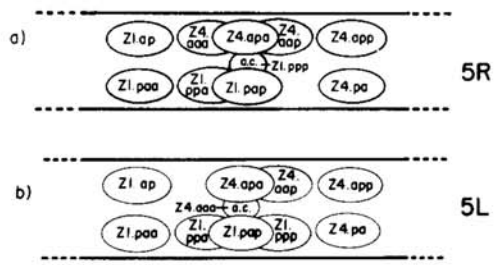

FIG. 6. 5R and 5L somatic primordia, dorsal view. The two alternate cell arrangements are related to each other by a 180° rotation around the dorsal–ventral axis passing through the anchor cell (ac). Anterior is to the left.

FIG. 7. Hermaphrodite somatic structures at the mid-L4 stage; Nomarski optics. a. sp., anterior spermatheca; p. sp., posterior spermatheca, ut., uterus; v., vulval lumen.

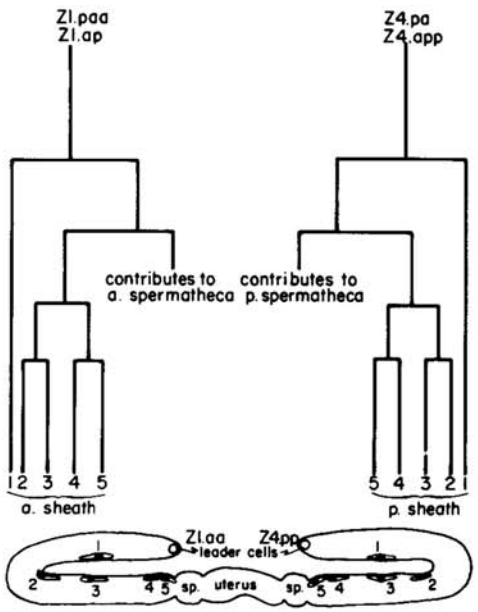

FIG. 8. Correlation of lineage with adult position of the hermaphrodite sheath cell nuclei. Z1.paa and Z1.ap both follow the indicated lineage pattern to give rise to the anterior sheath. Z4.pa and Z4.app give rise to the posterior sheath. Thus, two descendants, one from each precursor cell, are found at each position (1, 2, 3, 4, and 5). After a sheath cell arises, it becomes flat and begins to migrate distally onto the germ line arm. The positions marked in the diagram were ascertained by a combination of direct observation of this migration and tracing the positions of cells in serial 1-μm sections of gonads embedded in Epon. The identity of the cells in positions 4 and 5 is known by direct observation. The identity of cells in position 1 is assigned because the distal daughters of the sheath precursor cells arise early in the lineage and their positions are already quite distal along the germ line arm when the other sheath cells arise. Identity of cells in positions 2 and 3 simply follows the positions of these cells for the first few hours after they arise. An exchange in their positions at a later time is possible, but has not been observed.

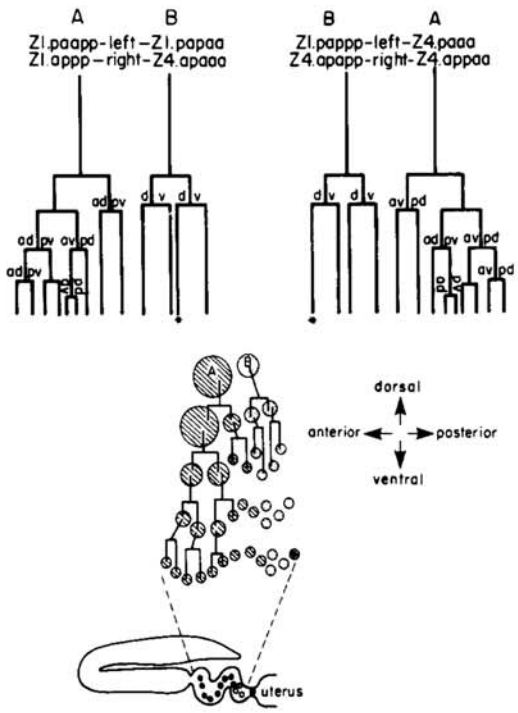

FIG. 9. Schematic of spermatheca development. Four cells contribute descendants to each spermatheca. Two cells (A) each contribute nine, and two cells (B) each contribute three. The formation of only half of the spermatheca (either the right half or the left half) is depicted diagrammatically. The shaded circles represent A descendants, and the stippled circles represent B descendants. The starred cells form the core of the spermathecal–uterine junction.

divide in the hermaphrodite fashion, but both daughter cells subsequently moved posteriorly to the distal tip to join Z4.p. In this case, Z1.aa and Z1.ap both acquired distal tip cell character; neither divided again, and both exhibited the small, oval nucleus which is characteristic of distal tip

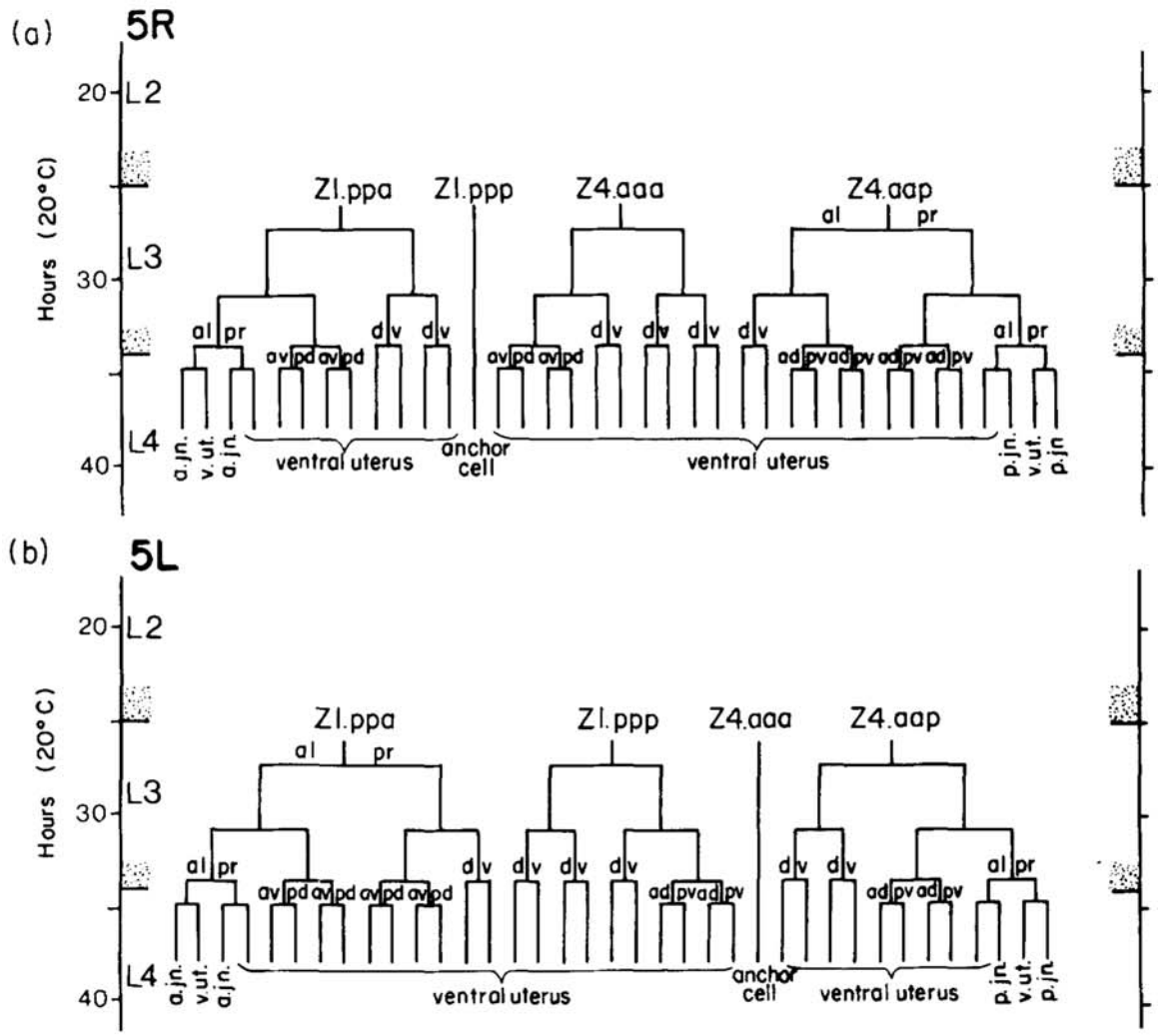

FIG. 10. Alternate lineages of hermaphrodite lineage. Z1.ppa, Z1.ppp, Z4.aaa, and Z4.aap follow one lineage from the 5R configuration (a) and another lineage from the 5L configuration (b). The two alternate lineages are related by twofold rotational symmetry. a. jn., anterior spermathecal-uterine junction; p. jn., posterior spermathecal-uterine junction; v. ut., ventral uterus.

cells in males.

Z1.p and Z4.a undergo two rounds of divisions to generate a cluster of eight somatic cells at the anterior tip of the developing gonad. The first round is asymmetrical in that the anterior daughters are larger than the posterior daughters. These daughters all divide symmetrically in the second round. The eight resulting daughters form the male somatic primordium (Fig. 15).

The male somatic primordium exhibits a variability in the positions of two cells. Either Z1.paa becomes the linker cell, and Z4.aaa becomes a vas deferens precursor cell, or vice versa (Fig. 16). The linker cell occupies the anterior tip position in the developing male gonad. The linker cell appears to serve a leader function during male gonadogenesis, preceding the developing gonad throughout its elongation. Three vas deferens precursor cells lie just posterior to the linker cell. The positions of these cells around the anterior-posterior axis are not fixed for any of the cells, and are interchangeable among the cells as they enlarge during L2. Four seminal vesicle precursor cells lie just posterior to the anterior block of the three cells described above. The positions of these four cells are also not fixed around the anterior-posterior axis as they enlarge during L2. The germ line compo-

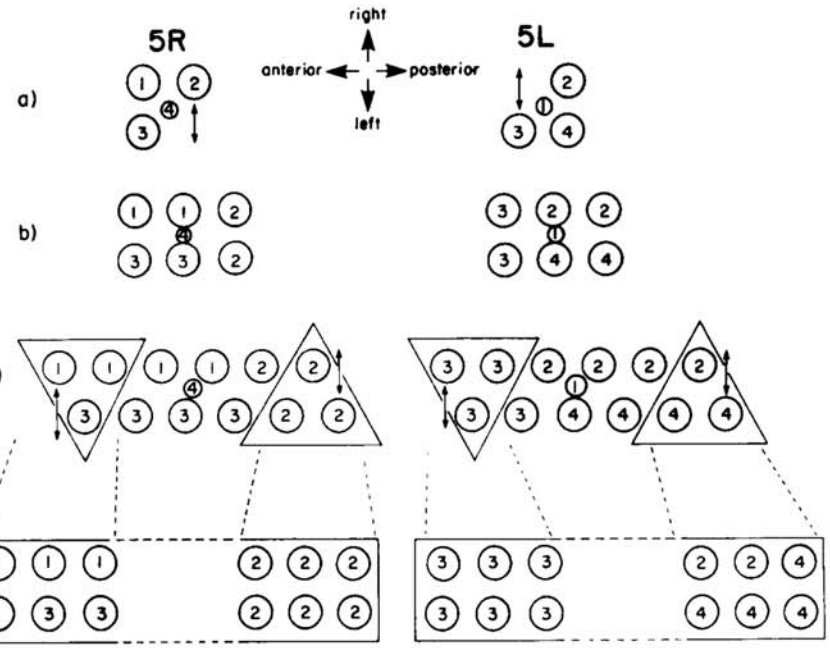

FIG. 11. Comparison of development of the ventral uterus from 5R and 5L primordia. Dorsal view. Cell 1, Z4.aaa; cell 2, Z4.aap; cell 3, Z1.ppa; cell 4, Z1.ppp. Descendants of these cells are also marked by the same number. (a) Position of cells 1, 2, 3, and 4 in 5R and 5L primordia. 2 divides left–right from 5R (arrow), but 3 divides left-right from 5L (arrow). (b) In both cases, symmetry is restored. (c) One round of divisions later. The left row of cells has shifted posteriorly somewhat with respect to the right row of cells. Cells divide left–right and restore bilateral symmetry. The cells not included in triangles divide dorso-ventrally in the next round of divisions, and are therefore left out of the next figure. (d) Similar structures arise from 5R and 5L primordia, but the ancestry of cells that occupy equivalent positions in the structure is different.

nent of the developing male gonad lies posterior to the somatic primordium.

Amitotic Period

During the second larval stage, the cells of the somatic primordium grow significantly in size, but they do not divide. Growth of the developing gonad proceeds anteriorly by continuous divisions of the germ line cells. No posterior elongation is apparent. The developing gonad elongates from 25 to about 65–70 μm long during this period.

Late Mitotic Period

Seven somatic cells generate a total of 53 cells during the late mitotic period of the male. Divisions begin at the end of L2 continue through L3, and finish shortly after the L3–L4 molt. The cells gradually assume their adult appearance during L4. The somatic structures of the male include the seminal vesicle which holds mature sperm and the vas deferens which provides a passage for the sperm to the exterior via the cloaca (Fig. 17).

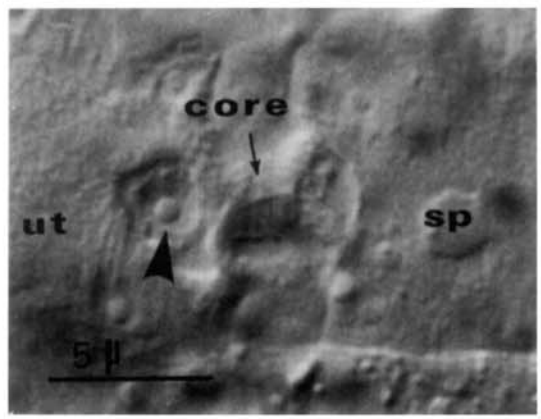

FIG. 12. Spermathecal–uterine junction; Nomarski optics. This focal plane shows the junctional core and the nucleus (arrowhead) of one of the two cells that appear to form the core. ut, uterus; sp, spermatheca.

(a) Seminal vesicle lineage. Four cells in the male somatic primordium each contribute five cells to the seminal vesicle. The divisions of these precursor cells follow a simple pattern of four serial asymmetrical

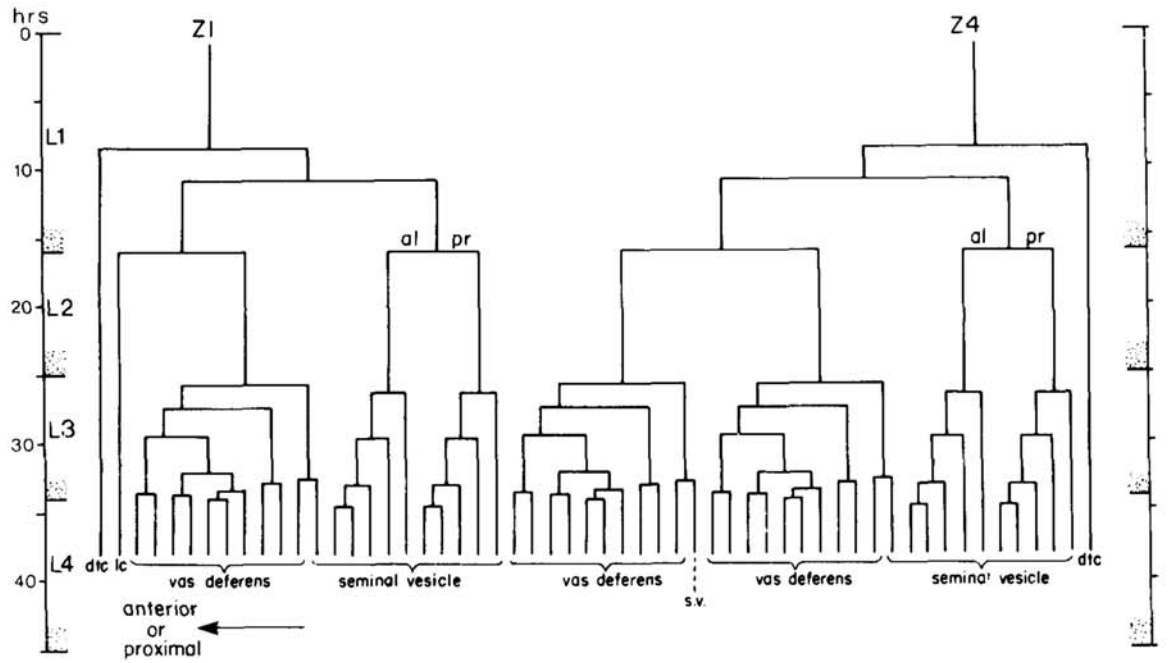

FIG. 13. One of the two alternate male lineages of gonadal somatic precursor cells Z1 and Z4. In the other lineage, Z4.aaa and Z1.paa exchange lineages so that Z4.aaa becomes the linker cell, and Z1.paa gives rise to 10 vas deferens cells and one seminal vesicle cell. Divisions give rise to an anterior daughter and a posterior daughter unless otherwise indicated. The fates of descendants are shown either individually or in groups: dtc, distal tip cell; lc, linker cell; s.v., seminal vesicle.

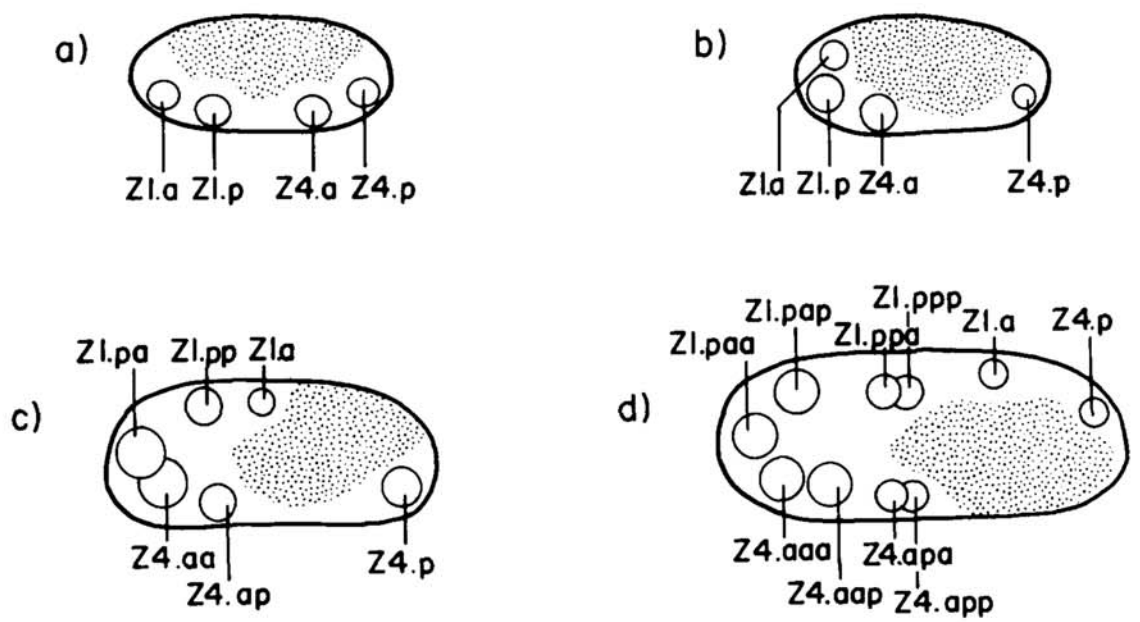

FIG. 14. Spatial arrangement of male Z1 and Z4 descendant nuclei during the early mitotic period. All diagrams are lateral views with anterior to the left and dorsal above. The space occupied by Z2 and Z3 descendants is depicted by stippling. (a) position of nuclei just after the first division of Z1 and Z4, (b) position of same nuclei after migration has started, (c) and (d) positions of nuclei after subsequent rounds of divisions. Note that Z1.a moves along the dorsal margin of the gonad toward the posterior pole.

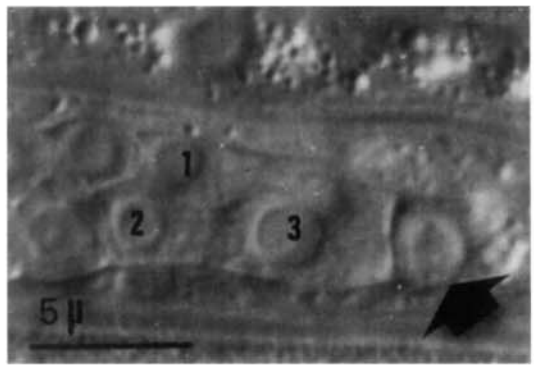

FIG. 15. Male somatic primordium; Nomarski optics. The linker cell (arrow) occupies the anterior tip of the primordium. Posterior to the linker cell is a large vas deferens precursor cell (3) and posterior to that are two smaller seminal vesicle precursor cells (1 and 2). This anterior to posterior arrangement is invariant. However, since the positions of the three vas deferens precursor cells, and of the four seminal vesicle precursor cells, are variable around the long axis of the developing gonad, the numbered cells cannot be lineally identified unless their lineage had been followed from the L1 stage.

divisions that produce one larger and one smaller daughter in each round. The smaller cells do not divide further. The resultant 20 cells constitute the inner, apparently secretory layer of the seminal vesicle. Their cytoplasm becomes granular during L4 and small blebs appear at the lumenal surface as they mature. The outer layer of the seminal vesicle comprises three large, very thin cells. These cells are the most distal daughters of the three cells that give rise to the vas deferens (Fig. 13). They arise just proximal to the developing seminal vesicle. After these three cells arise, they begin to enlarge, flatten, and spread over the developing seminal vesicle to encapsulate it.

(b) Vas deferens lineage. Three cells in the male somatic primordium each contribute 10 descendants to the vas deferens and one descendant to the seminal vesicle. These three cells undergo a series of asymmetrical divisions, each of which generates one larger and one smaller daughter. In this lineage, the smaller cells each undergo one mitosis before differentiating. The vas deferens is a complex secretory tube consisting of a variety of cell types. Preliminary studies show at least three cell types based on morphological differences in secretory granules (Wolf and Kimble, unpublished observations). However, a detailed elucidation of the cellular anatomy is not complete, and study of the correlation between adult cells, cell types, and lineal descendants within the vas deferens is still in progress.

(c) Linker cell. During L4, the linker cell joins the vas deferens to two E cell descendants (Sulston and Horvitz, 1977) in the male tail. Just prior to the final molt, the linker cell undergoes the morphological changes typical of cell death. This cell death opens the passageway between the lumens of the vas deferens and the cloaca.

DISCUSSION

Comparison of Gonadogenesis in Hermaphrodite and Male Worms

The two gonadal somatic progenitor cells present in the newly hatched worm can either follow a hermaphrodite or a male developmental pathway. If the hermaphrodite pathway is selected, the structures that are made display a twofold rotational symmetry, and 143 cells are generated. If the male pathway is selected, the structures made are asymmetrical, and only 56 cells are generated. The hermaphrodite and

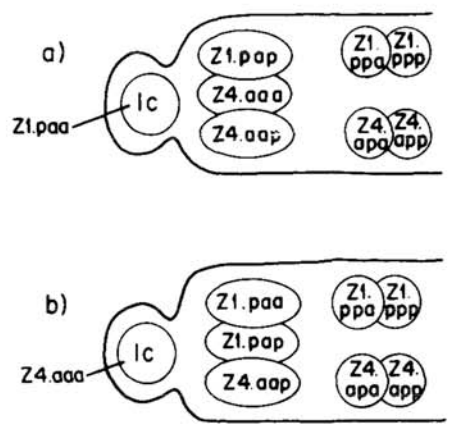

FIG. 16. Alternate male somatic primordia. In (a) Z1.paa has become the linker cell (1c), whereas in (b) Z4.aaa has become the linker cell. Thus, Z1.paa and Z4.aaa have alternate fates.

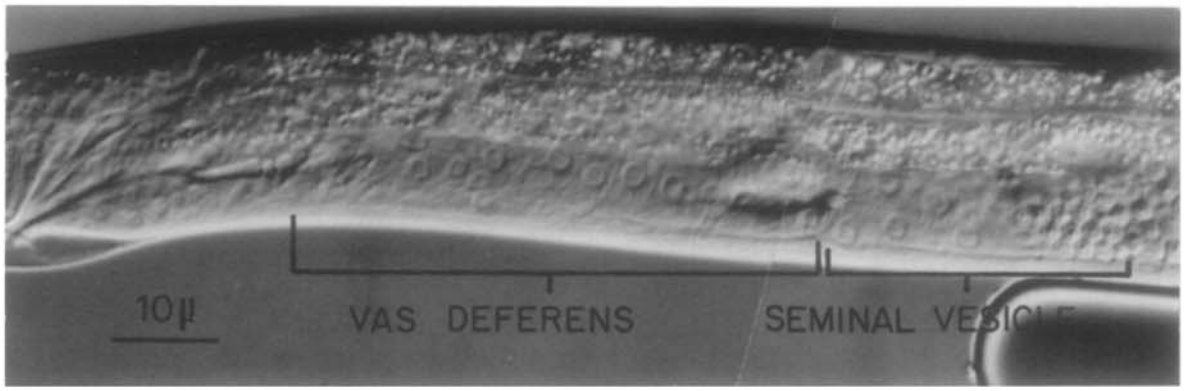

FIG. 17. Male somatic structures in late L4 stage; Nomarski optics. Anterior is to the right, and dorsal is above.

male developmental programs may be compared as follows.

(1) The arrangement and morphological appearance of the four cells in the gonadal primordium present in newly hatched worms is identical in both sexes. The somatic progenitor cells, Z1 and Z4, are located at the anterior and posterior tips of the primordium, respectively. The germ line progenitor cells, Z2 and Z3, occupy the region between Z1 and Z4. The primordium exhibits twofold rotational symmetry.

(2) Z1 and Z4 develop according to a temporal pattern that is essentially identical in hermaphrodites and males. During the second half of L1, Z1 and Z4 undergo a series of divisions that gives rise to 12 cells in the hermaphrodite and 10 cells in the male. During L2, these cells increase significantly in size without dividing further. By late L2, a somatic primordium takes shape due to migration and enlargement. During L3 and the first part of L4, the cells in this somatic primordium undergo extensive divisions. Cells that have stopped dividing differentiate morphologically to the characteristic adult appearance.

(3) The number and orientations of divisions in the early mitotic period are nearly the same in hermaphrodites and males. This lineage differs in the two sexes only by a single division of each somatic progenitor cell. Thus, in hermaphrodites, Z1.a and Z4.p undergo a division that usually does not occur in males. However, in one male, we observed that Z1.a divided in a hermaphrodite fashion. Since extra divisions of this sort have never been observed at any other point in the Z1–Z4 lineages of either sex, this extra division of Z1.a suggests that the hermaphrodite and male early lineages share a common program of control. For example, Z1 and Z4 may be programmed to divide in both sexes according to a single pattern with repression of one division in males.

An important difference in the early events of hermaphrodite and male gonad development occurs after the first division of Z1 and Z4. In the male, Z4.a begins its anterior migration, disrupting the twofold rotational symmetry of the primordium. This is the first morphological indication of the asymmetry that characterizes the rest of male gonad development. By contrast, hermaphrodites maintain the primordial symmetry throughout gonadogenesis.

(4) The three cells in the Z1–Z4 lineages that do not divide after the period of early divisions arise from equivalent positions in the early lineage trees and have similar development fates in hermaphrodites and males (Fig. 18). In both sexes, two of these cells become distal tip cells, and the third cell, either the anchor cell or linker cell, serves to join the gonadal lumen with the animal's exterior. The anchor and linker cells also share the characteristic of varia-

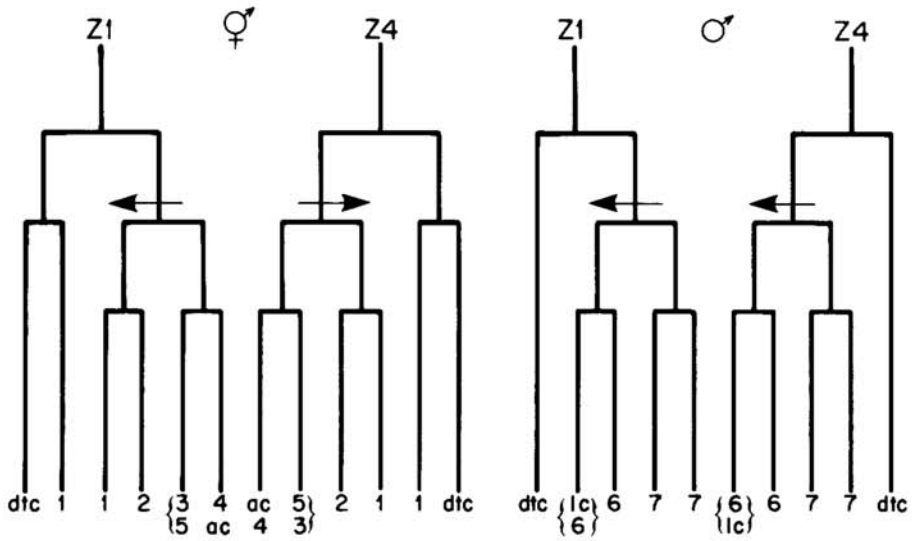

FIG. 18. Comparison of the hermaphrodite and male Z1 and Z4 early division patterns. The arrows indicate asymmetric divisions, and point to the larger of the two daughters in each case. The numbers refer to the developmental fates of the cells that arise from the early divisions: 1, sheath–spermatheca lineage; 2, dorsal uterus–spermatheca lineage; 3, 4, and 5, ventral uterus lineages; 6, vas deferens lineage; 7, seminal vesicle lineage; dtc, distal tip cell; ac, anchor cell; lc, linker cell. See text for further explanation.

ble ancestry. Either Z1.ppp or Z4.aaa can become the anchor cell in hermaphrodites, and either Z1.paa or Z4.aaa can become the linker cell in males.

In hermaphrodites, the distal tips move away from the original primordium site, and the most proximal point, marked by the anchor cell and developing vulva, is located where the primordium had been in the L1. In males, the future proximal end moves away, led by the linker cell, and the distal tip is found at the primordium site. Thus, the polarity of the distal–proximal axis of the hermaphrodite is opposite that of the male with respect to the direction of elongation of the gonad. However, in both sexes the spatial and temporal polarity of gamete maturation is toward the somatic structures, and in this sense, they are equivalent. The distal tip cells and the proximal anchor or linker cell might be a reflection of the early establishment of the distal–proximal axes, or they themselves might function to establish the distal–proximal axes of the gonad.

(5) A homologous relationship can be drawn between individual hermaphrodite and male somatic structures by similarities in their positions, lineages, and functions. The most distal somatic structures in both sexes consist of thin, flat cells which encase maturing gametes: the sheath in hermaphrodites and the seminal vesicle in males. In both cases four cells in the somatic primordium each contribute five cells toward that structure, albeit through different lineage patterns. The male vas deferens and the hermaphrodite spermatheca are the next structures in line from distal to proximal. Since the spermatheca houses sperm, and can be considered a male component in the hermaphrodite, and since the lineages are very similar that give rise to these structures, they may be homologous structures. Electron microscopic studies have also revealed an ultrastructural similarity between the vas deferens and the spermatheca (Wolf and Hirsh, unpublished observations). The uterus has no obvious counterpart in the male lineage.

Comparison of gonadogenesis in hermaphrodites and males leads to the striking conclusion that similar developmental programs are followed to generate structures that differ in their properties of symmetry and terminal differentiation. Thus, the de-

cision to make a hermaphrodite or male gonad probably pivots on a limited number of critical modifications in a fundamental program. This hypothesis is reminiscent of Ohno's hypothesis that the genetic control of mammalian sex differentiation is simple and depends on the presence or absence of testosterone during a particular period of development (Ohno, 1971). The genetic control of sex differentiation in *C. elegans* is also simple. After intensive searching for mutants that alter sex differentiation, only three genes have been identified that transform XX individuals into males (Hodgkin and Brenner, 1977), and only two genes have been identified that transform XO individuals into hermaphrodites (Nelson *et al.*, 1978; Hodgkin, personal communication).

The temporal control of the decision to make either a male or a hermaphrodite gonad has been investigated in a temperature-sensitive transformer mutant that produces males in XX worms (Klass *et al.*, 1976). The critical period of temperature sensitivity in this mutant extends from 3 hr before hatching to 12 hr after hatching (25°C). Thus, 12 hr after hatching is the first point in development at which a shift to restrictive temperature cannot cause any of the worms to make a male gonad. Since the first sign of sexual dimorphism occurs at about 9 hr (25°C) after hatching, the morphological divergence in the two programs may indicate a point of irreversible commitment to one developmental pathway.

Comparison of the Z1–Z4 Lineages to Other Lineages in C. elegans

Lineage patterns. Sulston and Horvitz (1977) discuss several standard lineage patterns that were observed in the nongonadal lineages. These patterns are also seen in the gonadal lineages. In fact, the entire male lineage can be described in terms of these basic patterns of division.

One of the simpler patterns is characterized by a stem cell repeatedly dividing asymmetrically to give rise to another stem cell and a differentiated cell that does not divide again. An archetypal example of stem cell logic is seen in the lineage of the seminal vesicle. Stem cell logic is also found in the early embryonic lineage of *C. elegans* (Deppe *et al.*, 1978) and in the postembryonic lateral hypodermal lineages (Sulston and Horvitz, 1977).

A more complex type of stem cell pattern has been suggested from histological studies of neuroblast development in *Drosophila* (Poulson, 1950). In this variation, the stem cell gives rise to another stem cell and a "pre-differentiated cell" that will divide one more time before differentiating. The vas deferens lineage in *C. elegans* provides an example of this pattern. The spermathecal lineage of the hermaphrodite begins to divide according to this pattern, but then diverges from it. A similar pattern is seen in the ventral nerve cord lineage of *C. elegans* (Sulston and Horvitz, 1977). Thus, this pattern may represent a basic mechanism of increasing cell number in different tissues and different organisms.

The early lineage patterns of hermaphrodites and males represent a third basic, but more variable lineage type. A pattern identical to the male early lineage gives rise to each of the rays in the male tail, and similar patterns are seen in the lineages of the posterior lateral ganglia and the lumbar ganglia (Sulston and Horvitz, 1977). Sulston and Horvitz (1977) compare this type of pattern to an insect pattern (Lawrence, 1966) and suggest that it reflects a developmental program common to both organisms.

The standard patterns of cell division observed in *C. elegans* imply the existence of simple programs by which cell number is increased. These programs may or may not direct a differential segregation of developmental potential to the daughters that arise in the lineages as will be discussed under *Mechanisms of Determination.*

Temporal control of division. Z1 and Z4 follow a temporal pattern of divisions that

is also found in other postembryonic lineages. This pattern is characterized by two periods of divisions separated by a period when no divisions occur. Two nongonadal lineages follow the same pattern (Sulston and Horvitz, 1977). The mesoblast cell, M, undergoes a series of divisions during the last half of L1 followed by a migration of certain of its early descendants during L2 (hemaphrodites) or L3 (males). These cells divide further in L3 to generate the sexual musculature of the vulva or the male tail. The P precursor cells of the ventral nerve cord follow a similar pattern in which a few of the descendants which arise in L1 divide again in L3.

The feature common to cells that divide first in L1 and then in L3 is their involvement in the development of the reproductive system. The function of this delay in development is open to speculation. A full elaboration of the sexual structures might be deferred until after the decision is made in L2 (Cassada and Russell, 1975) to bypass the dauer larval pathway and complete the sexually mature form. Fewer gonadal cells would be economical of the resources necessary to maintain the dauer larva until it finds a new source of nutrients. On the other hand, the L3 developmental delay may serve to ensure the coordinate development of the sexual tissues.

Mechanisms of Determination

Ancestry vs position. A fundamental question that arises out of lineage studies is how cells are led to diverge in their capacity for differentiation. Two mechanisms were proposed by Roux (1888). Cells might become committed to a particular pathway by "self-differentiation" (or cell ancestry) or by "correlative dependent differentiation" (or cell–cell interaction). If a cell is determined by ancestry, cell divisions must mediate a segregation of developmental potential to daughter cells. If a cell is determined by position, cell divisions might only serve to increase the number of cells. A third possibility is that both cell ancestry and cell position play significant roles in the determination of cells in development. For example, a segregation of cytoplasmic determinants during early cleavages in the embryo might restrict a cell's ability to differentiate to one subset of pathways, and cell position might convey to a cell which specific program it must follow among the available choices. Or, the determinants sequestered to one daughter rather than the other might allow that cell to respond differentially to external signals.

Invariance in cell lineages is regarded as a principle of nematode development, both classically (Boveri, 1899) and currently (Sulston, 1976; Sulston and Horvitz, 1977; Deppe *et al.*, 1978). Such invariance only demonstrates that the developmental program is rigidly controlled. The inability of cells to alter their program after manipulation, such as isolation of blastomeres (e.g., Wilson, 1925) or ablation of neighboring cells (Sulston and Horvitz, 1977) shows that cells are irreversibly committed, but does not reveal how that commitment was established. In most lineages, the invariance observed in division pattern and cell fate also corresponds to an invariance in cell position. When both lineage and position of descendant cells are invariant, the influence of cell ancestry and/or position on cell fate is extremely difficult to assess. However, the limited variability that is observed in essentially invariant developmental programs can be utilized to explore the significance of position and ancestry to cell determination.

The most common pattern of variability seen in the postembryonic lineages of *C. elegans* involves two cells, each of which is capable of following one of two alternative lineages. The two cells occupy one of two alternative positions, but once a position is assumed, the cell follows a lineage pattern that corresponds to that position.

The nongonadal lineages provide several examples of such variability (Sulston and Horvitz, 1977). Two pairs of cells in the male tail exhibit alternative lineages corre-

lated with position. Certain pairs of ventral cord precursor cells follow different lineages depending on their anterior–posterior position after migration into the cord from the left and right sides of the worm. In addition, two cells in the male gonadal lineage exhibit alternative lineages. The more anterior of the pair of cells becomes the linker cell, whereas the more posterior follows a typical vas deferens lineage pattern.

In hermaphrodites, a more complex situation is found. The positions of only two cells (Z1.ppp and Z4.aaa) are variable, but the lineages of four cells are altered (Fig. 10). Concurrent with the establishment of the hermaphrodite somatic primordium at the end of L2, the four cells that give rise to the ventral uterus assume one of two configurations. These four cells, as a group, follow one of two alternative lineage patterns which corresponds to the configuration in the individual primordium. This group phenomenon suggests that neighboring cells in some way can influence each other's fates.

Another explanation of the hermaphrodite variability might be that the two somatic progenitor cells can randomly assume the anterior or posterior positions during the embryonic formation of the four-celled gonadal primordium. If only one of the somatic progenitor cells were capable of producing an anchor cell, for example, and if that cell could become either Z1 or Z4, the observed variability would result.

A third explanation of the 5R and 5L alternatives might have been that a random population consists of two subpopulations, one programmed to follow the 5R pattern and one programmed to follow the 5L pattern. This cannot be the case because these programs are not clonally inherited; individuals whose developmental pattern was recorded as 5L or 5R and were then cloned gave rise to progeny that followed both 5L and 5R patterns.

Thus, two hypotheses remain that might explain the unique variability observed in the hermaphrodite Z1–Z4 lineage. Either two somatic progenitor cells are irreversibly committed during embryogenesis and can interchangeably become Z1 or Z4, or the interaction of the cells in the somatic primordium affects their subsequent lineage pattern. It may be possible to distinguish between these two explanations by laser ablation of Z1 or Z4 in a large number of animals. If Z1 and Z4 are irreversibly committed cells, an anchor cell should arise in approximately one half of such experimental animals. If Z1 and Z4 are not irreversibly committed, an alteration in the development of the remaining cell may be seen. If some form of regulation occurs and cell interaction proves to be the most reasonable explanation for the variability in the lineages of four of the cells in the somatic primordium, one might postulate that the rearrangement of cells to form a somatic primordium represents a change in position for all the cells involved, and that cell–cell interaction might influence the developmental fate of all the cells concerned.

Coordinates and developmental fates. Whether two daughters are determined because they have acquired a specific cytoplasm or a specific position, some kind of coordinate system is an absolute requirement for information to be conveyed differentially to daughter cells. An anterior daughter might follow a different pathway from its posterior sibling due to a differential segregation of cytoplasmic determinants to the two daughters. However, the difference might just as reasonably be due to a different environment imposed on the daughter cells, whether that is mediated through interaction with its neighbors or an externally imposed gradient. Thus, lineage data do not reveal the mechanisms by which the fate of one daughter becomes different from the fate of its sibling. Yet, lineage data demonstrate that the fate of a cell does correspond to the spatial relationship in which the siblings arise, and therefore, shows that the coordinate exists and is significant to the fate of the daughter cells.

The male Z1–Z4 lineage provides evidence that at least the last half of male gonadal development proceeds according to its own coordinate system rather than the organismic coordinate system. The late divisions of the male begin just before the gonad makes its 180° turn. Since there is some variability in the correlation between when a cell divides and when the gonad makes its bend, a cell may divide before the turn, in the turn, or after the turn. The daughter nearer the leading or proximal edge would be an anterior daughter in the first case, a dorsal daughter in the second, and a posterior daughter in the last case. Yet, that daughter does not vary with respect to further divisions or differentiation. Thus, distal–proximal coordinates along the gonadal axis seem to be more relevant to these divisions than the coordinates of the worm. Since the initial growth of the male gonadal primordium is only anterior and never posterior, it seems likely that the organismic coordinate system influences this first developmental step in the male. The twofold rotational symmetry exhibited by the hermaphrodite gonad does not contradict the idea that the gonadal coordinate system may be independent of the organismic coordinate system.

One principle that has emerged from the information on position and cell fate in the lineages of Z1 and Z4 is that the symmetry of the structure in which a particular division takes place is correlated with the symmetrical relationship between the fates of Z1 descendants and the fates of Z4 descendants (Fig. 18). In both hermaphrodites and males, the fates of the daughters of the first division follow the twofold rotational symmetry of the four-celled gonadal primordium. Thus, Z1.a is equivalent to Z4.p, and Z1.p is equivalent to Z4.a. A comparison of the fates of Z1 descendants with the fates of Z4 descendants shows that, in hermaphrodites, cells that are equivalent in developmental fate occupy positions that are related by twofold rotational symmetry (Fig. 18). The morphology of the hermaphrodite developing gonad also retains the twofold rotational symmetry. By contrast, the male developing gonad becomes morphologically asymmetrical soon after the first division. And, in males, equivalent cells in the Z1 and Z4 lineages are related asymmetrically along the anterior–posterior axis after the first division (Fig. 18).

In hermaphrodites, the only cells in the somatic primordium that are not positioned according to twofold rotational symmetry have lineages that are not related to each other by twofold rotational symmetry. So, Z1.ppa is not lineally equivalent to Z4.aap, and Z1.ppp is not equivalent to Z4.aaa. However, these four cells can assume one of two configurations, 5R and 5L, that are related by twofold rotational symmetry, and the lineages followed by the four cells in 5L are related to the lineages of the four cells in 5R by twofold rotational symmetry.

The significance of the correlation observed between the morphological symmetry displayed by the developing gonad and the developmental symmetry exhibited by the fates of Z1 and Z4 descendants is purely a matter of speculation at the present time. The correlation might be coincidental, it might be a reflection of two independent responses to an underlying coordinate system, or it might involve a direct cause and effect relationship.

A second principle that emerges from the Z1–Z4 lineages is that no structure (e.g., uterus, anterior spermatheca, vas deferens) develops as a cell clone. Clonal development is defined by two criteria (Crick and Lawrence, 1975). First, a cell must contribute all its descendants to a single structure. Second, a structure must consist of only the descendants of a single clone. In the development of the gonadal somatic structures, each structure consists of descendants from more than one cell in the somatic primordium, and in most cases, each cell in the somatic primordium contributes to more than one structure. Such nonclonal development of structures is also typical of organisms whose lineages were followed in

classical studies. For example, most structures in the trochophore larvae of the annelid *Nereis* (Wilson, 1892) and the mollusk *Crepidula* (Conklin, 1897)—apical rosette, prototroch, gut—are derived from descendants of all four progenitor cells that arise from the first two divisions of the egg.

In conclusion, the Z1–Z4 lineages are particularly advantageous for addressing certain key developmental questions:

(1) The unique variability in the hermaphrodite lineage strongly suggests that cell–cell interaction affects cell fate during gonadogenesis. Alternatively, the two somatic progenitor cells might be irreversibly commited during embryogenesis and assume the Z1 or Z4 position interchangeably in the gonadal primordium. The laser ablation of Z1 or Z4 will test the commitment of the unablated cell and therefore may distinguish between the two models.

(2) The two developmental programs available to Z1 and Z4 raise questions about how the genetic control of the programs is organized, and how differences in symmetry are established. The genetic control is accessible since *C. elegans* is amenable to mutational analysis and since gonadogenesis defective mutants can be obtained (Hirsh and Vanderslice, 1976). However, the necessity for a selection method for mutants that specifically affect morphogenesis has become apparent (Kimble, 1978). The establishment of symmetry is a much more elusive problem, but the morphological change in symmetry that is reflected in the behavior of four cells in the male may provide a key to how that change is mediated.

(3) The significance of nonclonal development of the gonadal somatic structures may also be approachable in this system. One guess is that cell–cell interaction is important to the evolution of complexity during development. It should be possible to alter the composition of the cells of the somatic primordium by laser ablation so that only one precursor cell is left for a particular structure. If cell interaction between two precursor cells is critical to differentiation, such a change should cause a change in the pattern of differentiation of the precursor cell.

The Z1–Z4 lineages provide a model of morphogenesis and differentiation that is sufficiently complex so the principles involved will probably be applicable to the development of most higher eukaryotes. Yet, the system is simple enough that the behavior of individual cells can be studied as they proceed through the sequential steps leading to the adult form. Our current goal is to understand the extents to which ancestry and cell interaction play a role in the determination of individual cells. Ultimately, we hope to exploit this system to elucidate the genetic control of the cells' progression through each step in gonadogenesis.

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