Centrosome Movement in the Early Divisions of *Caenorhabditis elegans*: A Cortical Site Determining Centrosome Position

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Abstract. In *Caenorhabditis elegans* embryos, early blastomeres of the P cell lineage divide successively on the same axis. This axis is a consequence of the specific rotational movement of the pair of centrosomes and nucleus (Hyman, A. A., and J. G. White. 1987. *J. Cell Biol.* 105:2123–2135). A laser has been used to perturb the centrosome movements that deter- mine the pattern of early embryonic divisions. The results support a previously proposed model in which a centrosome rotates towards its correct position by shortening of connections, possibly microtubules, be- tween a centrosome and a defined site on the cortex of the embryo.

THROUGHOUT the development of many invertebrates, the axes of cell division are defined, and the specifica- tion of these axes will be essential for correct develop- ment (Wilson, 1925). However, the basic processes by which a specific pattern of division is established during develop- ment remain obscure. During the division of a cell the cleav- age furrow bisects the mitotic apparatus (Conrad and Rappa- port, 1981), therefore a specific pattern of cleavage requires a mechanism for positioning mitotic spindles. A mitotic spindle forms between two centrosomes (Sluder et al., 1985; Hyman and White, 1987), and in early *Caenorhabditis ele- gans* embryos it appears that the central mechanism for es- tablishing a defined pattern of division is defined and repro- ducible movements of centrosomes before cell division (Hy- man and White, 1987).

These early divisions follow a stereotyped pattern of divi- sion (Nigon et al., 1960; Sulston et al., 1983), and the cen- trosomes are easily followed in living specimens (Nigon et al., 1960; Hyman and White, 1987). There are two different patterns: in the early AB lineage (Sulston et al., 1983), each blastomere divides at 90° with respect to its mother generat- ing an orthogonal pattern. The early P lineage blastomeres (Sulston et al., 1983) divide successively on the same axis (Laufer et al., 1980). Separation of the daughter centro- somes across the surface of the nucleus generates the or- thogonal pattern of division (Hyman and White, 1987). In those blastomeres that divide successively on the same axis, the centrosomes again migrate apart over the surface of the nucleus onto the transverse axis, but then the diametrically opposed centrosomes and nucleus rotate as a unit to lie on the longitudinal axis of the embryo, the future axis of divi- sion (Hyman and White, 1987). A specific pattern of centro-

some–nuclear movement therefore establishes the division axes of the embryo. During rotation in some blastomeres one centrosome moves towards a defined region of the cell cor- tex, a position retained for the rest of the cell cycle (Hyman and White, 1987). In this paper, I have perturbed centrosome movement using a laser in order to look at the relationship between centrosome movement and this region of the cortex.

Materials and Methods

Nematode Strains and Maintenance

Wild-type (N2) *Caenorhabditis elegans* (strain Bristol) was cultured at 20°C on agar plates with *Escherichia coli* strain OP50 as a food source (Brenner, 1974).

Examination of Embryos

Embryos were examined by light microscopy using Nomarski differential- interference contrast microscopy (Sulston and Horvitz, 1977). Embryos were placed on a thin pad of agarose and covered with a coverslip and sealed with Vaseline. Development was recorded using a Hamamatsu C2400 video camera together with an RCA (Lancaster, PA) time-lapse video recorder.

Laser Irradiation of Embryos

In all experiments, irradiation was performed using a Photochemical Re- search Associates, Inc. (Ontario, Canada) LN 1000, pulsed nitrogen laser, connected to an LN 100 dye laser. The dye used was 7-amino-4-methyl Cou- marin, wavelength 450 nm (Photochemical Research Associates, Inc.). The differential effects of laser irradiation can be obtained by setting up the laser power to make a very transient bubble in the cytoplasm, ∼1 μm^3. However, the experiments were generally performed successfully at much lower laser power, in which no visible damage was seen to the cytoplasm. Centrosome movement was followed until the centrosomes were in their required posi- tion. A graticule was used to position the laser beam. The laser was then fired at a pulse rate of twice per second. Irradiation was stopped as soon as an affect on centrosome movement was seen. In the experiments stopping rotation, this required an average of about one hit, but this was very depen-

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dent on the available power. Laser irradiation at the two cell stage, either on the centrosomes or at random positions in the cytoplasm, had no effect on the cell cycle or the development of the embryo: the embryos developed normally and hatched. Irradiation of the anterior cortex of P_1 caused a cytoplasmic bridge to form between AB and P_1, which resealed during cytokinesis, but these embryos did not develop properly. Other studies have destroyed centrosomes using a laser. The centrosomes of tissue-culture cells were irradiated with a continuous beam from an argon ion laser (Berns et al., 1977) and a 266-nm UV laser (Rattner and Berns, 1976).

Laser Permeabilization of Embryos

If the embryos were to be fixed after laser irradiation, they were mounted as follows. Embryos were cut from gravid hermaphrodites, collected with a micropipette, and clustered on a coverslip coated with 0.1% polylysine. The coverslip was inverted and the embryos covered with a drop of fixative after which the coverslip was inverted over a depression slide containing the fixative.

Fixation of Embryos

After laser irradiation, the beam of the laser was immediately positioned over the anterior end of the eggshell. The power of the laser was adjusted so that a large hole was blown in the eggshell. This had the double advantage of allowing the fixative in quickly, and also insuring penetration of the antibodies. Paraformaldehyde cannot be used as a fixative by eggshell penetration because it gradually kills the embryos, nor could methanol, because the manipulations required to take the coverslip from the microscope are beyond the time course of the experiment. Embryos were permeabilized in 0.6% glutaraldehyde, but prolonged fixation in glutaraldehyde prevented penetration of antibodies. To overcome this problem, the embryos were immediately removed from the glutaraldehyde and rinsed in isotonic medium consisting of 4% sucrose and 0.1 M NaCl. The osmolarity of the culture medium is critical, and is tested by determining whether the embryo swells or shrinks after laser permeabilization of the eggshell (Priess and Hirsch, 1986). The embryos were then placed in 2% paraformaldehyde in isotonic medium. The paraformaldehyde seemed to prevent the over-fixation caused by glutaraldehyde. After 15 min, excess paraformaldehyde was removed, and the coverslip placed in methanol at −20°C for 4 min. The embryos were rinsed in PBS, and treated with four changes of sodium borohydride (Sigma Chemical Co., St. Louis, MO) at 10 mg/ml. The loss of autofluorescence was monitored under a fluorescence microscope.

Immunofluorescence

Embryos were rinsed in three changes of PBS. The slides were then incubated for 1 h in YL1/2 (Kilmartin et al., 1982) washed in PBS with 0.1% Tween 20 and incubated in FITC–anti–rat IgG (Sigma Chemical Co.), which had been affinity purified by elution with glycine, pH 1.9, for 1 h and washed again. Specimens were mounted in phenylenediamine (Sigma Chemical Co.), and observed with a Carl Zeiss Inc. (Thornwood, NY) axioplan microscope equipped with epifluorescence, or a confocal microscope (White et al., 1987).

Data Analysis

Although the background using this protocol was higher than seen with some other fixation protocols, the protocol allowed rapid fixation after irradiation. The smallest time interval between irradiation and fixation was ∼6 s. The average time was ∼10 s, by which time the centrosome had moved back towards the transverse axis. Sections were photographed using a confocal microscope. To quantify the loss of microtubules in the irradiated area, I counted the number of microtubules in the defined quadrants around the irradiated centrosome, the unirradiated centrosome, and around centrosomes of unirradiated embryos. In particular I counted the number and length of microtubules in the region that corresponded to the irradiated region of the embryo. Quantification of the data was problematical because the centrosomes were at different distances from the anterior cortex in different embryos. I therefore measured the length of all the microtubules as a function of their possible length before touching the cortex.
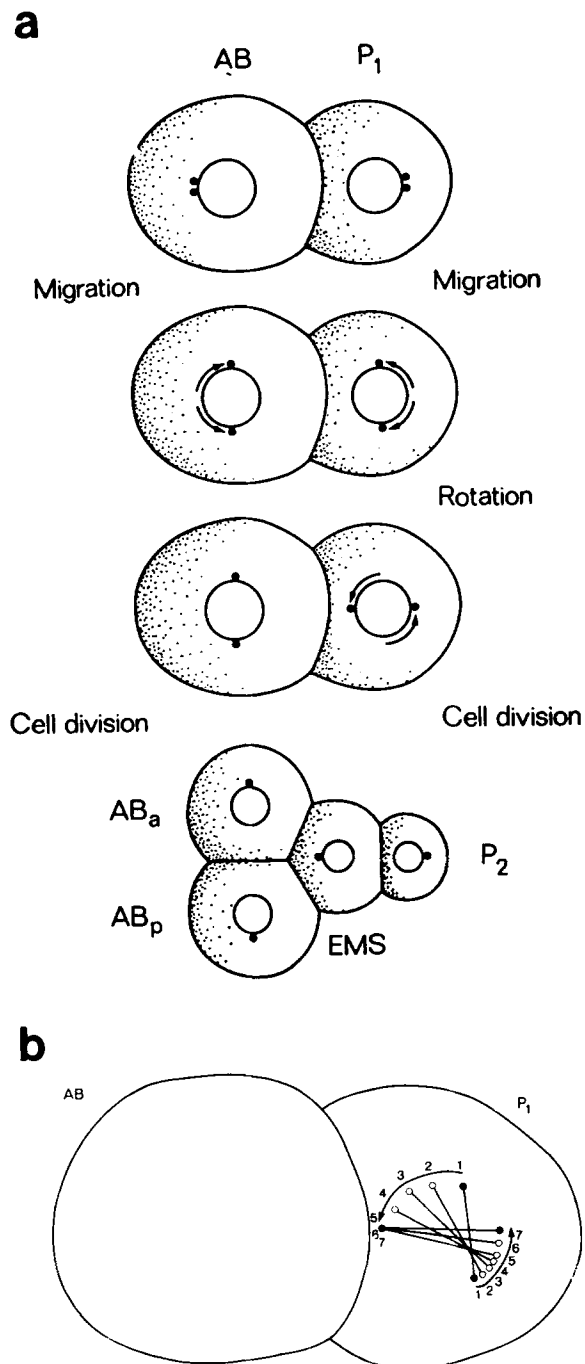
Figure 1. (a) The pattern of centrosomes at the two cell stage. Both AB and P_1 have inherited a centrosome from the division of P_0. In AB, the centrosomes split and migrate apart to lie on the transverse axis of the embryo where they will form the poles of a transverse spindle. In P_1, after centrosome migration, the centrosomes rotate through 90° onto the longitudinal axis of the embryo where they form the poles of a longitudinal mitotic spindle. (b) The specific pattern of centrosome movement during rotation in P_1, from Hyman and White (1987). The circles represent the centrosomes and the lines represent the distance to the center of the nucleus. Right is posterior, left is anterior. The numbers 1–7 represent time-points at 10-s intervals. Most of the movement at rotation takes place by movement of one centrosome towards the anterior cortex.

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Results

Background

The zygote P_0 divides on the longitudinal axis of the embryo to give the daughter cells AB and P_1 (Sulston et al., 1983). The blastomeres of the AB lineage divide with an orthogonal pattern, whereas the P blastomeres, P_0, P_1, P_2, EMS, and E divide successively on the longitudinal axis of the embryo. The centrosome movements responsible for successive divisions on the same axis have been determined (Hyman and White, 1987), and will be described here for the two cell stage, AB and P_1. After division of P_0 to give P_1 and AB, the nucleus in each daughter cell forms between the centrosome and the site of the P_0 cleavage furrow. The centrosome splits and the daughter centrosomes migrate apart across the surface of the nucleus until they lie on the transverse axis of the embryo (Fig. 1 *a*). In AB the centrosomes remain on the transverse axis, and the spindle is established on the same axis. After migration in P_1, the pair of centrosomes and nucleus rotate through 90° to lie on the longitudinal axis (Fig. 1 *a*). Rotation has random direction and takes place in ∼1 min out of the total P_1 cell cycle of 12 min at 20°C. The pattern of centrosome movement during rotation has been precisely defined. During rotation, the centrosomes stay diametrically opposed, with fixed positions on the nucleus. The direction of rotation is random. The posterior centrosome stays relatively still while the anterior centrosome moves toward, and then lies next to, the anterior cortex of P_1 (Fig. 1 *b*).

### Centrosome Rotation Normally Has Random Direction, but Damaging of One Centrosome Defines the Direction

Just before rotation of the centrosome–nuclear complex onto the anterior–posterior axis in P_1, both centrosomes lie equidistant from the anterior pole (Fig. 1 *b*). Rotation of the centrosome–nuclear complex could occur by preferential movement of one of the centrosomes towards the anterior or posterior pole or there could be equal preference for either pole. To test these possibilities, I irradiated one of the centrosomes during centrosome migration in P_1.

I followed the separation of the centrosomes by Nomarski

microscopy until they were almost diametrically opposed on the nucleus on the transverse axis of the embryo. One centrosome was then irradiated with the laser, and the movements of both centrosomes were followed. In all 22 embryos tested, the unirradiated centrosome rotated to the anterior cortex. In Fig. 3 *a*, the right centrosome has been irradiated with the laser, and in Fig. 3, *b–d*, the other centrosome has rotated towards the anterior (bottom) cortex. In unirradiated P_1 blastomeres, a centrosome chosen at random would rotate towards the anterior cortex in half of the embryos examined (Hyman and White, 1987). For instance, in Fig. 3 *a*, the left centrosome would move to the anterior cortex in 50% of untreated embryos. However, in 100% of embryos in which the right-hand centrosome had been damaged, the left-hand centrosome rotated to the anterior cortex. Similarly if the left-hand centrosome was irradiated, the right-hand centrosome would always rotate to the anterior cortex. The paths of centrosome movement after these experiments were normal: the centrosome–nuclear complex pivoted around the damaged centrosome, while the other centrosome moved to the anterior cortex.

The specificity of the irradiation was checked by irradiating posterior to either centrosome, which did not affect its ability to move to the anterior cortex. Irradiation during centrosome migration on the nuclear membrane equidistant between the two centrosomes, either anterior or posterior, had no effect on the direction of rotation. To assess the effect of rotation on the centrosome, staining the embryos with antitubulin antibody showed that the structural correlation with the functional effect was that the number and length of the microtubules in the irradiated centrosome was greatly reduced compared with the unirradiated centrosome (Fig. 2). The effect on microtubule distribution was no longer obvious at the time of spindle formation (data not shown).

A similar study was conducted on the rotation in the zygote P_0 (Nigon et al., 1960; Albertson, 1984; Hyman and White, 1987). This pattern of centrosome movement is similar to that in P_1. The location of one centrosome stays relatively unchanged while the other rotates towards the anterior pole (data not shown). One centrosome was then irradiated with the laser after pronuclear apposition (Albertson, 1984) but before rotation. In 11 out of 12 cases, the undamaged centrosome rotated towards the anterior cortex. None of
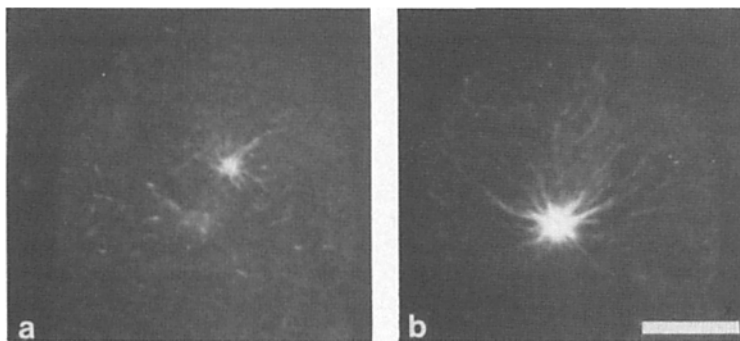
Figure 2. Antitubulin antibody staining of a P_1 cell after extensive irradiation of one centrosome. Posterior is up, only P_1 is shown. This figure illustrates a particularly severe case of damage. Other embryos had less damage to their centrosomes. The embryo was surrounded with glutaraldehyde and fixed immediately after centrosome irradiation by puncturing the egg shell with the laser. The embryos were stained with an antitubulin antibody (Kilmartin et al., 1982). (*a*) The centrosome has been irradiated; (*b*) the unirradiated centrosome of the pair. The staining pattern shows that the irradiated centrosome in *a* has a smaller focus of tubulin staining, and had fewer microtubules at the time of fixation. The energy used in these experiments did not irreversibly destroy the centrosome: a centrosome irradiated during prophase formed the pole of a mitotic spindle, and in general no defect was detectable in the morphology of the spindle. If the centrosome was irradiated a large number of times, chromosome segregation was occasionally affected; i.e., the occasional chromosome was lost from the spindle and formed its own micronucleus. Bar, 10 μm.

Table I. Movement of Nucleus and Transverse Centrosomes Before Rotation

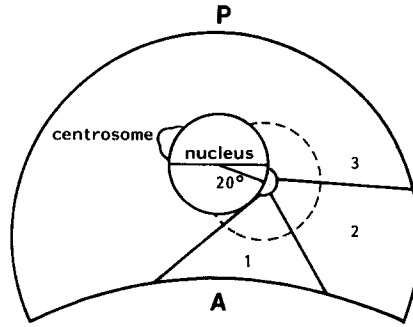| Embryo	Distance*	Distance‡
	μm	%
1	3	42
2	3.5	50
3	1	14
4	1	14
5	0	0
6	1.5	21
7	2.5	36
8	1	14
9	2	29

D, Distance moved by transverse centrosomes and nucleus before rotation begins. This table represents data taken from nine P_1 embryos recorded in succession.
* The distance moved in micrometers.
‡ The distance moved as a percentage of the total possible movement before the nucleus and transverse centrosomes would hit the anterior cortex.

Figure 4. Quadrants of P_1 defined by a graticule. A diagram illustrating how the cell was divided up into imaginary quadrants. A graticule with a cross was used to defined the lines crudely, and all the experiments in quadrant *1* were effectively done by irradiating in a straight line between the centrosome and the cortex.

these irradiations had any effect on the future development of the embryo. Therefore, it appears that direction of rotation in two blastomeres, P_0 and P_1, can be changed from random to predictable by irradiation of a centrosome before division.

Another question that could be addressed by damaging one centrosome concerned the initial anterior movement of the centrosome–nuclear complex before rotation but after centrosome separation, a movement noticed during the course of these experiments: towards the end of centrosome separation, the nucleus and centrosomes initially move towards the anterior cortex without any rotational movement.

After this initial movement, which varies considerably from embryo to embryo (Table I), the centrosome–nuclear complex rotates. It seemed possible that this movement occurs because both centrosomes are initially engaged by the rotational machinery. To test this, the anterior movement of the nucleus was examined in P_1 cells in which one centrosome had been damaged at the end of centrosome migration. The result was that the anterior movement was abolished, and rotation occurred immediately after migration (compare the position of the nucleus before rotation in Fig. 3 *b* with the position of the nucleus in Fig. 5 *b* before rotation). As a further test, one centrosome was damaged during the anterior movement of the nucleus and transverse centrosomes: in all six embryos tested, rotation started immediately after irradi-
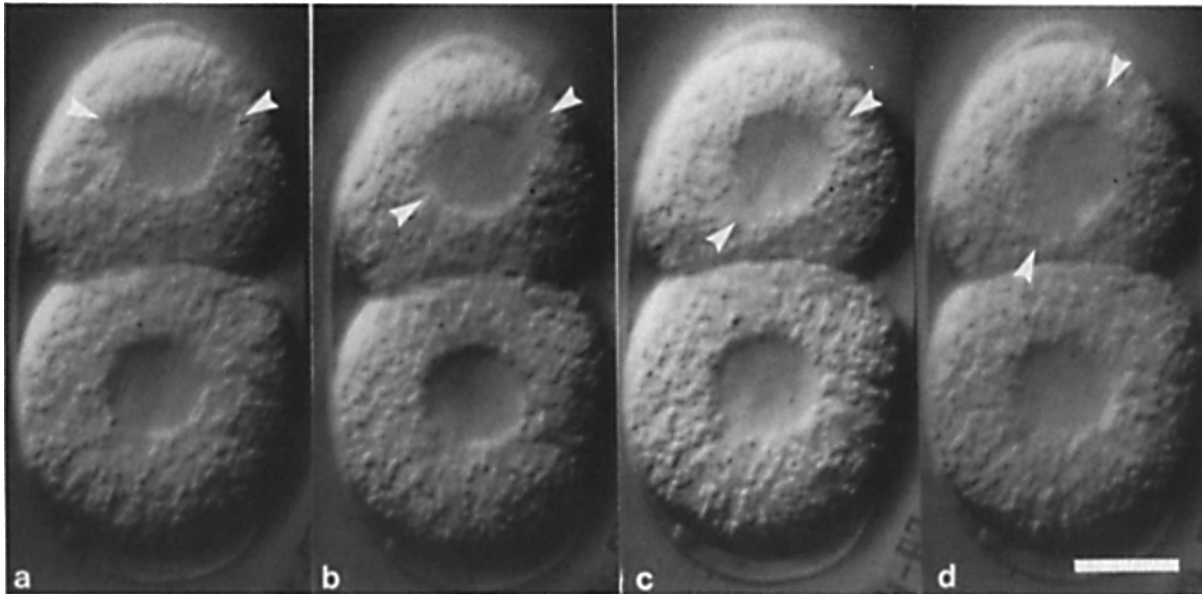
Figure 3. Nomarski microscopy at the two cell stage illustrating rotation of the centrosome–nuclear complex after laser irradiation of one centrosome in P_1. P_1 is the top cell. Centrosomes are illustrated by arrowheads. (*a*) The centrosome on the right has been irradiated. (*b*) 15 s, the left-hand centrosome has rotated towards the anterior cortex. (*c* and *d*) The left-hand centrosome has touched the anterior cortex. Close observation will show that the irradiated centrosome has stayed relatively still during rotation. Irradiation of the centrosome causes damage as revealed by microtubule staining in Fig. 2. However, it is possible to influence the direction of rotation routinely with less damage to the centrosome, suggesting that the laser interferes with the dynamics of microtubule assembly from the centrosome rather than causing whole-scale destruction. In all these experiments, the centrosome recovered from irradiation to organize one pole of a mitotic spindle, and the development of the embryo was unaffected. Bar, 10 μm.

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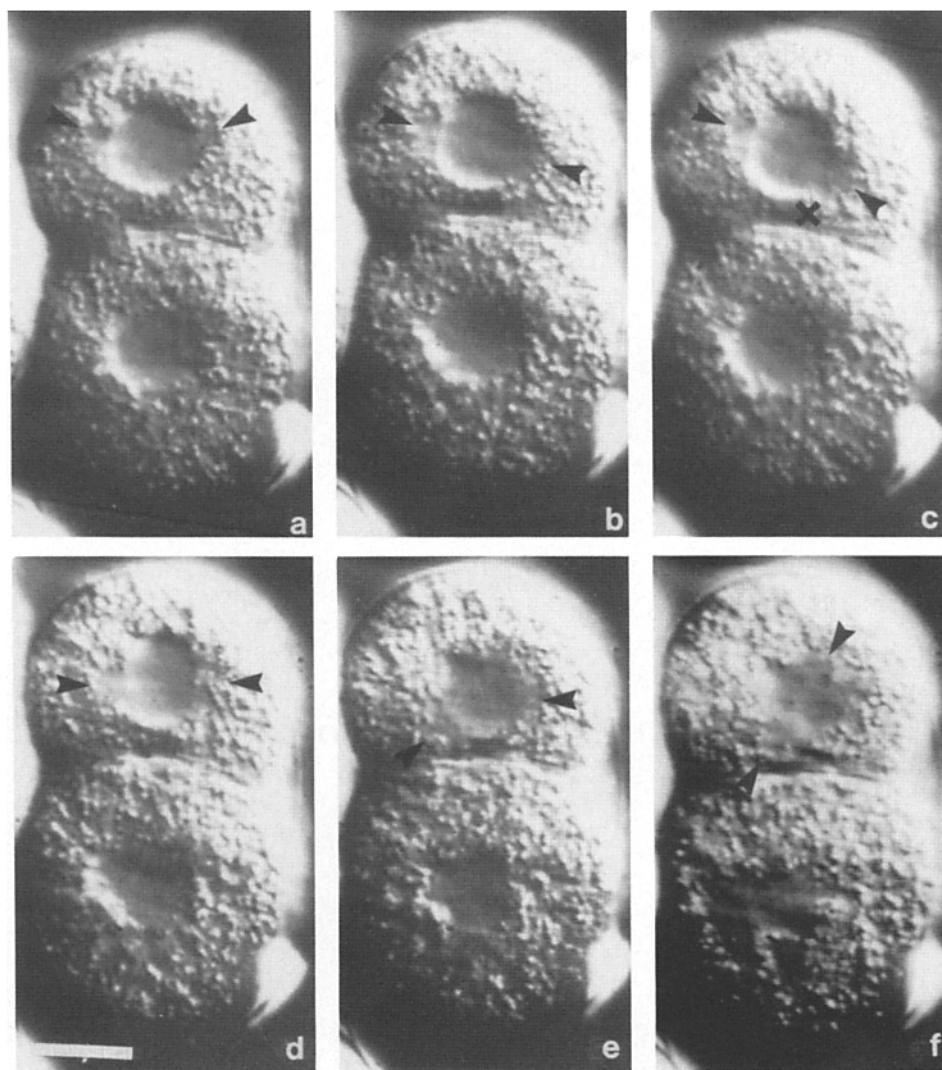
Figure 5. Stopping and changing direction of rotation by laser irradiation in quadrant *1* (Fig. 4). Posterior is up, anterior down. P_1 is the posterior cell, AB the anterior cell. Centrosomes are shown by arrowheads, irradiation position by a cross. (*a*) The centrosomes are transverse after migration. (*b*) The left centrosome has started rotation. (*c*) Rotation has been stopped by laser irradiation. (*d*) The centrosomes have returned to the transverse axis. (*e*) The other centrosome has rotated to the anterior cortex. (*f*) Rotation is complete. Rotation was unaffected by irradiation around the posterior centrosome, or at any other position in the cell including the nucleus, except quadrant *1*. Irradiation around AB centrosomes had no effect on their placement. Irradiation on the anterior cortex at the predicted final position of the anterior centrosome stopped rotation but also resulted in cell fusion and a small cytoplasmic connection being established between AB and P_1. Bar, 10 μm.

ation, and the unirradiated centrosome moved towards the anterior cortex (data not shown).

The Rotational Movement of the Anterior Centrosome Stops After Irradiation in Front of Its Leading Edge

The experiments in the previous section show that a single functional centrosome will preferentially localize on the anterior pole of P_0 and P_1 (Fig. 3). Two simple ways of gener-

ating the force which causes a centrosome to move toward the anterior cortex could be a repulsion of the centrosome from the posterior cortex, or an attraction toward the anterior cortex. To distinguish between these possibilities, different parts of the cytoplasm were irradiated in order to interfere with the connections between the cytoplasm and the cortex.

The experiments were performed when the anterior centrosome had rotated 20° from the transverse axis. To quantify these experiments, quadrants of the cell were defined
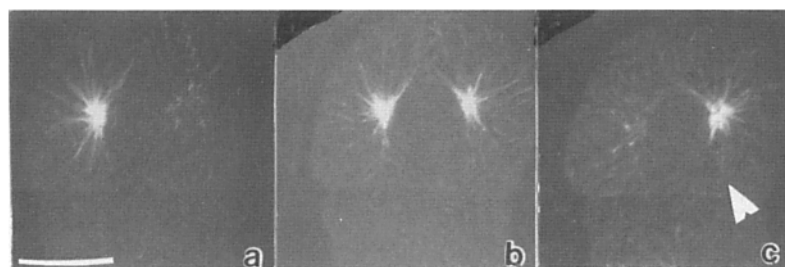
Figure 6. Microtubule distribution after laser irradiation has stopped rotation in P_1. P_1 is up (posterior). The figure illustrates an embryo irradiated during rotation. Rotation stopped, the centrosome went back to the transverse axis, and the embryo was fixed at ~10 s and stained with an antitubulin antibody. Shown here are three sections taken with a confocal microscope. (*c*) The same section as the original laser irradiation, and the white arrowhead marks the position of quadrant *1* where the embryo was irradiated. Very little microtubule staining is seen extending from the centrosome into this region, and this effect was reproducibly seen in most of the 17 embryos treated in the same way. (*b*) A section in which the centrosome in *c* is going out of focus and the other centrosome is coming into focus. *a* shows the other centrosome. *a* and *b* are illustrative of the fact that all the sections that contained the left centrosome in focus had microtubules extending towards the anterior cortex. Bar, 10 μm.

using a graticule (see diagram in Fig. 4). Irradiation between the anterior centrosome and the anterior cortex (quadrant *1*, compare Figs. 4 and 5) prevented rotation in 63 out of 65 embryos tested (Fig. 5). In Fig. 5 *c* rotation was stopped by irradiation in quadrant *1* marked by the black cross. Irradiation in quadrants *2* and *3* stopped rotation in only 2 out of 50.

As a further test, the distance from the centrosome at which the irradiation could stop rotation was investigated. A circle was defined with its center at the centrosome and a radius of $\sim$5 μm (Fig. 4). Rotation was stopped in 10 out of 10 embryos irradiated in quadrant *1* (Fig. 4) between the circumference of the circle and the cortex of P_1.

Irradiation Between the Anterior Cortex and the Anterior Centrosome Causes Loss of Microtubules in the Irradiated Region

It has previously been shown that microtubules are intimately involved in rotational movements of both P_0 and P_1 (Strome and Wood, 1983; Hyman and White, 1987). To assess the effect of cytoplasmic irradiation on the distribution of microtubules, 17 embryos were irradiated between the anterior cortex and the centrosome, and fixed as quickly as possible after rotation had stopped. The embryos were stained with an antitubulin antibody (Kilmartin et al., 1982). The stained embryos were photographed and the distribution of microtubules traced (Fig. 6). Measuring the length of a microtubule as a ratio of its actual length to its possible length before touching the cortex, showed that the length of the ratio was $\sim$40% in an irradiated region compared with a similar unirradiated region. Embryos were also fixed 30 s after rotation had stopped. By this time, the distribution appeared similar to that of untreated embryos (data not shown).

Dynamics of Rotation Revealed by Laser Irradiation

To assess the dynamic relationship between the centrosome and the cortex, I investigated whether rotation would restart after it had been stopped by laser irradiation, and if so what was the direction of rotation upon restarting. Tracing the movement of the centrosomes showed that in all embryos so irradiated, the centrosome–nuclear complex started rotation again. However, before this, the centrosomes often became aligned on the transverse axis again (Fig. 5 *d*). I initially thought that the centrosome became aligned on the transverse axis again because the posterior centrosome had maintained some connections during rotation, and now these connections could cause rotation in the other direction. I performed two tests of this hypothesis. First, the position of the unirradiated centrosome was traced during the experiment. To my surprise, I found that, in nearly all embryos examined, the unirradiated centrosome stayed in place while the irradiated centrosome moved back to the anterior cortex: movement of the anterior centrosome began back towards the transverse axis at an angular velocity (1.5 $\pm$ 0.4 deg/s) approaching that of the original rotation (2.1 deg/s), as if a spring compressed during rotation was released by irradiation. Second, I ablated the posterior centrosome during rotation of the anterior centrosome: I assumed that if the posterior centrosome maintained connections, it would slow down the velocity of rotation. However, irradiation of the posterior centrosome had no affect at all on the velocity of rotation of the anterior centrosome.

If the centrosomes sometimes returned to their prerotation position at the transverse axis (Fig. 5 *d*) there was then a 50% chance of the previously posterior centrosome rotating to the anterior cortex (Fig. 5 *e*). In other words, the direction of rotation was again randomized, as was the case for the initial rotation direction: after the irradiated centrosome had had its connections interfered with, each centrosome then had an equal opportunity of establishing connections. If irradiation was continued close to the original anterior centrosome after rotation had stopped, then the original posterior centrosome moved towards the anterior cortex in 100% of cases. This change of direction was extremely rapid, with an average of 17 s for rotation to start again; the longest time for restarting was 25 s. It was further possible to change the direction of centrosome movement a number of times in a single P_1 blastomere, so that eventually the nucleus and transverse centrosomes made contact with the anterior cortex of P_1. The mitotic spindle was then established between transverse centrosomes. The mitotic spindle continued to make rotational movements throughout the remainder of the cell cycle ($\sim$4 min is available in the cell cycle between rotation and cytokinesis), and rotation went to completion unless stopped by irradiation.

In some embryos in which rotation had been initially stopped, both centrosomes moved around the nucleus towards the anterior cortex so that they were no longer diametrically opposed on the nuclear surface (Fig. 7 *d*), showing that both centrosomes can concurrently engage the rotational machinery. One centrosome would eventually become the anterior centrosome, while the other centrosome would move around the nucleus to become the posterior centrosome (Fig. 7, *e* and *f*). In conclusion, both centrosomes appear to maintain a dynamic relationship with the anterior cortex of P_1, and laser irradiation can bias the rotation in favor of either centrosome.

The Appearance of a Membrane Invagination Correlates with the Rotation of the Centrosome

During the course of experiments to change the direction of rotation, a membrane invagination often appeared on the anterior cortex of P_1 (Fig. 8). When investigated carefully, this same invagination was occasionally seen during normal rotation and correlated temporally with the initial anterior movement of transverse centrosomes and nucleus. During rotation, the anterior centrosome moves toward the invagination, and eventually lies next to it. Furthermore, if the microscope is focused on the invagination whilst the centrosome is out of focus, the centrosome generally moves into this focal plane. The invagination remains present throughout the remainder of the P_1 cell cycle. The invagination remains with the future AB_p (the posterior AB daughter), during positioning of the future AB daughters by the skewing of the AB spindle (Fig. 8, *c* and *d*) (Priess and Thomson, 1987). If rotation had been blocked and the spindle was established between transverse centrosomes, then the rotational movements of the spindle continue towards the invagination. However, only the spindle pole near the invagination will make rotational movements. Irradiation at the invagination always stopped rotation. This invagination is indicative of tension on the cortex and suggests that rotational movements are generating tension on the cortex.
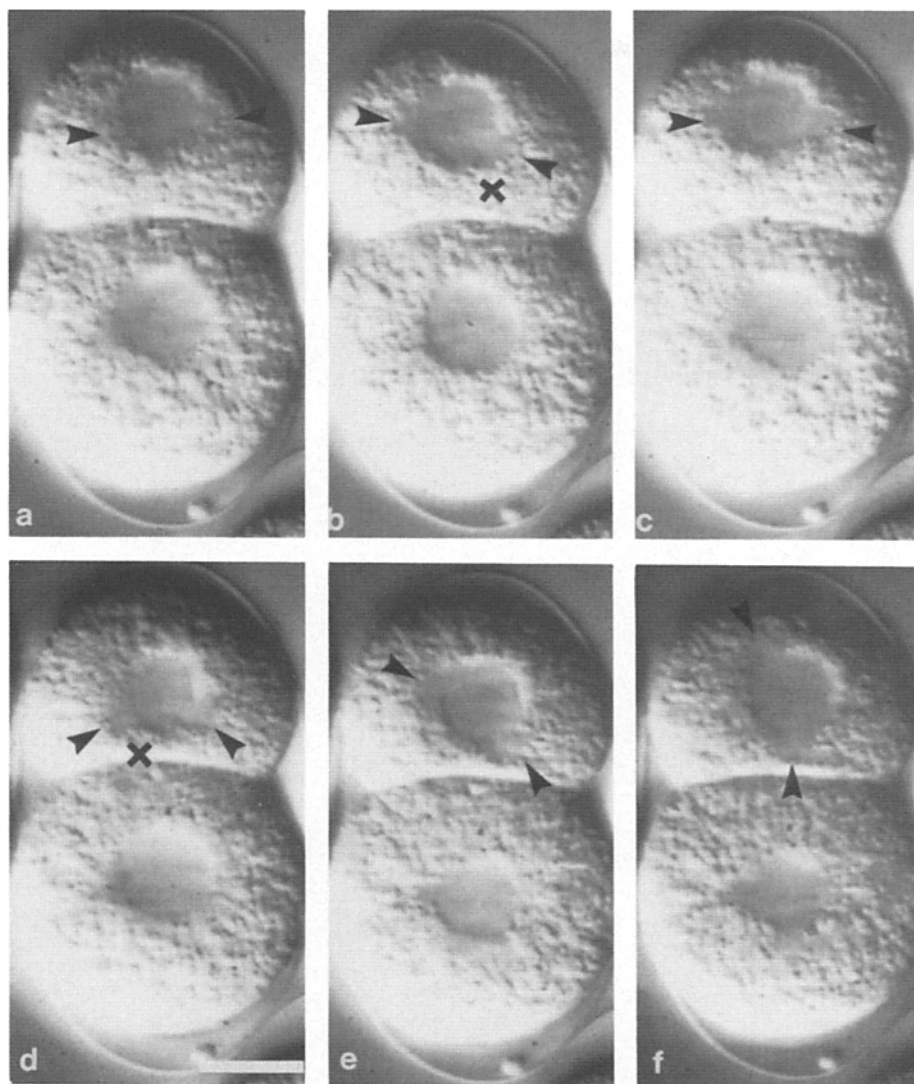
Figure 7. Concurrent movement of both centrosomes to the anterior cortex. Posterior is up, anterior is down. P₁ is posterior, AB anterior. Centrosome position is shown by arrowheads, the position of laser irradiation by a cross. (*a*) The centrosomes are transverse. (*b*) 15 s, rotation of the right-hand centrosome has been stopped by laser irradiation. (*c*) 28 s, the centrosomes have returned to the transverse axis. (*d*) 1 min, both centrosomes have moved around the nucleus to the anterior cortex. One centrosome was then irradiated. (*e*) 1.15 min, the centrosome has moved away from the cortex. (*f*) 1.45 min, rotation is complete. Bar, 10 μm.

Discussion

Hertwig's development of Sach's law (Wilson, 1925), states that the axis of the mitotic figure typically lies on the long axis of the cell, and from this it follows that the axis of division of a cell will depend on its long axis. Establishing a pattern of division that deviates from this law requires a mechanism for specifically positioning mitotic spindles. During the early embryonic divisions of *C. elegans*, the division axes of one set of blastomeres depend on specific rotational movements by the pair of centrosomes and the nucleus; in these blastomeres, the final positions of the centrosomes determine the division axis. The experiments in this paper have sought to localize the force-generating elements that move the centrosomes and nucleus from the transverse axis onto this longitudinal axis, using localized interference with a laser beam.

Previous investigations had shown that one centrosome at random in P₁ moves from the transverse axis towards the anterior cortex, and the current experiments have considered whether rotation occurs because one centrosome at random is pulled to the anterior cortex. I have demonstrated that, although in untreated embryos a centrosome will move to the

anterior cortex 50% of the time, if one centrosome is irradiated the other will always move to the anterior cortex: the direction of rotation has been changed from random to predictable. This says that the preferential position for a centrosome during rotation is on the anterior cortex, but that one centrosome at random takes up this position. Laser irradiation around the centrosome during rotation was consistent with the idea that this centrosome is pulled towards the anterior cortex and it seemed likely that the laser is interfering with connections between the centrosome and the anterior cortex which mediate rotation. The tension produced probably generates the invagination occasionally seen just before and during rotation. Therefore, it appears that the process by which a P₁ blastomere sets up a specific division axis involves connections established between a centrosome and a defined site on the cortex of the embryo.

The most likely connections causing this tension are microtubules. Previous experiments had suggested that microtubules are the cytoskeletal component necessary for both rotation in P₀ and P₁ (Strome and Wood, 1983; Hyman and White, 1987). The experiments in this paper also suggest that microtubules could be the key component. After rotation is stopped, the same centrosome can rotate again within
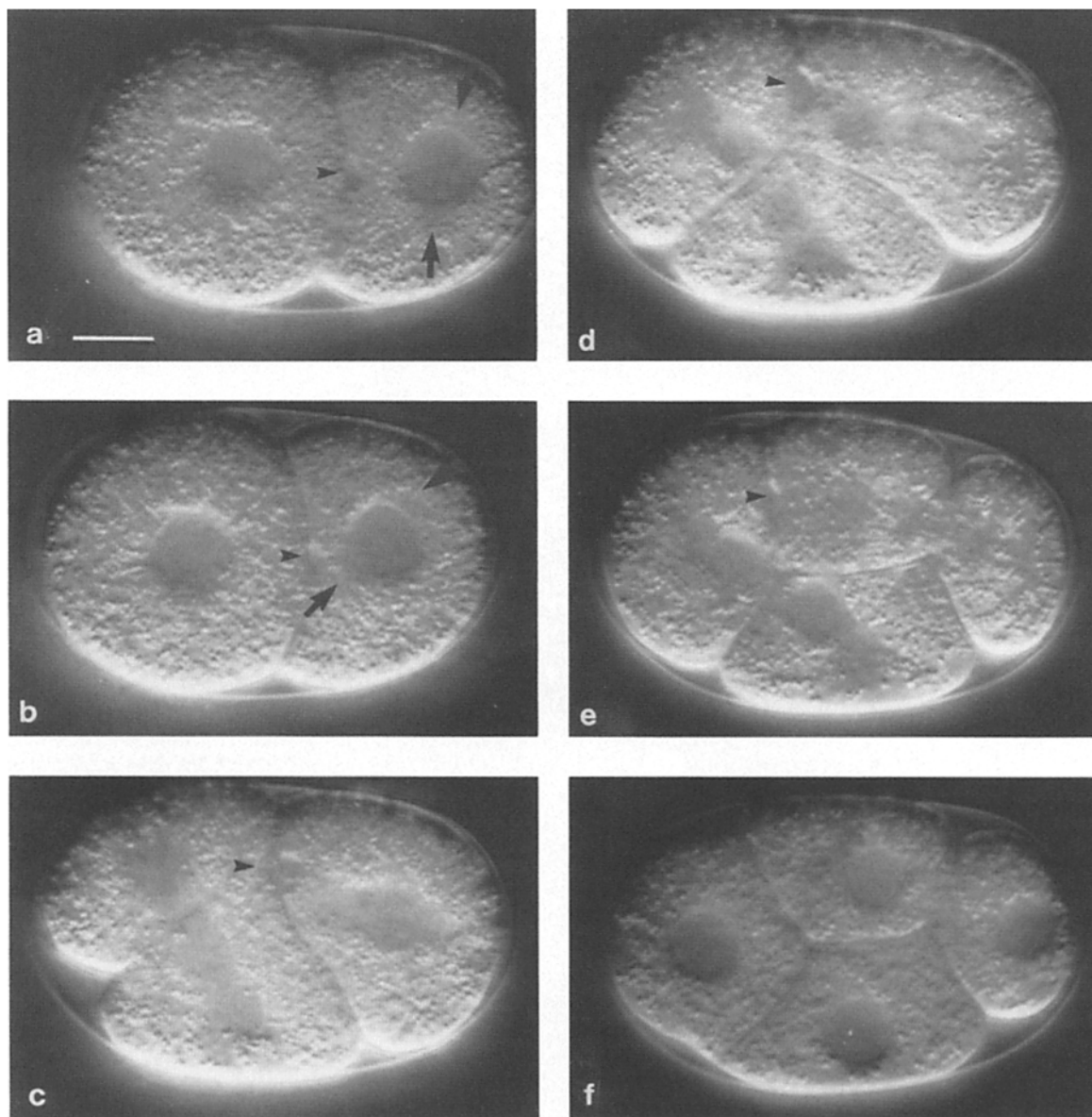
Figure 8. Appearance of a cortical invagination during rotation in P_1. Anterior is left, posterior is right. (*a*) The invagination has appeared at prophase. The arrow marks the site of the invagination. This invagination is consistent with exerting tension on the cortex during rotation. The cognoscenti of anaphase B forces may wish to note the skewing of the spindle in *c* when it becomes too large for the eggshell. If the eggshell is removed, this skewing does not occur, but continues to stay in the transverse axis and deform the egg. This is more consistent with pushing rather than pulling forces.

15 s. The ease with which the centrosomes can be steered in different directions and recover after irradiation suggests that the laser is interfering with a dynamic and changing mechanism rather than a fixed structure. Microtubules are structures that are dynamic enough to reestablish a connection and mediate rotation in this time interval (Mitchison et al., 1985). Microtubules in the irradiated region are depleted, although the damage was very variable from embryo to embryo, and studies in other systems have shown that microtubules can be sheared by laser irradiation (Ainst and Berns, 1981). Actin may also play a role in mediating rotation, since cytochalasin D inhibits rotation in P_1. However, the experiments in this paper have shown that the rotations in P_0 and P_1 seem to follow the same pattern even though rotation in P_0 is completely unaffected by the addition of cytochalasin D (Strome and Wood, 1983). It therefore seems unlikely that

the mechanism for generating the torque on the centrosome–nuclear complex is due directly to actin microfilaments.

A simple mechanism of rotation would be that microtubules are continually nucleated by the centrosomes and when the microtubules of one centrosome at random are caught by a site on the anterior cortex, shortening of the microtubules produces a torque on the centrosome–nuclear complex that mediates rotation (Hyman and White, 1987). However, the mechanism appears not to be that simple. Data presented here suggest strongly that both centrosomes initially engage the rotational machinery. Firstly, the nucleus and transverse centrosomes move to the anterior cortex before rotation itself starts; when one centrosome was irradiated during this movement, rotation led by the other centrosome started immediately. This strongly suggests that there is competition

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between the centrosomes initially for the site, and that biasing this competition by irradiating one centrosome initiates rotation. Secondly, laser perturbation experiments occasionally resulted in the pair of centrosomes moving from the transverse axis around the nucleus towards the anterior cortex.

One is therefore forced to consider a mechanism by which both centrosomes initially engage the rotational machinery, but one centrosome eventually establishes a stronger connection and wins out. If connections are continually made and broken, then once rotation begins further rotation would be heavily biased in favor of the rotating centrosome. The results presented here, whereby the centrosome–nuclear complex travels a variable distance towards the anterior cortex before rotation starts and very occasionally the nucleus and transverse centrosomes do touch the anterior cortex, are consistent with this random model. If this model is correct, then small perturbations of microtubule dynamics by the laser would be expected to bias the direction of rotation as is experimentally seen.

There is no evidence as to the molecular nature of this site. One clue is that it may be associated with the midbody, the position where the cleavage furrow pinched off the spindle in the previous division. The centrosome that moves during rotation in P_0, P_1, and P_2 always moves in the direction of the midbody left by the previous cell division. Perhaps translocator molecules become associated with the midbody, which can bind microtubules and exert a force to mediate rotation. A possible way to approach the nature of this site is by genetic analysis, since mutants can be isolated in which rotation does not occur.

It seems that spindle positioning is a process of key importance during development (Wilson, 1925). There are few documented cases of specific mechanisms for positioning spindles. At the four cell stage, the sea urchin spindle migrates to the future site of micromere formation thus setting up an asymmetric division (Dan, 1979), and other asymmetric divisions are set up by migration of the spindle (Nigon et al., 1960; Dan and Inoue, 1987). In all these cases, the spindle appears to move with one of the centrosomes leading. The ability of centrosomes to make directed movements via their astral microtubules was first documented by Aronson (1971), who suggested that nuclear movements in embryogenesis could occur via astral microtubules; and there is strong evidence that pronuclear migration occurs by a traction force between the microtubules from the sperm centrosomes, situated on the sperm pronucleus, and the female pronucleus (Hamaguchi and Hiramoto, 1986). The role of astral microtubules in actually positioning meiotic spindles on the cortex of the embryo has been demonstrated in the *Chaetopterous* embryo where micromanipulation experiments have shown that the meiotic spindle is anchored and can migrate to a position on the cortex (Lutz et al., 1988). It is probable that this positioning is due to dynamic microtubules. The rotational movements described here differ somewhat from meiotic anchorage, in that a specific centrosome–nuclear movement sets up the division axis before nuclear envelope breakdown. However, it is likely that a common mechanism of spindle positioning during embryogenesis may involve cortical sites which interact with microtubules nucleated from centrosomes to specifically position the centrosome and therefore the mitotic spindles.

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