Cell polarity in early *C. elegans* development

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SUMMARY

The polarization of the embryonic axes is a key event in embryogenesis, being one of the earliest manifestations of the shape and form of the organism. The acquisition of polarity by individual blastomeres is one of the earliest indicators of commitment to a particular pathway of differentiation. These phenomena have been studied in the development of *C. elegans* both at the cellular and organismal level. This review summarizes what is known about how polarity is established in the blastomeres of this organism, how the division axes of polarized cells are determined, and how the embryonic axes are set up.

Key words: *C. elegans*, cell polarity, blastomere, embryonic axis formation

INTRODUCTION

Most animals have a fairly asymmetric body form. The anteroposterior axis is generally the most obviously polar, exhibiting the highest degree of asymmetry, the dorsoventral axis is generally intermediate in this respect and the left-right axis is often superficially symmetrical, although it is rarely so in detail. On the other hand the fertilized egg has little obvious symmetry.

Many differentiated cells exhibit an obvious polarity; epithelial cells have clearly defined apical and basal domains and many neurons have spatially separated axonal and dendritic regions. The cells of an embryo in the early stages of development are not so obviously polar, yet many exhibit polarity in that they divide asymmetrically, giving rise to daughters that differ in their states of determination and often also in size. The question can then be posed: is the polarity of the organism a consequence of the combined polarities of all the asymmetric divisions during embryogenesis, or is a co-ordinate system set up in the early embryo which acts to specify the polarities of embryonic cell divisions? Polarity in the developing embryo of the nematode *Caenorhabditis elegans* has been studied both at the organismal and individual blastomere level by a variety of means including embryonic manipulations, cell biological characterization, and genetic analysis.

In this review we discuss experiments that address polarity in *C. elegans* blastomeres. The first section discusses how the embryonic axes are established and the state of axial polarity in early embryonic cells. In the second section we discuss a cell interaction that specifies polarity in the responding cell. The third section reviews the case in *C. elegans* for which most is known about the acquisition of cell polarity: the establishment of polarity in the zygote. Lastly, we discuss how cell division axes are set up in polarized cells.

EARLY *C. ELEGANS* DEVELOPMENT

Early development in *C. elegans* is shown in Figs 1 and 2 (see Wood, 1988 for review). The sperm enters at the future posterior end of the embryo, opposite the end where the oocyte pronucleus resides. The oocyte pronucleus, which was arrested in meiotic prophase, resumes meiosis and both polar bodies are extruded. After meiosis, contractions of the anterior membrane occur and a transient pseudocleavage furrow forms near the center of the egg. During this time the oocyte pronucleus migrates posteriorly toward the sperm pronucleus. The sperm pronucleus also migrates somewhat toward the oocyte pronucleus, and the two meet in the posterior half of the egg. The pronuclei break down and first cleavage divides the egg into a larger anterior cell (AB) and a smaller posterior cell (P$_1$). This cleavage is unequal because of the posterior placement of the mitotic spindle. AB then divides with the spindle initially set up orthogonal to the anteroposterior axis. During anaphase B, the elongating spindle skews towards the anteroposterior axis so that one daughter, ABa, is positioned anterior to the other, ABp. A few minutes later, P$_1$ divides along the anteroposterior axis to form a larger anterior cell (EMS) and a smaller posterior cell (P$_2$). Divisions in the AB lineage continue along axes orthogonal to the preceding division axes, while divisions of the P$_1$ progeny tend to place them in an anterior-posterior line, though constraints of the eggshell skew them somewhat off this axis. Gastrulation begins at the 28-cell stage; Ea and Ep are the first cells to migrate into the center of the embryo. By the time gastrulation ends approximately 550 cells have been generated and most embryonic cell divisions are complete. The embryo elongates inside the eggshell until it is folded in three. During this period of elongation, muscle cells begin to function and the embryo begins to twitch. At approximately 12 hours after fertilization, the L1 larva hatches from the eggshell.
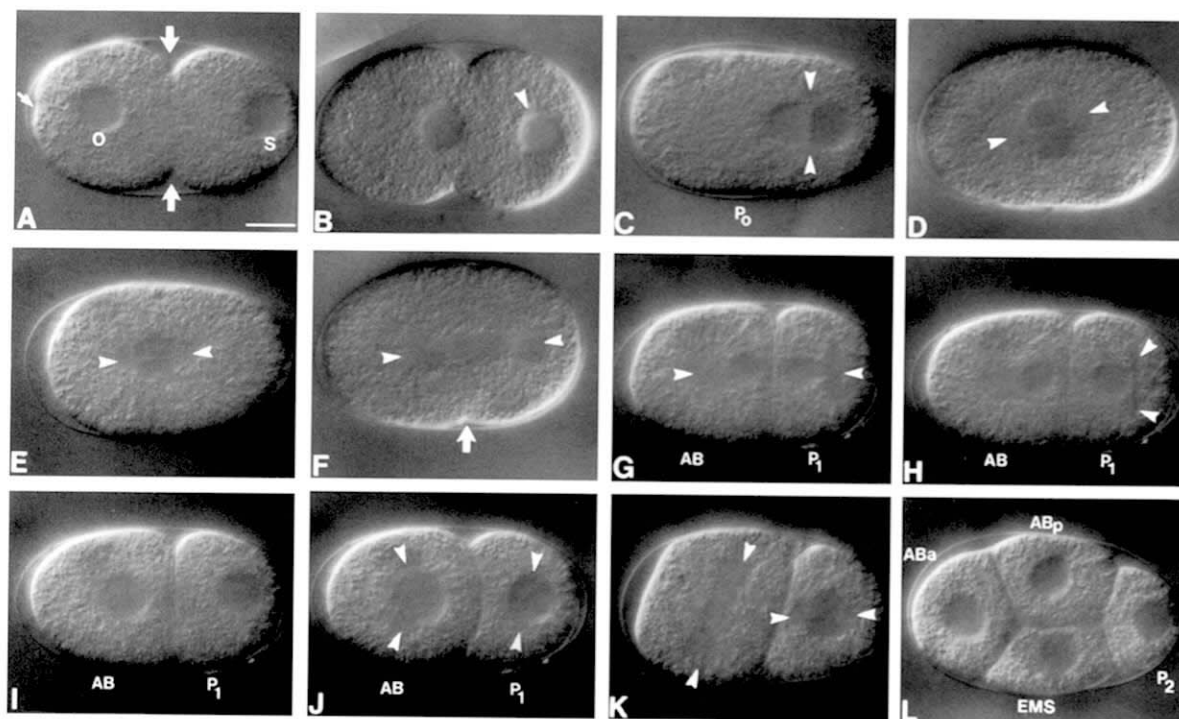
Fig. 1. Development from pseudocleavage through the four-cell stage, showing the positions of centrosomes (arrowheads) at each stage. The centrosome/nucleus complex rotates through 90° in P_0 (C and D) and in P_1 (J and K). In A, "O" marks the oocyte pronucleus, "S" the sperm pronucleus, pseudocleavage furrows are marked by large arrows, and the small arrow marks a membrane invagination. Large arrow in F marks the forming first cleavage furrow. Anterior is to the left.
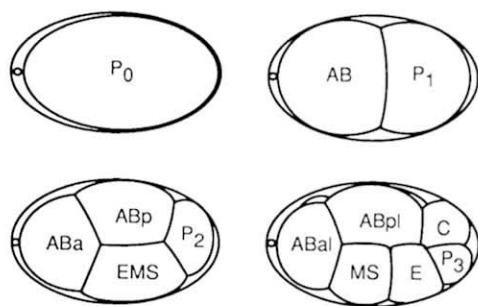

Fig. 2. Names of the blastomeres through the eight-cell stage. In the eight-cell stage embryo, ABar is the cell behind ABal, and ABpr is the cell behind ABpl. Anterior is to the left.

Segregation of fate

Unlike many other animals, in *C. elegans* early fate segregation does not establish the three germ layers (ectoderm, mesoderm and endoderm). Most tissues are assembled from bits of several distinct lineages. Up to the final cell division there are still mother cells that give rise to two daughters whose fates are normally associated with differing germ layers. The lineage is not completely chaotic though: the ectoderm forms largely from progeny of AB, Cpl and Cpr, the mesoderm forms largely from progeny of MS, D, Cal, and Car, the endoderm forms completely from the progeny E, and the germline from P_4 (Fig. 3).

CELL POLARITY AND ESTABLISHMENT OF THE EMBRYONIC AXES

All three embryonic axes in *C. elegans* are established by the six-cell stage. For each axis, evidence is reviewed on how the axis is established, and whether blastomere polarity might play a role in determining the axis.

The anteroposterior axis

Anteroposterior asymmetries are evident soon after fertilization (Fig. 4); they include the ruffling of the anterior membrane, the polarized flows of cortical and cytoplasmic material (Nigon, 1960; Hird and White, 1993) and the localization of P granules at pseudocleavage (Strome and Wood, 1982, 1983). At first cleavage, the asymmetries include the posterior positioning of the mitotic apparatus, and the difference in the size and shape of the two asters (Albertson, 1984), which has been shown to be position-dependent and not due to intrinsic differences between the asters (Hyman and White, 1987). The cell biology of the events that establish polarity in the zygote will be discussed in a later section. Here we discuss the anteroposterior asymmetries apparent in the egg at the time of fertilization, as candidates for the initial cue for establishing anterior-posterior polarity.

Some minutes before each oocyte passes into the spermatheca, the maternal nucleus moves to the end of the oocyte opposite the end that will first enter the spermatheca. The end near the oocyte nucleus will become the anterior end of the embryo. The polar bodies, which are a useful
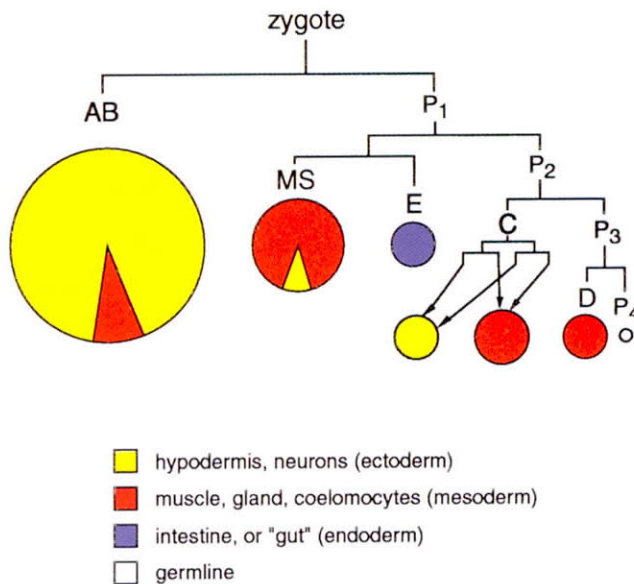
zygote

hypodermis, neurons (ectoderm)

muscle, gland, coelomocytes (mesoderm)

intestine, or "gut" (endoderm)

germline

Fig. 3. Segregation of fate in *C. elegans* (after Sulston et al., 1983).

Fig. 4. Establishment of asymmetry in the zygote ("P_0"). The zygote is shown just after fertilization and during the end of pseudocleavage. P granules are shown in blue, cortical actin in orange. ooc, oocyte pronucleus; sp, sperm pronucleus, with associated centrosomes.

mark of the place where the oocyte nucleus underwent meiosis, are normally found at this end of the embryo following fertilization, but occasionally can be found at the posterior end. The finding that the location of the oocyte nucleus at the time of meiosis can vary with respect to the future anterior-posterior axis suggests it may not act as a cue for determining the anterior-posterior axis.

As the oocyte enters the spermatheca, a sperm enters at the end opposite the maternal pronucleus. Experimentally altering the position of the mitotic apparatus in early blastomeres can generate cortical and cytoplasmic flows similar to those of pseudocleavage, whose directions are predicted by the position of the mitotic apparatus (Hird and White, 1993). This finding raises the possibility that the sperm asters (analogous to the mitotic apparatus) could direct the flows seen during pseudocleavage, and by doing so may establish the anteroposterior axis (Hird and White, 1993). However, the sperm entry point becomes anterior rather than posterior in some other nematodes, such as *Turbatrix aceti* (Pai, 1928) and *Panagrellus redivivus* (Goldstein, unpublished data).

Another possible cue for establishing the anteroposterior axis could be a pre-existing asymmetry in the cytoskeleton or of determinants in the unfertilized oocyte. However no asymmetric distributions of materials have been found in the oocyte by ultrastructural or molecular data.

Reversal of anteroposterior polarity in the germ line precursor P_2

Early cleavages in nematodes follow a stem cell-like pattern. The zygote (P_0) cleaves unequally, forming a larger anterior daughter (AB) which contributes to the somatic tissues, and a smaller posterior daughter (P_1) which continues to cleave in a stem cell-like pattern. P_1 reiterates this pattern, dividing to form a larger anterior daughter (EMS) which contributes to somatic tissues, and a smaller posterior daughter (P_2)
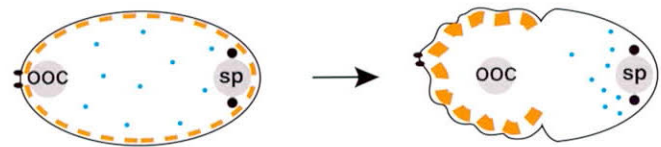
which continues to divide as a stem cell. Stem cell-like divisions continue for two more cycles, however their polarity varies between different nematode species: in some nematodes the smaller stem cell-like daughter continues to form posteriorly, making the germ line founder cell (P_4) the posterior-most daughter. In *C. elegans* and some other nematodes, a reversal of polarity takes place such that P_3 and P_4 come to lie anterior to their somatic sister cells. Schierenberg (1987) has shown that a posterior fragment of P_0 or P_1 retains the ability to divide in a stem-cell like pattern. However this ability moves out of the posterior region of P_2. If a posterior fragment of P_2 containing the nucleus and centrosomes is extruded early in the cell cycle, the fragment continues to divide as a stem cell, whereas if it is taken late in the P_2 cell cycle, it divides equally. The result suggests that there is a site in P_2 responsible for germline polarity, which changes position midway through P_2's cell cycle, placing the stem cell-like daughter to the anterior in the cleavages that follow.

The dorsoventral axis

The daughters of AB, ABa and ABp, give rise to distinct lineages. In an experiment designed to test whether the ABa and ABp blastomeres are initially equivalent, Priess and Thomson (1987) switched the positions of ABa and ABp by exerting pressure on one side of AB as it divided. If ABa and ABp fates were determined prior to the division of AB, then this operation would have caused inappropriate lineages to be executed in that part of the embryo. However, a normal worm developed, demonstrating that ABa and ABp are equivalent cells, in that each has the potential to differentiate as the other normally does. The subsequent differences in their fates must come from cell interactions.

The experiment also altered the dorsoventral axis of the four cell stage (Fig. 5). After the manipulation the EMS blastomere was on the side that would have become dorsal if unmanipulated, and a daughter of AB was on the side that would have become ventral if unmanipulated. This reversed the dorsoventral axis of the developing worm but gave an otherwise normal worm. Hence the differences between blastomeres along the dorsoventral axis are established by the four-cell stage.

This experiment provides evidence on the determination of dorsoventral polarity within each blastomere: each of the four blastomeres probably was not rotated 180° by the manipulation, yet the embryo's axis reversed and a normal worm developed, suggesting that dorsoventral polarity at this stage is a function only of the blastomeres' positions and not of a polarity within each of the cells. This point may
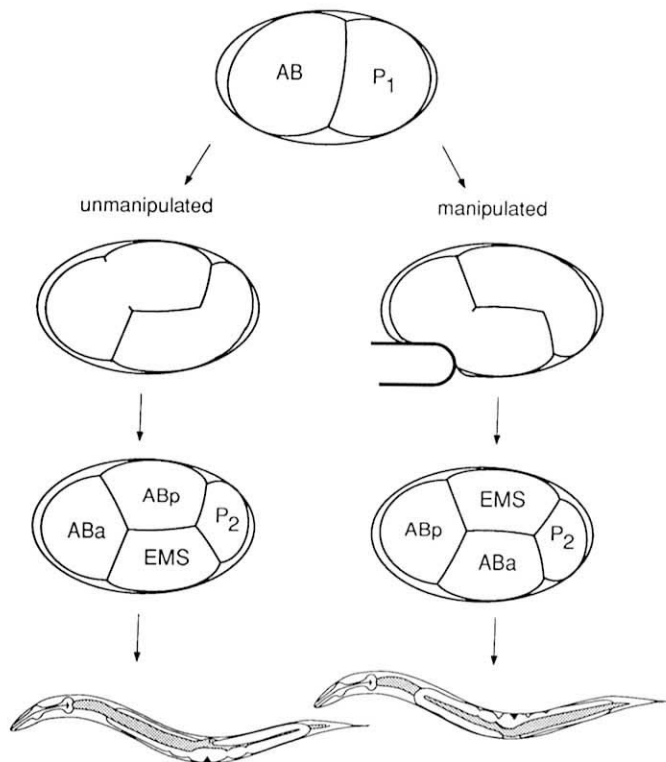
Both form normal, identical worms whose dorso-ventral axis is predicted by the configuration of the four-cell stage.

Fig. 5. Experiment demonstrating that ABa and ABp are developmentally equivalent, and that altering the positions of cells at the four-cell stage alters dorsoventral polarity. Anterior is to the left. The manipulation switched the positions of ABa and ABp and also generated a four cell stage that was "upside-down" (note for example the position of EMS). A normal worm developed. Note also that the manipulation reverses the left-right axis, yet a normal worm develops (not a left-handed worm), indicating that left-right polarity is not yet determined at the four-cell stage (after Priess and Thomson, 1987).

normal worm   "left-handed" worm

Fig. 6. Experiment demonstrating that left-right asymmetries are established via the skewing of ABa and ABp division axes and that ABa's and ABp's left side progeny are equivalent to their right side progeny. Embryos are viewed from their dorsal sides (hence EMS is hidden) with anterior to the left. Note for example the positions of the gut (grey) and gonad (white) in the anterior half of the worm. In the unmanipulated worm the gut is to the left of the gonad; in the manipulated worm the gonad is to the left (after Wood, 1991).

be illustrated by contrasting this case with an animal in which a functional polarity has been shown to exist in early blastomeres. In the starfish *Asterina pectinifera*, rotating one of the blastomeres of the two-cell stage 90° or 180° causes axis rotation in half the resulting embryo, resulting in twinned or double partial embryos (Kuraishi and Osanai, 1989).

The left-right axis

Left-right asymmetries are first apparent in *C. elegans* at the six-cell stage with the synchronous division of ABa and ABp. As these cells divide, an invariant skewing of their spindles causes the AB progeny on the left side of the embryo (ABal and ABpl) to shift slightly anteriorly relative to the two AB progeny on the right (ABar and ABpr). Wood (1991) has shown that the left-right handedness of the worm is established via this shift. When the left side progeny of AB were pushed posteriorly at the six-cell stage these cells executed lineages appropriate for the right hand side. Similarly, the cells on the right hand side (now positioned
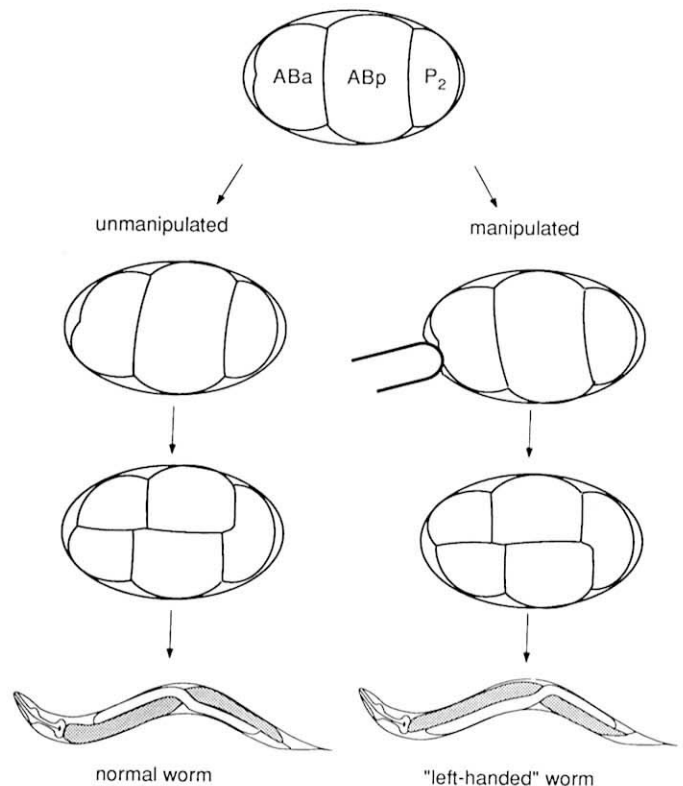
anteriorly as a result of the manipulation) executed left hand lineages. A worm developed but structures normally found on the worm's right side had developed on the left, and vice versa (Fig. 6).

That handedness could be reversed without reversing the left-right positions of blastomeres demonstrated that AB's left side progeny are initially equivalent to AB's right side progeny. The eventual differences between the fates of the left and right AB progeny must be established later via interactions with other cells. The skewing of the AB progeny at the six-cell stage causes AB cells on the right and left sides to have different neighbors later in embryogenesis: these differences in contacts, and hence differences in interactions, are probably responsible for left right differences. It is not known why the spindles in ABa and ABp always skew in the same direction in the unmanipulated embryo.

This experiment also demonstrates that each of the early blastomeres have not yet determined their left-right axes: reversal of the left-right axis could be accomplished without reversing the left-right polarity of each blastomere by rotating it 180°.

A POLARIZING INDUCTIVE INTERACTION

The blastomere repositioning experiments of Priess and Thompson (1987) and Wood (1991) discussed above suggested that AB cell fates are established largely via inductions. Induction has also been found to be required for establishment of gut fate in the E lineage, via an induction which functionally polarizes EMS.

The EMS blastomere divides longitudinally to form two cells with distinct fates, E and MS. The progeny of E form the entire gut of the worm. The progeny of MS contribute to the posterior half of the pharynx, some anterior body wall muscle, and some neurons. Blastomere isolation and recombination experiments have shown that gut is specified in E via an induction that occurs during the four-cell stage between P_2 and EMS (Goldstein, 1992). Isolating the EMS cell early in the four-cell stage prevented gut differentiation. Placing an early-isolated EMS back in contact with the P_2 cell rescued the gut defect, whereas contact with ABa or ABp did not.

Blastomere recombination experiments have shown that gut induction establishes a functional polarity in EMS (Goldstein, 1993). In a normal embryo P_2 contacts the posterior side of EMS, from which the E blastomere will form. When P_2 is removed, gut does not form and both of EMS's daughters take on MS-like lineage timings. This is not an artefact of manipulation, as replacing P_2 after removing it rescues gut specification and the timing defect in E. In an experiment designed to test whether the other side of EMS (the presumptive MS side) can respond to gut induction, P_2 was removed from a four-cell stage embryo and was placed onto the opposite side of EMS. In this case the blastomere that normally would have been E took on an MS-like lineage timing, and gut formed from the descendants of what normally would have been the MS blastomere (Fig. 7). The experiment shows that P_2 establishes gut fate in the side of EMS it contacts, and suggests that the two sides of EMS are otherwise equivalent in developmental potential.

Mutants in which the gut does not form (Morton et al., 1992; T. Stiernagle, D. C. Sigurdson, and J. Shaw, personal communication) may be useful in determining how gut induction establishes a functional polarity in EMS. In these mutants both daughters of EMS take on MS-like lineage timings, as occurs when P_2 is removed.

Gut induction occurs within a single cell cycle, specifying gut fate in one side of a single cell, EMS. Previous work has suggested that inductions generally take hours or days, and establish the fates of large groups of cells, however most of the work on timing of inductions has been done in vertebrates (Jacobson, 1966; Jones and Woodland, 1987). In invertebrate embryos there are several inductions which may occur quickly and affect the fate of only part of a single cell (Clement, 1962; Nishida and Satoh, 1989), however little is known yet about the timing of inductions in invertebrates.

ESTABLISHMENT OF POLARITY IN THE ZYGOTE

First cleavage in *C. elegans* generates two cells, AB and P_1, which differ in size, cell cycle period, cell contents, and cell fates. The events that occur before first cleavage are being studied to elucidate how an asymmetric division is accomplished and how cellular components are segregated.

Segregation of germ-line specific granules

Germ-line specific granules, termed P granules, have been detected in *C. elegans* by immunofluorescence (Strome and Wood, 1982) and by electron microscopy (Wolf et al., 1983). P granules are distributed uniformly throughout the egg's cytoplasm at the time of fertilization. During pseudo-cleavage they coalesce and become redistributed posteriorly, where they will be inherited by P_1 at first cleavage. The P granules are inherited similarly by the successive germline precursors P_2, P_3 and P_4. The redistribution of the P granules is achieved as early as prophase of each division. Although the function of P granules is unknown, they serve as a useful model for investigating how cellular contents are asymmetrically partitioned into particular daughter cells.

The role of microfilaments in generating asymmetry in P_0

Microfilament-based and microtubule-based motors are known to move cellular components within cells (Zalokar,
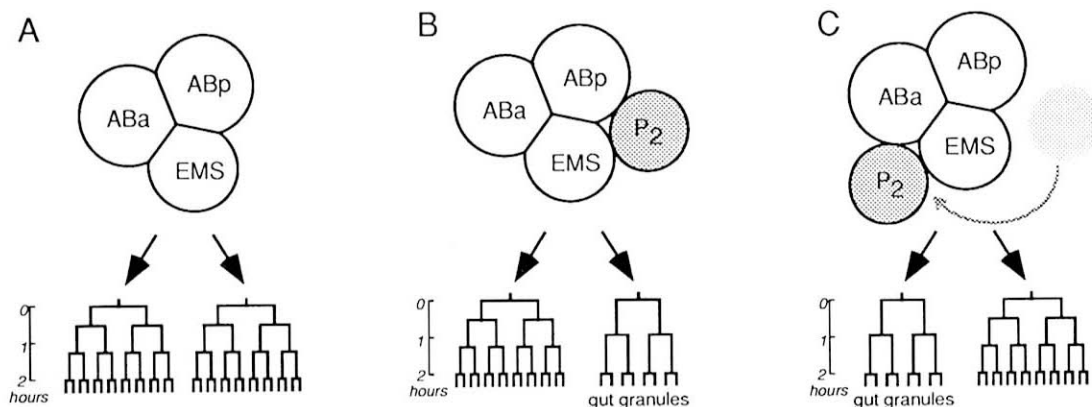

Fig. 7. Experiment demonstrating that either side of EMS can respond to gut induction. (A) P_2 removed. (B) P_2 removed and put back in place. Resulting lineage timings and site of gut differentiation are as in unmanipulated embryos. (C) P_2 moved to the opposite side of EMS. The side of EMS contacting P_2 is the one that takes on the slower E-like lineage and forms the gut (after Goldstein, 1993).

1974; Beckerle and Porter, 1983). Strome and Wood (1983) have tested whether microfilaments or microtubules are required for any of the motile events that occur in the first cell cycle. Treating fertilized eggs with inhibitors of microtubule polymerization prevented only the migration of the pronuclei toward each other, whereas P granule segregation to the posterior, pseudocleavage, and contraction of the anterior membrane occurred normally. Treatment with microfilament polymerization inhibitors did not affect pronuclear migration, but did prevent signs of asymmetry in the zygote. P granules coalesced but did not segregate posteriorly, and pseudocleavage and contraction of the anterior membrane did not occur.

The distribution of microfilaments in oocytes and early embryos has been investigated by Strome (1986). In oocytes and also just after fertilization actin microfilaments are found predominantly in the cortex, as a mesh of fibers and foci. As pseudocleavage occurs and the P granules are redistributed to the posterior pole, foci of actin concentrate progressively in the anterior side.

In order to determine when microfilaments are required for generating asymmetry, Hill and Strome (1988) pulsed embryos with a microfilament inhibitor (cytochalasin D) at three distinct times in the first cell cycle. Embryos pulsed for 2 to 10 minutes before psuedocleavage and pronuclear migration went on to segregate P granules and cleave normally. Embryos pulsed during pseudocleavage and pronuclear migration did not show normal signs of asymmetry in the zygote: P granules coalesced but were not segregated posteriorly, and the mitotic spindle formed in the centre of the zygote and either stayed central or drifted slightly in either direction, leading to a two-cell stage with equal or nearly equal-sized blastomeres. Embryos pulsed after pseudocleavage (and hence after P granule segregation) but before cytokinesis maintained the asymmetric distribution of P granules and cleaved normally. Hence microfilaments are required only briefly late in the first cell cycle to generate asymmetry in the zygote. This is the time that pseudocleavage occurs, the anterior membrane contracts, P granules are redistributed posteriorly, and pronuclear migration occurs. Microfilaments are not required before this time, nor are they required after this time to maintain asymmetry.

The role of microfilaments in segregating developmental information

The localization of developmental potential has been investigated by disrupting microfilaments as described above (Hill and Strome, 1988) and then studying the developmental consequences (Hill and Strome, 1990). Pulsing embryos before or after the critical interval did not affect subsequent development. Pulsing embryos during the critical interval led to either normal development or developmental defects consistent with abnormal establishment of embryonic asymmetry. In those embryos where early cleavages appeared normal, tissue differentiation also occurred normally, whereas in those where early cleavages were abnormal, differentiation rarely occurred. In the abnormally cleaving embryos early cell divisions occurred in a way that suggested either a duplication of the anterior half of the embryo (no small P_2-like cell at the four-cell stage, all four cells equal-sized), a duplication of the posterior half (P_2-like cells at both ends), or a complete reversal of polarity (P_2-like cell at the anterior end). The position of the P granules at this stage was consistent with this interpretation, as were the cell cycle times. The finding that cell size, cell cycle times, and positioning of the P granules were correlated in these embryos suggests that a single microfilament-mediated event establishes embryonic asymmetry, late in the first cell cycle.

Cortical and cytoplasmic flows during pseudocleavage

Hird and White (1993) have examined the cortex in live embryos during pseudocleavage and found that cortical material flows anteriorly. This is the time when foci of actin microfilaments found in the cortex become concentrated anteriorly (Strome, 1986) and is also the critical period when microfilaments are required to generate asymmetry in the zygote (Hill and Strome, 1988, 1990). Both the cortical and cytoplasmic flows of pseudocleavage have been found to be microfilament-mediated: flows occur with a velocity consistent with actin-mediated particle movements, and can be prevented by depolymerizing microfilaments but not by depolymerizing microtubules. One reasonable model that all of these observations suggest is the following: during pseudocleavage there is a flow of cortical actin towards the anterior of the egg where it assembles into a contractile network. In order to balance this cortical flow, cytoplasm flows in the opposite direction, i.e. towards the posterior. This flow is driven by the continuous contraction of the anterior cortex. This bulk cytoplasmic flow could sweep determinants posteriorly where they could be anchored and inherited by the posterior daughter.

Cytoplasmic and cortical flows also occur at the two-cell stage (Hird and White, 1993). In P_1, cortical material flows anteriorly and cytoplasmic material (including the nucleus and centrosomes) flows posteriorly, around the time when P granules are known to become redistributed posteriorly. These flows do not depend on the presence of intact microtubules, but may require microfilaments. P_1 then goes on to divide unequally. Such flows do not occur in AB, which undergoes equal cleavage and gives rise to daughter cells with equivalent developmental potential (Priess and Thomson, 1987). In this case, the flows in P_1 may arise from the close proximity of the posterior aster at first cleavage to the cortex.

Control of flow polarity

Hird and White (1993) have shown that many aspects of pseudocleavage can be replicated in later blastomeres. Treating blastomeres with the microtubule polymerization inhibitor nocodazole before cytokinesis causes the mitotic apparatus to be formed close to the cortex; when treated cells attempt to divide, there is a flow of cortical and cytoplasmic material in opposite directions, as in pseudocleavage. The direction of subsequent flows could be predicted by the position of the attenuated mitotic apparatus: cortical material always flowed away from it and cytoplasmic material always flowed towards it. In addition, a transient furrow is formed, the cortex distant from the mitotic
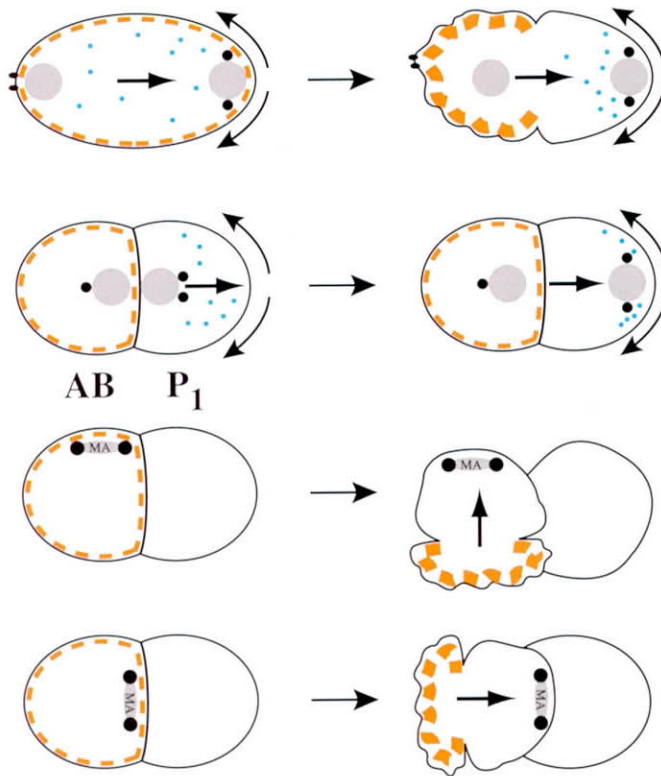
Fig. 8. From top: In the one-cell embryo, a simultaneous flow of cytoplasmic and cortical material takes place (arrows) during pseudocleavage. The cytoplasmic component of the flows may localize cytoplasmic factors, such as P granules (blue dots). The flows may be driven by the redistribution of an actin based contractile network (shown in orange). In the two-cell embryo, there is a simultaneous flow of cytoplasmic and cortical material in P₁. This cell then divides unequally to give daughters of distinct fates. The cytoplasmic flow may localize factors (such as P granules, blue) to the posterior of the cell. It may also be responsible for the posterior migration of the nucleus seen at this time. AB divides equally and does not have flows. The bottom two embryos show the cytoplasmic flow patterns that result from treatment with nocodazole prior to cytokinesis. AB does not normally have flows (see above) but exhibits the same flows as pseudocleavage when it attempts to divide in the presence of nocodazole. Note also the formation of a furrow and the membrane contractions just as in pseudocleavage. The polarity of the flows in drug treated AB is determined by the position of the nocodazole attenuated mitotic apparatus (MA). The actin contractile network is shown (orange) (after Hird and White, 1993).

apparatus undergoes a series of contractions, and actin becomes heavily concentrated in these contracting regions, as in pseudocleavage (Fig. 8). The results show that an eccentrically placed mitotic apparatus will direct flows in early blastomeres, suggesting that pseudocleavage may be brought about by the asters of the sperm pronucleus (analogous to the mitotic apparatus) inducing a cortical flow away from them and a cytoplasmic flow toward them.

Genes required for cytoplasmic reorganization

Four genes required for cytoplasmic localization have been identified by Kemphues et al. (1988), in screens for non-

conditional maternal effect lethal mutations. Each of the *par* mutants (for cytoplasmic <u>par</u>titioning) go through many cell divisions and often produce some differentiated cell types, but show defects in the redistribution of P granules and cortical microfilaments (Kemphues et al., 1988; Kirby et al., 1990; Morton et al., 1992). Cleavage orientations and blastomere sizes are often abnormal.

Specific defects vary between the four par mutants. In *par-1* mutants first cleavage generates two daughters of nearly equal size, and the P granules are distributed equally among early blastomeres. Pharyngeal muscle and body wall muscle both differentiate, but the gut does not. In *par-2* mutants P granules are either incompletely localized or not localized at all, and first cleavage generates two equal-sized daughters. Gut differentiation occurs rarely, while other tissues differentiate normally. In *par-3* mutants the P granules are incompletely localized or not localized, and microfilaments are not redistributed anteriorly during pseudocleavage, however differentiation of gut and other tissues occurs. In *par-4* mutants the cells of the two and four-cell stages are normal in size, however, P granules are distributed equally or nearly equally, and tissue differentiation is widely affected. Gut and body wall muscle cells differentiate only rarely, and pharyngeal muscle cells differentiate in fewer than half of the embryos. The defects suggest that *par* gene products are part of a system for intracellular localization.

ASYMMETRIC DISTRIBUTION OF A DETERMINANT IN EMBRYOS

The *skn-1* gene is required to specify the fate of the EMS blastomere (Bowerman et al., 1992). Bowerman et al. (1993) have shown recently that the SKN-1 protein is distributed unequally at the two-cell stage. SKN-1 is present from just before first cleavage through the eight-cell stage, accumulating to much higher levels in the nuclei of P₁ and its progeny than in AB and its progeny.

Mothers homozygous for maternal-effect mutations in the *mex-1* gene produce embryos in which SKN-1 accumulates in both AB and P₁ (Bowerman et al., 1993), and the AB lineage produces descendants with MS-like fates (Mello et al., 1992), suggesting that the *mex-1* gene product is involved in unequally distributing SKN-1 to P₁. *par-1* (Kemphues et al., 1988) is also required for the unequal distribution of SKN-1 at the two-cell stage (Bowerman et al., 1993).

At the four cell stage both EMS and P₂ have high levels of SKN-1, yet mutations in *skn-1* affect only EMS and not P₂. SKN-1 activity appears to be repressed in P₂ by *pie-1*: mothers homozygous for maternal-effect mutations in *pie-1* produce embryos in which the distribution of SKN-1 protein is normal (Bowerman et al., 1993), but P₂ takes on an EMS-like fate (Mello et al., 1992).

DETERMINATION OF CELL DIVISION AXIS IN POLARIZED CELLS

If an early blastomere partitions cytoplasmic components, such as P granules, along a particular axis, then it is crucial
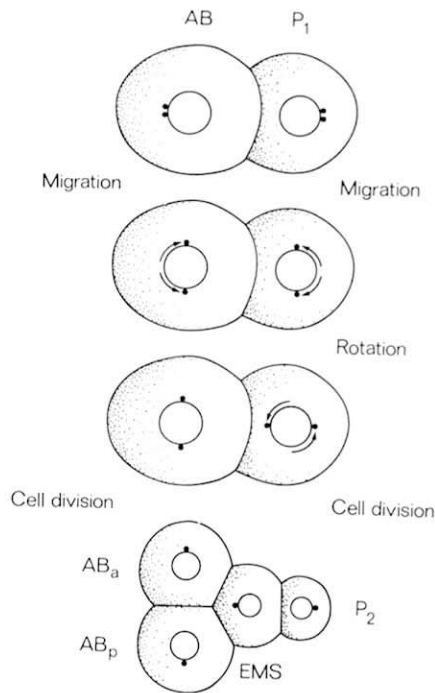
Fig. 9. Migration of centrosomes in AB and P₁, and rotation of centrosome-nuclear complex in P₁. The embryo is shown without the constraining eggshell. AB divides at right angles to the preceding anteroposterior division axis to give the equivalent daughters ABa and ABp. P₁ divides along the anteroposterior axis. This division is unequal and gives rise to EMS and P₂ (from Hyman and White, 1987, with permission).

that the subsequent division is along the same axis so that only one daughter cell inherits the segregated material. Cells normally divide at right angles to the preceding division as a consequence of the migration pattern of the centrosomes (which form the poles of the spindle) during interphase (Hyman and White, 1987), so an active mechanism is needed to impose alternative division axes. In a number of *C. elegans* blastomeres the diametrically opposed centrosomes rotate onto the anteroposterior axis prior to the assembly of the spindle. These cells therefore divide along the same axis as their mothers.

Hyman and White (1987) studied the rotational choreography of the centrosomes and the attached nucleus in P_0, P_1, P_2 and EMS (Fig. 9). In P_0, P_1 and EMS the centrosomes rotate through 90 degrees; in P_2 they rotate 45 degrees onto the same axis as the preceding segregation of the P granules. The centrosomes and nucleus in P_0 and P_1 move some distance anteriorly prior to rotation, then one centrosome, apparently at random, leads the rotation with the complex pivoting around the other centrosome. The leading centrosome often ends up very close to a discrete region of the anterior cortex; a membrane invagination is occasionally seen at this site indicating that tension is being exerted on the cortex. Rotation requires the presence of microtubules, and antitubulin immunofluorescence shows that microtubules extend from the centrosomes to the anterior cortex during rotation.

Hyman (1989) studied the dynamics of nuclear/centrosome rotation in P_1 using a laser microbeam. It was shown that disruption of either the rotating centrosome or of microtubules between the anterior cortex and the rotating centrosome stops rotation. Rotation resumes shortly after, but the other centrosome leads. Laser ablation at the membrane invagination also blocks rotation. These results have generated the following model for P_1 centrosome rotation (Hyman, 1989): the centrosomes (initially at 90 degrees to the anteroposterior axis) extend microtubules to a capture site on the anterior cortex where their ends are stabilized. The microtubules exert tension on this site, and pull the centrosomal-nuclear complex anteriorly, initially without rotation. A "tug of war" then ensues where one centrosome finally establishes a stronger connection to this site than the other. This generates torque, causing one centrosome to swing onto the axis. As centrosomes are diametrically opposed to each other on the nucleus, the rotation places the other centrosome along this axis. Microfilaments also play a role in this process, possibly by keeping the cortical site localized to a discrete spot (S. N. H., unpublished observations). Candidates for providing the rotational force include kinesin-like microtubule motors located either at the centrosomes themselves or at the anterior cortical site. There is immunocytological evidence that kinesin-like proteins are present at the centrosomes and at the rotational site in some blastomeres (S. N. H., unpublished observations). Definitive identification of the rotational machinery may come from analysis of mutants defective in centrosome rotation (Lesilee Simpson Rose, personal communication).

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