Proc. Natl. Acad. Sci. USA
Vol. 75, No. 1, pp. 376–380, January 1978
Developmental Biology

Cell lineages of the embryo of the nematode *Caenorhabditis elegans*

(metazoan model organism/determinate cleavage/stem cell autonomy/cell division synchrony/cell cycle clocks)

UWE DEPPE, EINHARD SCHIERENBERG, THOMAS COLE, CHRISTIAN KRIEG, DAVID SCHMITT, BONITA YODER, AND GÜNTER VON EHRENSTEIN*

Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, 3400 Göttingen, Federal Republic of Germany

Communicated by William B. Wood, September 29, 1977

ABSTRACT **Embryogenesis of the free-living soil nematode *Caenorhabditis elegans* produces a juvenile having about 550 cells at hatching. We have determined the lineages of 182 cells by tracing the divisions of individual cells in living embryos. An invariant pattern of cleavage divisions of the egg generates a set of stem cells. These stem cells are the founders of six stem cell lineages. Each lineage has its own clock—i.e., an autonomous rhythm of synchronous cell divisions. The rhythms are maintained in spite of extensive cellular rearrangement. The rate and the orientation of the cell divisions of the cell lineages are essentially invariant among individuals. Thus, the destiny of cells seems to depend primarily on their lineage history. The anterior position of the site of origin of the stem cells in the egg relates to the rate of the cell cycle clock, suggesting intracellular preprogramming of the uncleaved egg. We used a technique that allows normal embryogenesis, from the fertilized egg to hatching, outside the parent under a cover glass. Embryogenesis was followed microscopically with Nomarski interference optics and high-resolution video recording.**

In this paper we describe the cell lineage pedigree, orientation, and rhythm of cell divisions, in the embryo of the free-living soil nematode *Caenorhabditis elegans*, of 182 cells starting from the fertilized egg. Our aim is to elucidate the control of embryonic development in a simple metazoan model system. Brenner (1, 2) has shown that *C. elegans* has particular advantages as an experimental organism, including great simplicity and favorable genetics. It should be possible to analyze regulation by making genetic lesions—e.g., chemically induced mutations (3). Temperature-sensitive developmental mutants including embryonic-arrest mutants have been isolated (4). Of course, the characterization of the cellular and subcellular phenotype of mutants that are defective in embryogenesis is informative only if one already understands the normal developmental sequence at this level. Embryogenesis produces a juvenile *C. elegans* (hatchee) having only about 550 cells at hatching. The mature hermaphrodite is 1 mm long and has about 800 somatic cells, excluding the gonads. Recently, postembryonic cell lineages and development after hatching have been worked out (5, 6).

Classical studies established the striking determinate cleavage of nematode eggs and led to the concept of cell lineages (7, 8). The cell lineages were deduced by noting mitotic figures, cell and nuclear size, and other morphological features in fixed eggs. In *Parascaris equorum*, lineage to the 102-cell egg has been determined (9), as well as the origin and development of tissues to the 802-cell stage, by following mitoses and differentiation of groups of cells (10). Similar studies have been reported in other nematodes (11–14). Preliminary studies on early embryogenesis in *C. elegans* have been reported (15, 16).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Embryonic development of *C. elegans* is rapid, taking only half a day. The eggs are only about 30 × 60 μm. Because the eggs are transparent, the internal structure in intact living specimens can readily be examined. Observation and recording on video tape of the development of eggs under a light microscope with Nomarski interference optics allows one to trace the divisions and migrations of hundreds of cells individually.

MATERIALS AND METHODS

Strains and Culturing. NG agar, M9 buffer, M9 medium, and S medium have been described (2). Wild-type *C. elegans* var. Bristol, designated N2, was obtained in 1973 from Sydney Brenner and has been maintained at 16° on a lawn of *Escherichia coli* strain OP 50 on agar since then (2).

Preparation of Eggs for Observation. Eggs start to cleave inside the hermaphrodite parent about 30 min after fertilization and are laid 1–2 hr later. Embryos encased in their impermeable egg shell were allowed to develop outside the parent as follows. Adults with less than 20 eggs were dissected with a scalpel to release the eggs. Eggs with two to four cells were selected and transferred to a microscope slide with filter-sterilized S medium or tap water. A cover glass (24° × 36 mm) was added and ringed with petrolatum to avoid evaporation and pressure on the egg; care was taken to trap some air bubbles for aeration. This technique allows the eggs to develop normally; the time from the two-cell stage until hatching was 13 ± 1 hr at 20°–22°. This agrees well with observations of populations on agar plates (17).

Microscopy and Recording on Video Tape. A Zeiss universal microscope equipped with Nomarski differential interference contrast optics was used at ×1600 magnification. Because of the rapid succession of cell divisions and large cell numbers, analysis in real time is difficult. Therefore, the microscope was fitted with a TV camera (Ikegami Tsushinki Corp., New York model CTC 6000) coupled to a videotape recorder (International Video Corp., Sunnyvale, CA, model 711 P, 1-in. tape) and a monitor screen. Recordings were made at room temperature (20°–22°) with two heat filters on the microscope light source.

The cell lineages reported here were analyzed from tapes of two eggs recording the complete development until the animals started moving inside the egg shell. In addition, embryogenesis was recorded and analyzed in 18 other individuals from fertilization to the 30- (10 cases), the 54-, 60-, 75-, and 87- (3 cases), and the 100- (2 cases) cell stages, respectively. We also analyzed the E-cell lineage in six more individuals to make certain that all 8 E-cells divide in going to 16. After recording was terminated, it was ascertained that, under the cover glass, all 26 animals hatched and moved normally.

* To whom reprint requests should be addressed.

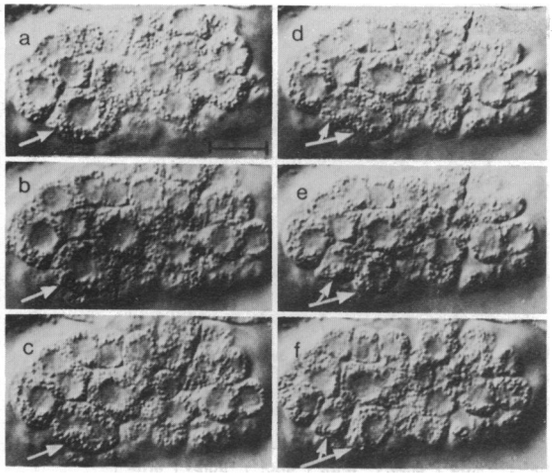

FIG. 1. Cell division of D.a into D.aa and D.ap (see Fig. 2 for nomenclature). Sequential photographs of a 100-cell embryo, left ventral view; anterior is at right. Nomarski optics. (*a*) Late interphase, nucleus swollen; (*b*) prophase, nuclear membrane breaks down; (*c*) metaphase plate; (*d*) anaphase; (*e*) telophase; (*f*) interphase. The time between *a* and *e* is 5 min. (Bar = 10 μm.)

For analysis we chose eggs oriented in an identical fashion. We used a standard orientation in which the two AB descendants are anterior and dorsal, respectively, the P_1-cell posterior, and the EMSt-cell ventral. During recording, the focus was moved systematically up and down through the entire egg, producing a series of 5–10 "optical sections."

Analysis of the Video Tapes. For analysis, the video tapes were played back repeatedly over the monitor. Despite some loss of fidelity in the video system, cell nuclei, cell boundaries, mitotic figures, and cytoplasmic streaming could be seen. Cells were followed individually, and the time and direction of their divisions were noted. Because cell boundaries are difficult to identify reliably, the analysis is based primarily on the nuclei and for early stages on mitotic figures. Later on, such figures are difficult to see and the two daughter nuclei can only be located after the nuclear membranes have re-formed. Cell movements are slow (maximally, 2 μm/min), easy to follow, and do not interfere with the construction of the lineage tree.

RESULTS

Cell Division. In *C. elegans*, all embryonic cell divisions are cleavage divisions. Therefore, the cells get progressively smaller. The overall process of cell division as observed with the Nomarski technique is quite uniform throughout embryogenesis (Fig. 1) and similar to that described for postembryonic division (6). After completion of cell division, the two daughter cells round off; but they never detach completely from one another. Except for divisions generating stem cells, the two daughters are approximately equal in size, as nearly as can be estimated with the Nomarski technique. We did not observe any cell death, as has been reported postembryonically (6).

Stem Cells and Cell Lineages. Unique to nematodes, the first cleavage separates the embryo into an anterior (AB) and a posterior (P_1) cell (15). AB is the stem cell for the primary ectoderm. In *C. elegans* we can define a stem cell as one whose descendants arise via synchronous and equal divisions. Five additional stem cells are established by the next divisions of the P_1 lineage (Fig. 2 *lower*). In these divisions the daughters are unequal in size, and the smaller (P_1–P_4) is the precursor of the germ line as in other nematodes (7–14). The other product of the P_1 cell division is EMSt, the precursor for endoderm, mesoderm, and stomodeum. It divides into separate stem cells for endoderm (E) and mesoderm and stomodeum (MSt). We have no evidence in *C. elegans* for another division generating separate M and St stem cells for mesoderm and stomodeum as seen in other nematodes. The other products of the P_2 and P_3 cell divisions, respectively, are the stem cells for secondary ectoderm (C) and secondary mesoderm (D) (Fig. 2 *lower*). All six stem cells and their daughters divide equally and synchronously a number of times (Fig. 2). However, the offspring of each stem cell divide at a different characteristic rate. It follows that the number of successive equal cell divisions is different for the various stem cell lineages.

Later we see asynchronous and asymmetrical divisions in the C and E lineages. In the C lineage, one member of each of the four daughter pairs of the third division divides later than the other one. Possible differences in size of these cells must be further studied. In the E lineage, the fourth division is asynchronous and the 16 offspring are not all equal in size.

Endogenous Cell Cycle Clocks and Rounds of Cell Division. The cells of a given lineage have their own inherent rhythm of cell divisions, endogenous cell cycle clocks. Cells that descend from the same stem cell divide at the same rate. The cells of the AB lineage have the shortest cell cycle and the cells of the P lineage the longest (Fig. 3).

Related to the autonomy of the cell cycle rates, rounds of cell division can be defined. Each doubling of the cells of the six lineages constitutes a round of cell division. Each round of cell division is initiated by a doubling of the AB descendants, followed by the division of MSt, C, E, and D in that order (Fig. 3). Another consequence is that asynchrony between lineages increases, so that each succeeding round or apparent wave of mitosis across the embryo takes longer to complete. This eventually leads to an overlap of the division rounds; the sixth division round starts before the previous round is complete, as do all later rounds (Fig. 3).

Within a lineage there seem to be time retardations. In the fifth round, the first one involving all the stem cells, the division of P_4 is retarded; presumably, it only divides once more during embryogenesis. The doubling of the E lineage from 8 to 16 cells in the seventh round also occurs much later than expected. Four of these 16 E cells divide once more, giving a total of 20 E cells at hatching (unpublished data).

Stem Cell Position and Waves of Mitosis. In addition to the temporal regularity of cell divisions, another regularity emerges, as a result of the anterior–posterior arrangement of the original stem cells within the egg. A more anterior position corresponds to a shorter cell cycle, and apparent waves of mitosis pass over the cleaving egg from anterior to posterior. These waves are already expressed at the two-cell stage, in which the anterior AB cell divides ahead of the posterior P_1 cell (Fig. 4*a*).

This anterior-posterior sequence of divisions according to position of the original stem cell is maintained when the descendants of a lineage are later displaced from their site of origin. For example, during gastrulation, the E cells move into the interior of the embryo anterior to the C and some MSt cells, yet they maintain their position in the division sequence (Fig. 4*e*). The AB descendants expand to cover almost the entire egg from the anterior to the posterior pole (Fig. 4*f*) but maintain their position in the original division sequence: AB, MSt, C, E, D, P_4.

The more anterior a stem cell is at formation, the faster its division rhythm will be. Therefore, we propose that the region of egg cytoplasm each stem cell receives at its birth is critical to its determination.

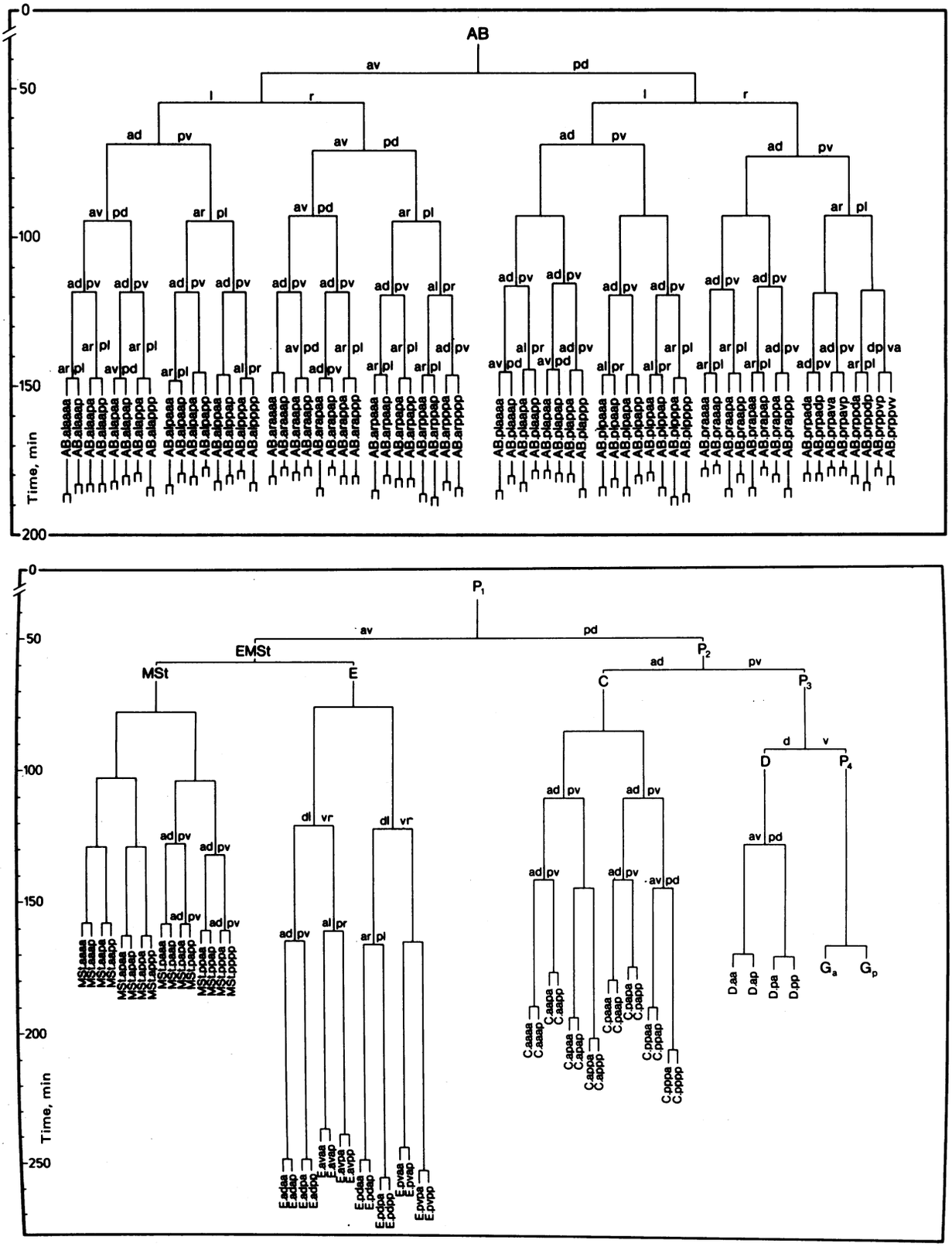

FIG. 2. Cell lineages from 2 to 182 cells. (*Upper*) AB lineage; (*Lower*) MSt, C, E, D, and P lineages. The times shown are for one individual. All or part of the lineage trees were followed in 25 more individuals. Fertilization and egg passage through the spermatheca occur at 0 min. P_0, the zygote (not shown), divides by an anterior-posterior (a-p) division into AB and P_1 35 min after fertilization. Divisions are a-p unless otherwise indicated. Most of the divisions of the 64 AB descendants into 128 are predominantly a-p but the exact directions have not been determined. Nomenclature is adapted from the postembryonic lineage (6). Stem cells are named with upper case letters. We assume that tissue destiny of cell groups will be as in other nematodes with analogous lineages and use the letters introduced by Boveri (7, 8): AB, ectoderm; C, secondary ectoderm; D, secondary mesoderm; E, endoderm; MSt, mesoderm and stomodeum; P_4, primordial germ cell; and G, germline precursor cells. P_1–P_3 are the sisters of the other stem cells. Stem cell daughters are named by adding a period and one lower-case letter per division (6). This

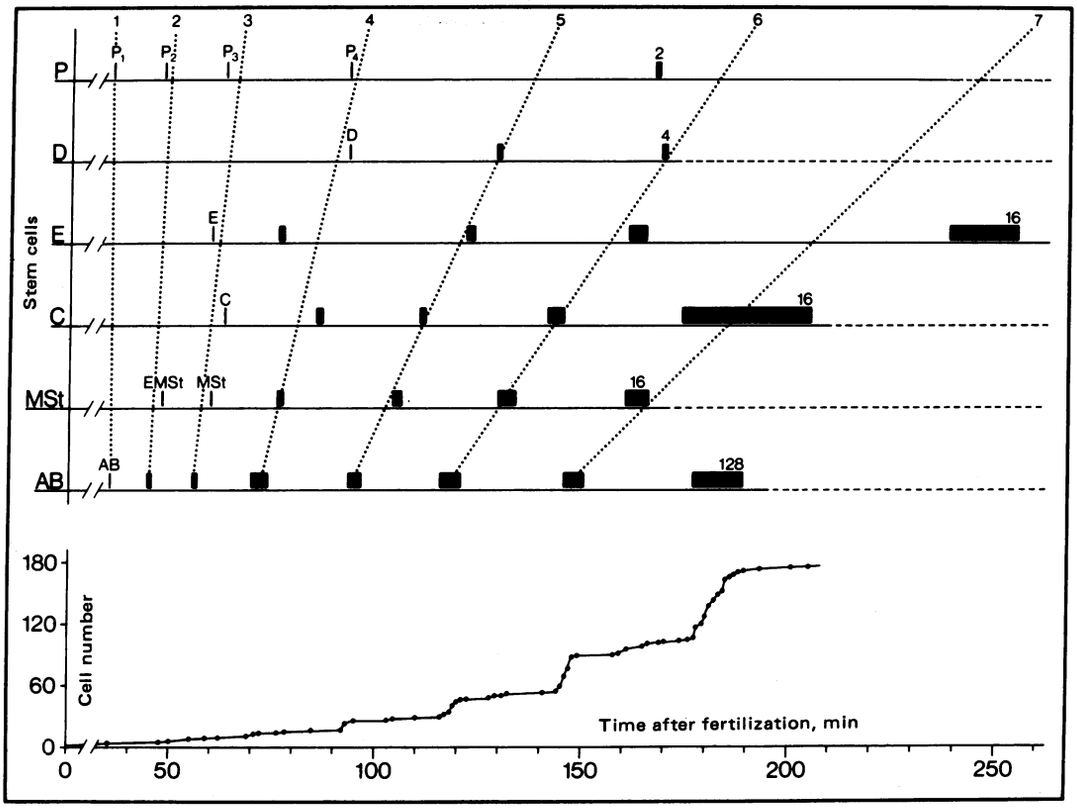

FIG. 3. (*Top*) Cell division times and rounds of cell division. (*Bottom*) Total number of cells. Data are taken from the egg shown in Fig. 2. On each line the cell division events for one stem cell lineage are given. The order from top to bottom is by length of cell cycle. The time when a stem cell arises is indicated by a single line and its name. [The zygote (P_0) is not shown.] Each black box indicates the time from the division of the first to the division of the last cell of a lineage. The number above the last box of each lineage indicates the number of cells of that lineage present after that division. Dotted lines indicate rounds of cell division. Dashed lines indicate lineages that have not yet been followed.

DISCUSSION

The egg of *C. elegans* has several favorable features for observation of the events of embryogenesis—transparency, ability to develop normally outside the parent, small size and cell number, and concomitant rapid development. We have added the advantages of Nomarski interference optics and high-resolution video recording and have so far succeeded in following 182 cells in living embryos. Analysis of this part of embryogenesis reveals several general principles.

The embryonic cell lineages are generally invariant. The major axis direction of every cell division was identical in all individuals. The total development time to a given stage varied by less than 3% from the mean in all individuals studied.

Each of the six stem cell lineages has its own internal clock—i.e., an autonomous rhythm of essentially synchronous cell divisions. The rhythms are maintained in spite of extensive cellular rearrangement. The invariance and the position-independent autonomy of the lineages are consistent with a model in which the destiny of cells depends only on their lineage history. Such a mechanism was also suggested for the postembryonic cell lineages of *C. elegans* (6).

The anteriorness of the site of origin of the stem cells in the egg relates to the setting of the rhythm of the cell cycle, suggesting preexisting organization of the egg. Indeed, the anterior-posterior and dorsal-ventral axes of the embryo are already recognizable at the first division of the zygote (unpublished data). These observations are consistent with a model of intracellular preprogramming. A map of the destiny of the various regions of the egg can therefore be constructed. External positional signals (e.g., gradients) may lead to differential organization of the egg cytoplasm or egg cortex. But the apparent anterior initiation and anterior-to-posterior propagation of the later waves of cell divisions do not require extrinsic positional signaling. Rather, we favor a model involving internal clocks within lineages. The positional values of the cells later on are seen as coincidental, because the cells are already fixed on a program involving timing, independent of position.

The fertilized egg begins development with a period of cleavage division–cell proliferation, with few visible signs of cellular differentiation. An exception is the E lineage, in which the characteristic autofluorescence becomes clearly visible when there are eight E descendants (unpublished data). Each stem cell gives rise to a predetermined number of cells by a series of synchronous and symmetrical divisions. The state of determination is clonally inherited—i.e., passed on to all the offspring of a given stem cell. We already know from preliminary studies on later embryos that differentiation, the final expression of this determination, sets in only after cells withdraw from the proliferative cell cycle. An early sign that a given lineage is approaching its final cell number may be the lengthening of its cell cycle as exemplified by the retardation of the division of the P lineage in the fifth round of cell division and of the E lineage in the seventh round (Fig. 3).

letter represents its position immediately after division relative to its sister cell. Anterior-posterior sisters are designated by "a" and "p," dorsal-ventral by "d" and "v," and left-right by "l" and "r." In oblique divisions, only the predominant axis is indicated. Lineage tree branches are labeled by the same letters; the "a," "d," or "l" sister is always put on the left branch, and two letters are allowed for oblique divisions.

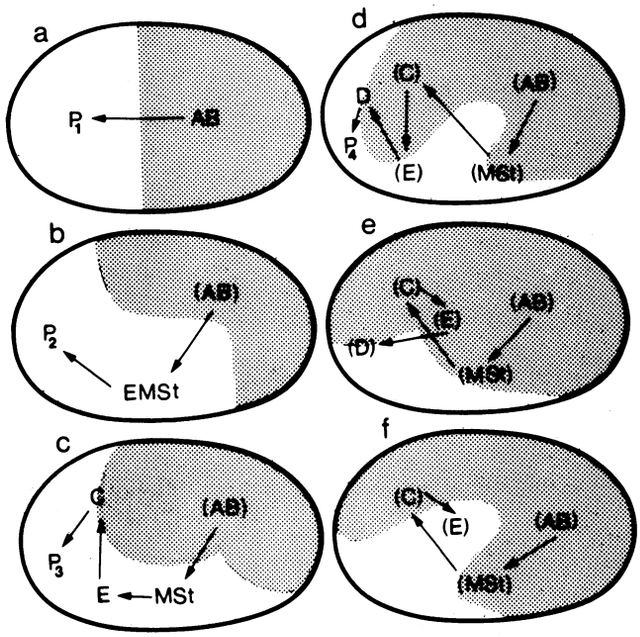

FIG. 4. Sequence of cell divisions projected onto the egg. *a–f* indicate the sequence in the second to the seventh division round, respectively. Eggs are in standard orientation (anterior, right; dorsal, top). Stem cells are named as in Fig. 2; parentheses denote their descendants. The stippled area is the region occupied by the AB descendants (in two-dimensional projection). The E descendants move inside and anterior during gastrulation (between stages *d* and *e*).

It is perhaps worth noting that in *Parascaris equorum*, *Ascaris lumbricoides*, and other nematodes, chromatin diminution and elimination occurs in all cells except the germ line precursors (18–20). If it occurs in *C. elegans*, diminution is not so extensive and is not quantitatively detectable (21).

Analysis becomes increasingly difficult as the cleavage divisions produce progressively smaller cells. But we hope to follow the lineage of selected tissues cellwise and of other tissues at least groupwise to the end of embryogenesis to relate lineages to the eventual tissues and organs and confirm our assignment of stem cell names (i.e., destinies) according to Boveri (7, 8). Classical studies suffered from many uncertainties, particularly in lineage relationships, inevitably encountered when following cells in groups and only in fixed eggs. Thus, very few assignments of cells to particular adult structures have remained unchallenged (7–14). The tracing of individual cells continuously in living embryos removes this uncertainty. In later stages of embryogenesis, when shaping of the organism and structural and functional specialization of cells occur essentially without cell division, ultrastructure is of central importance. These stages can best be studied from electron microscopic series.

As the hatched worm, *C. elegans* is bilaterally quite symmetric. This symmetry is derived by left–right cell divisions and by equivalent cells moving to matched left–right positions. But, in contrast to *Ascaris* (7–10), the early embryo of *C. elegans* has almost no elements of bilateral symmetry. Consistent with this, we find few predominantly left–right divisions (Fig. 2), although some divisions have a left–right component; this minor component is the most difficult axis to assess in our standard orientation. Cellular migrations occur either between or in connection with concomitant cell divisions.

We thank Dr. R. Cassada for many helpful critical discussions. U.D. was supported by an assistantship of the Deutsche Forschungsgemeinschaft, SFB 33. B.Y. received a research fellowship and E.S. a predoctoral fellowship of the Max Planck Society.

1. Brenner, S. (1973) *Br. Med. Bull.* **29**, 269–271.
2. Brenner, S. (1974) *Genetics* **77**, 71–94.
3. von Ehrenstein, G. (1973) *Jahrb. Max Planck Ges.* (München), pp. 38–60.
4. Hirsh, D. & Vanderslice, R. (1976) *Dev. Biol.* **49**, 220–235.
5. Sulston, J. E. (1976) *Philos. Trans. R. Soc. London Ser. B* **275**, 287–297.
6. Sulston, J. E. & Horvitz, H. R. (1977) *Dev. Biol.* **56**, 110–156.
7. Boveri, T, (1893) *Sitzungsber. Ges. Morphol. Physiol.* **8**, 114–125.
8. Boveri, T. (1899) *Festschrift Kupffer* (G. Fischer, Jena), pp. 383–430.
9. Zur Strassen, O. (1896) *Arch. Entwicklungsmech. Org.* **3**, 26–105, and 133–190.
10. Müller, H. (1903) *Zoologica (Stuttgart)* **17**, 1–30.
11. Spemann, H. (1895) *Zool. Jahrb. Abt. Morphol.* **8**, 301–317.
12. Ziegler, H. E. (1895) *Z. Wiss. Zool. Abt. A* **60**, 351–410.
13. Martini, E. (1903) *Z. Wiss. Zool. Abt. A* **74**, 501–556.
14. Pai, S. (1928) *Z. Wiss. Zool. Abt. A* **131**, 293–344.
15. Nigan, V., Guerrier, P. & Monin, H. (1960) *Bull. Biol. Fr. Belg.* **94**, 131–202.
16. Hirsh, D., Oppenheim, D. & Klass, M. (1976) *Dev. Biol.* **49**, 200–219.
17. Byerly, L., Cassada, R. C. & Russell, R. L. (1976) *Dev. Biol.* **51**, 23–33.
18. Boveri, T. (1887) *Anat. Anz.* **12**, 688–693.
19. Tobler, H., Smith, K. D. & Ursprung, H. (1972) *Dev. Biol.* **27**, 190–203.
20. Moritz, K. B. & Roth, G. E. (1976) *Nature* **259**, 55–57.
21. Sulston, J. E. & Brenner, S. (1974) *Genetics* **77**, 95–104.