Transitory localized aggregation of actin in *Caenorhabditis elegans* blastomeres with oriented asymmetric divisions

James A. Waddle\(^1\)*, John A. Cooper\(^2\) and Robert H. Waterston\(^1\)

Departments of Genetics\(^1\) and Cell Biology and Physiology\(^2\), Washington University School of Medicine, St Louis, MO 63110, USA

\(^1\)Author for correspondence

**SUMMARY**

During *Caenorhabditis elegans* embryogenesis, specific cells in the P\(_1\) lineage rotate their duplicated centrosome pair onto the anterior-posterior axis; this rotation is correlated with and necessary for a differential inheritance of cytoplasmic determinants in the daughter cells. Centrosome pair rotation is sensitive to inhibitors of actin and microtubule polymerization and may require microtubule attachment to a specific cortical site. We show that actin and the barbed-end binding protein, capping protein, transiently accumulate at this cortical site, possibly by assembly onto persistent remnants of previous cell divisions. Based on these observations, we propose a model for the molecular basis of centrosome rotation that is consistent with the dependence of rotation on actin filaments and microtubules.

Key words: centrosome, actin, *Caenorhabditis* embryogenesis, cell division, asymmetric division

**INTRODUCTION**

Cell fate specification in the early nematode embryo is controlled by cell-intrinsic and cell-extrinsic mechanisms (Bowerman et al., 1993; Cowan and McIntosh, 1985; Laufer et al., 1980; Mello et al., 1992; Priest et al., 1987; Priess and Thomson, 1987; Schnabel, 1991). One cell-intrinsic mechanism is the segregation of specific cytoplasmic determinants to one of two daughter cells. In *Caenorhabditis elegans*, maternally supplied determinants are unequally distributed to the posterior daughter as early as the 2-cell stage (Bowerman et al., 1993). In addition, cytoplasmic granules (P-granules) are asymmetrically distributed prior to mitotic spindle alignment and cleavage (Hyman and White, 1987; Strome and Wood, 1983). These observations indicate that early blastomeres possess mechanisms to ensure the polarized distribution of cytoplasmic components prior to cell division.

One important step in cytoplasmic partitioning is the proper orientation and placement of the mitotic spindle and cleavage plane (Strome, 1993). In early *C. elegans* embryogenesis, developmental potential is normally segregated along the anterior-posterior (a-p) axis (Bowerman et al., 1993; Cowan and McIntosh, 1985; Laufer et al., 1980; Sulston et al., 1983). Therefore, correct partitioning of determinants requires that cell divisions also occur along the a-p axis so that daughter cells receive the appropriate cytoplasm.

Control of cleavage plane orientation is evident in the embryonic divisions of many organisms including *C. elegans* (Allen and Kropf, 1992; Hyman and White, 1987; Palmer et al., 1992). In both AB- and P\(_1\)-derived cells, duplicated centrosome pairs migrate until they are diametrically opposed and orthogonally aligned relative to the previous spindle axis. However, subsequent to centrosome migration in P\(_1\) and its early descendants, centrosome pairs also rotate until aligned on the a-p axis (Hyman and White, 1987); such directed centrosome rotation does not occur in the early AB lineage in normal embryos.

Drug studies suggest that rotation in P\(_1\) and its descendants requires both actin filaments and microtubules (Hyman and White, 1987). In addition, laser irradiation experiments suggest a cortical region near the midbody is a likely attachment site for astral microtubules which participate in centrosome rotation (Hyman, 1989; Hyman and White, 1987). The nature of the actin structure(s) required for rotation was unclear.

To gain insight into the mechanism(s) by which actin participates in these early events, we examined the distribution of actin and actin capping protein (CP) during *C. elegans* embryogenesis. Previously we reported the identification and characterization of the genes and cDNAs encoding the \(\alpha\) and \(\beta\) subunits of *C. elegans* actin capping protein (Waddle et al., 1993). CP binds to and regulates actin polymerization in vitro and colocalizes with actin filament barbed ends in vivo (Caldwell et al., 1989; Casella et al., 1987). Thus, the location of CP in *C. elegans* embryos might provide clues to the organization of actin filaments during early nematode development.

In this study, we show that CP and actin transiently accumulate at a cortical site that is required for rotation of centrosome pairs onto the a-p axis (Hyman, 1989). Using wild-type and mutant (Kemphues et al., 1988) embryos, we show that the formation of the transient CP-actin complex correlates with centrosome pair rotation. In addition to the transient CP-actin complexes, early *C. elegans* embryos also contain persistent,
phalloidin-stained rings generated at prior cell divisions. The location of the cell division remnants suggests that the rings might be used for assembly of the transient CP-actin complexes.

MATERIALS AND METHODS

Nematode strains and culture methods

The maintenance and handling of nematodes was carried out using standard procedures (Brenner, 1974). The wild-type Bristol strain N2 and the par-3 mutant KK237 (lon-1(e185)par-3(e2074);3Dp3 III) (Kemphues et al., 1988) were used in this study.

Protein and antibody methods

Nematode CP was purified from a yeast strain overexpressing both nematode CP subunits (Waddle et al., 1993). Two rabbits (R-2821 and R-2822; East Acres Biologicals, Southbridge, MA) were injected into the right peritoneal lymph node with 50 µg of capping protein in Freund's complete adjuvant. Three weeks later, the animals were boosted by injection into the left peritoneal lymph node with incomple adjuvant containing 50 µg of CP. Three weeks later, a final subcutaneous boost of 100 µg was delivered.

Antibodies specific for CP were recovered from serum using a two-step approach. Serum was first applied to a CNBr-Sepharose-4B column coupled to total soluble protein made from yeast strain YJW7 (Waddle et al., 1993), which lacks the endogenous yeast CP subunit genes CAP1 and CAP2. Serum not bound to this column was then passed over a column containing bacterially expressed CP fusion proteins (Waddle et al., 1993). The column was washed in 10 volumes of PBS prior to elution in 0.1 M glycine pH 2.5. Eluted antibodies were immediately neutralized with one-tenth volume of 1 M Tris-HCl pH 8.0 and desalted on a 10-DG column (BioRad, Richmond, CA) equilibrated in PBS.

Procedures for electrophoresis, immunoblotting and the preparation of total SDS-soluble nematode extracts were carried out as described previously (Waddle et al., 1993).

Fixation and immunofluorescence microscopy of wild-type embryos

Localization experiments were performed with thousands of asynchronously growing embryos fixed and stained in batch. For immunofluorescence microscopy, embryos were fixed by two different methods. Unless specified otherwise, all manipulations were done at room temperature (22-25°C). For anti-actin and anti-CAP double staining, embryos were collected and fixed using Method I (Barstead and Waterston, 1991), a protocol optimal for anti-CP and anti-actin staining but not suitable for anti-tubulin staining.

To preserve components of the actin and microtubule cytoskeletons simultaneously (Method II), we fixed embryos in a cocktail containing 75% methanol, 3.7% formaldehyde (Fisher), 0.5x PBS and 0.1 mM EDTA for 15 minutes, followed by a 5 minute 100% methanol extraction. While anti-actin staining is slightly better when 100% methanol is used as the sole fixative, we used Method II for anti-tubulin and anti-CAP double stains because, without the formaldehyde step, methanol alone does not preserve components of the actin cytoskeleton (Waddle and Waterston, unpublished observations).

In most experiments, embryos fixed by either Method I or Method II were double stained with rabbit polyclonal antibodies against CP and a mouse monoclonal antibody directed against either actin (mAbC4; Lessard, 1988; ICN, Costa Mesa, CA) or β-tubulin (N.357, Amersham; Arlington Heights, IL). mAbC4 gives an immunofluorescence pattern almost indistinguishable from that of phalloidin (Strome, 1986). There are at least five conventional actin genes in C. elegans; four described previously (Krause et al., 1989) and a newly identified member (L. Schriefer, J. Waddle and R. Waterston, unpublished). In addition, three divergent actin-related proteins have been identified (J. Lees-Miller and D. Helfman, personal communication). All five C. elegans conventional actins contain the epitope for mAbC4; in contrast, reactivity of mAbC4 with the C. elegans actin-related proteins is unknown.

For antibody staining, embryos prepared by Methods I and II were rehydrated in three washes of PBS (containing 0.5% Tween 20 and 0.02% sodium azide) and one wash in blocking buffer (10% normal goat serum, 1% BSA in PBS). Primary antibody incubations were done for 1-2 hours using 2.5 µg/ml of affinity-purified rabbit anti-CP and 1:100 dilution of either mouse anti-actin or mouse anti-β-tubulin in blocking buffer. Unbound antibodies were removed by four washes in PBS and secondary antibodies (rhodamine-conjugated donkey anti-rabbit Ig; fluorescein-conjugated donkey anti-mouse; Chemicon, Temecula, CA) were applied at 1:100 in blocking buffer for 1-2 hours. Embryos were then washed three times in PBS, once in PBS containing 0.1 µg/ml DAPI and resuspended in 90% glycerol, 0.02 M Tris-HCl pH 8.0, 0.2 M DABCO.

To stain cell division remnants with phalloidin, a third fixation method was used (Method III). A 50 µl pellet of embryos was fixed 5 minutes in 1.5 ml of 3% formaldehyde in 0.1 M sodium phosphate, pH 7.0, 0.1 mM EDTA and then extracted with 100% acetone for 5-10 minutes. The embryos were then rehydrated in 75, 50 and 25% acetone, rinsed three times in PBS, and stained for 30 minutes in 0.5-1.0 ml of 6.6 nM rhodamine-phalloidin (Molecular Probes). Unbound phalloidin was removed by two washes in PBS and one wash in PBS containing 100 ng/ml DAPI; stained embryos were mounted as described above. For unknown reasons, early embryos prepared by Method III are not stained by the antibodies used in this study. Similarly, methanol extraction prevents phalloidin binding to embryos prepared by fixation Methods I and II.

A modified version of fixation Method II was used to collect and stain small numbers of par-3 mutant embryos. Strain KK237 was selfed and F1 Lon animals were identified. Such animals have lost the duplication that covers the par-3 locus and are thus homozygous for par-3. About 50 gravid Lon adults were placed on a subbed slide and a coverslip was applied with slight pressure to release the embryos and make contact between the eggs and the two glass surfaces. The mounts were either placed in liquid N2 (Strome, 1986) or frozen at −70°C for 15 minutes. The coverslips were pried off with a scalpel and embryos attached to the slide were fixed by Method II above.

Stained embryos were photographed using TM4X-400 film and a Zeiss Axioscope equipped with a 63x 1.25 N.A. Neofluor objective using automatic exposure with a reciprocity correction of 3 and an ASA setting at 200.

RESULTS

Background

A complete description of the embryonic cell lineage of C. elegans was reported ten years ago (Sulston et al., 1983). A brief review of the early cleavage pattern is presented here as background for the observations made in this study. During early C. elegans development, a series of unequal, anterior-posterior (a-p) cleavages of the germ-line precursor cells P0, P1, P2 and P3 generates five embryonic blast cells (AB, MS, E, C and D) and the germ-line progenitor P4 (Fig. 1A). First cleavage generates a larger anterior daughter, AB, and a smaller posterior daughter, P1. Early descendants of these two cells adopt different cleavage patterns (Fig. 1B). The equal, synchronous divisions of the AB lineage show an orthogonal pattern; successive divisions occur 90° to the previous cleavage. In contrast, unequal, asynchronous divisions in the P1 lineage occur on the same axis at successive divisions; this pattern is maintained by directed rotation of duplicated cen-
C. elegans capping protein

Initial immunolocalization of CP in 1- and 2-cell embryos revealed two classes of CP staining: a general cytosolic (Fig. 4B) and cortical distribution (not shown) in all cells, and a distinct structure located in a midfocal plane on the anterior cortex of P1 (arrowheads, Fig. 4E,H; see Fig. 1 for cell positions). Throughout this study, the focal plane of the microscope and film exposures were adjusted to optimize visualization of the midfocal plane CP complex; in such views, the cortical distribution of CP and actin reveals all cell boundaries whereas the general cytosolic staining is barely visible.

The location of the distinct CP complex in P1 is spatially coincident with a proposed site of microtubule attachment required for rotation of centrosome pairs onto the a-p axis (Hyman, 1989). To determine whether the cortical CP complex is present during centrosome pair rotation in P1, embryos were fixed and costained with anti-β-tubulin and anti-CP antibodies (Fig. 4). 2-cell embryos fixed and stained just prior to and during centrosome pair rotation in P1 (note centrosome positions in Fig. 4D and G, respectively) show increased CP accumulation on the anterior cortex of P1 (arrowheads in Fig. 4E,H). In contrast, 2-cell embryos that still contain midbody microtubules or have not yet duplicated the initial P1 centrosome (Fig. 4A) contain only the general cytosolic and uniform cortical staining (Fig. 4B).

To learn more about the organization of actin at the cortical CP complex and to determine the cell specificity and time of appearance of the CP structure, we examined the distribution of CP and actin in one and two-cell embryos at various points in the cell cycle. Nuclear morphology, as revealed by DAPI

![Fig. 1. Review of early nematode lineage, cleavage pattern specification and cell positions in the developing embryo. (A) A series of unequal cleavages in the P-lineage generates five embryonic founder cells and the germ-line precursor P4 (boxed cells). Cleavages within the AB lineage are symmetric and cell-cycle times are synchronous. In contrast, daughter cells in the P1 arm of the lineage divide asynchronously. (B) Cells in the AB and P1 lineage adopt different cleavage patterns. After first cleavage, each daughter inherits a centrosome (small filled circle). These duplicate and each migrates 90° around the nucleus until they are diametrically opposed. The AB cell sets up a spindle and cleaves perpendicularly to the a-p axis. In contrast, prior to spindle formation in P1, the centrosomes rotate 90° until they lie on the a-p axis (Hyman and White, 1987). The next division in the AB-lineage is also 90° to the previous cell division and occurs on the l-r axis. Because embryonic cell divisions occur within the confines of the eggshell, the final cell positions partially obscure the absolute orientation of each division (Fig. 1C) (Hyman and White, 1987; Laufer et al., 1980; Schierenberg, 1987; Sulston et al., 1983).](image-url)
staining, was used to determine the relative position of each cell in the nuclear cycle. By this method, individual cells could be unambiguously assigned to one of six nuclear intervals: interphase, prophase, prometaphase, metaphase, anaphase and telophase. Additional staging information was obtained by assessing the position of cells in the microtubule cycle (Hyman and White, 1987) using an anti-tubulin antibody (not shown).

For the experiments described below, we used hundreds of static images to reconstruct the distribution of actin and CP on a cell-to-cell basis in the early embryo. For each cell stage (e.g. 1, 2, 4, 6, 7 and 8), we examined more than 25 embryos at each of the six nuclear intervals for each cell within such an embryo.

Previously, the distribution of actin from fertilization through first cleavage was examined using phalloidin (Strome, 1986) and an anti-actin antibody (mAbC4; Lessard, 1988); these reagents reveal identical patterns of cortical actin fibers and dots in 1-cell *C. elegans* embryos (Strome, 1986). Using mAbC4 and fixation Method I (see Experimental Procedures), we observe the same actin distribution at the 1-cell stage (not shown) as that reported previously (Strome, 1986). Similarly, the pattern of actin and CP in 2-cell embryos at interphase (Fig. 5A-C) or early prophase (Fig. 5D-F) resembles the cortical pattern of 1-cell embryos; this distribution reveals the cell boundaries in AB and P1 cells in these midfocal plane views.

At late P1 prophase (Fig. 5I), a CP-actin complex forms on the anterior cortex of P1 near the midbody (Fig. 5G,H). Initially, the complex assembles as a flat, 4-6 μm button located at the posterior cortex of AB and the anterior cortex of P1. At the level of resolution of the light microscope, it is not clear whether CP and actin initially accumulate on the cortex of both AB and P1, or only on the P1 cortex. As P1 enters prometaphase (Fig. 5L), CP and actin accumulation on the anterior cortex of P1 reaches a maximum (Fig. 5J,K). In addition, the morphology of the CP-actin complex changes from a flat button to a conical shape with the vertex pointing toward the P1 nucleus (compare Fig. 5G to J); the thickness of

---

**Figures: 2 and 3.** Specificity of anti-Nematode Capping Protein antibodies. **Fig. 2** Total SDS-soluble nematode extracts were run on a 10% polyacrylamide gel and stained with Coomassie brilliant blue (lane 1) or blotted and probed with 0.5 μg/ml of preimmune IgGs (lane 2) or affinity-purified rabbit anti-CP antibodies (lane 3). Relative molecular mass standards are shown to the left (×10^{-3}). Arrows indicate the position of the CP α and β subunits. **Fig. 3.** 1-, 2- and 4-cell embryos fixed by Method I and costained with 1:100 dilution of anti-actin mAbC4 as a positive control for permeability and fixation (A,D,G), 2.5 μg/ml CP preimmune IgGs (B,E,H) and DAPI (C,F,I). All are midfocal planes. Scale bar is 10 μm.
The CP-actin complex along the a-p axis changes from about 0.5 µm to as much as 4 µm. Eventually the complex is pushed off center by the telophase movements in AB (Fig. 5M-O) so that it comes to lie closer to one of the two AB daughter nuclei. The cortical CP-actin complex disappears prior to anaphase in P1 (Fig. 5P-R) and is never observed at later times in the P1 nuclear cycle.

Transient CP-actin complexes form at prophase in other cells of the P1 lineage

The timing, cell-specificity and location of the transient CP-actin complex in 2-cell embryos coincides with the properties of centrosome rotation in the P1-lineage (Hyman, 1989; Hyman and White, 1987). A causative role for the CP-actin complex in directed centrosome rotation predicts that other cells that rotate their centrosomes onto the a-p axis should also assemble CP-actin complexes on their respective cortices during the prophase to metaphase interval. In the early embryo, these structures should be observed in the early P1 descendants P2 and EMS, but not ABa and ABp (Fig. 1).

To test this prediction, we examined stained embryos at times before, during and after rotation in specific cells. In early 4-cell embryos (Fig. 6C), CP and actin are distributed in a general cytosolic and cortical pattern very similar to that observed at interphase in 2-cell embryos (compare Figs 5A,B to 6A,B). Later in the 4-cell stage, a CP-actin complex forms on the anterior cortex of EMS (Fig. 6D,E) as it enters prophase (Fig. 6F); in more than 25 embryos examined at this stage, no complexes formed on the posterior of EMS. The CP-actin complex in EMS disappears prior to metaphase (Fig. 6I); by this time, a CP-actin complex forms on the anterior of P2 (Fig. 6G-I). The CP-actin complex on the anterior of P2 is also short-lived and disappears prior to anaphase in P2 (Fig. 6J-L). Finally, we have not detected CP-actin complexes associated with any portion of the ABa or ABp cortex within the prophase to metaphase interval of the nuclear cell cycle; such transient complexes appear to be restricted to P1 and its descendants.

A CP-actin complex forms in both cells of 2-cell par-3 mutant embryos

A role for the CP-actin complex in centrosome pair rotation also predicts that mutant cells that undergo ectopic rotations might inappropriately assemble CP-actin complexes. We tested this hypothesis by staining embryos produced from animals homozygous for the maternal-effect lethal mutation par-3 (Kemphues et al., 1988). In par-3 mutant embryos, an abnormal, symmetric first cleavage generates two cells that
Fig. 5
**Fig. 5.** CP and actin distribution at the 2- to 4-cell transition. Wild-type embryos fixed by Method I and stained with anti-actin (A,D,G,J,M,P), anti-Cp (B,E,H,K,N,Q) and DAPI (C,F,I,L,O,R). Anterior is to the left. (A-C) 2-cell embryo just after first cleavage; the nuclei are at interphase. (D-F) Early prophase in AB and P1, the chromosomes are just starting to condense (F). (G-I) At late prophase in P1, a flat button of CP and actin forms on the anterior cortex of P1. (J-L) By prometaphase in P1, the intensity of staining at the cortical site increases and invaginates toward the posterior of P1. (M-O) The complex persists into metaphase of P1 but is consistently pushed off-center by the nuclear movements in AB. (P-R) At anaphase in P1, the complex has disappeared and the staining has returned to the interphase pattern. All views are midfocal plane. Arrowheads point to the DAPI-stained polar bodies; these are out of focus in C, L and O. Scale bar is 10 µm.

**Fig. 6.** Formation of CP-actin complexes during prophase in EMS and P2. Wild-type 4-cell embryos fixed by Method I and stained with anti-actin (A,D,G,J), anti-Cp (B,E,H,K) and DAPI (C,F,I,L). Anterior is to the left in all cases. (A-C) Lateral aspect of an early 4-cell stage embryo, all nuclei at interphase. (D-F) Ventral aspect of a 4-cell embryo; only the ABa, EMS and P2 nuclei are seen in this focal plane. EMS is at prophase (middle nucleus); the anterior-most spot in E shows CP but not actin staining of the polar body in the ABa cell. A CP-actin complex appears on the anterior cortex of EMS where it abuts ABa (arrow in E); all other cells show uniform cytosolic and cortical actin and CP staining. (G-I) Lateral aspect of a 4-cell embryo prior to metaphase in EMS and late prophase in P2; only the anaphase ABa, prometaphase EMS and prophase P2 nuclei are seen in this focal plane. A CP complex appears on the anterior cortex of P2 where it abuts EMS (arrow in H); all other cells show uniform cytosolic and cortical actin and CP staining. (J-L) Ventral aspect of a 6-cell embryo focused on the lower focal plane. The P2 cell at the posterior is dividing; all cells show uniform actin and CP staining. Scale bar is 10 µm.
divide synchronously after both daughters rotate their centrosomes onto the a-p axis. We examined more than 50 par-3 mutant, 2-cell embryos in the prophase to metaphase nuclear interval; in each case, a CP-actin complex formed on the cortex of both cells in 2-cell embryos (Fig. 7A-F). The ectopic structure in the anterior cell always assembles near the midbody and as a mirror image of the CP-actin complex in the posterior cell.

Persistent, phalloidin-stained rings mark prior cell divisions

While developing fixation methods suitable for both phalloidin and antibody staining of early embryos, we uncovered conditions that reveal phalloidin-stained, ring-shaped structures shared by sister cells. The detection of these structures requires formaldehyde fixation followed by 100% acetone extraction (see Experimental Procedures), a treatment that presumably extracts less stable phalloidin-binding components including most of the cortical actin cytoskeleton in early nematode embryos (compare the cortical and cytosolic actin staining in Figs 5 and 6 to that in Fig. 8).

The time of appearance, position, morphology and number of phalloidin-positive rings within embryos suggest that these organelles are persistent remnants from prior cell divisions. For the remainder of this report, we will refer to the rings as cell division remnants. Fig. 8 shows the position and number of cell division remnants in a 2-, 4- and 7-cell embryo. Three important observations can be made from this experiment. First, in contrast to the transient CP-actin complexes described above, the cell division remnants are relatively permanent; once they appear, they apparently persist for many cell cycles. For example, a given embryo always has one fewer ring than the number of cells; the number of rings equals the total number of individual cell divisions. Second, in contrast to the transient CP-actin complexes, the rings are found in daughter cells of both the AB and P1 lineages. Third, for cells in the early P1 lineage, the subcellular position of the cell division remnant is similar to the location of the transient CP-actin complexes that assemble during centrosome rotation (compare Fig. 5J to Fig. 8A). For example, the cell division remnant from first cleavage is located at a central site in the midfocal plane between AB and P1 in early 2-cell embryos (Fig. 8A, arrow). Later, nuclear movements in the dividing AB cell push the cell division remnant off center (not shown) in a manner similar to the transient CP-actin complexes temporally associated with centrosome rotation in P1 (Fig. 5M,N). Of interest is whether the cell division remnants are positioned at the base of the transient CP-actin complexes. Unfortunately, simultaneous observation of the persistent cell division remnants and the transient CP-actin complexes is problematic; current fixation and staining methods for the two structures are not compatible.

DISCUSSION

In C. elegans, segregation of developmental potential occurs along the a-p axis and is associated with successive, a-p cell divisions within the P1 lineage (Fig. 1; Bowerman et al., 1993; Cowan and McIntosh, 1985; Laufer et al., 1980; Sulston et al., 1983). The sequential a-p divisions are caused by directed rotation of centrosome pairs onto the a-p axis prior to each cell division (Hyman and White, 1987). Drug studies indicate centrosome rotation requires both actin filaments and microtubules (Hyman and White, 1987); rotation is also blocked by laser irradiation of the region between a proposed cortical micro-
tubule attachment site and the anterior centrosome (stippled box on the cortex of P₁, Fig. 1B; Hyman, 1989).

An examination of live embryos reveals that rotation occurs in a one minute interval within the 12 minute P₁ cell cycle (Hyman, 1989); a period temporally coincident with late prophase in P₁ (Hyman and White, 1987). We have shown that during centrosome rotation and late prophase in P₁ (Figs 4 and 5, respectively), an actin complex forms at or near the proposed microtubule attachment site. The apparent colocalization of the transient actin complex and the cortical site determining rotation (Hyman, 1989) is consistent with cytochalasin-sensitive assembly, positioning or function of the microtubule attachment site (Hyman and White, 1987).

Previous investigations of the mechanism of centrosome pair rotation in C. elegans are consistent with models in which centrosome pairs rotate in a specific direction by shortening connections between the centrosome and a defined site on the anterior cortex of cells (Hyman and White, 1987; Hyman, 1989). Laser irradiation, immunolocalization and drug studies further suggest that the centrosome-cortex connections include microtubules (Hyman and White, 1987; Hyman, 1989). Based on the latter observation, molecular models for centrosome pair rotation must include components that shorten microtubule connections; microtubule-based motors are obvious candidates for such components.

The force required to rotate centrosome pairs onto a new axis could be generated by at least two configurations of localized, microtubule-based motors: accumulation and tethering of minus-end directed motors at the cortical actin complex (Fig. 9A) or, conversely, plus-end directed motors at the centrosome (Fig. 9B). In each configuration, directed translocation of tethered motors toward a stabilized microtubule end decreases the distance between the centrosome and the cortex and causes rotation (Fig. 9A and B, compare cells on the left with those on the right).

Localized and tethered motor models for centrosome pair rotation predict that tension generated during centrosome rotation will be transmitted to the microtubule attachment site on the anterior cortex of P₁. Light and electron microscopic observations reveal that a centrally located region of the

---

**Fig. 8.** Persistent, cell division remnants bind phalloidin. Wild-type 2-(A,B), 4-(C,D) and 7-cell (E,F) embryos fixed by Method III and stained with rhodamine-phalloidin (A,C,E) and DAPI (B,D,F). Arrows mark the cell division remnant from first cleavage; arrowheads point to other remnants. Note that the bulk of the cytosolic and cortical actin is extracted by Method III (Compare 2- and 4-cell embryos in this figure with those in Figs 5 and 6). Anterior is left in all cases. All views are left lateral aspects; scale bar is 10 µm.
Studies using algae and yeast demonstrate that spindle orientation in organisms is consistent with the models proposed above. Directed centrosome rotations in algae (Allen and Kropf, 1992), sea urchin embryos (Dan and Ito, 1984) and grasshopper neuroblasts (Yamashiki and Kawamura, 1986) show an additional similarity to those in C. elegans because they are associated with unequal cell divisions. Additionally, studies of yeast dynein heavy chain function show that null mutations in the dynein gene disrupt spindle orientation during mitosis (Li et al., 1993; Eshel et al., 1993).

Fig. 9. Two general models for directed centrosome pair rotation. (A) Model for a motor tethered and localized to the CP-actin complex on the anterior cortex of P1. Cell on the left: a P1-derived cell shown after duplication and migration of the centrosomes (filled circles). Sometime during prophase, CP and actin (stippled region) accumulate on a pre-existing cell division remnant (filled rectangle on cortex). Motor molecules (two-headed structure) bind to the CP-actin complex, capture astral microtubules (thin lines) and translocate toward the minus-end of the microtubule (direction indicated by small arrow). Cell on the right: because the motors are tethered to the cortical site, they pull the microtubule toward the anterior of the cell. The tension imparted to the microtubule is transmitted to the anterior cortex giving rise to an invagination of the membrane (filled box on cortex) and a change in the shape of the CP-actin region (stippled region). Note that the minus-end of the microtubule is attached to or stabilized by the centrosome in this model. (B) Model for a motor tethered and localized to the centrosome and the plus ends of microtubules attached and stabilized by molecules at the CP-actin complex on the cortex; symbols represent the same components as in A. Cell on the left: a P1-derived cell shown after duplication and migration of the centrosomes transverse to the a-p axis. Sometime during prophase, CP and actin accumulate on a pre-existing cell division remnant; the CP-actin complex serves as a microtubule attachment and stabilization site. Motor molecules accumulate near the centrosome, bind nearby microtubules and translocate toward the plus end of the microtubule. Cell on the right: because the motor is tethered, the consequences are the same as those shown for the right cell in A. Note that in either model, microtubule depolymerization must occur at the motor proximal end of the microtubule. The size of the components are not to scale.

The kinesin superfamily of microtubule motors includes both plus-end and minus-end directed members (McDonald et al., 1990; Walker et al., 1990), while cytoplasmic dyneins are generally thought to be minus-end directed motors (McIntosh and Porter, 1989). Therefore, the localized motor in Fig. 9A could be a cytoplasmic dynein (Lye et al., 1987) or a minus-end directed kinesin such as the Drosophila ncd gene product (McDonald et al., 1990; Walker et al., 1990). In contrast, the centrosomally tethered motor in Fig. 9B must translocate toward the plus-end of the microtubule, an activity that has only been demonstrated by kinesins.

The transient actin complexes that form in cells of the P1 lineage could serve as binding sites for the microtubule motors shown in Fig. 9A or, alternatively, serve as attachment and stabilization sites for the plus ends of microtubules in Fig. 9B. The observation that CP is also a component of the transient actin complex suggests two possible roles for CP in centrosome rotation: nucleation, stabilization or positioning of actin filaments at the cortical microtubule attachment site or, alternatively, as part of the force-generating component that causes rotation.

Recent work has shown that the dynactin complex, in addition to dynein, is required for cytoplasmic dynein-dependent translocation of membrane vesicles on microtubules (Gill et al., 1991; Schroer and Sheetz, 1991). Ultrastructural and biochemical analysis of the purified dynactin complex reveals that its components include a filament that resembles F-actin as well as an actin-related protein (actin-RPV; J. Lees-Miller et al., 1992), CP and conventional actin (D. Schafer et al., manuscript in preparation). These new findings can be incorporated into a specific molecular model for centrosome rotation similar to the general mechanism outlined in Fig. 9A.

We propose that sometime during prophase, a dynactin complex associates with the cell division remnant on the cortex of P1 (Fig. 9A). The dynactin complex then binds to and tethers cytoplasmic dynein (Lye et al., 1987) to the cortical site (Fig. 9A). By chance, dynein binds to an astral microtubule growing out from one of the centrosomes and then attempts to translocate along the microtubule toward the minus end (Fig. 9A, arrow). Because dynein is tethered to the cortex by the dynactin complex, it pulls the connected centrosome toward the cortex causing an a-p alignment of the coupled centrosome pair (Fig. 9A, right cell). This specific model predicts the accumulation of dynein and the dynactin complex polypeptides at the CP-actin complex during rotation.

Investigations of mitotic spindle orientation in other organisms are consistent with the models proposed above. Studies using algae and yeast demonstrate that spindle orientation in these organisms also requires functional actin filaments and microtubules (Allen and Kropf, 1992; Palmer et al., 1992). Directed centrosome rotations in algae (Allen and Kropf, 1992), sea urchin embryos (Dan and Ito, 1984) and grasshopper neuroblasts (Yamashiki and Kawamura, 1986) show an additional similarity to those in C. elegans because they are associated with unequal cell divisions. Additionally, studies of yeast dynein heavy chain function show that null mutations in the dynein gene disrupt spindle orientation during mitosis (Li et al., 1993; Eshel et al., 1993).
Do persistent cell division remnants serve as assembly sites for the transient CP-actin complexes?

The general models for centrosome rotation (Fig. 9) depend on P1 lineage-specific assembly of a localized microtubule attachment site on the a-p axis; the cell division remnants described in this study are likely candidates for such assembly sites. Invariably, the CP-actin complex assemblies very close to, if not coincident with, the location of the cell division remnant (compare Figs 5 and 8). Because cell division remnants are present in all cells in the early embryo and cells in the AB lineage do not assemble transient CP-actin complexes, the assembly of CP-actin complexes must be positively regulated in P1-derived cells or negatively regulated in AB-derived cells. A model invoking cell division remnants as assembly sites for transient CP-actin complexes must include two levels of recognition. At one level, cell division remnants must contain proteins that serve as binding sites to directly assemble of the cortical CP-actin complex. However, another level of recognition is required in cells that contain more than one cell division remnant; such cells must distinguish between multiple remnants within a cell.

To illustrate the nature of the second level of recognition, we use the example of the EMS blastomere. EMS contains two cell division remnants (Fig. 8C); one on the anterior cortex derived from first cleavage and one on the posterior cortex generated upon division of P1. If the choice of which cell division remnant to use as an assembly site were random, CP-actin complexes should be detected on the anterior and posterior cortices of EMS with equal frequency. However, the CP-actin complex detected at prophase in EMS invariably formed on the anterior of EMS; this cortex contains the oldest cell division remnant in EMS.

Finally, CP-actin complexes are useful markers for the identification of cells that rotate their centrosome pairs onto the a-p axis; these markers may facilitate the characterization of mutations affecting spindle orientation. Additionally, we can now postulate that the anterior cortices of EMS and P2 contain microtubule attachment sites critical for centrosome rotation in these cells; laser ablation near these sites would be expected to cause inappropriate transverse cleavages (Hyman, 1989). The consequences of laser-induced, transverse divisions in EMS cause inappropriate transverse cleavages (Hyman, 1989). The maternal gene skn-1 encodes a protein that is distributed unequally in early C. elegans embryos. Cell 74, 443-452.

REFERENCES


(Accepted 13 May 1994)