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

A. A. Hyman

Laboratory of Molecular Biology, Medical Research Council, Cambridge CB2 0QG, England

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 determine 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, between 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 specification of these axes will be essential for correct development (Wilson, 1925). However, the basic processes by which a specific pattern of division is established during development remain obscure. During the division of a cell the cleavage furrow bisects the mitotic apparatus (Conrad and Rapport, 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 elegans* embryos it appears that the central mechanism for establishing a defined pattern of division is defined and reproducible movements of centrosomes before cell division (Hyman and White, 1987).

These early divisions follow a stereotyped pattern of division (Nigon et al., 1960; Sulston et al., 1983), and the centrosomes 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 generating 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 centrosomes across the surface of the nucleus generates the orthogonal 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 division (Hyman and White, 1987). A specific pattern of centrosome–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 cortex, 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 Research Associates, Inc. (Ontario, Canada) LN 1000, pulsed nitrogen laser, connected to an LN 100 dye laser. The dye used was 7-amino-4-methyl Coumarin, 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, \(\sim 1 \mu m^2\). 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 position. 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-
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 P₁ caused a cytoplasmic bridge to form between AB and P₁, 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% polylysin. 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 and 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 (Price and Hirsh, 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 YLI/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](image-url)

**Figure 1.** (a) The pattern of centrosomes at the two cell stage. Both AB and P₁ have inherited a centrosome from the division of P₀. 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 P₁, 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 P₁, 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 10-5 intervals. Most of the movement at rotation takes place by movement of one centrosome towards the anterior cortex.
Results

Background

The zygote P₀ divides on the longitudinal axis of the embryo to give the daughter cells AB and P₁ (Sulston et al., 1983). The blastomeres of the AB lineage divide with an orthogonal pattern, whereas the P blastomeres, P₀, P₁, P₂, EMS, and E divide successively on the longitudinal axis of the embryo. The centrosomes move 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 P₁. After division of P₀ to give P₁ and AB, the nucleus in each daughter cell forms between the centrosome and the site of the P₀ 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 P₁, 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 P₁ 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 P₁ (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 P₁, 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 P₁.

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 P₁ 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 P₀ (Nigon et al., 1960; Albertson, 1984; Hyman and White, 1987). This pattern of centrosome movement is similar to that in P₁. 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 P₁ cell after extensive irradiation of one centrosome. Posterior is up, only P₁ 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 1. Movement of Nucleus and Transverse Centrosomes Before Rotation

| Embryo | Distance* (μm) | Distance† (%) |
|--------|----------------|---------------|
| 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 P₁ 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 3. Nomarski microscopy at the two cell stage illustrating rotation of the centrosome-nuclear complex after laser irradiation of one centrosome in P₁. P₁ 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) 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.

Figure 4. Quadrants of P₁ 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.

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 P₁ 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 irrad-
Figure 5. Stopping and changing direction of rotation by laser irradiation in quadrant 1 (Fig. 4). Posterior is up, anterior down. P₁ 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 P₁. Bar, 10 μm.

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 P₀ and P₁ (Fig. 3). Two simple ways of generating 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 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. c 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.

Figure 6. Microtubule distribution after laser irradiation has stopped rotation in P₁. P₁ 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
The Journal of Cell Biology, Volume 109, 1989

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 P1 (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 P1 cell cycle. The invagination remains with the future ABp (the posterior AB daughter), during the positioning of the future AB daughters by the skewing of the AB spindle (Fig. 8, c and d) (Prise 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 stops rotation. This invagination is indicative of tension on the cortex and suggests that rotational movements are generating tension on the cortex.
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 *P₀* 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 P1. 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 P1. However, the experiments in this paper have shown that the rotations in P0 and P1 seem to follow the same pattern even though rotation in P0 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
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–nucleus 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 P₀, P₁, and P₂ 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 Chaetopterus 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–nucleus 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.

The author would like to thank John White and Mary Dasso for their great help and support during this project; and many colleagues at the Laboratory of Molecular Biology of the Medical Research Council and the University of California at San Francisco for stimulating conversations, especially Jim Priess, Richard Durbin, Tim Mitchison, and Nichol Thomson. I would also like to thank John Kilmaritn for providing unlimited supplies of YL1/2; and Leon Avery, Doug Kellog, Marc Kirschner, and Ruth Lehman for critical reading of the manuscript. Received for publication 14 February 1989 and in revised form 18 April 1989.

**References**

Ainat, J. R., and M. W. Berns. 1981. Mechanisms of chromosome separation during mitosis in Fusarium (Fungi imperfecti). New evidence from ultrastructural and laser microbeam experiments. J. Cell Biol. 91:446-458.

Albertson, D. 1984. Formation of the first cleavage spindle in nematode embryos. Dev. Biol. 101:61-72.

Aronson, J. F. 1971. Demonstration of a colcemid-sensitive attractive force acting between the nucleus and a center. J. Cell Biol. 51:579-583.

Berns, M. W., J. B. Rattner, S. Brenner, and S. Meredith. 1977. The role of the centrosomal region in animal cell mitosis. A laser microbeam study. J. Cell Biol. 72:351-368.

Brenner, S. 1974. The genetics of Caenorhabditis elegans. Genetics. 77:71-94.

Conrad, G. W., and R. Rappaport. 1981. Mitosis and Cytokinesis. A. Zimmermann and A. Forrer, editors. Academic Press, New York.

Dan, K. 1979. Studies on unequal cleavage in sea urchins. I. Migration of the nucleo to the vegetal pole. Dev. Growth & Differ. 21:507-535.

Dan, K., and S. Inoue. 1987. Studies of unequal cleavage in molluscs. II. Asymmetric nature of the two asters. Int. J. Invertebr. Reprod. Dev. 2:335-354.

Hamaguchi, M. S., and Y. Hiramoto. 1986. Analysis of the role of astral rays in pronuclear migration in sand dollar eggs by the colcemid-UV method. Dev. Growth & Differ. 28:143-156.

Hyman, A. A., and J. G. White. 1987. Determination of cell division axes in the early embryogenesis of Caenorhabditis elegans. J. Cell Biol. 105:2123-2133.

Kilmartin, 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.

Laufer, J., P. Bazzicapauso, and W. B. Wood. 1980. Segregation of development potential in early embryos of Caenorhabditis elegans. Cell. 19:569-577.

Lutz, D. A., Y. Hamaguchi, and S. Inoue. 1988. Micromanipulation studies of the asymmetric positioning of the maturation spindle in Chaetopterus sp. oocytes. I. Anchorage of the spindle to the cortex and migration of a displaced spindle. Cell. Motil. Cytoskeleton. 8:32-46.

Mitchison, T., L. Evans, E. Schulze, and M. Kirschner. 1985. Sites of microtubule assembly and disassembly in the mitotic spindle. Cell. 45:515-527.

Nigon, V., P. Guerrier, and H. Mohin. 1960. L'Architecture polaire de roeuf et les mouvements des constituents cellulaires au cours des premieres etapes du developement chez quelques nematodes. Bull. Biol. Fr. Belg. 93:131-202.

Pries, J., and D. Hirsch. 1986. Caenorhabditis elegans morphogenesis: the role of the cytoskeleton in elongation of the embryo. Dev. Biol. 117:156-173.

Wilson, E. B. 1925. The Cell in Development and Heredity. MacMillan Publishing Co., New York. 377 pp.