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Polyploid Tissues in the Nematode *Caenorhabditis elegans*

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During larval development, the number of somatic nuclei in *C. elegans* hermaphrodites increases from 558 to 959 (J. E. Sulston and H. R. Horvitz, *Dev. Biol.* **56**, 110-156, 1977; J. E. Sulston *et al.*, *Dev. Biol.* **100**, 64-119, 1983). At the same time, the animals increase about 60-fold in volume. We have measured the DNA contents of several classes of nuclei by quantitating the fluorescence of Hoescht 33258 stained DNA (D. G. Albertson *et al.*, *Dev. Biol.* **63**, 165-178, 1978). Probably all embryonic nuclei, including those of neurons, muscles, hypodermis, and intestine, are diploid at hatching. Neurons, muscles, and nondividing hypodermal nuclei remain diploid throughout larval development. The DNA content of the intestinal nuclei doubles at the end of each larval stage, reaching 32C by the adult stage. New hypodermal cells, generated by division of seam cells in the larval stages, undergo an additional round of DNA replication before fusing with the major syncytium (hyp7, Sulston *et al.*, 1983). Thus the larval hyp7 syncytium comprises a fixed number of diploid embryonic nuclei plus an increasing number of tetraploid postembryonic nuclei. Some of the endoreduplications that occur in the intestinal and hypodermal lineages of *C. elegans* may correspond to nuclear or cellular divisions in another nematode *Panagrellus redivivus* (P. W. Sternberg and H. R. Horvitz, *Dev. Biol.* **93**, 181-205, 1982). © 1985 Academic Press, Inc.

INTRODUCTION

DNA replication is usually a prelude to cell division, but, in many animals, some tissues can grow by increasing cell size and DNA content rather than cell number (White, 1973). These exceptional cases are interesting because they show how cell cycle events can be uncoupled during normal development.

The nematode *Caenorhabditis elegans* is widely used in studies of the genetic control of development. Cell divisions can be observed in living animals and the lineages of all cells are now completely known (Sulston and Horvitz, 1977; Kimble and Hirsch, 1979; Sulston *et al.*, 1980, 1983). Here we describe DNA endoreduplications that occur in intestinal and hypodermal nuclei during larval development.

METHODS

The developmental ages of individual hermaphrodite larvae were determined using differential interference contrast microscopy (Sulston and Horvitz, 1977). The animals were then transferred to gelatinized slides using a mouth capillary to expel each individual in about 1 μl of M9 buffer (Brenner, 1974). A clean 12 × 12-mm glass coverslip was laid over each animal and the slides were frozen for 10 min on dry ice. The frozen coverslips were pried off with a razor and the slides were immediately immersed in a fixative (6 parts absolute ethanol, 3 parts acetic acid, 1 part chloroform) and left overnight. Usually this procedure flattens and fractures the larvae so that only left or right half animals adhere to the slide.

The fixed slides were air dried, incubated with 20 μg/ml ribonuclease A in 2× SSC buffer for 1 hr at 37°C, rinsed in PBS, and stained with Hoescht 33258 according to Albertson *et al.*, (1978).

Images of fluorescent nuclei, obtained from an image-intensifying video camera mounted on a Zeiss epifluorescence microscope, were digitized, time-averaged, and stored using an Intellect 100 image processor. Subsequent calculations were performed by a VAX computer with an AED graphics display.

RESULTS

Background, Linearity, and Reproducibility of Fluorescence

Hoescht 33258, a dye which fluoresces strongly when bound to DNA, was used for microfluorometry of nuclear DNA (Albertson *et al.*, 1978). Fluorescent nuclei were recorded using an image-intensifying video camera and a picture digitizer which measured the intensity of fluorescence at each of 512 × 512 points in the image. Using a graphics display with a joystick, a rectangle was drawn around each nucleus with care to avoid overlap with neighboring nuclei. The total fluorescence of the nucleus was calculated by summing the fluorescence of all points within the rectangle and subtracting the background. The background fluores-

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cence was approximated by the linear function which gave the best least-squares fit to points along the boundary of the rectangle. Digitized pictures of fluorescent nuclei are shown in Fig. 1.

Nuclei of the same type, when recorded in a single picture, had standard deviations of less than 2% in good specimens (Fig. 1). Nuclei from a single animal, when recorded in several separate pictures, had deviations of 5–20% (Table 1).

The fluorescence was relatively insensitive to nuclear volume. Diploid hypodermal, muscle, and neuronal nuclei gave similar readings (Table 1) despite large differences in nuclear size. Extremely condensed, haploid, nuclei of sperm gave values nearly one-half those of neuronal nuclei (0.45 ± 0.06, $n = 13$; see also Albertson *et al.*, 1978).

Intestinal Nuclei

The embryonic intestine is a hallow tube made of 20 mononucleate cells. Near the end of the first larval stage, at the onset of lethargus, most of the intestinal nuclei divide (Sulston and Horvitz, 1977). The six anterior-most nuclei do not divide and, variably, any of the four posterior-most nuclei may also fail to divide. The intestinal cells and nuclei continue increasing in size without further divisions.

The intestinal nuclei are diploid at hatching and, with the exceptions noted above, replicate and divide near the beginning of the first lethargus (Table 1). As expected, 12 ($=2n$) bivalents can be seen at metaphase of these divisions. During the lethargus, these nuclei replicate again, becoming tetraploid. Unlike the nuclear division, endoreduplication occurs without obvious chromosome condensation. Another three endoreduplications, one in each larval stage, increase the ploidy of these nuclei to 32C by the final moult. The time of endoreduplication, measured carefully in third stage larvae (Table 1), is near the beginning of lethargus. Similarly, the six nondividing nuclei in the anterior intestine endoreduplicate once in each larval stage. These endoreduplications are diagrammed in Fig. 2.

Hypodermal Nuclei

The hypodermis is a sheet of cells which forms the outer surface of the animal and secretes the cuticle. The largest hypodermal cell, hyp7, is formed in the embryo by the fusion of 23 mononucleate hypodermal cells (Sulston *et al.*, 1983). During larval development in hermaphrodites, 18 lateral (H1, H2, V1–V6, and T) and 12 ventral (P1–P12) ectoblasts generate an additional 110 mononucleate cells which fuse with hyp7 (Sulston and Horvitz, 1977). By the adult stage, hyp7 contains 133 nuclei and is a cylindrical cell enveloping all of the body excepting the extreme head and tail. Smaller hypodermal cells cap the extreme head (hyp1–hyp6) and tail (hyp8–hyp11), respectively. These smaller cells, unlike hyp7, acquire no additional nuclei after hatching (Sulston and Horvitz, 1977).

The embryonic nuclei of hyp7, hyp8, hyp9, and hyp10, and presumably the other minor hypodermal cells, remain diploid throughout larval development (Table 1). In contrast, the hyp7 nuclei derived postembryonically from precursors V1–V4, and presumably the other lateral precursors, are tetraploid (Table 1). The nuclei

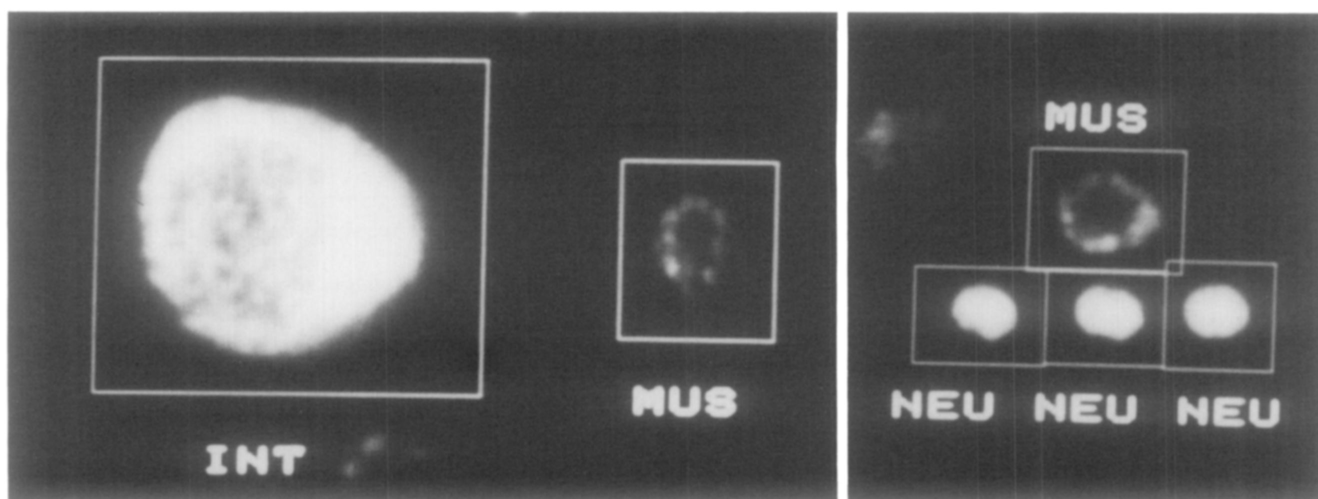

FIG. 1. Digitized images of fluorescent nuclei in a newly moulted adult (approx. 45 hr) stained with Hoescht 33258. DNA contents were calculated by summing the fluorescence in rectangular regions containing the nuclei and subtracting background fluorescence. The relative DNA contents of the nuclei in this example were, from left to right, 16.3, intestine (INT); 0.99 and 0.98, muscles (MUS); and, 0.99, 1.01, and 0.99, neurons (NEU). Magnification approximately 2000×.

TABLE 1
RELATIVE DNA CONTENTS OF LARVAL NUCLEI

Animal	Age[a] (hr)	Neurons[b]	Body muscles[b]	Embryonic hypodermis[c]	Intestine nondividing[d]	Intestine dividing[e]
1	5.0	—	1.00 ± 0.05 (4)	—	—	0.86 ± 0.12 (3)
2	8.5	1.00 ± 0.14 (3)	0.97 ± 0.14 (3)	—	—	—
3	8.5	—	1.00 ± 0.06 (7)	1.18 ± 0.20 (2)	—	—
4	10.5	1.00 ± 0.09 (2)	1.13 ± 0.06 (2)	1.16 ± 0.14 (2)	—	1.31 (1)
5	12.0	—	1.00 ± 0.12 (4)	0.65 (1)	—	1.83 (1)
6	14.0	1.00 ± 0.16 (8)	1.05 ± 0.10 (9)	0.78 ± 0.12 (2)	—	2.07 ± 0.22 (3)
7	14.5	1.00 (1)	—	1.02 (1)	—	—
8	15.5	1.00 ± 0.27 (4)	0.88 ± 0.24 (4)	1.08 ± 0.24 (4)	—	2.10 ± 0.36 (11)
9	17.0	1.00 ± 0.13 (3)	1.11 ± 0.09 (7)	1.03 (1)	2.10 (1)	2.37 ± 0.18 (4)
10	18.0	1.00 ± 0.12 (4)	0.97 ± 0.12 (12)	0.98 ± 0.16 (4)	—	1.81 ± 0.25 (5)
11	24.0	1.00 ± 0.09 (12)	—	—	—	4.7 ± 0.7 (4)
12	25.0	1.00 ± 0.13 (13)	1.11 ± 0.12 (11)	1.12 ± 0.10 (9)	—	4.8 ± 0.5 (7)
13	28.0	1.00 ± 0.05 (10)	0.95 ± 0.05 (11)	1.16 ± 0.19 (2)	4.2 ± 0.1 (2)	4.4 (1)
14	28.5	1.00 ± 0.03 (6)	0.87 ± 0.07 (17)	0.90 ± 0.06 (3)	4.4 ± 0.3 (4)	4.3 ± 0.3 (4)
15	29.5	1.00 ± 0.10 (5)	1.07 ± 0.10 (6)	—	4.0 ± 0.2 (2)	4.5 ± 0.3 (9)
16	32.0	1.00 ± 0.01 (2)	1.09 ± 0.05 (10)	0.96 (1)	8.9 ± 0.7 (4)	9.5 ± 0.7 (8)
17	33.0	1.00 ± 0.17 (22)	0.99 ± 0.17 (11)	—	8.8 (1)	8.2 ± 0.7 (8)
18	45.0	1.00 ± 0.05 (12)	0.88 ± 0.06 (3)	—	15.4 ± 1.3 (4)	15.5 ± 0.5 (3)
19	45.0	1.00 ± 0.05 (6)	1.02 ± 0.08 (17)	—	16.0 ± 1.3 (4)	18.0 (1)

Postembryonic lateral hypodermis[f]

Animal	Vn.a(sy)	Vn.p(se)	Vn.p$_p^a$(se)	Vn.p$_p^a$a(sy)	Vn.p$_p^a$p(se)	Vn.p$_p^a$pa(sy)	Vn.p$_p^a$pp(se)
1	—	—	—	—	—	—	—
2	2.31 (1)	1.05 (1)	—	—	—	—	—
3	1.99 ± 0.15 (3)	1.09 ± 0.10 (3)	—	—	—	—	—
4	2.18 ± 0.12 (2)	1.16 ± 0.14 (2)	—	—	—	—	—
5	1.91 ± 0.37 (2)	0.74 (1)	—	—	—	—	—
6	2.20 (1)	1.02 (1)	—	—	—	—	—
7	2.02 (1)	1.81 (1)	—	—	—	—	—
8	2.20 ± 0.18 (3)	2.32 ± 0.06 (3)	—	—	—	—	—
9	2.29 (1)	—	2.00 ± 0.22 (3)	—	—	—	—
10	2.23 ± 0.40 (2)	—	—	1.00 ± 0.07 (7)	0.96 ± 0.09 (5)	—	—
11	—	—	—	—	—	—	—
12	2.02 ± 0.26 (2)	—	—	2.31 ± 0.20 (5)	—	1.07 ± 0.16 (6)	1.15 ± 0.09 (5)
13	2.18	—	—	2.38 ± 0.07 (2)	—	2.03 ± 0.07 (2)	1.02 ± 0.01 (2)
14	1.86 ± 0.15 (2)	—	—	1.96 ± 0.09 (5)	—	1.97 ± 0.06 (5)	0.86 ± 0.03 (5)
15	—	—	—	—	—	—	—
16	2.16 (1)	—	—	2.16 ± 0.01 (2)	—	2.18 ± 0.03 (2)	1.87 ± 0.23 (2)
17	2.11 ± 0.15 (2)	—	—	1.92 ± 0.08 (3)	—	2.18 ± 0.30 (4)	2.22 ± 0.12 (4)
18	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—

[a] Developmental ages in hours after hatching at 20°C (Sulston and Horvitz, 1977). The four larval moults occur at 16.0, 25.0, 34.0, and 45.0 hr on this scale.

[b] Means and standard deviations of DNA contents normalized to neurons or body muscles. Numbers of nuclei measured are shown in parentheses.

[c] These values are from hyp7 nuclei derived from Caaa and Cpaa (Sulston *et al.*, 1983). Hyp7, hyp8, hyp9, and hyp10 nuclei derived from AB are also diploid in the larval stages.

[d] These values are from the six anterior-most cells of the intestine. No differences were observed between the four cells adjacent to the pharynx and the two cells posterior to them.

[e] The underline separates measurements made before/after the nuclear divisions. Nuclei of the four posterior-most cells, whose lineages are variable, are excluded.

[f] These values are from seam cells V1–V4. The underlines indicate that the cell has divided. We were unable to identify individual hypodermal nuclei in older larvae or adults, but judging by the number and position of diploid and tetraploid nuclei at these stages, the syncytial nuclei generated in the fourth larval stage are probably tetraploid and the seam nuclei remain diploid throughout larval development.

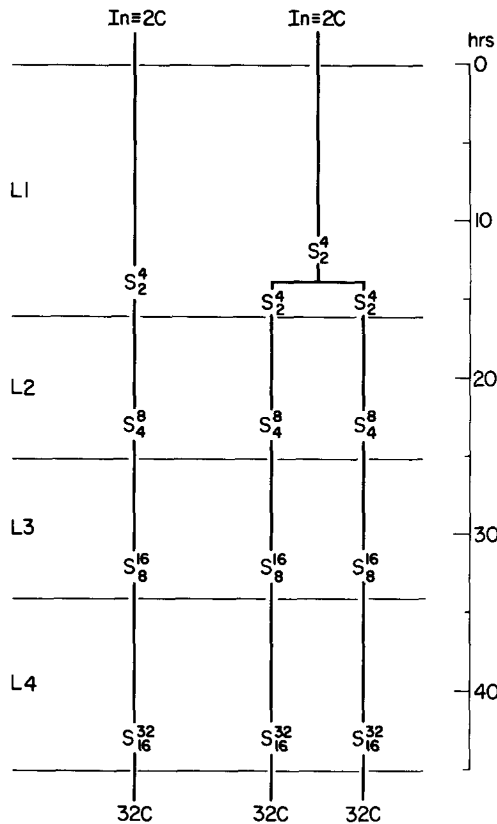

FIG. 2. Endoreduplications in the larval intestine. Time scale begins with hatching (0 hr) and continues through four larval moults (horizontal lines). Times of DNA synthesis are indicated by an S with a subscript and superscript to indicate the initial and final ploidy, respectively. The lineage on the left is for nondividing nuclei. The lineage on the right is for nuclei which divide near the end of the first larval stage (Sulston and Horvitz, 1977).

of these presumptive syncytial cells endoreduplicate soon after the cells are born (Table 1; diagrammed in Fig. 3). As in the intestine, endoreduplication occurs without obvious chromosome condensation. Then, around 3 hr after their birth, the cells fuse with hyp7 (Hedgecock and Thomson, 1982).

The ventral ectoblasts (P1–P12) also contribute 12 nuclei to hyp7 in hermaphrodites. Because our staining procedure favors lateral aspects, we did not obtain enough reliable measurements to establish the ploidy of these ventral nuclei.

Other Tissues

Neurons and body muscles are diploid throughout larval development (Table 1). Certain minor cell types, such as coelomocytes, were also found to be diploid in the early larval stages where they could still be recognized. We did not measure the DNA contents of the structural cells of the gonad. These are large cells, comprising several types (Kimble and Hirsch, 1979), and candidates for polyploidy. Unfortunately, they were difficult to recognize in our preparations and often obscured by germ nuclei. Finally, we have not checked for possible endoreduplications in the adult stage itself. Although no cell divisions occur in *C. elegans* adults (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979), many tissues continue to divide in adults of larger nematode species. Even in *C. elegans*, adults live for over a week and increase several-fold in volume during that time.

DISCUSSION

The transcriptional capacity and, indirectly, the size of cells are limited by their ploidy. Cell ploidy is determined by the number and ploidy of the nuclei. Cells with multiple nuclei can arise by nuclear division or cell fusion (Fig. 4). Nuclear divisions occur in the larval intestine (Sulston and Horvitz, 1977). Cell fusions occur in certain muscles of the pharynx and in the hypodermis (Sulston *et al.*, 1983; Sulston and Horvitz, 1977).

Nuclear ploidy can double by DNA replication without division (Fig. 4). For example, there is no cell

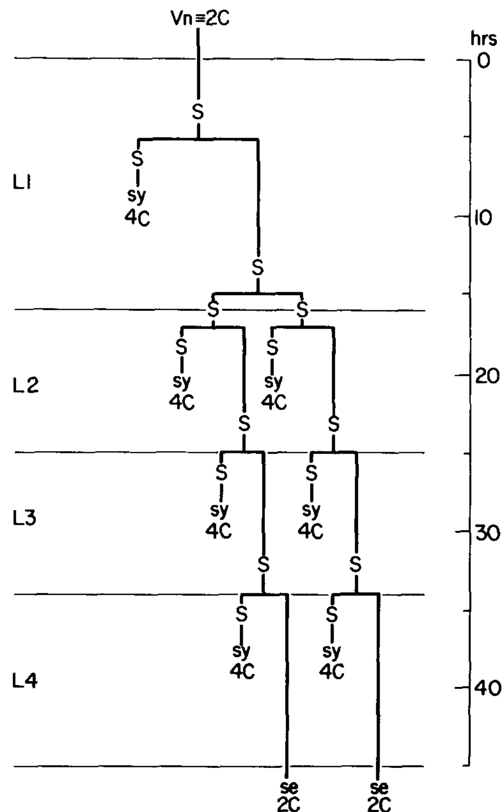

FIG. 3. Endoreduplications in the lateral hypodermis. DNA synthesis is indicated by an S. Nomenclature and cell lineages of seam (se) and hyp7 syncytial nuclei (sy) are from Sulston and Horvitz (1977).

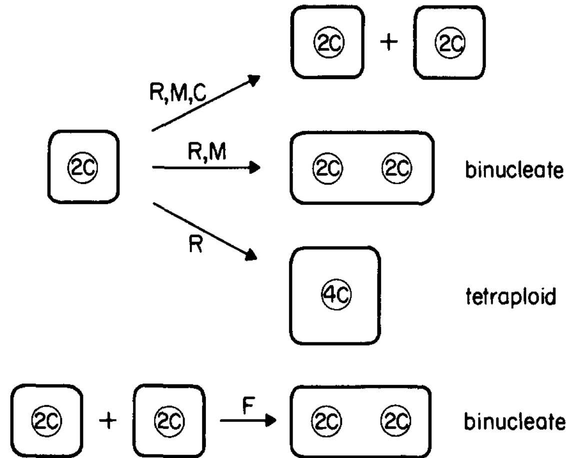

FIG. 4. In ordinary cell divisions, DNA replication (R), mitosis (M), and cytokinesis (C) occur coordinately and each diploid cell yields two diploid daughters. If replication and mitosis occur, but without cytokinesis, the cell becomes binucleate. If replication occurs with neither mitosis or cytokinesis, the ploidy of the nucleus doubles. Cell fusion (F) is a second way of generating multinucleate cells.

division in the larval intestine. Instead, a nuclear division, followed by four rounds of endoreduplication, generate binucleate cells containing the equivalent of 32 diploid genomes at the final moult. Similarly, a combination of endoreduplication and cell fusion raises hyp7 and 23 genome equivalents at hatching to over 200 equivalents in the adult. For comparison, the animals increase about 60-fold in total volume during the larval stages (Sulston and Horvitz, 1977).

Mutations in the *lin-5* gene disrupt mitosis and cytokinesis but not DNA replication in *C. elegans* larva (Albertson *et al.*, 1978). In these mutants, cycles of chromosome condensation and unsuccessful mitosis occur in blast cells that would normally undergo division. In consequence, they become large, polyploid, mononucleate cells and express aspects of the differentiation expected of their normal descendants. By comparison, endoreduplications in the wild type intestine and hypodermis occur without evident chromsome condensation or attempted mitosis. Hence, these normal endoreduplications probably diverge from the full cell division cycle at a step prior to the *lin-5* block.

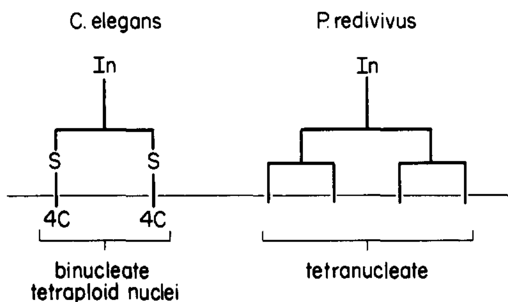

FIG. 5. Comparison of intestinal lineages of *C. elegans* and *P. redivivus* in the first larval stage. Horizontal line indicates time of moult.

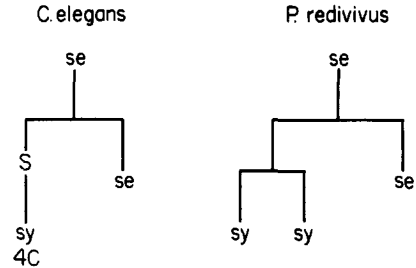

FIG. 6. Comparison of seam (se) cell divisions yielding syncytial (sy) cells in *C. elegans* and *P. redivivus*.

If the purpose of endoreduplication is simply to increase the number of genome copies available for transcription, nuclear division or cell division might serve as well. Indeed, the intestinal nuclei of *Panagrellus redivivus* undergo a second mitosis at about the time of the first endoreduplication in *C. elegans* (Sternberg and Horvitz, 1982). This suggests that the endoreduplication in *C. elegans* may be equivalent to a nuclear division in *P. redivivus* (Fig. 5). Similarly, in *P. redivivus* certain hypodermal precursors undergo an additional cell division before fusing with hyp7 (Sternberg and Horvitz, 1982). The endoreduplication that occurs in *C. elegans* may correspond to this complete cell division in *P. redivivus* (Fig. 6). A testable prediction is that intestinal and hypodermal nuclei at the equivalent stage in *P. redivivus* should be diploid rather than tetraploid.

It is interesting to consider the evolution of endoreduplication and its relation to ordinary cell division. Adults of large parasitic nematodes, such as *Ascaris lumbricoides*, grow a million-fold larger in volume than *C. elegans* and have several orders of magnitude more intestinal and hypodermal nuclei. The larval and adult lineages in these nematodes are unknown, but it is conceivable that the additional nuclei arise by continued division of the same precursors found in *C. elegans*. Their common ancestor may have had an intermediate number of cells and imprecise control of postembryonic cell number. In the evolution of smaller nematodes, such as *C. elegans*, where size and development time are at a premium, these postembryonic lineages may have been greatly pruned and, where possible, proliferative cell divisions abbreviated to nuclear divisions or endoreduplications.

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