Cell Contacts Orient Some Cell Division Axes in the *Caenorhabditis elegans* Embryo

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**Abstract.** Cells of the early *Caenorhabditis elegans* embryo divide in an invariant pattern. Here I show that the division axes of some early cells (EMS and E) are controlled by specific cell–cell contacts (EMS–P_2 or E–P_3 contact). Altering the orientation of contact between these cells alters the axis along which the mitotic spindle is established, and hence the orientation of cell division. Contact-dependent mitotic spindle orientation appears to work by establishing a site of the type described by Hyman and White (1987. *J. Cell Biol.* 105:2123–2135) in the cortex of the responding cell: one centrosome moves toward the site of cell–cell contact during centrosome rotation in both intact embryos and reoriented cell pairs. The effect is especially apparent when two donor cells are placed on one side of the responding cell: both centrosomes are “captured,” pulling the nucleus to one side of the cell. No centrosome rotation occurs in the absence of cell–cell contact, nor in nocodazole-treated cell pairs. The results suggest that some of the cortical sites described by Hyman and White are established cell autonomously (in P_1, P_2, and P_3), and some are established by cell–cell contact (in EMS and E). Additional evidence presented here suggests that in the EMS cell, contact-dependent spindle orientation ensures a cleavage plane that will partition developmental information, received by induction, to one of EMS’s daughter cells.
Centrosomes appear to be aligned during rotation by the shortening of astral microtubules that run from the centrosome to the cortex, pulling one of the asters to a specialized site in the cortex. Evidence for this derives from the distribution of microtubules during centrosome rotation and from experiments in which microtubule-depolymerizing or -stabilizing drugs were shown to inhibit rotation (Hyman and White, 1987). Additionally, laser ablation of sites in the cell between the leading centrosome and the proposed localized site in the cortex perturbs centrosome movements, whereas laser ablation of other sites in the cell has no such effect (Hyman, 1989).

In this paper I describe experiments which show that some of the sites used to align mitotic spindles are induced by cell–cell contacts between specific cells.

Materials and Methods

All experiments used wild-type C. elegans (N2 Bristol strain), which were cultured as described by Wood (1988) on agar plates.

Methods for removing eggshells and vitelline membranes and for culturing cells have been described by Edgar and Wood (1993). To place P2 cells in contact with EMS cells in random orientations, eggshells and vitelline membranes were removed, and cells were then isolated from each other at various times during the four-cell stage, which lasts 15 min. P2 and EMS cells were then placed in contact. The cells stick immediately upon contact. Cell cycle times cited here are measured from the time that cytokinesis began in a cell. Cells were isolated from one embryo at a time and were placed in contact only with other cells from the same embryo (except in the experiment shown in Fig. 6 A). Time-lapse videomicroscopy was performed on a multiplanep image-recording system as described by Hird and White (1993). At least five cases were assembled for each experiment; results were invariably as indicated, unless otherwise stated.

Centrosomes were identified in live cells and time-lapse recordings by the clearing of yolk granules, which grow as asters form, and by the occasional fast movements of cytoplasmic granules toward the centrosome, as described by Hyman and White (1987).

Isolated cells were identified on the basis of size. In the two-cell stage AB is larger than P1. In the four-cell stage ABa and ABp are indistinguishable by size, but each is larger than EMS, and EMS is larger than P2. Of the cells of the eight-cell stage, E is only slightly smaller than MS and cannot be distinguished from it, but P3 is markedly smaller than C. Cells have distinct cell cycle periods, which confirmed identifications of isolated cells.

The random orientation of cell pairs was established by two methods. First, live observations and time-lapse recordings at high magnification showed that the cells did not move around each other or rotate in place after being placed in contact. Whole cell rotation would be apparent, but cytoplasmic granules can easily be traced in the recordings; this is critical to rule out a role for cell repositioning in the results. Second, in both fixed and live cell pairs, random orientation was confirmed by visualizing the site of an asymmetry within the cells—the position of the centrosomes (see Fig. 8). Isolated cells were prepared for observation by mouth pipetting them into culture medium on a coverslip. Clay feet were applied to the corners of the coverslip to prevent flattening the embryos, and the coverslip was inverted onto a slide. The edges were sealed with Vaseline to prevent dessication. In time-lapse recordings of centrosome rotation in intact embryos, the embryos were viewed with the EMS blastomere (the future ventral side) facing upward, as centrosome movements in EMS occur in a frontal plane. This was accomplished by mouth pipetting embryos into egg salts on 0.1% poly-L-lysine-coated coverslips and maneuvering the embryo into the appropriate orientation by pushing fluid out of the mouth pipette as the embryo sunk onto the coverslip. The coverslip was given clay feet, inverted over a slide, and sealed with Vaseline as previously described. During rotation, the centrosomes and nucleus appeared to rotate as a unit, as cytoplasmic granules at the surface of the nucleus moved in concert with the centrosomes.

Cell division axes were estimated to 15° intervals by comparing the angles of cell divisions, observed at 2,000× (M3C Kombibietero dissecting microscope; Wild, Heerbrugg, Switzerland) to a protractor. The arrows in Figs. 2 and 3 represent the axis of the mitotic spindle in each dividing cell relative to the position of cell–cell contact. The axis of the mitotic spindle was inferred from the positions of cells during and immediately after cytokinesis. The arrow pointing directly above the plane of contact between the
two cells (see, for example, the arrow with 11 cases in Fig. 2 B) represents the cases in which cytokinesis occurred in a plane perpendicular to and directly through the plane of contact with the other cell. This led to three cells, each contacting the other two, arranged in a roughly equilateral triangle. The arrow pointing directly above the dividing cell (see, for example, the arrow with 9 cases in Fig. 2 B) represents the cases in which cytokinesis occurred in a plane perpendicular to, but not through, the plane of contact with the other cell. This led to three cells in an L-shaped configuration, with one daughter of the dividing cell not contacting the other cell.

Gut differentiation was assayed using a standard marker for gut differentiation, birefringent rhadhin granules, under polarizing optics (Babu and Siddiqui, 1980).

Nocodazole (Sigma Chemical Co., St. Louis, MO) was used at a concentration of 10 μg/ml in culture medium. Embryos were permeabilized to nocodazole by removing the eggshell and vitelline membrane. This concentration prevents centrosome rotation in all blastomeres examined (Hyman and White, 1987).

To fix cells for immunofluorescence, cells were washed twice in a simplified culture medium and fixed in 2% paraformaldehyde in simplified culture medium, to minimize cell damage as cells were transferred to fixative. The simplified culture medium consisted of 840 μl of stock salts solution (0.7 M NaCl, 0.3 M KCl), 1 ml of 0.25 M Hapes buffer, pH 7.4, 100 μl of 100 mg/ml galactose, 40 μl of 0.5 M disodium phosphate, 100 μl of base mix stock solution (Edgar and Wood, 1993), and 1,320 ml of water and was derived from the culture medium protocol described by Edgar and Wood (1993). Paraformaldehyde was prepared by first dissolving paraformaldehyde at 20% in water at 60°C, with ~50 μl of 5 M NaOH per 5 ml of water. This solution was diluted 1:10 in the simplified culture medium. Fixed cells were then washed through two changes of M9 buffer (Wood, 1988) by mouth pipetting and were pipetted into M9 buffer on 0.1% polylysine-treated slides. Intact embryos were fixed in methanol-acetone at -20°C as described by Hyman and White (1987).

The YL1/2 antibody (kind gift of J. Kilmartin, MRC-LMB, Cambridge) was used to visualize microtubules (Kilmartin et al., 1982) by standard methods (Hyman and White, 1987), except for the fixation procedures previously described.

Results

Division Axes in Cell Pairs

P2 cells were placed in contact with EMS cells in the first 5 min of the EMS cell cycle. This randomized the orientation of contact between these two cells (see Materials and Methods). EMS then cleaved in a consistent orientation relative to where P2 was placed, which left EMS's two daughters and P2 placed along a single axis (Fig. 2 A). This result suggested that contact with P2 orients the EMS cell division axis.

Each of the cells of the four-cell stage, A, B, P2, and P2, were then tested similarly in all pairwise combinations, and cell division axes were followed. Of these four cells, only P2 had the ability to orient another cell's division axis and only EMS could have its division axis oriented (Fig. 3, A-F).

Selected cells of the eight-cell stage were juxtaposed. The daughters of EMS (E and MS) were placed in contact with the daughters of P2 (P3 and C) in the first 5 min of the eight-cell stage. The division axes of both E and MS were affected by cell contact: E and MS divided in a consistent direction relative to where P3 and C were placed, regardless of the orientation in which the cells were initially placed in contact (Fig. 3, G and H). In normal embryos, the P3 cell contacts E (see Fig. 1). Both C's ability to orient the division axis of MS and MS's competence to have its division axis oriented by contact with P2 or C appear to be latent, as the appropriate cell contacts are not made in normal embryos. The division axes of P2 and C appeared to be unaffected by contact with E and MS, as they divided in random orientations relative to the positions of E and MS (data not shown).

The Timing of the Cell–Cell Interaction

The time at which P2 must be in contact with EMS to orient its division axis was determined. When P2 and EMS were isolated in the first 5 min of EMS's cell cycle and P2 was placed in a random position on EMS, EMS then cleaved in a consistent direction relative to where P2 was placed (Fig. 2 A). When the same experiment was performed in the last 9 min of EMS's cell cycle, EMS cleaved in random orien-
tations relative to where P2 was placed (Fig. 2 B), suggesting that the division axis has been fixed by this time. The time when P2 affects EMS's future division axis appears to be 5–6 min into the P2 and EMS cell cycles, 9–10 min before EMS cleaves (Fig. 2, A–C, asterisks).

Next, it was determined whether an EMS cell that has no cell contact at this time (9–10 min before EMS cleaves) can have its division axis oriented by placing a P2 cell in contact soon after this time (this experiment is similar to the previous one, only here no cell is in contact with EMS 9–10 min before it cleaves; the difference is illustrated in Fig. 2, B and C). EMS divided in random orientations (Fig. 2 C).

These results indicate that to orient EMS's division axis, P2 must contact EMS at a "critical time" (Fig. 2, A–C, asterisks), 9–10 min before EMS cleaves. It remains possible that P2 might also need to contact EMS after the critical time, for example, until rotation is completed. Regardless of whether or not contact is made at the critical time, placing P2 at a new position on EMS after this time cannot affect EMS's cell division axis.

Time-Lapse Videomicroscopy of Centrosome and Nuclear Movements

The pattern of centrosome movements in cells of intact early embryos has been documented extensively by Hyman and

Figure 3. (A–F) Pairwise combinations of cells from four-cell embryos; compare with Fig. 2 A, in which cell contact aligns a cell division axis. Cells were isolated in the first 5 min of the four-cell stage and were then placed in contact in pairs. The number at the end of each arrow represents the number of cases that divided with their mitotic spindles along the axis indicated by the arrow (see Materials and Methods). ABx represents ABa or ABp, as these cells were not distinguished from each other. The effect of P2 on EMS (shown in Fig. 2 A) was specific: (A) ABx contact does not orient EMS's division axis, and (B) P2 could not orient the division axis of an ABx cell. (C–F) No other cell pairs led to specifically oriented cell division axes. (G and H) Experiments using cells from eight-cell embryos. (G) EMS cells were isolated 2–7 min before EMS cleaved. EMS was allowed to cleave twice, and the division axes of E and MS were recorded. The division axis of the MS-like daughter is depicted on the left; the E-like daughter is on the right. Which daughter was which was determined by watching cell division times as described by Goldstein (1993). (H) E and MS cleave in consistent orientations when placed in contact with P3 and C. P2 and EMS cells were isolated 2–7 min before EMS cleaved. After both P2 and EMS had cleaved, the two daughters of P2 were placed in contact with the two daughters of EMS as shown. The direction in which each cell cleaved was recorded. Results are shown for EMS's two daughters. Placing P3 and C in positions other than that depicted altered E and MS cleavage directions as expected (three cases; not shown). P3 and C divided in random orientations relative to the positions of E and MS (not shown). Note that using EMS cells isolated 10–12 min before cleaving, which produces two MS-like lineages (Goldstein, 1995), gave identical results (nine cases; not shown), indicating that the MS-like cells produced from uninduced EMS cells can respond similarly.

Figure 4. Centrosome rotation in EMS in intact embryos. Ventral view shows rotation of the centrosome–nucleus complex. Centrosomes are marked by arrowheads and are apparent as clearings of yolk granules in still photographs of carefully focused specimens. Anterior is to the left. Bar, 10 μm.
White (1987). Here I focus on centrosome movements in the EMS blastomere. Centrosome movements were followed by time-lapse videomicroscopy in intact embryos, reoriented P2-EMS cell pairs, EMS cells with two P2 cells placed in contact with them, and isolated EMS cells.

In intact embryos (Fig. 4), the centrosome that EMS inherited duplicated on the anterior side of the nucleus (the side away from P2), and the two resulting centrosomes migrated to opposite sides of the nucleus. Then the diametrically opposed centrosomes and the nucleus rotated as a unit through 90°. This moved the centrosomes from left–right positions (either side of the embryo's sagittal plane) to anterior–posterior positions on the nucleus. Rotation occurred over 2-3 min and finished 3-4 min before cytokinesis began. The centrosome-nucleus complex moved posteriorly during rotation such that by the end of rotation, EMS's posterior centrosome lay close to its posterior cortex, and the mitotic spindle was set up somewhat posterior of the cell's center.

P2 and EMS cells were juxtaposed in random orientations before the critical time (Fig. 5). During rotation in each EMS cell, a centrosome appeared to be captured at the site of cell-cell contact: the nucleus–centrosome complex in EMS rotated and moved toward P2, with one centrosome leading. Depending on where P2 had been placed, anywhere between 0° and 90° of rotation occurred before one centrosome reached the site of cell–cell contact.

Two P2 cells were placed near each other in contact with an EMS cell before the critical time (Fig. 6 A). Both EMS centrosomes appeared to be captured by the two sites of cell–cell contact, and the centrosome–nucleus complex moved to an eccentric position in the cell, toward the P2 cells.

EMS cells were isolated before the critical time and were left in isolation (Fig. 6 B). The first phase of centrosome movement, migration to opposite sides of the nucleus, occurred normally; however, the second phase, rotation of the centrosome–nucleus complex, did not occur. The mitotic spindle then formed in the absence of rotation, leading to a division that was presumably orthogonal to the previous division.

In the time-lapse recordings an additional nuclear movement was noted, the significance of which is not known. Immediately after the EMS cell division in P2–EMS cell pairs apposed before the critical time, the nucleus in one daughter of EMS (the daughter that contacted P2) moved toward the P2 cell. This movement occurred during ~1 min after nuclear envelope formation. No such movement occurred in the other daughter of EMS. This movement has been seen previously in normal embryos by Schierenberg (1987). Schierenberg (1987) also found that this movement requires contact with the P2 cell, a finding confirmed here in recordings of isolated EMS cells, in which the movement did not occur in either daughter. A similar movement occurred after E and MS cell divisions in each daughter cell that contacted the P2 or C cells. This occurs in normal embryos in the daughter of E contacting P2 (Schierenberg, 1987). These movements might be dependent on alignment of the axis of cell division preceding the movement, or possibly only on cell contact immediately after division; these possibilities were not tested here.

**The Role of Microtubules**

To determine whether cell contact–dependent rotation in EMS requires intact microtubules, EMS and P2 cells isolated before the critical time were placed in contact in random orientations and were then cultured in 10 μg/ml nocodazole to depolymerize microtubules. Nocodazole treatment started between the two phases of centrosome movement—after migration but before rotation normally occurs in EMS.

![Figure 5. Centrosome rotation in P2-EMS cell pairs apposed before the critical time. Three examples are shown, through three time points each. P2 is the smaller cell and EMS is the larger cell in each photo. P2 was placed at various distances from the closer centrosome, leading to nearly 90° of rotation in A, about 15° in B, and 0° in C. The centrosomes are not readily apparent in some still photos; for details on how centrosome positions were identified, see Materials and Methods. Centrosomes are marked by arrowheads. Bar, 10 μm.](image-url)
No rotation occurred in the nocodazole-treated cells (Fig. 6 C).

The EMS nucleus drifted to a seemingly random edge of the cell, the nuclear membrane broke down, and an attenuated spindle was set up. The cell then extended out from the site of the attenuated mitotic apparatus as cytoplasmic streaming occurred, much as described previously by Hird and White (1993) in nocodazole-treated P2 and AB cells. Extension generally occurred from two sites in EMS, near each centrosome. After extending to several times its normal length, the cell retracted and rounded up, and a similar extension and streaming then occurred in P2 after its nucleus broke down. Extensions and retractions continued with nuclear cycles.

**Anti-Microtubule Immunofluorescence in Embryos and Cell Pairs**

Hyman and White (1987) have shown microtubule distributions in lateral views of fixed, intact embryos. I have examined frontal views of fixed, intact embryos to visualize both asters during rotation in EMS and isolated P2-EMS cell pairs as previously described and then fixed at various times through both phases of centrosome movement, migration and rotation. In the intact embryos (Fig. 7) centrosome positions and microtubule distributions were essentially as described by Hyman and White (1987). In the isolated P2-EMS cell pairs (Fig. 8), the initial positions of the centrosomes confirmed that the experiment initially randomized cell orientations (Fig. 8, A-C). In the later stages (after the mitotic spindle formed), one centrosome in EMS lay near the site of P2-EMS contact (Fig. 8 F, arrowhead), as in the intact embryos (Fig. 7 C, arrowhead). During cytokinesis, the spindle remained close to the site of P2-EMS contact, with one centrosome closely apposed to the cortex at this site (Fig. 7 D and Fig. 8 G). The centrosome positions revealed by anti-microtubule immunofluorescence confirm the results reported on centrosome positions in live embryos.

**The Relationship between Division Axis Orientation and Gut Induction in EMS**

In *C. elegans* the gut founder cell (E) is established by an interaction between the P2 and EMS cells of the four-cell stage. Contact with P2 makes one side of EMS (the putative E side) differentiate differently from the other side (Goldstein, 1992, 1993, 1995). The relationship between gut induction and spindle orientation in EMS was examined, as both require contact between P2 and EMS. Gut cell fate and spindle orientation are both induced at approximately the same time (Fig. 2, asterisks). If P2 and EMS are not in contact at this time, placing P2 and EMS back in contact can no longer rescue spindle orientation, but can still rescue gut induction. In the manipulations shown in Fig. 2 C, P2 does not affect spindle orientation; however, gut differentiation generally does occur and always in the daughter of EMS contacting P2 (data not shown). This result demonstrates that gut induction can occur in the absence of spindle orientation and thus does not depend on proper orientation of the EMS mitotic spindle.

These cases (Fig. 2 C) were additionally examined to de-

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*Figure 6.* (A) Two P2 cells placed near each other on an EMS cell. In the first frame one EMS centrosome has already moved toward one of the P2 cells. The following frame shows EMS’s other centrosome moving toward the other P2 cell, pulling the nucleus to an eccentric position in EMS and aligning the mitotic spindle along an axis perpendicular to that formed when only one centrosome is captured. The P2 cells are the two small, unlabeled cells. Six such cases were assembled in which two P2 cells were placed near each other in contact with an EMS cell. In one of these cases the two P2 cells captured only one EMS centrosome and the EMS spindle axis was aligned perpendicular to the plane where the two P2 cells contacted EMS; the other five cases resulted as shown. (B) EMS cell isolated in the first 5 min of its cell cycle: no rotation occurred. (C) Nocodazole-treated P2-EMS cell pair: no rotation occurred, and the cell extended from the site of the attenuated mitotic spindle. The centrosomes are not readily apparent in some still photos; for details on how centrosome positions were identified, see Materials and Methods. Centrosomes are marked by arrowheads. Bar, 10 μm.
etermine whether all spindle orientations are compatible with gut induction. When EMS's cleavage furrow formed directly through the site of P₂-EMS contact, gut differentiation did not occur (0/10 cases). When EMS cleaved in various other planes, gut differentiation did occur (14/14 cases). Although P₂ does not need to orient EMS's spindle to induce gut fate in EMS, cleavage directly through the site of P₂-EMS contact appears to be incompatible with gut induction. A similar conclusion was suggested by Schierenberg (1988), who noted that gut differentiation sometimes does not occur after manipulations that cause a more or less transverse EMS cell division.

in F. The asters in P₂ are not captured by the site of cell-cell contact and end up in random orientations, as seen in G. Note that the net of cytoplasmic microtubules disappears as the asters form. Bar, 10 μm.

Figure 7. Anti-microtubule immunofluorescence of the EMS cell of intact embryos through centrosome rotation (A-C), metaphase (C), and anaphase (D). The mitotic spindle in EMS is near the plane of P₂-EMS cell contact in metaphase, indicated by the arrowhead in C. Ventral view; anterior is to the left. Bar, 10 μm.

Figure 8. Anti-microtubule immunofluorescence in P₂-EMS cell pairs, through migration (A-C), rotation (D-F), and EMS cell division (G). Each cell pair was placed in contact before the critical time and was fixed some minutes later. In each photo the P₂ cell is the smaller cell and is on the left. The original position of P₂ on EMS (before dissociation of the intact four-cell embryo) can be inferred by the positions of the duplicated centrosomes in EMS before migration is completed—on the side of EMS opposite to where the centrosomes begin their migration to opposite sides of the nucleus; hence the manipulation repositioned P₂ ~ 120° in A, 150° in B, and 15° in C, confirming that cells were in fact reassOCIated in various orientations. As in intact embryos, the mitotic spindle in EMS is near the plane of contact with P₂, as indicated by the arrowhead.
Cell-Autonomous Centrosome Rotation in P1 and P2

To determine whether centrosome rotation occurs cell autonomously in P1 and P2, these cells were isolated in the first 5 min of their cell cycles, and centrosome movements were recorded. Both phases of centrosome movements, migration and rotation, occurred in isolated P1 and P2 cells. Approximately 90° of rotation occurred in each cell. The result suggests that unlike EMS and E, centrosome rotation occurs in P1 and P2 independently of contact with other cells. Additional support derives from the experiments in which P1 and EMS were placed in contact in random orientations, as EMS's spindle axis was aligned with P1, yet P2 divided in random orientations (Figs. 3 and 5). This shows that the mitotic spindle axis of P2 is not aligned by contact with EMS. Similar observations were made in the P3 cell in experiments in which P2 and C were placed in contact with E and MS (Fig. 3).

Discussion

These experiments revealed a role for cell–cell interactions in determining cell division axes in the early C. elegans embryo. In some cells (EMS and E) specific cell–cell contact is involved in specifying normal division axes. Contact appears to induce a site in the cortex that attracts a centrosome, causing centrosome–nuclear rotation and aligning the mitotic spindle. Other cells (P1, P2, and P3) were found to establish such sites cell autonomously.

Centrosome movement to a specialized site in the cortex in C. elegans blastomeres bears some similarity with the movement of the microtubule-organizing centers (MTOCs) in the budding yeast Saccharomyces cerevisiae. In both cases microtubules emanating from an MTOC appear to attach to a specialized site in the cortex, the MTOC moves toward this site, and this movement requires intact microtubules and actin microfilaments (Hyman and White, 1987; Sullivan and Hufnaker, 1992; Palmer et al., 1992). MTOC movement in yeast also occurs in two distinct modes: cell autonomously during bud formation and by an extracellular cue during mating. Numerous components likely to be involved in MTOC movement have been identified in yeast by identifying proteins that assemble at the cortical site where cell growth occurs and by generating mutations that prevent the establishment of cell polarity or alter the orientation of polarization. Some of the mutations affect both cell-autonomous polarization in budding and the response to an external cue during mating, and others affect only one mode or the other (see reviews by Chant, 1994; Chevenet, 1994). Proteins likely to be involved in forming the complex that provides the force for rotation include actin capping protein (CP), an actin-related protein homologous to a component of the vertebrate dynactin complex, and a microtubule motor protein, dynein (Li et al., 1993; Eshel et al., 1993; Muhua et al., 1994).

The nature of the cortical site in C. elegans has been investigated by generating antibodies to candidate site components (Waddle et al., 1993, 1994). In C. elegans, actin and CP transiently localize to the cortical sites toward which rotation occurs, during the period in the cell cycle when rotation is occurring (Waddle et al., 1994). The actin–CP complexes appear to form on the midbodies (the persistent remnants of cell divisions). Some cells contain two midbodies; in these cases, only one actin–CP complex forms, at the older midbody. In most cells, rotation occurs toward this site. In the EMS cell this site is in the anterior cortex; however, rotation is not directed toward the anterior. Rotation occurs instead toward a site in the posterior cortex, bordering the P2 cell, suggesting that the actin–CP complex is not involved in rotation in this cell. A broader disc-shaped actin–CP complex has been seen on the other side of EMS (at the P2–EMS cell border) and at the P2–E and P2–Ep borders (J. A. Waddle and R. H. Waterston, personal communication). The patterns suggest that these broad complexes might be responsible for cell contact-dependent centrosome rotation, as they are found between cells shown here to interact in this context (P2–EMS and P2–E), and the smaller complexes might be responsible for cell-autonomous centrosome rotation in cells such as P1, P3, and P3.

Some of the mutations affect both cell-autonomous polarizations. In the EMS cell this site is in the anterior cortex; however, rotation is not directed toward the anterior. Rotation occurs instead toward a site in the posterior cortex, bordering the P2 cell, suggesting that the actin–CP complex is not involved in rotation in this cell. A broader disc-shaped actin–CP complex has been seen on the other side of EMS (at the P2–EMS cell border) and at the P2–E and P2–Ep borders (J. A. Waddle and R. H. Waterston, personal communication). The patterns suggest that these broad complexes might be responsible for cell contact-dependent centrosome rotation, as they are found between cells shown here to interact in this context (P2–EMS and P2–E), and the smaller complexes might be responsible for cell-autonomous centrosome rotation in cells such as P1, P3, and P3. This suggestion predicts that additional broad actin–CP complexes could be induced, for example, by placing cells such as C and MS in contact. Unfortunately, the current cell fixation methods required for preserving isolated cells and for detecting the broad actin–CP complexes are incompatible.

In the P0, P1, P2, and P3 cells, cytoplasmic germline-specific granules, termed P granules, are segregated to one side of each cell before division (Strome and Wood, 1982, 1983). The mitotic spindle is oriented along the same axis along which P granule segregation occurs; thus only one daughter cell inherits the P granules at each division.

The EMS cell also appears to have a polarity before it divides, which it acquires via an embryonic induction. In addition to orienting EMS's cell division axis, contact with P2 appears to polarize EMS with respect to the developmental information it contains: P2 is required during the four-cell stage for EMS to produce gut cells from its E lineage (which derives from the side of EMS that P2 contacts); either side of EMS can give rise to the gut via contact with P2, and in the absence of contact with P2, both of EMS's daughters differentiate along a default state normally taken only by E's sister, MS (Goldstein, 1992, 1993, 1995). Thus contact between P2 and EMS serves two roles, both aligning EMS's mitotic spindle and inducing a cell fate change in one side of EMS.

The relationship between gut induction and mitotic spindle orientation in EMS was examined. When EMS is isolated early in its cell cycle, neither gut induction nor the spindle orientation effect occurs. Placing P2 in contact with EMS soon after this time still rescues gut induction, but can no longer rescue the spindle orientation effect (Fig. 2 C). In these cell pairs, EMS divides in various orientations, and gut differentiation generally occurs. Additionally, when P2 is placed in contact with EMS near a centrosome (Fig. 5 C), no centrosome–nuclear rotation occurs, yet gut cell differentiation occurs. These results show that mitotic spindle orientation and centrosome–nuclear rotation need not occur for gut cell fate to be induced in EMS, ruling out a model for gut induction in which the effect of P2 is simply to align the mitotic spindle in a way that divides presegregated developmental information between E and MS. Additional evidence against such a model comes from an experiment showing that moving P2 to the opposite side of EMS causes EMS's
other daughter to produce the gut cell lineage (Goldstein, 1993).

There is one spindle orientation that appears to be incompatible with gut induction: when the EMS cleavage furrow forms directly through the site of cell-cell contact, gut differentiation does not occur. Hence spindle orientation in EMS appears to ensure that cleavage occurs in a plane that will partition developmental information, received via induction from P2, to one of EMS's daughter cells.

The results suggest a model in which P2 has two effects on EMS: it induces gut in the side of EMS it contacts and ensures that EMS's spindle axis is aligned in a way such that only one of EMS's daughters inherits this information (Fig. 9). These two effects appear to be separable, in that mitotic spindle orientation need not occur for gut to be induced. It is possible, however, that both effects are the results of a single ligand–receptor interaction between P2 and EMS and that the interaction can cause spindle orientation for only a short period, whereas it can cause gut induction for a longer period. Once potential signals are identified, whether P2 presents two signals or one should be testable by presenting these to EMS cells in culture. The identification of signals and receptors might additionally aid in identifying other cell contacts that orient division axes later in development.

Other inductions of cell fate also have effects on cell division axes (see, for example, Hill and Sternberg, 1993). In these cases inducing cells might have two direct effects on responding cells, affecting both division axes and cell fates. Alternatively, the effects on cell division axes might be secondary effects of cell fate changes: for example, changes in cell fate could affect cell division timing and hence might lead to a change in the geometry of the surrounding cells when a cell is dividing.

Two results suggest that in the cells requiring specific cell contacts, cell–cell contact establishes a cortical site for centrosome rotation, rather than simply affecting the position of a site that is already present in the cell: first, in the absence of P2, no centrosome rotation occurred in EMS and EMS divided in an orthogonal (AB-like) manner. Second, when two P2 cells were placed near each other on an EMS cell, both centrosomes were captured by the two sites of cell–cell contact, revealing that a cell is capable of forming two sites of centrosome capture.

In conclusion, results presented here show that the division axes of some cells (EMS and E) are specified by contact with specific neighbors. Contact-dependent mitotic spindle orientation appears to work by inducing a localized site in the cortex of the responding cell that attracts a centrosome, aligning the mitotic spindle. The dynamics of centrosome movements and the requirement for microtubules suggest that the site established by cell contact is of the type described by Hyman and White (1987) and Hyman (1989). Other cells (P1, P2, and P3) appear to establish such sites cell autonomously. In the EMS cell, two effects (polarized developmental information received via induction and orientation of the mitotic spindle) are coupled by two types of interactions with the same cell, P2, allowing cell division to partition developmental information.

I thank J. White, S. Hird, E. Schierenberg, R. Arkowitz, and J. Hodgkin for helpful comments on the manuscript and J. Kilmartin for antibodies. Nematodes were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources. This work was supported by American Cancer Society and Human Frontiers Science Program postdoctoral fellowships.

Received for publication 22 December 1994 and in revised form 13 February 1995.

References
Albertson, D. G. 1984. Formation of the first cleavage spindle in nematode embryos. Dev. Biol. 101:61–72.
Allen, V., and D. L. Kropf. 1992. Nuclear rotation and lineage specification in Pelvetia embryos. Development. 115:873–883.
Babu, P., and S. Siddiqui. 1980. Genetic mosaics of Caenorhabditis elegans: a tissue-specific fluorescent mutant. Science (Wash. DC). 210:330–332.
Chant, J. 1994. Cell polarity in yeast. Trends Genet. 10:328–333.
Chenevert, J. 1994. Cell polarization directed by extracellular cues in yeast. Mol. Biol. Cell. 5:1169–1175.
Dan, K. 1979. Studies on unequal cleavage in sea urchins. I. Migration of the nucleus to the vegetal pole. Dev. Growth Diff. 21:527–535.
Dan, K., and S. Ito. 1984. Studies of unequal cleavage in molluscs: I. Nuclear behavior and anchorage of a spindle pole to cortex as revealed by isolation technique. Dev. Growth Diff. 26:249–262.
Edgar, L. G., and W. B. Wood. 1993. Nematode embryos. In Essential Developmental Biology: A Practical Approach. C. D. Stern and P. W. H. Holland, editors. IRL, Oxford University Press. Oxford. 11–20.

Eshel, D., I. A. Urrestarazu, S. Vissers, J.-C. Jauniaux, J. C. van Vliet-Reedijk, R. J. Finta and I. R. Gibbons. 1993. Cytoplasmic dynein is required for normal nuclear segregation in yeast. Proc. Natl. Acad. Sci. USA. 90:11172–11176.

Freeman, G. 1983. The role of egg organization in the generation of cleavage patterns. In Time, Space and Pattern in Embryonic Development. W. R. Jeffery and R. A. Raff, editors. Academic Press, Inc., New York. 171–196.

Goldstein, B. 1992. Induction of gut in Caenorhabditis elegans embryos. Nature (Lond.). 357:255–257.

Goldstein, B. 1993. Establishment of gut fate in the E lineage of C. elegans: the roles of lineage-dependent mechanisms and cell interactions. Development. 118:1267–1277.

Goldstein, B. 1995. An analysis of the response to gut induction in the C. elegans embryo. Development. 121:1227–1236.

Goldstein, B., S. N. Hird and J. G. White. 1993. Cell polarity in early C. elegans development. Development. (Suppl.):279–287.

Hertwig, O. 1884. Das Problem der Befruchtung und der Isotropie des Eies, eine Theorie der Vererbung. Jenaische Zeitschrift, XVIII.

Hill, R. J., and P. W. Sternberg. 1993. Cortical and cytoplasmic flow polarity in early embryonic cells of Caenorhabditis elegans. J. Cell Biol. 121:1343–1355.

Hyman, A. A. 1989. Centrosome movement in the early divisions of Caenorhabditis elegans: a cortical site determining centrosome position. J. Cell Biol. 109:1185–1193.

Hyman, A. A., and J. G. White. 1987. Determination of cell division axes in the early embryogenesis of Caenorhabditis elegans. J. Cell Biol. 105:2123–2135.

Kil martin, J. V., B. Wright, and C. Milstein. 1982. Rat monoclonal antitubulin antibodies derived by using a new nonsecreting rat cell line. J. Cell Biol. 93:576–582.

Li, Y.-Y., E. Yeh, T. Hays, and K. Bloom. 1993. Disruption of mitotic spindle orientation in a yeast dynein mutant. Proc. Natl. Acad. Sci. USA. 90:10096–10100.

Lutz, D. A., Y. Hamaguchi, and S. Inoué. 1988. Micromanipulation studies of the asymmetric positioning of the maturation spindle in Chaetopterus sp. oocytes: 1. Anchorage of the spindle to the cortex and migration of a displaced spindle. Cell Motil. Cytoskel. 11:83–96.

Muhua, L., T. S. Karpova, and J. A. Cooper. 1994. A yeast actin-related protein homologous to that in vertebrate dynactin complex is important for spin-