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Developmental strategies during early embryogenesis of *Caenorhabditis elegans*

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I. INTRODUCTION

How the complex, multicellular structure of an organism is generated from the information contained in the uncleaved egg is a central question in developmental studies.

Nematodes are particularly suitable for studying this question. A unique combination of favourable properties, including transparent eggshell, normal embryogenesis under the microscope outside the mother, small number of cells and rapid, reproducible development made nematodes classic models for developmental biologists (for reviews see Chitwood & Chitwood, 1974; von Ehrenstein & Schierenberg, 1980).

In addition to the attractive features mentioned above, the free-living soil nematode *Caenorhabditis elegans* (Fig. 1) is also well suited for analysis of the genetic control of development (Brenner, 1974) unlike the classically studied parasitic nematode *Parascaris equorum* (*Ascaris megalocephala*).

Recently cellular (e.g. Sulston, Schierenberg, White & Thomson, 1983) and genetic (e.g. Sternberg & Horvitz, 1984) aspects of development have been studied extensively in *C. elegans*.

The pattern of early embryogenesis in *Ascaris* and *C. elegans* appears to be typical for nematodes in general and includes the following features.

(i) Cells are determined (lose totipotency) very early. The first division of the zygote generates two different cells with restricted developmental potential (Boveri, 1899; Deppe *et al.* 1978; Figs 2, 3).

(ii) Development proceeds in an essentially 'mosaic' fashion. The pathway of differentiation is thought to be generally dictated by intrinsic factors, although cases of limited intercellular regulation have been found in later embryos (Stevens, 1909; Boveri, 1910*a*,*b*; zur Strassen, 1959; Laufer, Bazzicalupo & Wood, 1980; Sulston *et al.* 1983).

(iii) From the beginning of embryogenesis a germline is present (see below). The germline is separated early from the soma through a series of unequal cleavages (Boveri, 1899; Figs 2, 3).

Key words: *Caenorhabditis*, nematodes, determination, polarity, prelocalization, symmetry, laser microbeam.

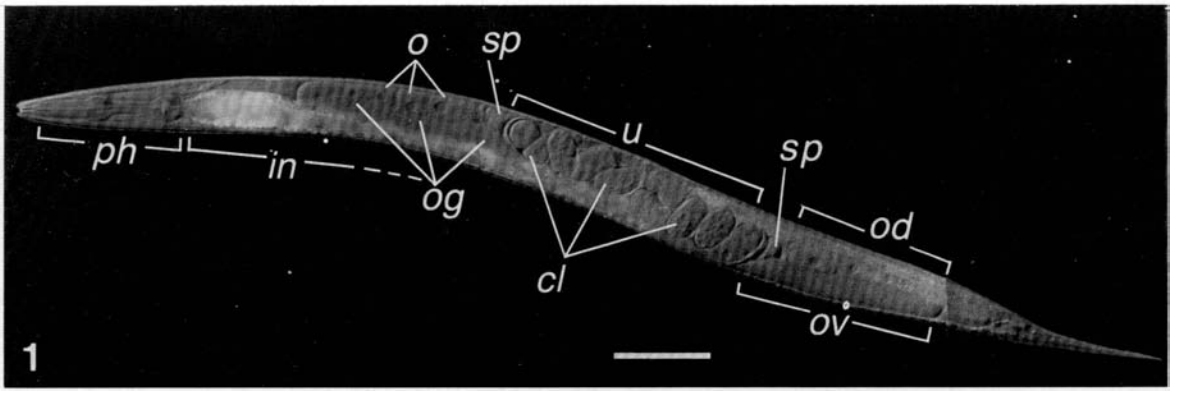

Fig. 1. Young, gravid adult hermaphrodite of *C. elegans*. The gonad consists of two reflexed tubes containing oogonia (*og*) which develop to mature oocytes (*o*). These are fertilized in the spermatheca (*sp*), start embryogenesis in the uterus (*u*) and are laid eventually through the vulva (not visible). *ph*, pharynx; *in*, intestine; *cl*, cleaving eggs; *ov*, ovary; *od*, oviduct. Bar, 100 μm.

In *C. elegans* the early phase of embryogenesis, when the framework for further development is laid down with the sequential formation of five somatic founder cells and the primordial germ cell, takes place within the first hour of cleavage. No indication of newly transcribed mRNA has been detected (as poly(A)$^+$ material) in *C. elegans* during this early period (Hecht, Gossett & Jeffery, 1981). Thus, it is assumed that at least these first important steps of development are controlled by maternal gene products.

Much of our present view of early development, not only in nematodes, is based on the ingenious work by Boveri (e.g. 1899; 1910*a*). Some of his observations and conclusions are therefore briefly summarized below.

Concept of germline and soma

From the two cells generated with the first division of the zygote, one of them (or its immediate daughters) will pass through the process of 'chromatin diminution' (loss of chromatin during mitosis; for a recent review of molecular aspects see Tobler, Müller, Back & Aeby, 1985), while the other will not. This latter cell will again cleave into one daughter, which will lose parts of its chromosomes, and another one which will preserve the full chromatin content. This process is repeated two more times. The result of this peculiar behaviour is that all cells except one carry a reduced amount of chromatin. Boveri interpreted this observation on fixed and stained *Ascaris* eggs as the visible manifestation of a germline (with full chromatin content) from which the somatic cells (with reduced chromatin content) are set aside *via* a series of unequal cleavages. However, even in nematodes, chromatin diminution seems to be an exception rather than the rule. In *C. elegans* it is not detected. Nevertheless the concept of germline and soma appears to apply to all nematodes. The early lineage trees are identical for *C. elegans* and *Ascaris* (Fig. 3).

Concept of cytoplasmic determination of cells

Uncleaved *Ascaris* eggs were allowed to divide while being centrifuged. While most of them developed normally, some produced with their first division two cells of equal size and behaviour. Analysis of subsequent cleavage pattern and chromatin diminution revealed that both cells behaved like a germline cell P_1 (Boveri, 1910*b*). He never observed eggs with two AB-like cells.

Boveri's interpretation of this result was that in those eggs that were oriented with their anterior–posterior axis exactly in the direction of the centrifugal force, the cleavage furrow formed at right angles to its normal position (the normal rotation of the cleavage spindle through 90° is prevented). Assuming that cytoplasmic components form parallel layers from anterior to posterior, both cells would receive identical amounts of each layer.

Screening large quantities of non-manipulated eggs, he occasionally found one that had obviously been fertilized by two sperm. In a simultaneous double-mitosis four cells were generated from the zygote. He discerned three different types of resulting 4-cell stages: (a) 2 AB-like and 2 P_1-like cells, (b) 1 AB-like and 3 P_1-like cells, (c) 3 AB-like and 1 P_1-like cells.

From these (and other supportive) observations he made the following conclusions.

(i) Determination of different cells does not result from differential segregation of chromosomes but depends on differential segregation of cytoplasmic 'quality'. The resultant differences could be either of an absolute or a relative kind.

(ii) Determination is not due to the differential segregation of 'organ-forming regions' but occurs stepwise *via* binary decisions. The critical determinative step is not cell division itself but a process that is normally tightly coupled to it.

(iii) Although development of the *Ascaris* embryo proceeds in a strictly mosaic (cell autonomous) fashion, fragments of any cell (including the uncleaved zygote) are able to regulate and behave like the complete cell. Thus it is not the structure of a cell but the complex of independent cells that represents a mosaic.

(iv) The polar organization of the germline cell is (or at least can be) newly established prior to cleavage.

In the following sections some results from recent work on *C. elegans* are described which can be correlated to Boveri's experiments and which confirm the continuing relevance of his conclusions.

II. ESTABLISHMENT OF POLARITY IN THE EARLY EMBRYO

In any embryo the presence of an appropriate polarity is a basic prerequisite for the generation of different cells. It has been suggested that a basic polarity is transferred from one generation to the next by means of cytoplasmic continuity in the germline (e.g. zur Strassen, 1959; Nieuwkoop, 1977). On the other hand a number of examples has been reported in which axis formation in developing eggs has been affected experimentally (e.g. Ziomek & Johnson, 1980; Black & Gerhart, 1985). This does not preclude the existence of an inherited polarity but indicates that if it exists, it can be overridden by external influences.

No signs of polarity are seen in the oocytes of *C. elegans* under the light microscope, except that the nucleus can be more or less displaced from the centre of the cell (particularly in the most mature oocyte prior to fertilization; Fig. 1). Germline-specific structures ('P-granules'), as identified with fluorescent antibodies, are randomly distributed in the cytoplasm of the oocytes (Strome & Wood, 1982). The egg of *C. elegans* is fertilized at the pole that enters the spermatheca first and that will become the posterior pole of the embryo (Ward & Carrel, 1979). However, it remains unclear whether sperm penetration induces the anterior–posterior (a–p) polarity of the fertilized egg (Albertson, 1984). About half an hour after fertilization the pronuclei appear at opposite poles (Fig. 2A). At that time the egg clearly shows a visible a–p polarity (Fig. 4A): both polar bodies are usually extruded at the anterior pole, a temporary constriction (pseudocleavage) bisects the egg into an anterior region with strong cytoplasmic streaming including formation of pseudopodia and a posterior region which remains as stiff as before fertilization. The oocyte pronucleus migrates posteriorly towards the sperm pronucleus (Fig. 2B) which initially appears to be fixed to the posterior periphery. They meet in the posterior region, migrate together towards the centre, rotate through 90° and fuse to form the zygote (Fig. 2C,D). Immediately the first division starts, generating a larger somatic cell AB and a smaller, posterior germline cell P_1 (Fig. 2E,F). The polarity expressed in the ability to cleave unequally (along the a–p axis) is inherited from one germline cell to the next, while somatic cells (with one early exception, see section V) cleave apparently equally (members of individual cell lineages express synchronous cell cycle periods; Fig. 2; Deppe *et al.* 1978; Schierenberg, 1984*a*).

However, the polar organization of germline cells seems not to be a static feature; rather it is evidently newly established after each unequal cell division, as suggested by Boveri (1910*a*) for *Ascaris*.

One indication that this is also the case in *C. elegans* is the fact that the 'P-granules' gather at one pole only prior to mitosis (see section III) but are distributed all over the cell during interphase. An even stronger argument is the observation that the direction of cleavage polarity is reversed (in the germline cell P_2) as an integral part of normal development (Schierenberg, 1985; unpublished data). Thus P_3 and P_4 are positioned anterior to their somatic sister cells in contrast to P_1 and P_2. This reversal of polarity becomes particularly obvious in the cleavage pattern of posterior fragments of P_0 and P_1 leading to partial 'twins' (Fig. 4B,C).

What could be the developmental significance of this reversal phenomenon? The reversal of polarity may occur in order to preserve the contiguity between the intestinal precursor cell(s) and the germline (otherwise P_3 would take the position of C, see Fig. 2). During gastrulation the two daughters of the primordial germ cell P_4 follow the E-cells and migrate into the centre of the egg (see Schierenberg, 1984*a*). During later embryogenesis the two germ cells send protrusions into the intestinal cells. It has been speculated that the germ cells, which have to execute an extensive postembryonic programme of proliferation, may be nursed by the gut cells (Sulston *et al.* 1983). In fact a visible affinity between intestinal precursors and

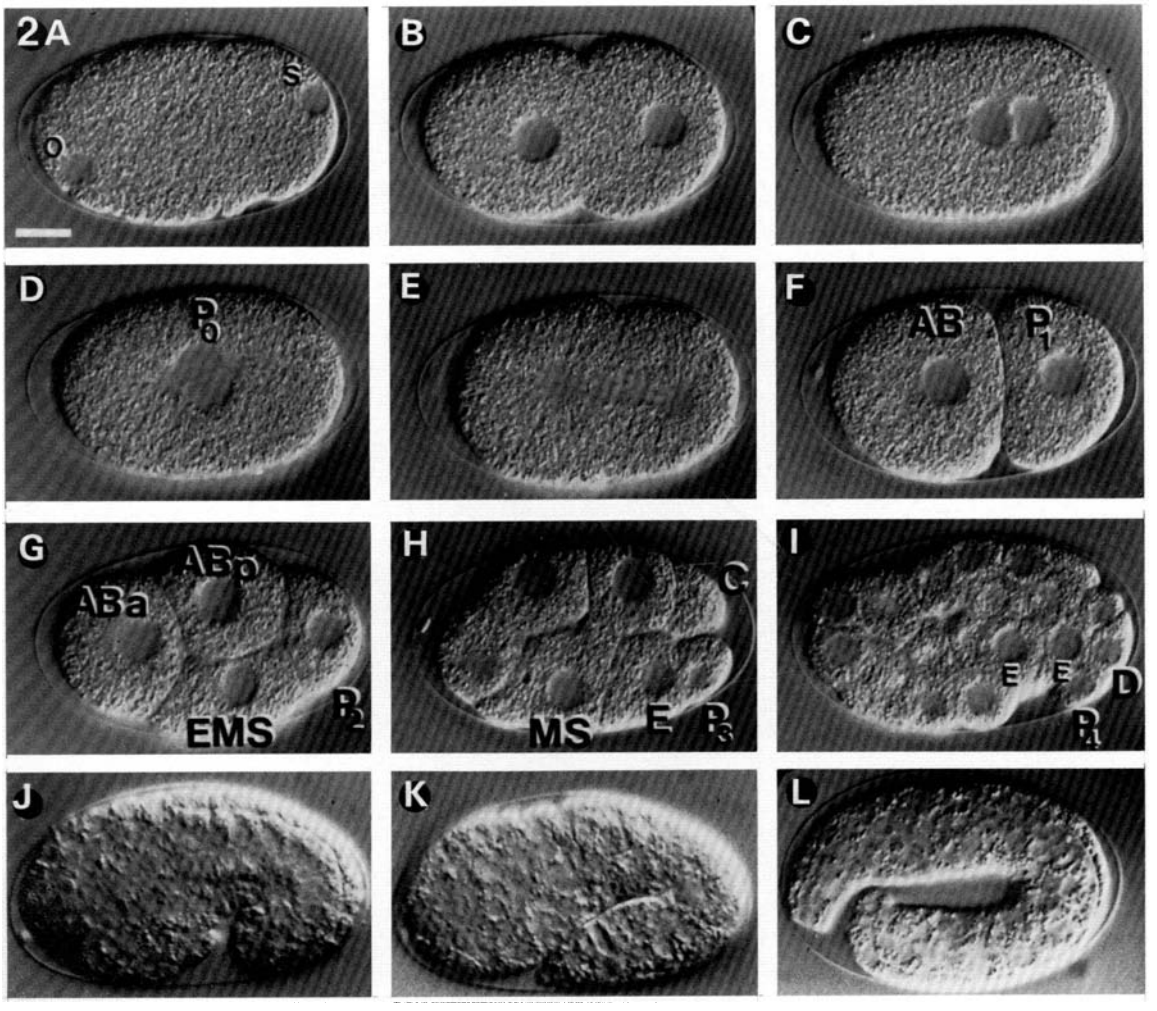

Fig. 2. Embryonic development of *C. elegans*.

(A) About 30 min after fertilization the oocyte (*o*) and sperm (*s*) pronuclei appear at opposite poles.

(B,C) The oocyte pronucleus migrates towards the posterior pole to meet the sperm pronucleus while a temporary 'pseudocleavage' takes place.

(D) After migration back to the centre they rotate and fuse to form the zygote P_0.

(E,F) Immediately afterwards the first cleavage starts to form a larger anterior somatic founder cell AB and a germline cell P_1.

(G) After the division of AB into ABa (anterior) and ABp (posterior), P_1 cleaves unequally into a somatic cell EMS and a new germline cell P_2.

(H) The division of both AB cells and EMS is followed by the unequal cleavage of P_2 into a somatic founder cell C and a new germline cell P_3.

(I) Then the somatic cells divide in the order AB, MS, E and C. Concomitantly with the synchronous duplication of the eight AB cells the last unequal cleavage in the embryonic germline generates the somatic founder cell D and the primordial germ cell P_4. Soon afterwards gastrulation starts with the immigration of the two E-cells (gut precursors).

(J) Several hours later morphogenesis starts with an indentation at the ventral side.

(K,L) Elongation leads eventually to the formation of a worm, which hatches about 12 h after fertilization (at 25 °C). Orientation: anterior, left; dorsal, top. Bar, 10 μm.

germline cells has been observed during early embryogenesis and a specific cell–cell interaction between them can be inferred from the altered development of partial embryos (see section V).

III. STRUCTURAL AND FUNCTIONAL PRELOCALIZATION

In two different ways it could be demonstrated that a prelocalization takes place in the embryo of *C. elegans* prior to first cleavage.

Around the time when the two pronuclei meet (Fig. 2C) the P-granules accumulate in the posterior region of the egg and are thus incorporated only into the germline cell P_1. The alteration from apparently random cytoplasmic distribution to prelocalization at one pole is repeated during each cell cycle of the germline cell until the primordial germ cell P_4 (see Figs 2, 3) is formed. When this cell divides both daughters receive P-granules in approximately equal amounts (Strome & Wood, 1982, 1983; Yamaguchi, Murakami, Furusawa & Miwa, 1983). Thus, the P-granules mark the pathway of the germline in *C. elegans* with the same precision as chromatin nondiminution (see section I) does in *Ascaris*. Besides this structural prelocalization a functional prelocalization has also been concluded from the following kind of experiments.

Using a laser microbeam coupled to a microscope (Schierenberg, 1984) portions of the egg can be removed (Laufer & von Ehrenstein, 1981; Schierenberg & Wood, 1985). If more than approximately 25% of the cytoplasm (including

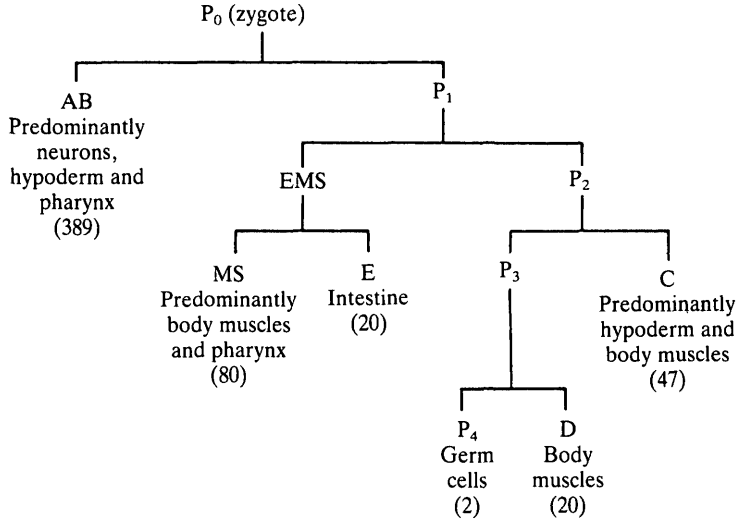

Fig. 3. Schematic early cell lineage tree of *C. elegans*. A series of unequal cleavages in the germline results in the formation of five somatic founder cells (AB, MS, E, C, D) and the primordial germ cell P_4. Each founder cell constitutes a cell lineage. A cell anterior relative to its sister is placed on the left arm of a lineage branch. The tissues to which each cell lineage contributes predominantly (AB, MS, C) or exclusively (E, D, P_4) are indicated below cell names. The numbers of surviving cells produced in each cell lineage during embryogenesis are given in parenthesis (adapted from Schierenberg, 1984*b*).

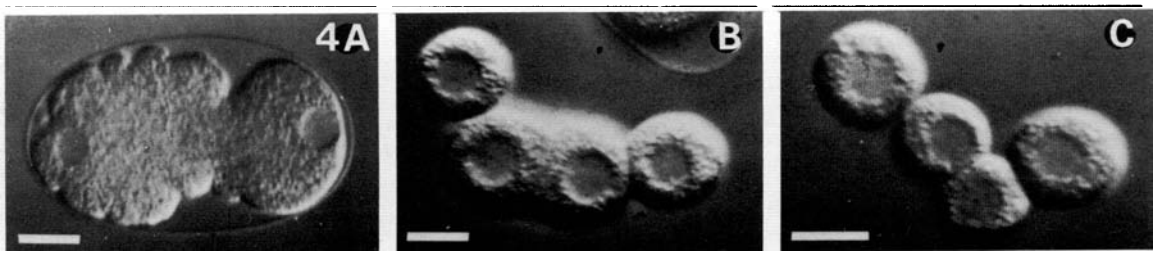

Fig. 4. Polarity in *C. elegans* embryos.

(A) 1-cell embryo. Prezygote stage. Oocyte pronucleus (left) has just started to migrate towards posterior. Pseudocleavage separates ruffled anterior from smooth posterior region. Orientation: anterior, left.

(B) Development of a posterior fragment of P_0. Its first division generated two cells of equal size and with the cleavage behaviour of P_1. Their unequal cleavages generate a 4-cell complex in which the partial twins are attached with their larger, EMS-like, cells.

(C) Development of a posterior fragment of P_1. Its first division generated two cells of equal size and the cleavage behaviour of P_2. Their unequal cleavages generate a 4-cell complex in which the partial twins are attached with their smaller, P_3-like, cells. Bars, 10 μm.

surrounding plasma membrane) is removed from the posterior pole of a 1-cell embryo it loses its potential to perform the typical stem-cell-like cleavages of a germline cell. Instead, like a somatic founder cell, it passes through a series of equal divisions. The removal of more than twice as much cytoplasm from the anterior pole does not interfere with the normal pattern of early cleavages. A small portion of cytoplasm extruded prior to pronuclear migration together with the male pronucleus and its centrioles from the posterior pole is able to cleave like a complete zygote (at least until the 24-cell stage), while even as much as two-thirds of total cytoplasm together with both pronuclei or the zygote nucleus extruded from the anterior pole of a 1-cell embryo can only perform equal (AB-like) cleavages (Schierenberg, 1985; unpublished data).

These experiments allow several conclusions. First, it becomes clear that the potential for germline division behaviour involves a non-nuclear quality which is localized in the posterior region of the 1-cell embryo prior to pronuclear migration.

Second, with regard to Boveri's view of intracellular regulation, a portion of a germline cell can only behave like the complete cell if it carries that critical region.

Third, the localization of germline-specific division potential at the posterior pole precedes the migration of germline-specific granules to that pole. Thus, P-granules are obviously not causally involved in determination of early germline-specific cell behaviour.

So far, only the early development of embryos derived from a zygote fragment has been observed in detail. Usually no hatching worm develops (for exceptions, see Laufer & von Ehrenstein, 1981). One reason for this is abnormal cell positioning. Preliminary analysis has given no indication that any specific developmental potential is lost if a major portion of cytoplasm is removed at the anterior

pole of the zygote (Schierenberg, unpublished data). This is consistent with Boveri's inference that no 'organ-forming regions' are prelocalized.

IV. SEGREGATION OF DEVELOPMENTAL POTENTIAL AND DETERMINATION OF CELLS

As a result of unequal cleavages in the germline, somatic founder cells with different developmental potential are generated (Figs 2, 3; for details, see Sulston *et al.* 1983). Three aspects will be considered here in this context.

(A) *The role of the cell cycle*

Laufer *et al.* (1980) demonstrated that cell division is not a necessary prerequisite for cell-specific differentiation in *C. elegans*. For instance, the typical birefringence of differentiated gut cells can be observed in the P_1-cell (which is a precursor of the founder cell for the gut, see Fig. 3) of a cleavage-blocked 2-cell stage. However, if different potentials are included in a single cell, e.g. muscle and gut, they seem to exclude each other and only one or the other is expressed (Cowan & McIntosh, 1985). The results of some recent experiments of mine support Boveri's notion that cellular determination involves a process which is not cell division itself but is normally intimately connected to it.

One centrosome was removed from the anterior pole of a zygote after the onset of first mitosis. In this way cell division was uncoupled from the continuing cell cycle. The first cleavage was delayed until another centriole had been synthesized during the following cell cycle. Consequently, the reversal of polarity in the germline (see section II) occurred too early with respect to the number of cells present (4 instead of 8) but at the correct time with respect to the number of already performed cell cycles (Fig. 5B). Based on this and other observations it has been hypothesized that the early phase of cellular determination may involve primarily a binary segregation of germline *versus* soma. According to this model, which somatic founder cell is actually generated at a certain division depends on the number of cell cycles the germline cell has already passed through (Schierenberg, 1985).

(B) *The role of the cleavage plane*

The generation of an organism not only requires specific functional differentiation of cells, but also their specific positioning. One strategy to reduce the complexity of this task is the formation of bilateral symmetry. The hatched juvenile of *C. elegans* expresses a high degree of bilateral symmetry. For the most part this is laid down during early embryogenesis within individual cell lineages (Sulston *et al.* 1983; Schierenberg, Carlson & Sidio, 1984). In the case of the somatic founder cells MS, C and D the first division generates two daughters, one of which will produce all structures of this lineage in the left half of the body and the other essentially the same cells (in a mirror-image pattern) in the right half. In *C. elegans* the plane of symmetry that separates left from right cells is initially not

identical to the long axis of the egg. This is approached stepwise with increasing cell number (Fig. 6).

The analysis of early development suggests that here only cleavages with anterior–posterior orientation of the cleavage spindle (like those in the germline) can generate cells of completely different potential. In contrast, only transverse

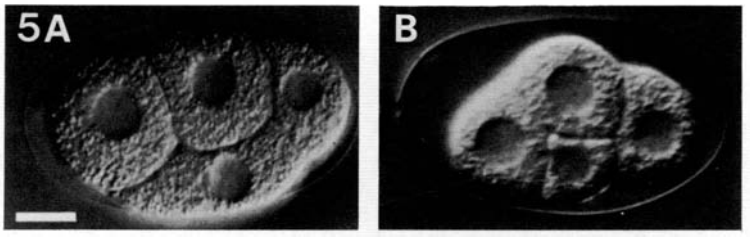

Fig. 5. Cell cycle and cell determination.
(A) Normal 4-cell stage. Small germline cell lies posterior to its sister.
(B) 4-cell stage after one centrosome had been removed from the zygote. Small germline cell lies anterior to its sister. Orientation: anterior, left; dorsal, top. Bar, 10 μm.

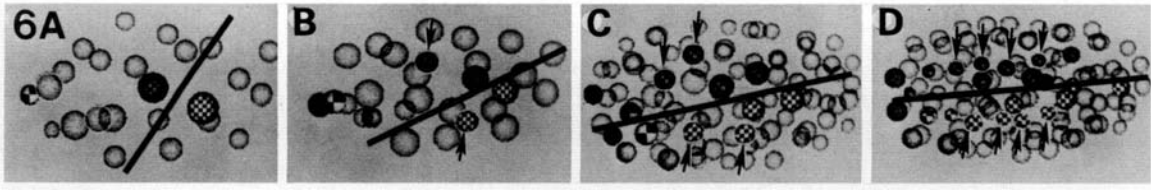

Fig. 6. Formation of bilateral symmetry. Computer reconstruction from living embryos. Ventral view. 24- (A), 28- (B), 87- (C), 102- (D) cell stages. Descendants of MS are marked with checkered patterns. MSa (light checkered) is the precursor for MS cells in the left half of the embryo. MSp (dark checkered) is the precursor for MS cells in the right half of the embryo (A). MSap, MSpp and their descendants are marked with arrows in addition (B–D). With increasing cell number the plane of symmetry (marked with a black line) separating left from right MS cells rotates towards the a–p axis of the egg (adapted from Schierenberg, 1984*b*).

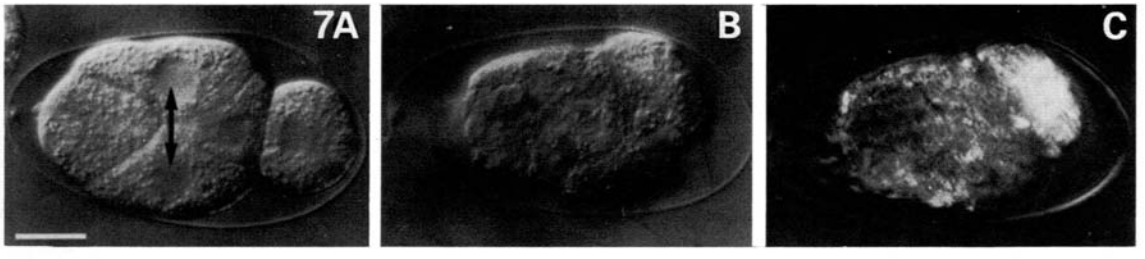

Fig. 7. Development after alteration of cleavage plane. Transverse division of EMS (arrows) after partial removal of AB (A). Terminal phenotype after development overnight (B) and corresponding pattern of autofluorescence (C). Orientation: anterior, left. Bar, 10 μm.

cleavages can produce cells of the same potential (e.g. those of MS, C and D) establishing bilateral symmetry.

In order to substantiate this inference, a cell was manipulated such that it cleaved perpendicular to the normal plane. After removal of part of the AB cell, EMS divided transversely rather than longitudinally (Fig. 7A). In several, but not all, cases the two daughters of EMS expressed a similar cleavage pattern and their descendants formed bilaterally symmetric cell groups on each side of the embryo. The widely spread autofluorescence (typical for the descendants of E forming the gut and equivalent to the birefringence described above) found later in the embryo (Fig. 7C) is consistent with the view that both descendants of EMS received developmental potential that is normally restricted to the progeny of E.

The pattern duplication found in fragments of germline cells (Fig. 4C,D) can also be interpreted analogously. Thus, in these cases the germline cells appear to follow a cleavage pattern that is typical for somatic cells as a strategy to form bilateral symmetry.

(C) *The developmental capacity of blastomere nuclei*

All data presented so far support the view that it is the quality of the cytoplasm (including plasma membrane) that very early shunts blastomeres into one or the other specific developmental pathway. Does this process of early cell determination include the nucleus?

To investigate this, cytoplasm of a 1-cell embryo was extruded from the anterior pole together with the zygote nucleus. The anterior fragment cleaved like an AB cell (see also section III). After two divisions of that fragment one of the newborn nuclei was allowed to slip back into the posterior, anucleate part of the zygote, which had remained in the eggshell. In this way, a new cell was formed which cleaved like a P_1 cell (Schierenberg, 1985) and was able to express markers of differentiation typical for P_1-descendants (e.g. muscle twitching). Thus, during the first two cell cycles (in an AB-like cytoplasmic environment) the nucleus did not lose its capacity to promote P_1-like development. This suggests that at least early blastomere nuclei remain totipotent in *C. elegans* and that the observed developmental restrictions of cells are induced (and maintained) by non-nuclear influences. The possibility remains that in this respect nematodes may not fundamentally differ from those systems in which transplantation of nuclei from differentiated cells revealed their capacity to promote development of a complete organism (Gurdon, 1974).

V. MOSAIC DEVELOPMENT AND EARLY CELL–CELL INTERACTION

Determination of cell fate in nematode embryos is believed to proceed in a cell-autonomous way (Boveri, 1910*a*; Laufer *et al.* 1980). However, this rule appears to have at least one exception (as do other rules during embryogenesis of *C. elegans*, see Sulston *et al.* 1983).

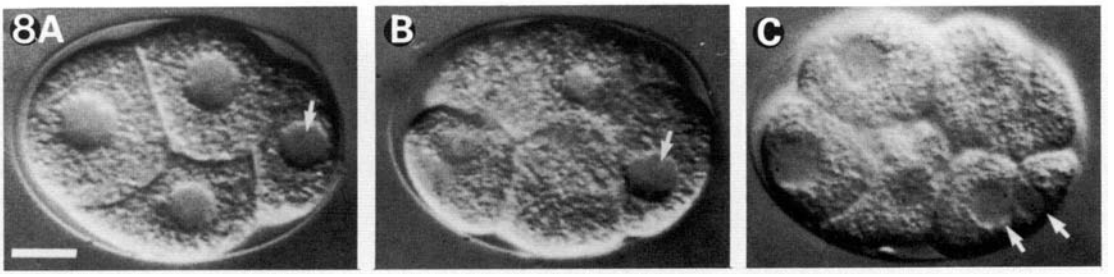

Fig. 8. Affinity between germline cell and gut precursor.
(A) 4-cell stage. Nucleus of P_2 close to posterior periphery (arrow).
(B) 6-cell stage. Nucleus of P_2 has been translocated (arrow).
(C) 8-cell stage. Nuclei of E and P_3 lie adjacent to each other (arrows). Orientation: anterior, left; dorsal, top. Bar, 10 μm.

In the 4-cell embryo the nucleus of P_2 is translocated from the posterior region of the cell (Fig. 8A) to the anterior–ventral periphery (Fig. 8B). After the division of EMS and P_2 the nuclei of E and P_3 lie eccentrically and side by side separated only by the cell membrane (Fig. 8C).

However, if the P_2 cell has been extruded at the 4-cell stage, the nucleus of E stays in the centre of the cell. Subsequently the cell cycle periods in the E cell lineage are similar (or even identical) to those of the MS lineage (Schierenberg, unpublished data), in contrast to normal development, where the E cells express considerably longer cell cycle periods (see Schierenberg & Wood, 1985). Also more than the normal 20 E descendants are produced when P_2 is extruded.

From all this it is concluded that a specific interaction takes place between P_2 and EMS. This interaction can be interpreted as a direct consequence of the polarity reversal described above (section II). The posterior region of the uncleaved egg exerts an attractive force. This causes the sperm pronucleus to remain there while the oocyte pronucleus migrates towards it (Fig. 4A) and further causes the P-granules to be prelocalized. Also in P_1 and P_2, the nuclei stay for most of the cell cycle close to the posterior periphery. After reversal of polarity the attractive force apparently arises from the region of P_2 adjacent to EMS. Both blastomeres cleave unequally into cells of different developmental potential. The nuclei of E and P_3 both react visibly to the attraction by adopting their peripheral location (Fig. 8C). In this way the unique behaviour of EMS, which is the only somatic sister of a germline cell that does not represent a somatic founder cell, but passes through an unequal cleavage to generate two of them (see Fig. 3), is explained.

VI. CLOSING REMARKS

Cell determination in early embryos of *C. elegans* obviously does not involve zygotic gene expression but rather appears to depend on when and how cytoplasmic segregation takes place. The case of cell–cell interaction discussed above may represent a paradigm of how secondary specification ('fine tuning') can occur.

It is consistent with the notion that here a permissive signal allows a cell to express one of its inherent options (see Holtzer, 1978).

The inability of many nematode cells to compensate for ablated cells (see Sulston *et al.* 1983) does not give any information about whether cells need (permissive or instructive) clues from outside to enter a specific developmental pathway. A detailed analysis of the developmental capacity of isolated early blastomeres is still lacking. With the complete (invariable) cell lineage in hand (Sulston *et al.* 1983) it should be possible now to do this. In the end it may well turn out that the general rules underlying 'mosaic' development in nematodes do not differ from those active in 'regulative' eggs. Determination of cells is a multi-dimensional process. Depending on the direction of division, either two cells of the same or of different subsequent cleavage behaviour arise. In addition time appears to be a critical parameter counted in numbers of cell cycles. Newport & Kirschner (1982) suggested a model for the onset of transcription in *Xenopus*, according to which a cytoplasmic factor is titrated against the DNA. It remains to be investigated whether a similar mechanism is involved in the determination of embryonic cells of *C. elegans*.

In his experiments Boveri could obtain *Ascaris* eggs with two cells which both behaved like a germline cell P_1 (no chromatin diminution). In contrast, King & Beams (1938) achieved diminution of *all* chromosomes after a longer period of strong centrifugation (preventing cleavage). The same result was reported recently by Moritz & Bauer (1984) to occur in *Ascaris* embryos developing under pressure. To explain their findings they postulate a 'presomatic activation' from the anterior pole which reaches all chromosomes and causes their diminution if no cell division takes place in time.

In section IV I have described an experiment in which a centrosome was removed from the zygote preventing cleavage for one cell cycle. Consequently reversal of polarity occurred too early with respect to cell number. Preliminary results (Schierenberg, unpublished data) indicate that this experiment can result in the complete failure of the egg to perform any germline-like cleavages (thus lacking a visible germline, like the manipulated *Ascaris* eggs mentioned above), provided the experiment is performed *before* fusion of the pronuclei.

This suggests that here also time is an important factor. The process(es) that prevent(s) the establishment of a germline may act only during a specific phase of the cell cycle.

Even for the case of the 'simple' pattern of nematode development we are only beginning to understand (or even identify) strategies involved in the determination of cells. Nevertheless, our detailed knowledge of cell lineages and the available techniques allow us to ask new questions and to continue to search for general rules. 'The task is to use macroscopic clues . . . to help guess the logical or formal structure of the decision processes – the epigenetic code – then, with the macroscopic functional description in hand to find the circuitry. Without the formal structure to provide the functional description . . . it will be very difficult to find or make sense of the molecular machinery' (Kauffman, 1975).

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