Cell Cycling and DNA Replication in a Mutant Blocked in Cell Division in the Nematode *Caenorhabditis elegans*

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The postembryonic development of the nematode *Caenorhabditis elegans* has been described at the level of individual cell lineages. A mutant of postembryonic development, *lin-5 II,* causes a failure of postembryonic nuclear and cell divisions. Mitosis in living animals is seen by light microscopy to proceed through prophase and nuclear envelope breakdown, but an abnormal-looking metaphase plate forms in the mutant, after which the interphase nuclear morphology reappears until the next attempted round of division. The precursor cells which give rise to the ventral nerve cord have been studied in *lin-5.* In the wild type these cells divide asymmetrically to give six descendants (one hypodermal cell and five neurons). In the mutant these precursors accumulate approximately **six** times the diploid quantity of DNA within a single nucleus, while attempting mitosis up to three times. These polyploid cells display characteristics of the cells they would have produced ordinarily.

INTRODUCTION

The lineages of all nongonadal cells formed after hatching in the nematode *Caenorhabditis elegans* have been described recently (Sulston and Horvitz, 1977). In nematodes the number of cells of each type is constant from animal to animal, therefore it is possible to identify almost any cell in any animal, and in the case of cells formed postembryonically to know that cell's genealogy. This has allowed the identification of mutants with altered cell patterns or numbers. One such mutant is described here that is blocked in nuclear and cell division. As in celldivision mutants of yeast (Hartwell *et al.,* 1974) and tetrahymena (Frankel *et al.,* 1976), certain cell processes continue in spite of the block. But unlike these unicellular organisms, the blocked nematode precursor cells would have given rise to cells of several different types by a known lineage in wild-type animals, so the fate of these blocked precursors can be examined with respect both to the cell cycle and differentiated state. The precursors to the ventral nerve cord were chosen for this purpose.

MATERIALS AND METHODS

Nematode strains. The culturing and general genetic methods used with *Caenorhabditis elegans* (var. Bristol) have been described (Brenner, 1974). The lineage mutant *lin-5 II (e1348)* was induced by ethyl methane sulfonate and was found by screening Feulgen-stained whole mounts of F1 clones of mutagenized animals. The mutant has been mapped to linkage group II, 0.2% to the right of *dpy 10 (e128).* The *lin-5* animals are obtained by segregation from the strain -E-H214 *(lin-5/dpy 10).* A second isolate, *e1457,* was shown by complementation to be an allele of *lin-5,* and is maintained in the strain E-H98. However, unless otherwise stated, in this paper *lin-5 will* refer to the allele *e1348. A* double mutant, *lin-5; nuc1* was obtained by segregation from *lin-5/ dpy-10; nuc-1. Nuc-1 X (e1392) is* deficient in endodeoxyribonuclease. The double mutant was constructed using E-11214 and E1392.

Cytology. Animals displaying the uncoordinated phenotype of *lin-5* were picked off the stock plates and placed on a petri dish without a bacterial lawn. When a

sufficient number of animals had been collected they were washed off the plate with M9 buffer and centrifuged, the buffer was removed, and 5 ml of fixative (6 parts absolute ethanol, 3 parts acetic acid, 1 part chloroform) was added. Worms were fixed overnight, then centrifuged and the excess fixative was removed. Suspended in a small volume of fixative, the worms were dropped onto glass microscope slides which had been dipped previously in a solution of bovine serum albumin (1 mg/ ml) and then were air dried. The nematodes were rehydrated for 5 min in PBS (0.15 M NaCl, 0.03 *M* KCI, 0.01 *M* phosphate, pH 7), then stained for 5 min in 0.3 µg/ml of Hoechst 33258 in PBS and rinsed for 1 min in H20. After air-drying, slides were mounted in citrate phosphate buffer, pH 4.

For fluorescent Feulgen staining, the method of Deaven and Petersen (1974) was followed using acriflavine as the fluorochrome.

Live animals . were mounted on agar slabs for Nomarski differential interference contrast microscopy as described previously (Sulston, 1976; Sulston and Horvitz, 1977).

Fluorescence microscopy and microfluorimetry. Slides were viewed in a Zeiss RA or Universal microscope fitted for transillumination with the dark-field Ultracondensor. For slides stained with Hoechst 33258, excitation filter UG1 and barrier filters *47* and -65 were used, and for the fluorescent Feulgen, excitation filter KP 500 and barrier filter 50 were used. Hoechst-stained animals were photographed with Ilford Pan F film.

Microfluorimetric measurements were made on the Universal microscope fitted with a phototube on the trinocular head. An aperture 1 mm in diameter was placed in the focal plane of one eyepiece and an identical aperture in the focal plane of the eyepiece of the phototube was aligned so that the same portion of the field was covered. When multiple readings were required on an elongated nucleus, it was aligned along a graticule in the third eye. piece so that positioning of the specimen with respect to the aperture could be made in reference to it. Fluorescence intensity was recorded from a digital readout of the phototube. For each nuclear reading, the background was recorded from a part of the worm adjacent to the nucleus. The method gives a ratio of neurons to sperm of 2.3 ± 0.4, even though the volume of these nuclei gives an approximate conden- sation ratio of 1:8.

Electron microscopy. Animals were prepared for electron microscopy as described by Ward *et al.* (1975). Morphology of ventral cord cells was reconstructed by tracing cell processes on electron micrographs of every third serial section as described by White *et al. (1976).*

RESULTS

Postembryonic Development of the Ventral Cord in C. elegans

The postembryonic development of the ventral nerve cord in C. *elegans* has been described (Sulston, 1976; Sulston and Horvitz, *1977).* In the first larval stage (L1) the ventral nerve cord increases in cell number from 15 "juvenile" motor neurons to 57 motor neurons. This is accomplished by the migration of precursor cells from their initial lateral position into the ventral cord where they divide asymmetrically (Fig. 1) to produce five neuronal descendants (cells *a-e* in Fig. 1) and a hypodermal cell (cell f in Fig. 1). The precursor cells insert themselves among the juvenile neurons, usually .in the pattern shown in Fig. 1. A whole mount of a larva fixed and then stained with Hoechst 33258 before the precursors have entered the ventral cord is shown *in* Fig. 2a. A larva with the adult complement of ventral cord cells is shown in Fig. 2b.

In contrast a whole mount of the mutant *lin-5* is shown in Fig. 2c. In place of the

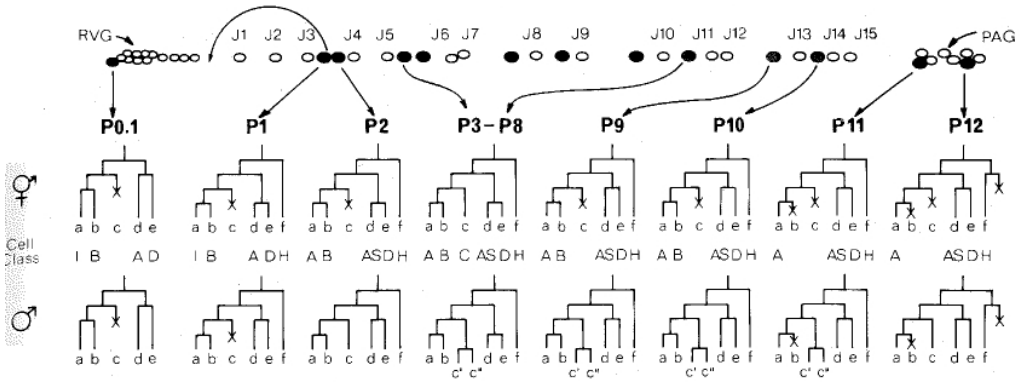


FIG. 1. Development of the ventral nerve cord. The number of neurons in the ventral nerve cord is increased by the migration of six pairs of precursors (•) into the juvenile array of ventral cord cells (0) about 8 hr after hatching. In addition, one neuroblast, P0.1, is present at hatching in the retrovesicular ganglion (RVG). Precursors P1-P12 divide in an anterior-posterior direction according to the same scheme. After the first mitosis, the anterior daughter of PI migrates forward into the RVG. Thus P0.1 and PI give rise to cells comprising part of the RVG, and P11 and P12 to part of the pre anal ganglion (PAG). Certain descendants die soon after being formed. These are the c and *b* cells at the ends of the nerve cord and are indicated by an X. In the male, all but two of the c descendants survive, and in the third larval stage the c cells of P3-P11 divide yet again. The neuron classes of the ventral cord have been assigned by connectivity and morphology from electron microscopic reconstruction (White *et al.,* 1976), and a correspondence between cell type and lineage has been shown (Sulston, 1976). Thus in both hermaphordites and males, descendant a is a type A neuron, *b* a type B neuron, c a type C neuron, d a type AS neuron, e a type D neuron, and f a hypodermal cell, H. In the RVG, the a descendant of P0.1 and P1 is an interneuron, I.

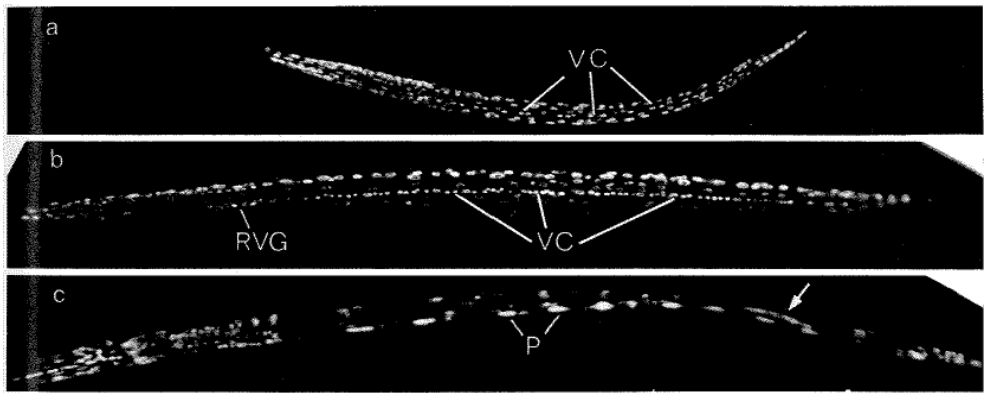


FIG. 2. Hoechst 33258-stained whole mounts. Whole mounts of larvae were fixed and then stained with Hoechst 33258, (a) First-stage larva showing 15 juvenile ventral cord (VC) neuronal nuclei. x 300. (b) Second-stage larva with complete ventral nerve cord (VC). At the anterior end of the cord 20 cells form the RVG. Hypodermal nuclei in the ventral cord may be distinguished from neuronal nuclei by being larger and by the prominent nonstaining nucleolus. x 300. (c) A larval *lin-5* animal. In place of the ventral cord, nine large nuclei (P) are interspersed with the condensed juvenile cord nuclei. In the lateral myoblast nucleus four nucleoli can be seen (arrow). x 540.

adult number of 57 ventral cord neurons one sees 15 small neuronal nuclei and 9 or 10 large nuclei interspersed between them. The pattern is similar to the juvenile cord shown in Fig. 1 with larger precursor nuclei and small, juvenile nuclei. Often the large ventral cord nuclei contain several nucleoli similar to the lateral myo

blast nucleus in Fig. 2c.

The numbers and morphology of nuclei present at hatching in C. *elegans* appear the same in the wild-type animals and in the mutant. The behavior of the mutant at hatching is also indistinguishable from that of the wild type. Mutant animals become uncoordinated during the second larval stage when they measure ~300 μm in length: They have difficulty moving forward and are almost incapable of moving backward.

Postembryonic lineages in C. *elegans* have been followed by watching the various blast cells perform their cell divisions (Sulston, 1976; Sulston and Horvitz, 1977). A series of events leading up to cell division can be seen in the light microscope, and these are shown for two cell cycles of the ventral nerve cord precursors in Fig. 3. Prophase, which lasts for about 4 min, is distinguished by the disappearance of the nucleolus and the appearance of coarse granularity in the nucleoplasm (34 and 115 min), the metaphase plate initially is formed within the nuclear envelope (38 min), which breaks down after a further 1 to 2 min (39 and 121 min). About 1 min later the metaphase plate splits and two cusps can be seen moving apart (anaphase, 122 min); the cleavage furrow separates the daughter cells (125 min) and the daughter nuclei appear (130 min). The nuclear envelope is absent for a total of 7 min (±2 min).

In contrast, Fig. 4 shows a series of two rounds of attempted cell division in a ventral cord precursor in *a lin-5* animal. Prophase appears normal, but there is no true metaphase. An imperfect plate may be formed (19 min) often in an inappropriate orientation. The nuclear envelope breaks down, but there is no visible anaphase movement, Eventually the coarse granularity and failed metaphase plate disperse; the nuclear envelope reforms, having been absent for 10 to 20 min or, exceptionally, for as long as 60 min. On rare occasions two nuclei are formed.

The interphase appearance of the nuclei in *lin-5* changes characteristically during development. The precursor appears nor

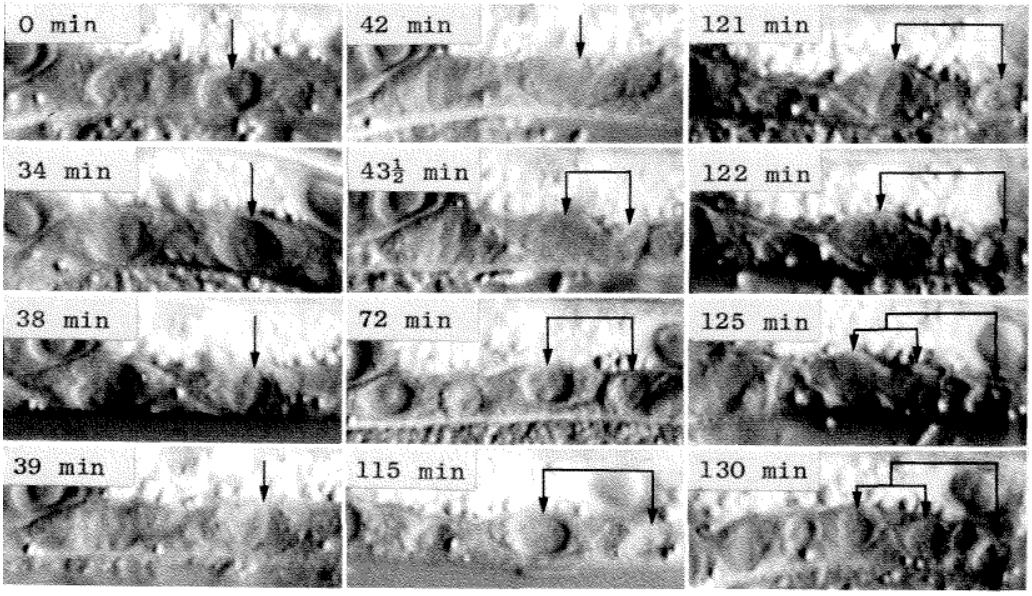


FIG. 3. Two rounds of division of the ventral cord precursor P8 (arrowed) in a wild-type animal. Nomarski, x 2100. 0 min, interphase; 34 min, prophase; 38 min, early metaphase; 39 min, metaphase, nuclear envelope broken down; 42 min, anaphase; 43.5 min, late anaphase; 72 min, interphase; 115 min, early prophase; 121 min, metaphase; 122 min, anaphase; 125 min, late anaphase; 130 min, interphase.

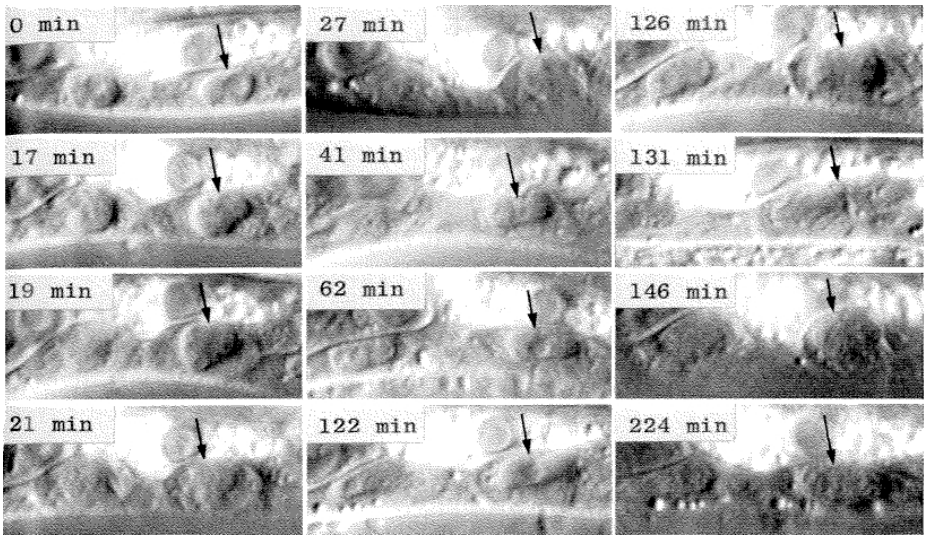


FIG. 4. Two rounds of attempted cell division in ventral cord precursor P8 in *a lin-5* animal. Nomarski, 2100. 0 min, interphase; 17 min, prophase; 19 min, attempted metaphase; 21 and 27 min, nuclear envelope broken down; 41, 62, and 122 min, interphase; 126 min, prophase; 131 and 146 min, nuclear envelope broken down; 224 min, interphase.

mal (0 min); after one round there is usually a large nucleolus, and the nucleus is often partially divided by a septum (41 and 62 min); subsequently the nucleus exhibits the fine granularity characteristic of a neuron (224 min). _The few nuclei which undergo only one round usually retain a large nucleolus and thus resemble mature hypodermal nuclei.

All postembryonic somatic blast cells undergo similar abortive cell divisions. Those blast cells that give rise to descendants which ordinarily migrate often display cytoplasmic movements that follow the path of one or more descendants. In some cases the whole cell may move (e.g., P1 migrates toward the RVG), and in some blocked precursors the nucleus may be pulled into an irregular shape by conflicting migrations (e.g., P12 and myoblast).

As mentioned earlier, *lin-5* animals are both uncoordinated and sterile. Light microscope examination of the postembryonic gonad development reveals that like somatic blast cells the structural cells of the gonad primordium repeatedly go through abortive divisions. The two germline nuclei, however, divide normally until the gonad contains about 14 cells and then they too begin to undergo abortive divisions.

Since abortive division cycles can be observed in *lin-5*, the number of times a cell attempts division can be counted, as though one were following the lineage of these cells. Table 1 summarizes observations made on the ventral cord precursors of five hermaphrodites and three males. Males of *lin-5* were obtained by repeated crosses, and therefore animals 4, 5, 6, and 7 are siblings in Table 1. In both hermaphrodites and males, precursors P1-P8 generally attempt mitosis three times. In hermaphrodites, however, precursors P9-P11, at the posterior end of the cord, go through fewer than three cycles, while in males P9 and P10 and usually P11 make three attempts to divide. At the extreme ends of the cord in both hemaphrodites and males, P0.1 in the retrovesicular ganglion (RVG)

TABLE 1

MITOTIC CYCLING OF *lin-5* PRECURSORS

Individual	Number of attempted mitoses												
	P0.1	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
♀													
1[a]	2	3	3	3	3	3	3	3	3	3	2	1	3–4
2[a]	2 died	3	3	3	3	3	3	3	3	3	3	2	3
3	2	2	1 {1–2[b] / 2	3	3	3	2 {1[c] / 1?	3	3	1	3	2	3
4[d,e]	2	3	3	1	3	3	3	3	3	2	2	1	3
5[d,e]	2	3	3	3	3	3	3	3	3	2	3	2	2
Mean number of cycles	2	2.8	3	2.6	3	3	3	3	3	2.2	2.6	1.6	3
Predicted DNA content[f]	4	7.2	8	6.4	8	8	8	8	8	4.8	6.4	3.2	8
♂													
6[a,d]	3	3	1	3	3	3	3	3	3	2	3	1	2
7[a,d]	2	3	4	3	3	3	3	3	3	3	3	3	3
8[a]	2	3	3	3	3	3	3	3	3	3	3	3	3
Mean number of cycles	2.3	3	3	3	3	3	3	3	3	2.7	3	2.3	2.7
Predicted DNA content[f]	5.2	8	8	8	8	8	8	8	8	6.8	8	5.2	6.8

[a] Observation began with precursors in the cord but divisions had not begun.

[b] At first mitosis, two nuclei were formed. The larger posterior nucleus cycled twice more, while the smaller anterior one cycled once and possibly twice more.

[c] At the second attempted mitosis, two nuclei were formed. The anterior cycled once again and the posterior one may have cycled again.

[d] Stock of ♀ and ♂ maintained by crossing. These are brother and sister larvae.

[e] Observation began before all precursors had migrated into the ventral cord.

[f] DNA content calculated for replication of all chromosomes based on mean number of cycles.

and P12 in the preanal ganglion (PAG) usually make two and three attempts, respectively. Occasionally a nucleus divides into two parts and then these cycle independently, as in animal 3 (Table 1). Approximately 120 min elapses between each abortive metaphase, compared with .the wild-type cell cycle time of 75 min; however, this difference may simply reflect the metabolic state of the animals. The number of attempted mitoses, however, is less than expected from the normal lineage of the precursors. The lineage sequence (Fig. 1) is timed so that the precursor to a/b and c and that for d and e divide at nearly the same time. Thus we might have expected a minimum of four abortive mitoses.

DNA Accumulation in Blocked Precursors

The quantity of DNA in precursor nuclei was determined relative to juvenile_ neurons (judged to be diploid relative to sperm, Table 2) by the fluorescent Feulgen microfluorimetric technique or routinely using Hoechst 33258-stained whole mounts of *lin-5*. The data are summarized in Table 2. For hermaphrodites. two alleles of *lin-5* were. tested and it was found that P3-P8 contain five to six, times the diploid

TABLE 2

RATIO OF HOECHST 33258 FLUORESCENCE OF PRECURSORS TO JUVENILE NUCLEI

Animal	Number of animals	P1 (n)[a]	P2 (n)	P3 (n)	P4 (n)	P5 (n)	P6 (n)	P7 (n)	P8 (n)	P9 (n)	P10 (n)	P11 (n)
lin-5 ♀ (e1348)	11	3.6±1.6 (10)	4.8±1.0 (10)	5.4±0.8 (10)	5.7±0.6 (11)	5.7±0.8 (11)	5.9±0.5 (10)	5.8±0.6 (11)	5.4±0.8 (10)	3.5±1.0 (9)	4.8±1.0 (10)	3.3±1.2 (5)
lin-5 ♂ (e1348) picked by light microscopy	9	–	5.3±1.0 (6)	5.1±0.9 (5)	5.5±1.1 (6)	5.2±0.4 (3)	6.4±0.3 (3)	5.9±0.9 (5)	5.5±1.1 (7)	5.9±0.8 (7)	6.0±1.8 (6)	–
lin-5 ♀ (e1457)	9	4.6±0.8 (7)	5.0±0.8 (9)	5.1±0.8 (9)	5.1±0.9 (9)	4.7±0.5 (7)	5.3±0.4 (7)	5.5±0.9 (8)	5.4±0.7 (7)	3.5±1.2 (7)	4.2±1.0 (8)	1.9 (1)
lin-5; nuc-1 ♀	21	5.5±1.0 (9)	5.5±1.3 (16)	5.4±1.0 (18)	5.8±0.9 (18)	5.6±0.8 (17)	5.9±1.0 (17)	5.8±0.9 (12)	5.2±1.0 (11)	4.2±1.8 (14)	4.3±1.4 (16)	3.7±1.0 (2)
lin-5 (e1348) Fluorescent Feulgen	15	5.3±0.6 (3)	6.2±1.7 (6)	6.5±1.2 (7)	5.0±0.7 (6)	5.8±1.7 (8)	5.4±0.9 (7)	5.5±1.7 (8)	6.8±2.2 (10)	4.8±2.6 (8)	6.7±1.4 (7)	–

[a] Numbers in parentheses refer to total number of readings made on each precursor.

amount of DNA while P1, P2, and P9-P11 have lower average amounts. Because P0.1 and P12 lie within ganglia it was not possible to determine their DNA contents. Often P11 is obscured by the PAG, too.

In contrast, male *lin-5* which were identified first by light microscopy and then fixed and stained displayed uniformly high DNA contents (five to six times) for all precursor nuclei. The number of readings for males is low because males rarely survive beyond the third larval stage, apparently because of failure in the development of the cloaca leading to constipation. Therefore, in males, in contrast to hermaphrodites, both the cycling and DNA content of P9 and P10 are similar to other ventral cord precursors. In hermaphrodites P9 and P10 are distinct from P3-P8 because one daughter cell dies.

Cell Death

A mutant *nuc-1* deficient in endodeoxyribonuclease causes remnants of cell death to remain in the ventral nerve cord as small condensed dots of material staining with nuclear stains (Sulston, 1976). In Fig. 5a a whole mount of a *nuc-1* animal shows the cell deaths from P9c and P10c in the posterior ventral cord. A double mutant of *nuc-1* and *lin-5* was made to see if the DNA content of P9 and P10 in hermaphrodites in contrast to males was being reduced by the triggering of DNases used in cell death. The data in Table 2 show that both *lin-5* and the double behave similarly and thus the removal of a DNase does not lead to increased amounts of DNA in P9 and P10. The final DNA content of P2 also appears to be the same in the double and in *lin-5;* however, the DNA content of P1 has increased slightly in the *lin-5; nuc-1* animals.

However, cytology of whole mounts of the double mutant shows that cell deaths can occur in these precursors as evidenced by small dots of DNA (Fig. 5b) similar to those seen in *nuc-1* animals (Fig. 5a). The "individual" cell deaths in *lin-5* appear to have been extruded from the precursor nucleus which remained intact. Death encompassing the whole precursor nucleus was also seen (Fig. 5c). Either type of cell death can be assigned as arising from a particular precursor by its position in the ventral nerve cord. Cell deaths were scored in *lin-5; nuc-1* animals and in animals heterozygous for *lin-5* but homozygous for *nuc-1* (Table 3). Deaths of precursors P0.1 and P1 were more frequently found than for other precursors whose lineage gives rise to cell death. Individual

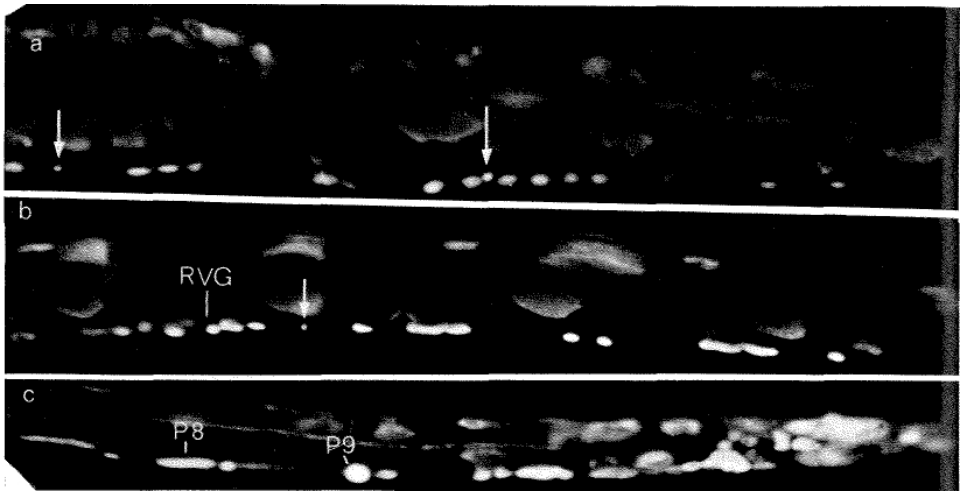


FIG. 5. Hoechst-stained ventral cords of *lin-5; nuc-1* double mutants. (a) Cell deaths (arrows) remaining in *a nuc-1* animal. The more anterior death to the left is P9c and the other P10c. Cell deaths may become smaller with age. x 1200. (b) Small death (arrow) probably extruded from P1 in the RVG. The "individual" cell deaths in *nuc-1* animals are similar to this one and are always smaller than neuronal nuclei as can be seen here. x 1200. (c) Death of the whole precursor P9. These deaths may be distinguished from surviving precursor nuclei such as P8 because of the very round shape and uniform fluorescence. x 1200.

TABLE 3

CELL DEATH

	Number of animals	Number and type of cell death associated with precursors									
		P0.1 (*n*)[a]		P1 (*n*)		P2 (*n*)		P9 (*n*)		P10 (*n*)	
		Cell	Precursor	Cell	Precursor	Cell	Precursor	Cell	Precursor	Cell	Precursor
lin-5; nuc-1		(27)		(27)		(29)		(30)		(29)	
	30	nd[b]	5	nd	9	0	2	2	1	2	0
Control (+/ *dpy-10*, +/ *lin-5; nuc-1*)		(41)		(41)		(44)		(43)		(44)	
	44	nd	0	nd	0	34	0	33	0	32	0

[a] Numbers in parentheses refer to total number of times deaths from the precursor could be scored.

[b] Individual cell deaths associated with precursors P0.1 and P1 in the RVG were not scored because these are often obscured by nuclei in the ganglion; nd = not done.

cell deaths were scored in the ventral cord only and, although present in *lin-5; nuc-1* animals, are infrequent compared to the control. Cell deaths were never seen in regions of the ventral cord that are occupied by precursors whose b and c descendants survive (P3-P8). Therefore, in spite of the fact that the precursors in *lin-5* exist as single polyploid cells, it is possible for the cell death characteristic of only certain of the descendant cell types to be expressed in part or all of the nucleus. Since cell deaths were associated only with the proper precursors, cell death might be considered to be an expression of one type of differentiation open to descendant cells.

Differentiation of Polyploid Cells

The descendants ordinarily made by these ventral cord precursors have been

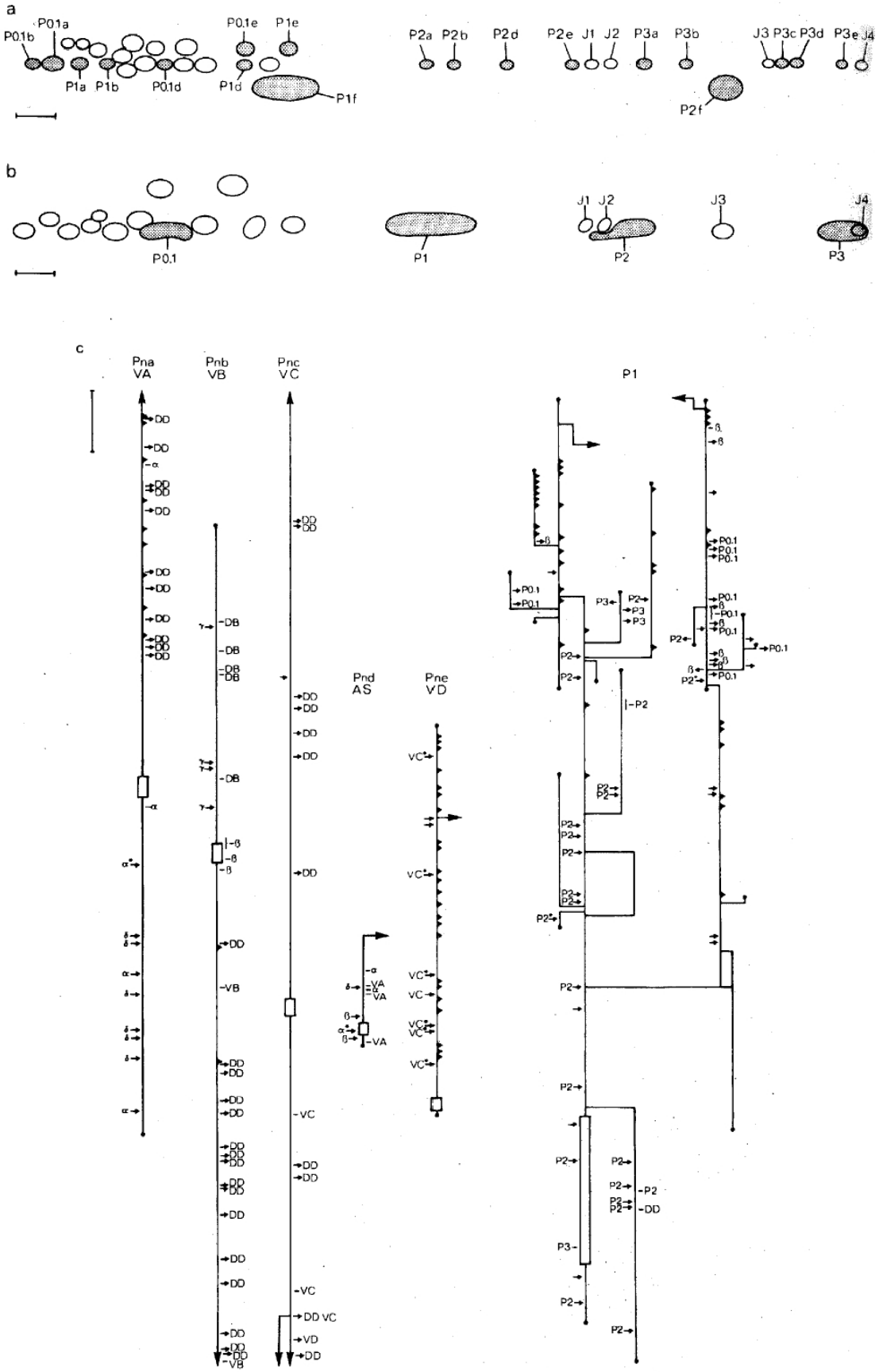
characterized by serial section reconstruction with electron micrographs (White *et al.*, 1976). It is possible to follow the lineage of an animal in the light microscope and then section that animal when it has reached adulthood. The lineage of a *lin-5* animal was followed, and the RVG and anterior ventral cord of one was reconstructed. Figure 6a shows a reconstruction of a wild type and Figure 6b, the region that was reconstructed of the mutant animal 1 of Table 1.

Most of the polyploid precursor nuclei lie in the positions to which they initially migrated. Comparison of Figs. 6a and b shows that the P1 descendants lie within the RVG, whereas in the *lin-5* animal the P1 nucleus is midway between the RVG and the first juvenile cell of the cord. The position of P1 in *lin-5* is variable: It is either found in the RVG or as in Fig. 6b. In addition, the precursor nuclei have an irregular amoeboid shape. A section through P3 in Fig. 7 shows that nuclei may have fingers of cytoplasm projecting into them. Also in this nucleus there were two mitochondria, one of which is included in the section in Fig. 7.

When the processes from the precursor nuclei were traced, it was found that each cell had at least eight major branches, and one cell as many as 16. In contrast wild-type ventral cord neurons are bipolar cells that rarely have even small branches (White *et al.*, 1976). A representative set of diagrams of the morphology and synapses of wild type ventral cord cells is shown in Fig. 6c and may be compared with the branched structure of P1 from the mutant animal. All precursors made neuromuscular junctions . (NMJs) (Fig. 7), and although branches from two or more precursors may be present in a given region of the cord only one branch makes NMJs at a time. In the wild type the same behavior is displayed by neurons within one class, but not between classes (White *et al.*, 1976). If the wild-type rules are being followed in *lin-5*, then in some way all the ventral cord precursors recognize each other as being of the same cell type.

Ventral cord motor neurons have been classed as A, B, C, AS, or D type on the basis of morphology and synaptic input (White *et al.*, 1976). These are summarized schematically in Fig. 8a. In the same way synapses made with the precursor cells of the *lin-5* animal were recorded as shown in Fig. 6c for P1, and each is summarized in Fig. 8b. The input to P0.1 is characteristic of A (receives from a interneurons) and D (receives from other motor neurons) type cells. In contrast, P2 behaves like A and B type cells, since it received from a and 0 interneurons and synapses on other motor neurons. Precursor P3 appears D-like, since it receives from other motor neurons. The presence of gap junctions between P2 and P3 suggests that these two might share some neuron class characteristics, and therefore P2 must also be D-like or P3, A- or B-like. Neither P2 nor P3 was reconstructed completely. Precursor P1 is a D type because it receives chemical synapses from other motor neurons and makes gap junctions to P3 (D type). However, in the wild type the a descendent of P1 is not a ventral cord motor neuron type, but an interneuron. At least one characteristic of this neuron is displayed by the P1 cell and so it is classed as a D/I in Fig. 8.

These observations on P0.1, P1, P2, and P3 of a single animal show that in general the polyploid precursors differentiate as their neuronal descendants would have done, but no one neuronal cell type alone is expressed. In a partial reconstruction of another *lin-5* animal it was found that P1 had become a part of the syncytial ventral hypodermis and had a nucleus typical of a hypodermal with a large nucleolus. Therefore these polyploid precursors are capable of expressing both neuronal and hypodermal cell characteristics, although neuronal characteristics are displayed more frequently (in all, five neuronal-like and


a
P01b
P01a
P01e
P1e
P2a
P2b
P2d
P2e
J1
J2
P3a
P3b
J3
P3c
P3d
P3e
J4
P1a
P1b
P01d
P1d
P1f
P2f
b
P0.1
P1
J1
J2
J3
J4
P2
P3
c
Pna
VA
Pnb
VB
Pnc
VC
Pnd
AS
Pne
VD
P1
DD
DB
VB
VC
VA
VD
P0.1
P2
P3

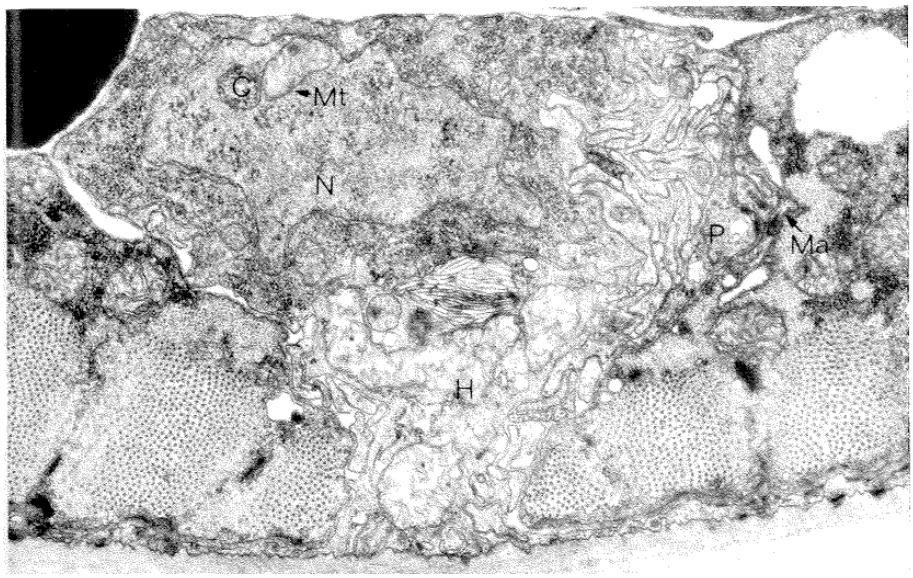


FIG. 7. Electron micrograph of a section taken through the ventral cord of *tin-5. A* precursor nucleus (N) sits over the hypodermal ridge (H). On either side of the cord muscle cells can be seen. Muscles send arms (Ma) to the right side of the nerve cord where neuromuscular junctions are made ordinarily with the ventral cord neurons, but here with a precursor cell branch (P). A mitochondrion (Mt) has been trapped within the precursor nucleus, adjacent to an in-pocketing of the cytoplasm into the nucleus (C). x 22,500.

one hypodermal *lin-5* nuclei have been reconstructed).

DISCUSSION

The *lin-5* mutation causes postembryonic cell and nuclear divisions to fail,. resulting in the formation of cells with large polyploid nuclei. We have shown that at least for the ventral nerve cord precursors the extent to which these cells become polyploid is limited, as are the number of rounds of mitosis. In spite of the mitotic block, correct timing of developmental processes is retained, and cells express variable aspects of the differentiated descendants they would have produced.

Most ventral cord precursors in *lin-5*

FIG. 6. Electron microscopic reconstruction of wild-type and *lin-5* ventral cords. (a) The positions of nuclei in the RVG and ventral cord were plotted relative to picture number from a series of transverse sections through a wild-type animal. Since the lineage of this animal was not followed prior to sectioning, the descendant cell names have been assigned from the electron microscopically determined neuron cell type according to the correspondence between lineage and cell type shown in Fig. 1. Bar = 10 µm. (b) The positions of nuclei in the RVG and ventral cord of the *lin-5* animal 1 of Table 1 plotted as in (a). Bar = 10 µm. (c) A typical set of reconstruction diagrams of the five descendant ventral cord neuron cell types VA, VB, VC, AS, and VD derived from a single wild-type precursor compared with the undivided precursor, P1 from the *lin-5* animal 1. The juvenile type A, B, and D neurons make neuromuscular junctions in the dorsal cord and so have been named DA, DB, and DD. The ventral cord interneurons are a, ,6, y, and 6 (White *et al.,* 1976). The *lin-5 P1* cell synapses with other precursor cells (P0.1, P2 and P3), juvenile neurons, and ventral cord interneurons. Bar = 10 µm. j , 1 , end of process; T , process continues anteriorly in the ventral cord beyond the region shown; 1 , process continues posteriorly;- h , right-hand commissure to dorsal nerve cord; Ft , left-hand commissure to dorsal nerve cord; A, neuromuscular junction; - , chemical synapse arrow points from the presynaptic to the postsynaptic cell; -, gap junction; *, diadic synapse; o , cell body.

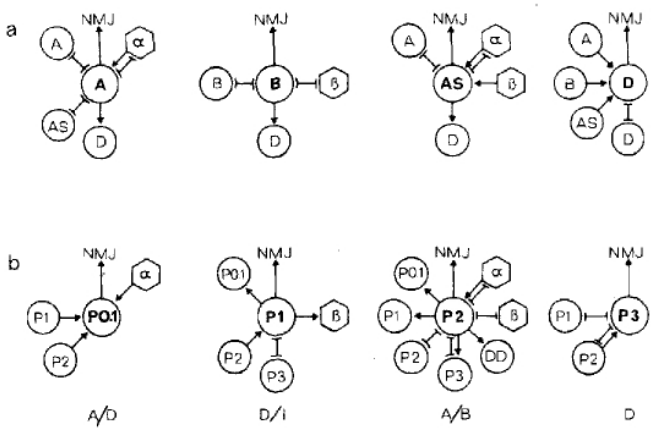


FIG. 8. Connectivity of ventral cord motor neurons. Wild-type ventral cord neurons (A, B, AS) have been classified according to their patterns of connections with ventral cord interneurons. In contrast, type D neurons do not receive from interneurons but receive chemical synapses from other motor neurons. All four types make gap junctions to members of their own class and make neuromuscular junctions (NMJ). (a) Wild type, (b) reconstruction of P0.1 through P3 of the lin-5 animal 1 of Table 1. 0, motor neuron;(), interneuron; ¯∴, chemical synapse; arrow points from the presynaptic to the post-synaptic cell; gap junction.

make three attempts at nuclear division. If each of these abortive divisions represents one traverse through the cell cycle, then three S-phases will have occurred in the precursors, which could result in a final DNA content of up to $(2n)^3$ or eight times a juvenile neuron's nuclear DNA content. The final DNA content of the precursors is, however, less than this, being approximately the sum of that of the descendents ordinarily produced by the precursors. Higher DNA contents were recorded from other blast cells that produce a greater number of descendants (unpublished observations). Therefore it is unlikely that the amount of DNA detectable in the ventral cord precursors is limited by the method. Similarly, the mutation itself does not cause the accumulation of DNA to stop at the same amount in all blast cells. This might suggest that the ability of a cell to replicate its DNA in *lin-5* animals is linked to the particular blast cell's lineage.

Possibly, DNA replication in *lin-5* nuclei follows the lineage strictly. Since the lineage is asymmetric, then once formed those sets of chromosomes that would have been segregated to the final descendants must be removed from the pool of replicating DNA, all contained in *lin-5* within a single nucleus. Models for distinguishing between sets of chromosomes have invoked chemical tagging (Holliday and Pugh, 1975) or segregation of oldest DNA strands (Cairns, 1975). It is not unreasonable to suppose that if such mechanisms are used to label cells for further cell division or cessation of cell division, then they might continue to operate in *a lin-5* nucleus, since it has been shown that satellite DNA sequences are selectively under-replicated in polytene chromosomes (Endow and Gall, 1975). Furthermore in mealy bugs in which the paternal chromosomes are heterochromatic, endoreduplication occurs with the maternal but not paternal sets (Nur, 1966). This and other examples of chromosome imprinting (Chandra and Brown, 1975) suggest that. DNA sequences, individual chromosomes, and sets of chromosomes may be handled differently within a single nucleus.

On the other hand, in most naturally occurring binucleate cells and experimentally formed multinucleate cells, replication and mitosis occur synchronously, even in experiments with nuclei taken from

cells in. different stages of the cell cycle, or nondividing cells (Johnson and Rao, 1971). It would appear that in these cells all chromosomes might be replicated. If all chromosomes are replicated in *lin-5* nuclei, then the cessation of DNA synthesis at a final DNA content of five to six times the diploid amount might result because precursor cells have sufficient resources for formation of only six daughter nuclei. If a depletion of some factor were to limit the extent of polyploidy, then partially replicated chromosomes would have participated in the third mitosis. A lack of precursors for initiating DNA replication after the third mitosis may prevent the fourth mitosis predicted by the lineage but not observed. To distinguish between these alternatives it would be necessary to determine the DNA content of *lin-5* nuclei as cell cycling proceeds, but such experiments are not feasible, because of the small size of the larvae and crowding in the ventral cord.

The polyploid ventral cord cells in *lin-5* animals are in a sense analogous to heterokaryons formed by cell fusion. When neuroblastoma cells and fibroblasts were fused, neuronal characteristics were observed variably (Davidson, *1974).* In *lin-5* the expression of variable multiple neuronal cell type characteristics is preferred over the hypodermal cell daughter type. In contrast to the highly polyploid *lin-5* cells, other mutants which fail in terminal nuclear divisions giving rise to tetraploid or binucleate cells display only one of the two possible daughter neuronal types (J. White, unpublished observations). In the run-out model discussed above, incomplete replication of chromosomes in the last Sphase will result in some DNA sequences being present in more copies than others. These are likely to vary from precursor to precursor and might then lead to the variation in differentiated character expressed by the precursor cells of *lin-5.*

The defect in *lin-5* animals appears to be a factor essential for cell division. Larvae, homozygous for *lin-5,* complete embryonic development perhaps because the required factor is supplied in the egg by the heterozygous mother. Therefore a limited amount of division continuing in the germ cells after hatching might be explained by the retention of maternal product by these cells. This would be possible because the primordial germ cells are set aside at the very early divisions of the egg (Deppe *et al., 1977),* and so they have undergone very few rounds of division relative to somatic cells in the egg. After hatching, when germ cell proliferation begins, normal division continues until the maternal product runs out.

Temperature-sensitive cell cycle mutants have been studied in yeast (Hartwell *et al., 1974),* tetrahymena (Frankel *et al.,* 1976), and tissue culture cells (Wang, *1974,* 1976). In these systems mutants which block nuclear division prevent further cell cycles from occurring at the restrictive temperature. This has led to the suggestion that nuclear division is necessary before a new cell cycle can be initiated (Hartwell *et al.,* 1974). In *lin-5,* cell cycling proceeds in spite of a block at metaphase and a failure to produce daughter nuclei. However, in the light microscope the other stages of mitosis can be seen, suggesting that the progression through mitosis without the proper segregation of the chromosomes into daughter nuclei is sufficient to initiate a new cell cycle in *lin-5.* The defect leading to failure of nuclear division in *lin-5* is unknown, but the improperly formed metaphase plate seen in the light microscope might suggest a failure of chromosome attachment to the spindle. It should be - possible to examine stages of mitosis in nematode ventral cords in the electron microscope and to combine this with isolation of other maternal effects and temperature-sensitive mutants in further studies of the process of nuclear division.

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