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.

THE precise placement of cell division planes in embryos is important for partitioning segregated cytoplasmic components to particular daughter cells (Whittaker, 1980). Cell division axes are specified by the orientation of the mitotic spindle: division occurs in the plane between the two asters, which are at the poles of the mitotic spindle (Rappaport, 1961; reviewed by Strome, 1993). In some cells, specific alignment of the mitotic spindle occurs by attaching an aster to a specialized site in the cortex of the dividing cell (Dan, 1979; Dan and Ito, 1984; Lutz et al., 1988; Allen and Kropf, 1992; Palmer et al., 1992; Hyman and White, 1987; Hyman, 1989). Cell division axes can also be oriented by cues external to the dividing cell, generally by constraining the cell's shape. Aspherical cells tend to divide their longest axis in two (Hertwig, 1884; Wilson, 1896), probably as a result of the asters' growing too large for their initial axis and then shifting positions to a longer axis as they continue to grow. Hence a physical barrier such as another cell or an extraembryonic envelope can affect cell division patterns (Wilson, 1896; Freeman, 1983). Cell shape can play a role even in complex division patterns: isolating a cell of the early leech embryo causes it to divide in an abnormal pattern, but its normal division pattern can be restored by contact with a synthetic bead that constrains the cell's shape as its neighbor normally does (Symes and Weisblat, 1992).

In this paper I present evidence that cell division axes can also be controlled by specific cell–cell contacts: in the *Caenorhabditis elegans* embryo, specific cell–cell contact induces a localized site in the cortex of some cells that causes rotation of the centrosome–nucleus complex, thereby defining the position of the mitotic apparatus and hence the cell division axis. In one cell type studied, the EMS cell, contact-dependent mitotic spindle orientation appears to be important for partitioning developmental information, gained via induction, to one of its daughter cells.

Cell Division Patterns in Early C. elegans Development

Two distinct patterns of cell division occur in the early *C. elegans* embryo (Fig. 1) (Hyman and White, 1987; for review, see Goldstein et al., 1993). One pattern, which occurs, for example, in the AB cell of the two-cell stage, is characterized by equal divisions along successively orthogonal axes. The mitotic spindle in AB is set up at a right angle to the mitotic spindle of the preceding division, and the AB cell division produces two equal-sized daughters. The other pattern, which occurs, for example, in the P_1 cell of the two-cell stage, is characterized by unequal (stem cell–like) di-

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visions that occur successively along the same axis. The mitotic spindle in P_1 is set up along the same axis as the mitotic spindle of the preceding division, and the P_1 cell division produces two unequally sized daughters: a larger daughter called EMS and a smaller one, P_2. EMS then also divides unequally, producing E and MS; and P_2 divides unequally, producing P_3 and C. P_3 divides again unequally, producing P_4, which is the germline founder cell, and D. Each of these divisions occurs roughly along the same axis as the preceding division, resulting in a line of cells, though the constraints of the eggshell skew them somewhat off this axis. Early cell cycles last typically 12–20 min at 20°C; a few of the cell cycles are longer.

Hyman and White (1987) have described in detail the centrosome movements that precede these cell divisions. Two phases of centrosome movements occur in P_0, P_1, and other cells that divide successively along the same axis, termed "migration" and "rotation" (Nigon et al., 1960; Albertson, 1984; Hyman and White, 1987). First, the replicated centrosomes separate and migrate to opposite sides of the nucleus. After migration, the centrosome and nucleus rotate as a unit through 90°. As a result, the mitotic apparatus is then set up along the same axis as in the preceding division. In AB and the other orthogonally dividing cells, only the first phase of centrosome movement, migration, occurs. This leaves each mitotic spindle along an axis orthogonal to the mitotic spindle of the previous division.

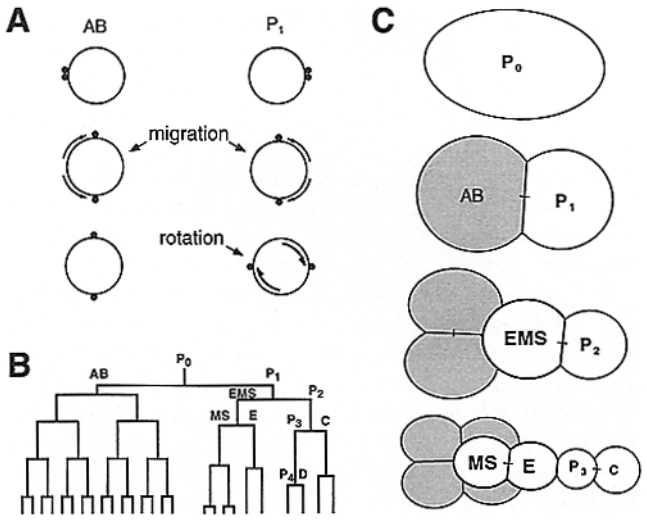

Figure 1. Early cleavage patterns and centrosome movements in *C. elegans*. (*A*) Two phases of centrosome movements. The first phase, migration of centrosomes to opposite sides of the nucleus, occurs in both cells of the two-cell embryo. The second phase, rotation of the centrosome–nuclear complex through 90°, occurs in the P_1 cell (*right*) but not the AB cell (*left*). Other cells in which the second phase occurs include P_0, P_2, P_3, EMS, and E. (*B*) Cell lineage through the 26-cell stage (~100 min after fertilization, at 20°C), after Sulston et al. (1983). The names of the six "founder cells" (AB, E, MS, P_4, D, and C) and the cells from which they derive are shown. (*C*) Diagrams of division axes in one-, two-, four-, and eight-cell embryos without the constraint of the eggshell, which normally skews cell placements. Sister cells are connected by short lines. The AB lineage (*shaded*) divides in successively orthogonal axes. The P_1 lineage divides successively along the same axis, producing a line of cells. Anterior is to the left.

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 P_2 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. P_2 and EMS cells were then placed in contact. The cells stick immediately upon contact. Cell cycle times cited here are measured to/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 multiplane 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 grows 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 P_1. In the four-cell stage ABa and ABp are indistinguishable by size, but each is larger than EMS, and EMS is larger than P_2. Of the cells of the eight-cell stage, E is only slightly smaller than MS and cannot be distinguished from it, but P_3 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, as 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 desiccation. 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% polylysine-treated 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 Kombistereo 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 rhabditin 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).

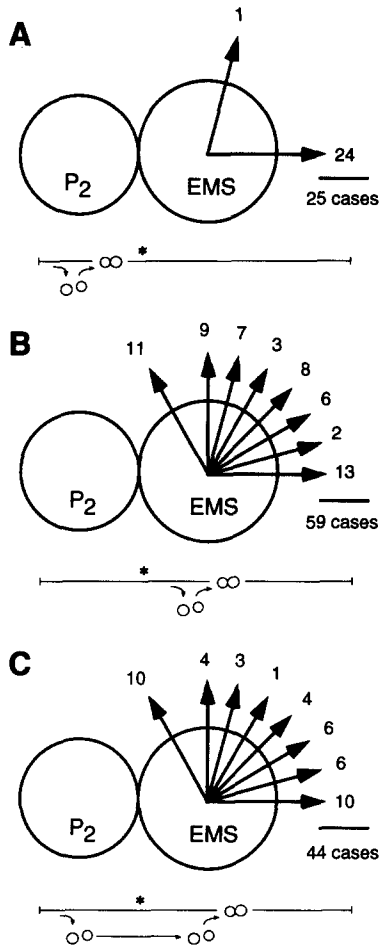

Figure 2. Division axes in P_2–EMS cell pairs. The number at the end of each arrow represents the number of cases that divided with their mitotic spindles along the axis indicated by the arrow (see Materials and Methods). The time line below each experiment represents the 15-min long EMS cell cycle, with an asterisk at the critical time, 5 min into the cycle. Each time line shows when the P_2 and EMS cells were in contact (on the time line) and when they were apart (below the time line; ~1-min long in *A* and *B* and longer in *C*). (*A*) P_2 and EMS placed together in random orientations, in the first 5 min of their cell cycles. EMS nearly always divided away from P_2. (*B*) Same cells, isolated and placed together later in their cell cycles. EMS then divided in various orientations relative to where P_2 was placed. (*C*) Same cells isolated in the first 5 min of their cell cycles, left in isolation past the critical time (*asterisk*), and then placed in contact in random orientations. EMS divided in various orientations relative to where P_2 was placed. As might be expected, in these cases, the normal pulling of the EMS nucleus toward P_2 before nuclear envelope breakdown did not occur (based on observations in five cases).

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 Hepes 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

P_2 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 P_2 was placed, which left EMS's two daughters and P_2 placed along a single axis (Fig. 2 *A*). This result suggested that contact with P_2 orients the EMS cell division axis.

Each of the cells of the four-cell stage, ABa, ABp, EMS, and P_2, were then tested similarly in all pairwise combinations, and cell division axes were followed. Of these four cells, only P_2 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 P_2 (P_3 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 P_3 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 P_3 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 P_3 or C appear to be latent, as the appropriate cell contacts are not made in normal embryos. The division axes of P_3 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 P_2 must be in contact with EMS to orient its division axis was determined. When P_2 and EMS were isolated in the first 5 min of EMS's cell cycle and P_2 was placed in a random position on EMS, EMS then cleaved in a consistent direction relative to where P_2 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 P_2 was placed (Fig. 2 *B*), suggesting that the division axis has been fixed by this time. The time when P_2 affects EMS's future division axis appears to be 5–6 min into the P_2 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 P_2 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, P_2 must contact EMS at a "critical time" (Fig 2, *A–C, asterisks*), 9–10 min before EMS cleaves. It remains possible that P_2 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 P_2 at a new position on EMS after this time cannot affect EMS's cell division axis.

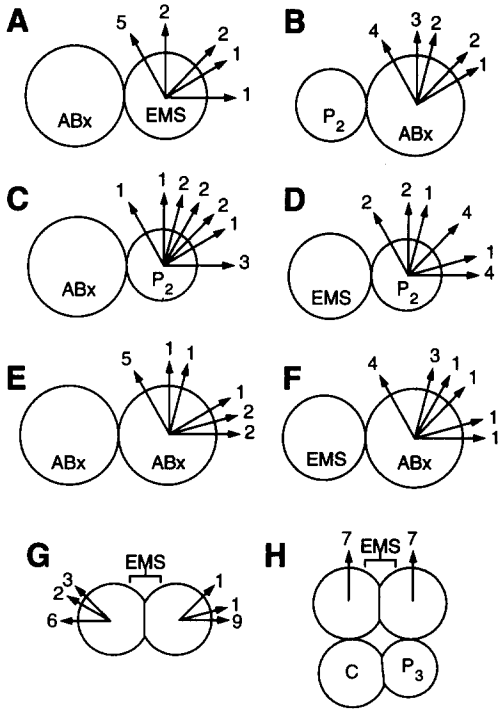

Figure 3. (*A–F*) Pairwise combinations of cells from four-cell embryos; compare with Fig. 2 *A*, in which cell contact aligns a cell division axis. Cells were isolated in the first 5 min of the four-cell stage and were then placed in contact in pairs. The number at the end of each arrow represents the 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 P_2 on EMS (shown in Fig. 2 *A*) was specific: (*A*) ABx contact does not orient EMS's division axis, and (*B*) P_2 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 P_3 and C. P_2 and EMS cells were isolated 2–7 min before EMS cleaved. After both P_2 and EMS had cleaved, the two daughters of P_2 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 P_3 and C in positions other than that depicted altered E and MS cleavage directions as expected (three cases; not shown). P_3 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.

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

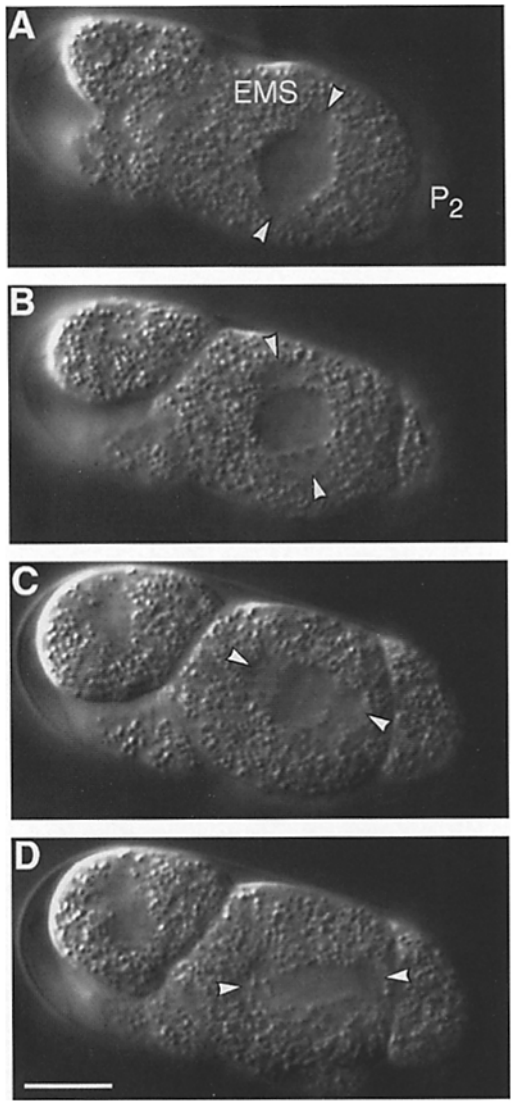

Figure 4. Centrosome rotation in EMS in intact embryos. Ventral view shows rotation of the centrosome–nucleus complex. Centrosomes are marked by arrowheads and are apparent as clearings of yolk granules in still photographs of carefully focused specimens. Anterior is to the left. Bar, 10 μm.

White (1987). Here I focus on centrosome movements in the EMS blastomere. Centrosome movements were followed by time-lapse videomicroscopy in intact embryos, reoriented P_2–EMS cell pairs, EMS cells with two P_2 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 P_2), 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.

P_2 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 P_2, with one centrosome leading. Depending on where P_2 had been placed, anywhere between 0° and 90° of rotation occurred before one centrosome reached the site of cell–cell contact.

Two P_2 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 P_2 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 P_2–EMS cell pairs apposed before the critical time, the nucleus in one daughter of EMS (the daughter that contacted P_2) moved toward the P_2 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 P_2 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 P_3 or C cells. This occurs in normal embryos in the daughter of E contacting P_3 (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 P_2 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.

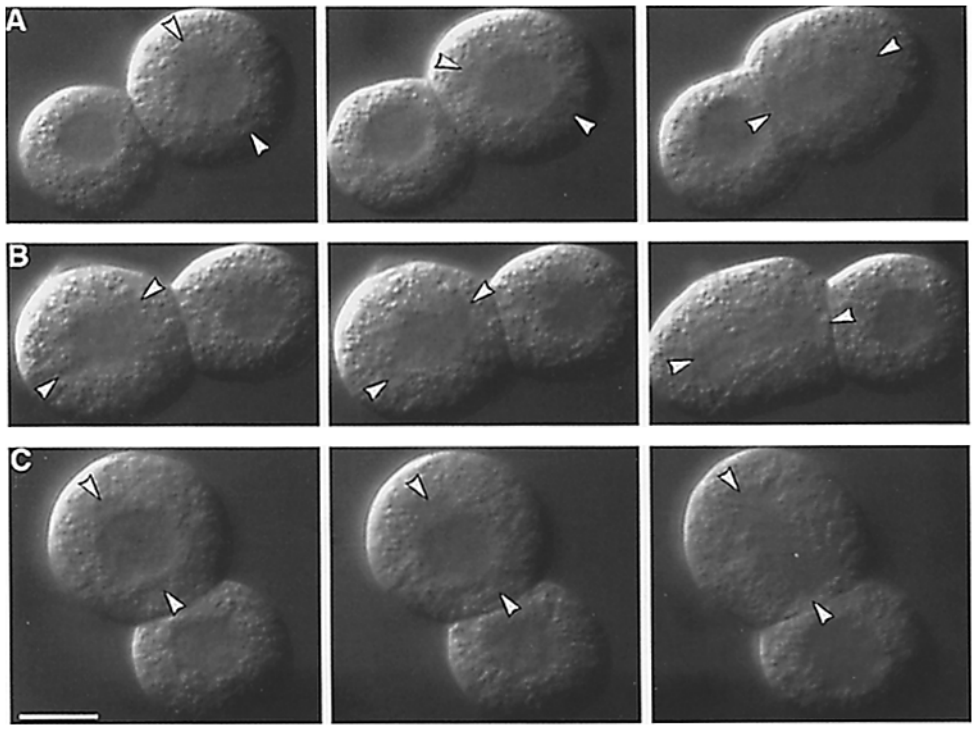

Figure 5. Centrosome rotation in P_2–EMS cell pairs apposed before the critical time. Three examples are shown, through three time points each. P_2 is the smaller cell and EMS is the larger cell in each photo. P_2 was placed at various distances from the closer centrosome, leading to nearly 90° of rotation 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.

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 P_1 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 P_2 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 P_2–EMS cell pairs apposed 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 P_2–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 P_2–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 P_2–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 P_2 and EMS cells of the four-cell stage. Contact with P_2 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 P_2 and EMS. Gut cell fate and spindle orientation are both induced at approximately the same time (Fig. 2, *asterisks*). If P_2 and EMS are not in contact at this time, placing P_2 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*, P_2 does not affect spindle orientation; however, gut differentiation generally does occur and always in the daughter of EMS contacting P_2 (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-

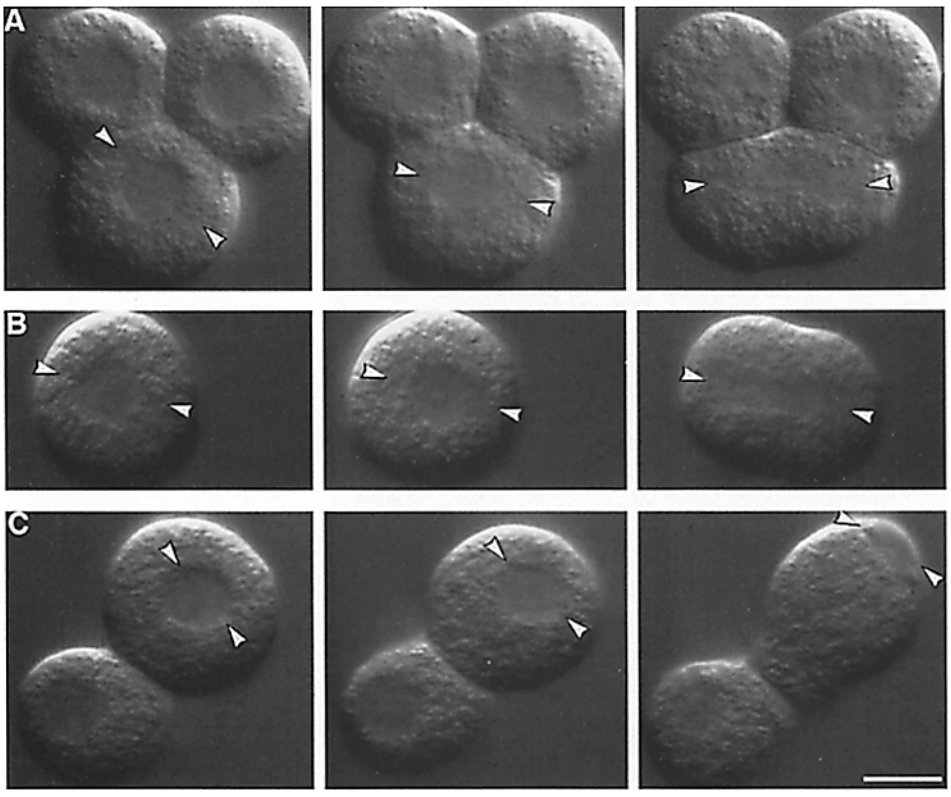

Figure 6. (*A*) Two P_2 cells placed near each other on an EMS cell. In the first frame one EMS centrosome has already moved toward one of the P_2 cells. The following frame shows EMS's other centrosome moving toward the other P_2 cell, pulling the nucleus to an eccentric position in EMS and aligning the mitotic spindle along an axis perpendicular to that formed when only one centrosome is captured. The P_2 cells are the two small, unlabeled cells. Six such cases were assembled in which two P_2 cells were placed near each other in contact with an EMS cell. In one of these cases the two P_2 cells captured only one EMS centrosome and the EMS spindle axis was aligned perpendicular to the plane where the two P_2 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 P_2–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.

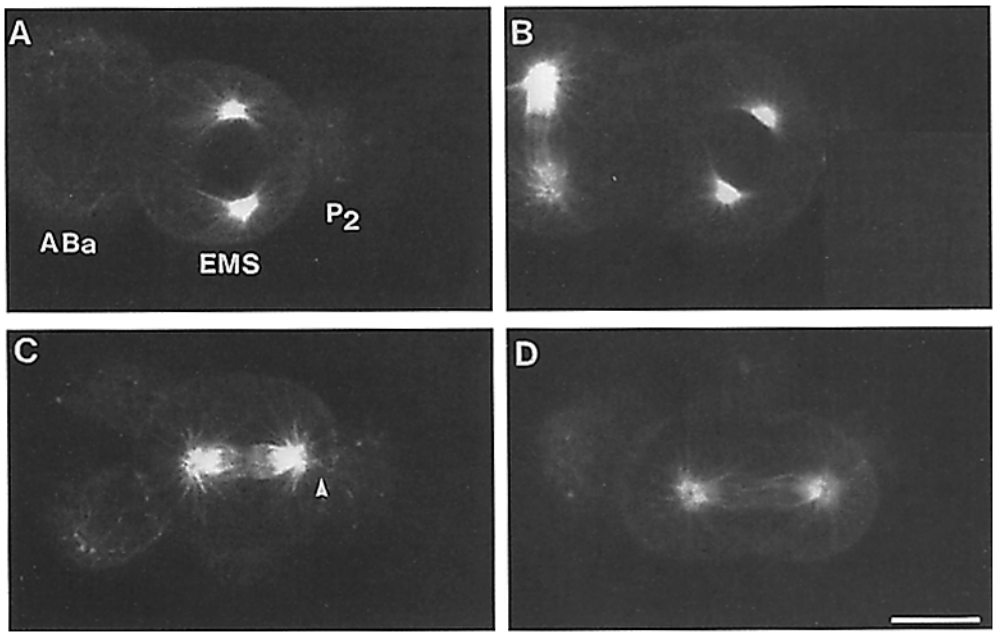

Figure 7. Anti-microtubule immunofluorescence of the EMS cell of intact embryos through centrosome rotation (*A–C*), metaphase (*C*), and anaphase (*D*). The mitotic spindle in EMS is near the plane of P_2–EMS cell contact in metaphase, indicated by the arrowhead in *C*. Ventral view; anterior is to the left. Bar, 10 μm.

termine whether all spindle orientations are compatible with gut induction. When EMS's cleavage furrow formed directly through the site of P_2–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 P_2 does not need to orient EMS's spindle to induce gut fate in EMS, cleavage directly through the site of P_2–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.

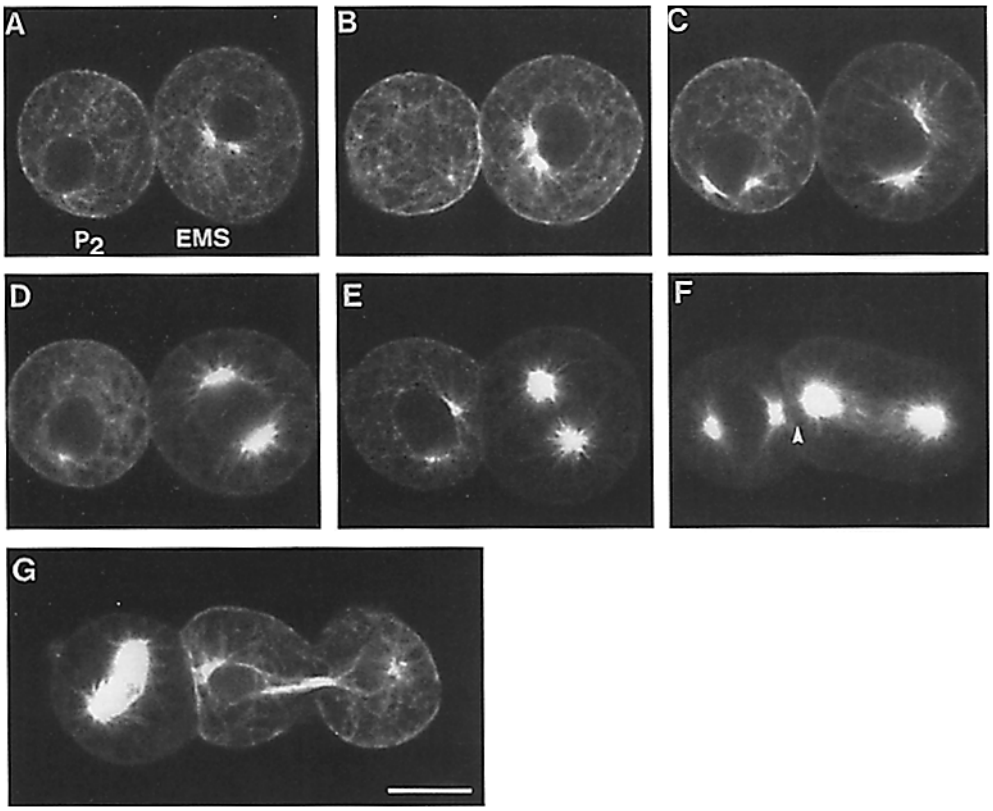

Figure 8. Anti-microtubule immunofluorescence in P_2–EMS cell pairs, through migration (*A–C*), rotation (*D–F*), and EMS cell division (*G*). Each cell pair was placed in contact before the critical time and was fixed some minutes later. In each photo the P_2 cell is the smaller cell and is on the left. The original position of P_2 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 P_2 ~ 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 P_2, as indicated by the arrowhead in *F*. The asters in P_2 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.

Cell-Autonomous Centrosome Rotation in P_1 and P_2

To determine whether centrosome rotation occurs cell autonomously in P_1 and P_2, 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 P_1 and P_2 cells. Approximately 90° of rotation occurred in each cell. The result suggests that unlike EMS and E, centrosome rotation occurs in P_1 and P_2 independently of contact with other cells. Additional support derives from the experiments in which P_2 and EMS were placed in contact in random orientations, as EMS's spindle axis was aligned with P_2, yet P_2 divided in random orientations (Figs. 3 and 5). This shows that the mitotic spindle axis of P_2 is not aligned by contact with EMS. Similar observations were made in the P_3 cell in experiments in which P_3 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 (P_1, P_2, and P_3) 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)[1] 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 Huffaker, 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; Chenevert, 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 P_2 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 P_2–EMS cell border) and at the P_3–E and P_4–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 (P_2–EMS and P_3–E), and the smaller complexes might be responsible for cell-autonomous centrosome rotation in cells such as P_1, P_2, and P_3. 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 P_0, P_1, P_2, and P_3 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 P_2 appears to polarize EMS with respect to the developmental information it contains: P_2 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 P_2 contacts); either side of EMS can give rise to the gut via contact with P_2, and in the absence of contact with P_2, 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 P_2 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 P_2 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 P_2 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 P_2 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 P_2 to the opposite side of EMS causes EMS's

1. *Abbreviation used in this paper*: CP, actin capping protein; MTOC, microtubule-organizing center.

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 P_2, to one of EMS's daughter cells.

The results suggest a model in which P_2 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 P_2 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 P_2 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.

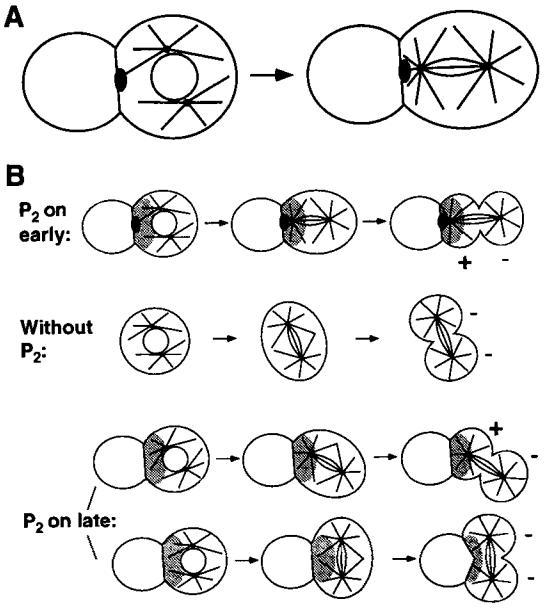

Figure 9. (*A*) Model for mitotic spindle orientation via cell–cell contact. Contact between specific cells establishes, in the cortex of one cell, a site (*black dot*) that captures astral microtubules, causing rotation of the centrosome–nuclear complex and hence alignment of the mitotic spindle. (*B*) In the EMS cell, P_2 both induces a cortical site (*black dot*) that orients the mitotic spindle and gut cell fate (*shading*) in one side of EMS. Plus and minus signs indicate whether gut differentiation occurs from EMS's daughter cells in each situation. (*P_2 on early*) Both effects occur in intact embryos or when P_2 is moved to a new site on EMS early in its cell cycle. (*Without P_2*) Neither effect occurs when EMS is isolated early in its cell cycle. (*P_2 on late*) When EMS is cultured in isolation through the critical time for spindle orientation, placing P_2 back in contact with EMS (Fig. 2 *C*) can still induce gut fate in one of EMS's daughters without affecting spindle orientation. In these cases, a division orientation that divides the region of cell–cell contact (and hence of gut specification) in two prevents gut differentiation (*bottom row*), whereas all other EMS spindle orientations are compatible with gut induction (*top row*). Note that when P_2 is placed in contact with EMS late in the EMS cell cycle (Fig. 2 *C*), EMS's nucleus does not move toward P_2 yet gut is induced, suggesting that gut induction does not require the asymmetric localization of the EMS nucleus.

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 P_2, no centrosome rotation occurred in EMS and EMS divided in an orthogonal (AB-like) manner. Second, when two P_2 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 (P_1, P_2, and P_3) 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, P_2, allowing cell division to partition developmental information.

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