

## An Analysis of the Role of Microfilaments in the Establishment and Maintenance of Asymmetry in *Caenorhabditis elegans* Zygotes

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Received July 20, 1987; accepted September 10, 1987

Microfilaments are needed to generate asymmetry during the first cell cycle in *Caenorhabditis elegans* zygotes. To investigate when and how microfilaments participate in this process, we have "pulsed" zygotes with the microfilament inhibitor cytochalasin D (CD) at different times during the cell cycle. We have shown that microfilaments are only required during a narrow time interval approximately three-quarters of the way through the first cell cycle for the manifestations of asymmetry that occur during and subsequent to this interval. When CD treatment spans this critical time interval, pseudocleavage, pronuclear migration, germ-granule segregation (all of which occur during the interval), and movement of the mitotic spindle to an asymmetric position (which occurs later in the cell cycle) are perturbed. In contrast, embryos briefly treated with CD before or after the critical time interval manifest normal asymmetry. Our results suggest that in *C. elegans* microfilaments participate in the generation of zygotic asymmetry by providing spatial cues and/or serving as a part of the necessary machinery only during a brief period in the first cell cycle, and are not required to maintain asymmetries that have already been established. © 1988 Academic Press, Inc.

### INTRODUCTION

Classical embryological experiments suggest that specification of cell fates in many systems is controlled by the unequal partitioning of cytoplasmic components present in the egg (reviewed in Davidson, 1986). Boveri (1910) showed that the differential distribution of cytoplasm controls the germ vs soma decision in the nematode *Ascaris*. Microsurgical studies on systems such as sea urchins, ascidians, and molluscs have shown that divisions that are obviously asymmetric at the morphological level also lead to the differential specification of cell fates (Horstadius, 1939; Wilson, 1925; Conklin, 1905). More recent studies in a variety of systems suggest that cytoskeletal structures participate in the cytoplasmic localization events described above (Jeffery and Meier, 1983; Dorresteijn *et al.*, 1987). The goal of our work is to use the asymmetric first division of *Caenorhabditis elegans* embryos to understand the process that regulates the distribution of developmental potential at a molecular level.

A variety of directed movements within the cell occurs during the first cell cycle of *C. elegans*, illustrating the asymmetric nature of the zygote at a morphological level. Approximately three-fourths of the way through the first cell cycle several events occur concurrently: contractions of the anterior membrane and the formation of a pseudocleavage furrow, the unequal migration of the pronuclei in the process of pronuclear meeting, the reorganization of foci of actin, and the segregation of germ-line-specific granules. Later in the

cell cycle, the mitotic spindle forms and becomes asymmetric in both structure and position, and finally the zygote undergoes unequal first cell cleavage (Nigon *et al.*, 1960; Hirsh *et al.*, 1976; Albertson, 1984; Strome and Wood, 1982; Strome, 1986). The two resulting blastomeres differ in size, content, and developmental fate (Kimble and Hirsh, 1979; Sulston *et al.*, 1983; Strome and Wood, 1983).

We have investigated the involvement of microfilaments in these processes by using the microfilament inhibitor cytochalasin D (CD)<sup>1</sup> to disrupt microfilament structure at different times during the first cell cycle. Strome and Wood (1983) previously demonstrated that continuous treatment of zygotes with CD beginning either before or during the early stages of pseudocleavage inhibits all of the manifestations of embryonic asymmetry we have described. Our experiments were designed to explore three different ways that microfilaments may be involved in establishing asymmetries: (1) Proper microfilament structure may be required before any of the events themselves take place to establish the proper cellular "context" for the events. (2) Proper microfilament structure may be required only during the execution of events. (3) Proper microfilament structure may be required after the events have occurred in order to maintain the asymmetries that have been generated. We tested these three possibilities by disrupting micro-

<sup>1</sup> Abbreviations used: R-Ph, rhodamine phalloidin; CD, cytochalasin D; ECM, embryonic culture medium; PBS, phosphate-buffered saline; DAPI, 4,6-diamidino-2-phenylindole.

filament structure before, during, and after zygotic events and analyzing subsequent development of the one-cell embryo. The results of our experiments show that proper microfilament structure is required during pseudocleavage, pronuclear migration, and germ-granule segregation in order for these events to occur normally ((2) above). Disruption of microfilaments before or after the actual occurrence of these events has no effect on them. Proper microfilament structure is required prior to the generation of an asymmetric spindle ((1) above). Microfilaments do not appear to be necessary to maintain asymmetries that have already been established. Thus, we have defined a single critical time interval in which proper microfilament structure is required for the correct execution of events that occur both during and after the interval.

#### MATERIALS AND METHODS

*Maintenance of C. elegans.* Wild-type *C. elegans* (N2) was grown at 16°C on NGM plates with *Escherichia coli* strain NA22 as a food source (Brenner, 1974).

*Analysis of the development of embryos treated with cytochalasin D.* Embryos were dissected from adult hermaphrodite gonads in a drop of embryonic culture medium (ECM; 118 mM NaCl, 40 mM KCl, 3.4 mM CaCl<sub>2</sub>, 3.4 mM MgCl<sub>2</sub>, 5 mM Hepes, pH 7.4, 50% fetal calf serum) (Edgar and McGhee, 1986) containing 2 µg/ml cytochalasin D (CD) (Strome and Wood, 1983) on a polylysine-coated glass slide. Embryos were overlayed with an 18 × 18-mm glass coverslip, with high vacuum grease applied along two edges to serve as a spacer. The embryos were permeabilized by applying gentle pressure to the coverslip. CD was removed by wicking fresh ECM without CD under the coverslip from one side to the other using a paper towel. While wicking, slight pressure was exerted on the coverslip to assure mixing of the solutions. Success of permeabilization was determined by three criteria: (1) the altered appearance of the cytoplasm that is characteristic of CD-treated embryos (Strome and Wood, 1983), (2) Nile blue staining of the membranes of blastomeres, and (3) the inhibition of known microfilament-dependent events (Strome and Wood, 1983). The effects of inhibitor on zygote development were assayed by monitoring single embryos at 16°C by Nomarski differential interference contrast microscopy.

*F-actin staining of embryos.* The distribution of actin filaments was visualized in embryos using the F-actin-specific stain rhodamine-phalloidin (R-ph) (Wulf *et al.*, 1979; Hoch and Staples, 1983). Embryos were isolated from adult hermaphrodites by a modification of the procedure of Johnson and Hirsh (1979); adult hermaphrodites were dissolved in 1% sodium hypochlorite/0.5 M KOH until the carcasses disappeared, after

which the embryos were harvested by centrifugation. Embryos were washed twice in M9 buffer (Brenner, 1974) and resuspended in ECM that contained 2 µg/ml CD. Permeabilization was achieved by passing the embryos through a 30-g syringe needle with moderate force (Cowan *et al.*, 1985). Permeabilized embryos were then exposed to different experimental solutions by centrifugation and resuspension. After appropriate experimental manipulations, embryos were fixed for 3 hr at 16°C with agitation in 1.5% paraformaldehyde, 0.1% glutaraldehyde dissolved in ECM. Embryos were extracted for 1 hr at room temperature in ECM containing 0.5% Triton X-100 and stained overnight at 16°C with agitation in 6.6 nM R-ph, 0.25% Triton X-100 in PBS. Embryos were then washed in five changes of PBS with the third change containing 1 µg/ml DAPI to stain DNA.

*Immunofluorescent staining of embryos.* Embryos were fixed for immunofluorescence staining as described by Strome and Wood (1982). Briefly, slides with embryos attached were frozen in liquid N<sub>2</sub>, the coverslips were removed, and the slides were immersed in cold methanol followed by cold acetone. Slides were then air dried. The distribution of cytoplasmic germ granules (P granules) was visualized by indirect immunofluorescence using a monoclonal antibody directed against *C. elegans* P granules (Strome and Wood, 1983). Mitotic spindles in embryos were stained using a monoclonal antibody generated against *Drosophila melanogaster* α-tubulin, generously given to us by Dr. M. T. Fuller (Piperno and Fuller, 1985). Staining was performed on fixed embryos by the procedure of Strome and Wood (1982). Briefly, fixed embryos were incubated at 4°C with 1.5% ovalbumin, 1.5% bovine serum albumin in PBS for 30 min and then with a monoclonal antibody for 12 hr at 4°C. Slides were washed twice in PBS, after which the embryos were incubated in fluorescein-labeled goat anti-mouse secondary antibody (U.S. Biochemicals) in PBS for 4 hr at 4°C and washed three times with PBS. DAPI was included in the second wash to stain nuclei. The samples were mounted in Gelvatol (Monsanto) mounting fluid.

*Photography and microscopy.* Fluorescence microscopy was performed on a Zeiss ICM405 inverted microscope equipped with epifluorescence optics and a 40× neofluar objective. Zygotes were examined with 515–560 nm epi-illumination for rhodamine fluorescence, 450–490 nm epi-illumination to visualize fluorescein, and 365 nm epi-illumination to visualize DAPI. Living embryos were monitored by Nomarski differential interference contrast microscopy using a 40× planachromat objective. Photographs were taken using Kodak Tri-X film at ASA 1600. Film was developed using Diafine two-bath developer (Acufine Inc., Chicago, IL).

## RESULTS

*Microfilament-Mediated Events during the First Cell Cycle*

After fertilization, a number of directed movements occur in the one-cell embryo of *C. elegans* (Nigon *et al.*, 1960; Hirsh *et al.*, 1976; Albertson, 1984; Strome and Wood, 1982, 1983; Strome, 1986; Wolf *et al.*, 1983). A review of these events is presented (Figs. 1 and 2) because they are the events that were monitored in experimental embryos. Approximately 1 hr after fertilization (at 16°C), the meiotic divisions of the egg pronucleus are completed at the anterior end of the zygote. This is followed by contractions of the anterior cell membrane, which will be referred to as early pseudocleavage (Fig. 2a). This contraction leads to a constriction perpendicular to the anterior-posterior axis that resembles a true cleavage furrow; we will refer to this stage as late pseudocleavage (Fig. 2e). During pseudocleavage, the egg pronucleus begins to migrate posteriorly. The sperm pronucleus slowly moves away from the posterior periphery, and the two pronuclei meet in the posterior hemisphere (Fig. 2e) and then migrate to the center of the embryo. Concomitant with pseudocleavage and pronuclear migration, germ-line-specific P granules, visualized by indirect immunofluorescence, become localized in the posterior cortex of the zygote (Fig. 2g). The mitotic spindle is initially positioned symmetrically along the anterior-posterior axis, but it becomes asymmetric in both position and structure as it grows. By anaphase the spindle is positioned slightly closer to the posterior end of the cell and the posterior aster appears flattened relative to the anterior aster (Fig. 2i). Cytokinesis occurs at a position determined by the position of the mitotic spindle and generates a small posterior cell (P1) and a larger anterior cell (AB; Fig. 2k).

By treating zygotes continuously with the microfilament inhibitor CD, Strome and Wood (1983) showed that all of the manifestations of asymmetry described above could be inhibited by the disruption of MFs. When zygotes are treated continuously with CD: pseudocleavage does not occur (Fig. 2b), the pronuclei meet

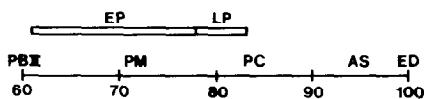


FIG. 1. The time course of events during the one-cell stage. Numbers below the line indicate developmental time in minutes after fertilization at 16°C (Kemphues *et al.*, 1986, and our measurements). PBII, extrusion of second polar body; PM, beginning of pronuclear migration; PC, conjunction of pronuclei; AS, movement of the spindle to an asymmetric position; ED, end of first cleavage. The bar above the line indicates the duration of pseudocleavage; EP, early pseudocleavage; LP, late pseudocleavage.

in the center of the zygote (Fig. 2f) rather than at the posterior end, P granules are located in the center of the zygote (Fig. 2h) rather than at the posterior cortex, and the mitotic spindle does not become asymmetrically positioned (Fig. 2j). As expected, cytokinesis is inhibited (Fig. 2l).

*Microfilament Structure in CD-Pulsed Embryos*

Strome (1986) previously described the distribution of microfilaments in single zygotes rendered permeable to the F-actin-specific stain rhodamine-phalloidin. Filamentous actin appears to exist in two populations, dots or foci of actin and actin fibers. Both the foci and the filaments are distributed homogeneously in the cortex of newly fertilized eggs, but as pseudocleavage occurs, the foci of actin become more concentrated in the anterior region of the zygote (Fig. 2c) (Strome, 1986).

For our experiments it was necessary to develop a procedure to permeabilize and stain large numbers of embryos (see Materials and Methods). Using this procedure, we have obtained results similar to those of Strome (1986). We observed the anterior concentration of foci described above in 22 out of 24 embryos (Fig. 3a). In the other two embryos the foci appeared to be somewhat more concentrated in the posterior of the embryo (see Discussion). We observed that as karyokinesis proceeds, the anterior concentration of foci becomes less pronounced and by telophase foci are again distributed throughout the zygote and are subsequently found in both blastomeres of the two-cell embryo.

We analyzed the microfilament structure in embryos that were "pulsed" with the inhibitor CD to determine if the inhibitor could be used successfully to transiently perturb microfilament structure during the first cell cycle. CD perturbs the normal actin array within 1 min of exposure as judged by R-ph staining. In 94 out of 103 embryos ranging in age from just after the completion of meiosis until about the 16-cell stage and including embryos in all of the time intervals we used experimentally, the arrays of cortical filaments and foci of actin found in cells disappeared after 1 min of treatment. Instead, F-actin was seen in the form of large spikes and aggregates throughout the cytoplasm (Figs. 2b and 3b) (Strome, 1986). The other 9 embryos contained spikes and aggregates as well as some foci and filaments. In no case did we see spikes and aggregates of F-actin in embryos that were permeabilized to culture medium lacking CD.

Upon removal of CD, embryos can reestablish normal microfilament arrays. This is most dramatically seen in zygotes that are treated with CD for 2 to 3 min during early pseudocleavage. When these embryos are allowed

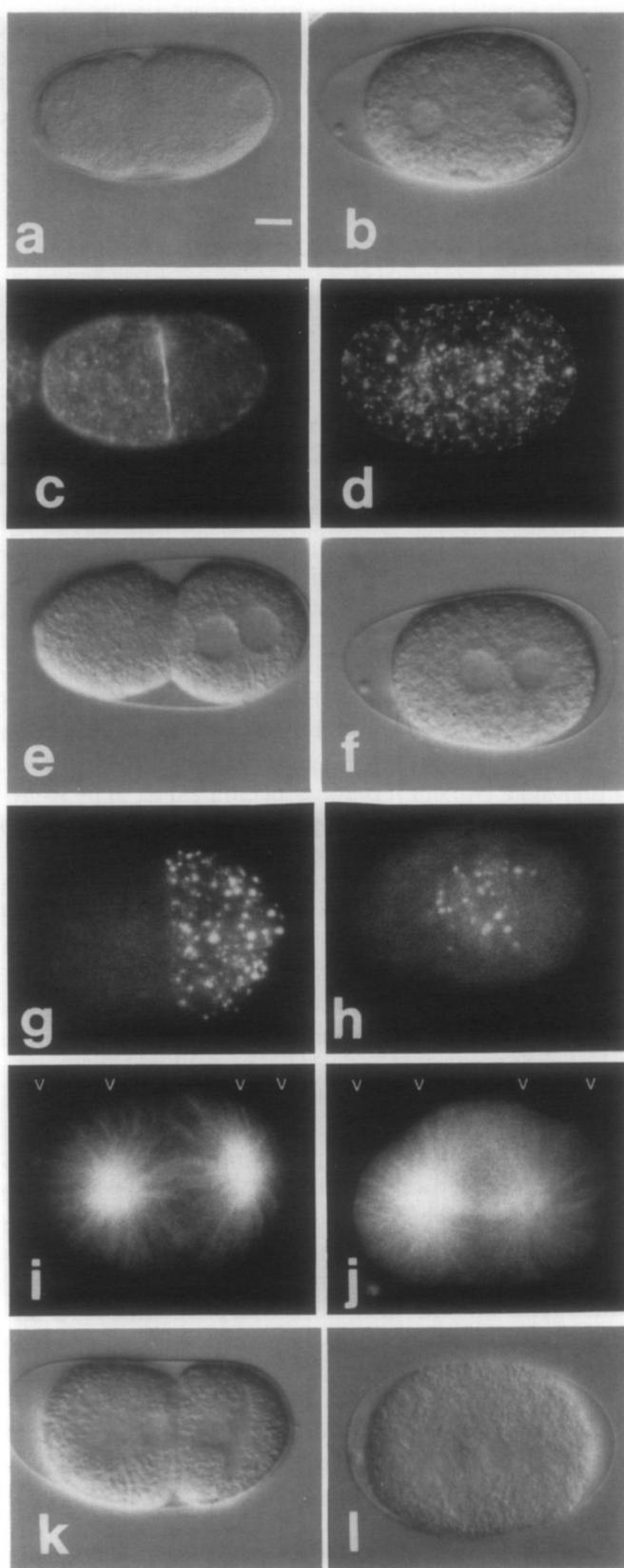


FIG. 2. Comparison of events that occur in normal embryos (panels on the left) and embryos treated continuously with 2 µg/ml CD after meiosis (panels on the right). In this and all other figures, anterior is to the left, and posterior is to the right. (a, b) CD treatment inhibits

to recover and are then fixed and stained with R-ph at or slightly before pronuclear meeting, we see that the F-actin is organized into a normal array of filaments and foci. Sometimes, foci in these pulsed embryos are somewhat larger and less numerous than those in treated controls. Nevertheless, in 36 out of 43 successfully pulsed and stained embryos, the foci were more concentrated in the anterior of the zygote (Fig. 3c), as in control embryos. Of those embryos that did not show an anterior concentration of foci: 3 embryos displayed no differential distribution of actin foci, and 4 embryos exhibited foci apparently more concentrated in the posterior end of the zygote (see Discussion). If development is allowed to proceed after such a CD pulse, we observe that the zygote is able to recover and cleave to form a normal asymmetric two-cell embryo (15 out of 15 cases). The ability of such pulsed zygotes to undergo cytokinesis indicates that microfilaments recover function as well as structure after the CD pulse. As further indication that brief CD treatment does not generally impair embryo viability, when such pulsed zygotes are incubated in culture medium, they develop similarly to permeabilized control zygotes; they undergo the proper series of early divisions, gastrulate, develop gut, muscle, and hypodermal differentiation markers, and undergo limited morphogenesis (D. Hill and S. Strome, manuscript in preparation).

F-actin staining of embryos "pulsed" later in the cell cycle also reveals the ability of actin filaments to recover from inhibitor treatment. Successfully pulsed embryos were able to recover from the inhibitor and undergo cytokinesis. Foci and fibers were seen in the newly formed two-cell embryo, with intense microfilament staining at the cleavage furrow (Figs. 3d and 3e). No differential distribution of foci was seen in the two-cell embryo, which is consistent with observations in untreated embryos.

#### Effects of CD Pulses on Zygotic Events

*Division of the cell cycle into intervals.* To gain insight into how microfilaments participate in the generation of zygotic asymmetry, we have used CD to transiently perturb microfilament organization during three specific time intervals that are marked by events in the

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pseudocleavage. (c, d) CD treatment disrupts the normal array of microfilaments. (e, f) Pronuclear meeting occurs in the posterior hemisphere of untreated embryos and in the center of CD-treated embryos. (g, h) CD treatment inhibits the posterior segregation of germ-line-specific P granules; instead they coalesce in the center of the zygote. (i, j) In CD-treated zygotes the mitotic spindle does not become asymmetrically positioned. Arrowheads indicate the positions of the center of the spindle asters and the cell periphery. (k, l) CD treatment inhibits cytokinesis. Bar = 10 µm.

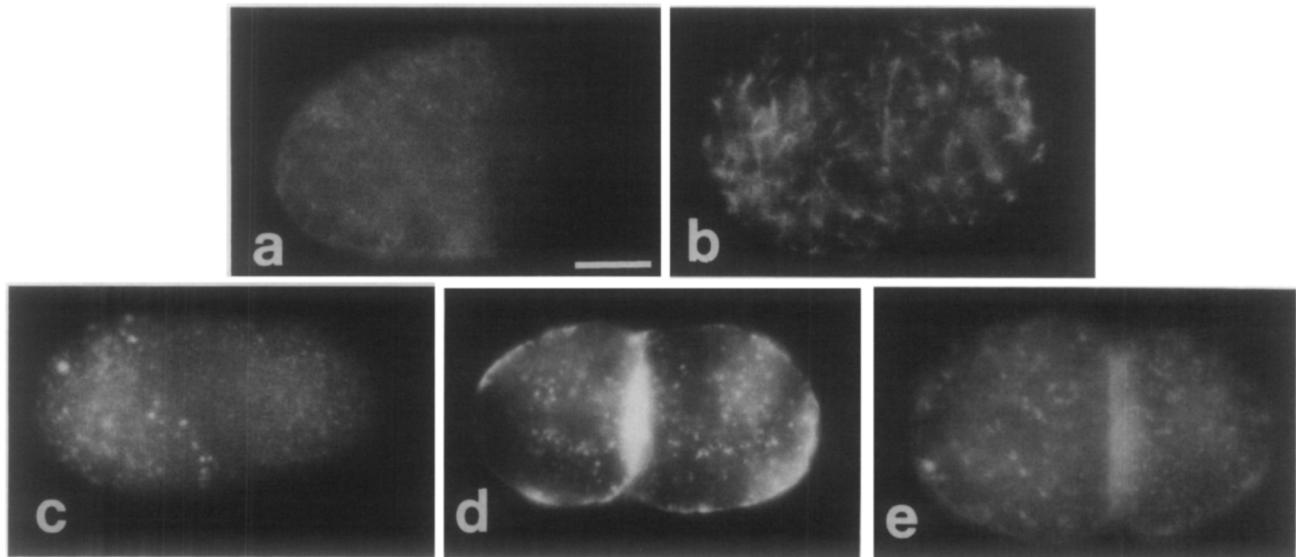


FIG. 3. Rhodamine-phalloidin staining of F-actin in untreated and CD-treated embryos. (a) Control embryo during pronuclear migration, showing the anterior concentration of actin foci. (b) Embryo treated with 2 µg/ml CD for 1 min during pronuclear migration, showing the appearance of spikes and aggregates. (c) Embryo "pulsed" with 2 µg/ml CD in the early interval (see text) and allowed to recover for 5 min. Anterior foci recover. (d, e) Embryos "pulsed" with CD in the middle and late time intervals, respectively, and allowed to recover and divide. The arrays of actin fibers and foci appear normal in both two-cell embryos. Bar = 10 µm.

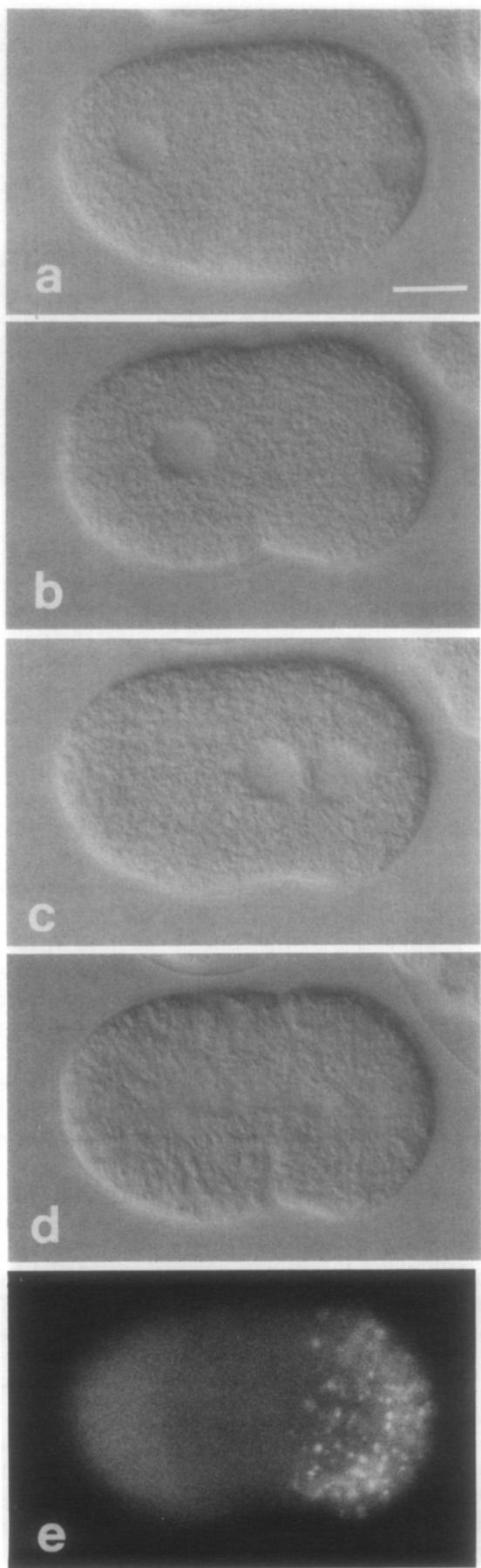
first cell cycle (see Fig. 1): (1) early, before pronuclear migration, when meiosis is being completed and early pseudocleavage is taking place; (2) middle, during pronuclear migration and late pseudocleavage; and (3) late, after pronuclear meeting until just before cytokinesis takes place.

*The early time interval: Transient perturbation of microfilament structure does not interfere with subsequent generation of embryonic asymmetry.* Embryos treated with CD before completion of the second meiotic division do not extrude a second polar body and instead contain two egg pronuclei; early pseudocleavage does not occur in these embryos. When CD is introduced after meiosis and during early pseudocleavage, all aspects of early pseudocleavage disappear (Fig. 4a). Embryos pulsed with CD for 2 to 10 min and allowed to recover from the inhibitor show complete reversal of the effects of CD. If CD is removed prior to when pseudocleavage would normally be complete, a contraction of the membrane occurs, which corresponds to the resumption of late pseudocleavage (Fig. 4b). In most cases, pseudocleavage appears normal, but in 1 out of 15 cases, the returning pseudocleavage was aberrant, with only a slight rippling of the membrane. After pseudocleavage, the egg pronucleus migrates and meets the sperm pronucleus in the posterior hemisphere of the embryo (Fig. 4c). The pronuclei move to the center of the embryo and a normal mitotic spindle forms. Cleavage generates a normal appearing two-cell embryo with a large AB cell and a smaller P1 cell (Fig. 4d; also see Fig. 7c for a diagrammatic summary of these events).

Immunofluorescent staining of P granules in the two-cell embryo resulting from this early CD treatment reveals that granules have been segregated normally and are located at the posterior cortex of the P1 cell (Fig. 4e; Fig. 7c).

We observed the above phenomena in 10 out of 10 embryos exposed to CD for 2 min and in 5 out of 5 embryos exposed to CD for 8 to 10 min. In every case, if the egg pronucleus had not yet begun the anterior to posterior migration at the time of washing, it met the sperm pronucleus at the posterior end of the zygote and all subsequent manifestations of asymmetry were established. We conclude from these experiments that in the period from the completion of meiosis until late pseudocleavage and the commencement of pronuclear migration, normal microfilament structure is not required for proper asymmetry to be expressed later in the first cell cycle.

*The middle time interval: Proper microfilament structure is necessary during pronuclear migration in order to generate embryonic asymmetry.* Upon treatment during the middle time interval, pseudocleavage disappears and the egg pronucleus migrates to the center of the embryo and stops. The sperm pronucleus migrates to meet the egg pronucleus in the center (Fig. 5a). At this point, 8 to 10 min after initiating CD treatment, CD was washed out of the embryos. Such pulsed embryos continue zygotic development, but do not display normal spindle asymmetry. In 7 of the 13 embryos analyzed, the spindle remained symmetrically positioned along the anterior-posterior axis, the P aster did not become



flattened, and cytokinesis led to the formation of a two-cell embryo with equal-sized blastomeres (Figs. 5e and 7d). In 6 of the 13 embryos analyzed, the spindle drifted slightly posteriorly or slightly anteriorly after it formed. In these embryos, the cleavage furrow still bisected the spindle and one of the blastomeres was slightly larger than the other (2 with a larger posterior cell as shown in Fig. 5b and 4 with a larger anterior cell). In all 13 embryos, P granules, visualized by indirect immunofluorescence, were found in the center of the embryo, distributed to both blastomeres (Figs. 5d, 5f, and 7d). Usually the granules were clumped directly at the junction of the blastomeres, but occasionally they were spread out along the membranes of both cells, which may be due to fixation of the embryos before cytokinesis was complete (Fig. 5f).

We conclude from these experiments that proper microfilament structure is required during the time of egg pronuclear migration and late pseudocleavage for the manifestations of asymmetry occurring during and subsequent to this time interval.

*Late time interval: Proper microfilament structure is unnecessary after pronuclear meeting to generate or maintain asymmetry.* To determine whether microfilaments are also necessary at the end of the first cell cycle in order to maintain the asymmetries already generated and to participate in setting up an asymmetrically positioned spindle, we exposed embryos to CD during the late interval and washed it out before cytokinesis would occur (Fig. 6). When embryos were treated with CD at pronuclear meeting, both pronuclei migrated to the center of the embryo, where the mitotic spindle was established. CD was washed out of the embryo 2 to 10 min after CD treatment was initiated, and in 15 out of 15 embryos the spindle became asymmetrically placed toward the posterior end of the zygote (Fig. 7e). Mitosis proceeded normally, and the embryo underwent a normal cleavage resulting in a large AB cell and a smaller P1 cell (Fig. 6a). In 15 out of 15 cases P granules were segregated normally to the posterior cortex of the P1 cell (Figs. 6b and 7e).

We conclude from these results that once MFs have carried out their function in the middle time interval they are no longer needed to maintain the asymmetries already established or to participate in positioning the mitotic spindle correctly.

FIG. 4. Embryo pulsed with CD in the early time interval, during the completion of meiosis and early pseudocleavage. (a) Upon treatment with 2  $\mu$ g/ml CD, pseudocleavage disappears. (b) After a 2-min exposure to CD, the embryo in (a) was washed with medium lacking CD. Pseudocleavage returns (b) and the pronuclei migrate and meet in the posterior hemisphere of the embryo (c). (d) The embryo undergoes an apparently normal asymmetric first cleavage. (e) The embryo shown in (d) was fixed and stained with anti-P-granule antibodies. P granules are localized in the posterior cortex of P1 after the first cell division. Bar = 10  $\mu$ m.

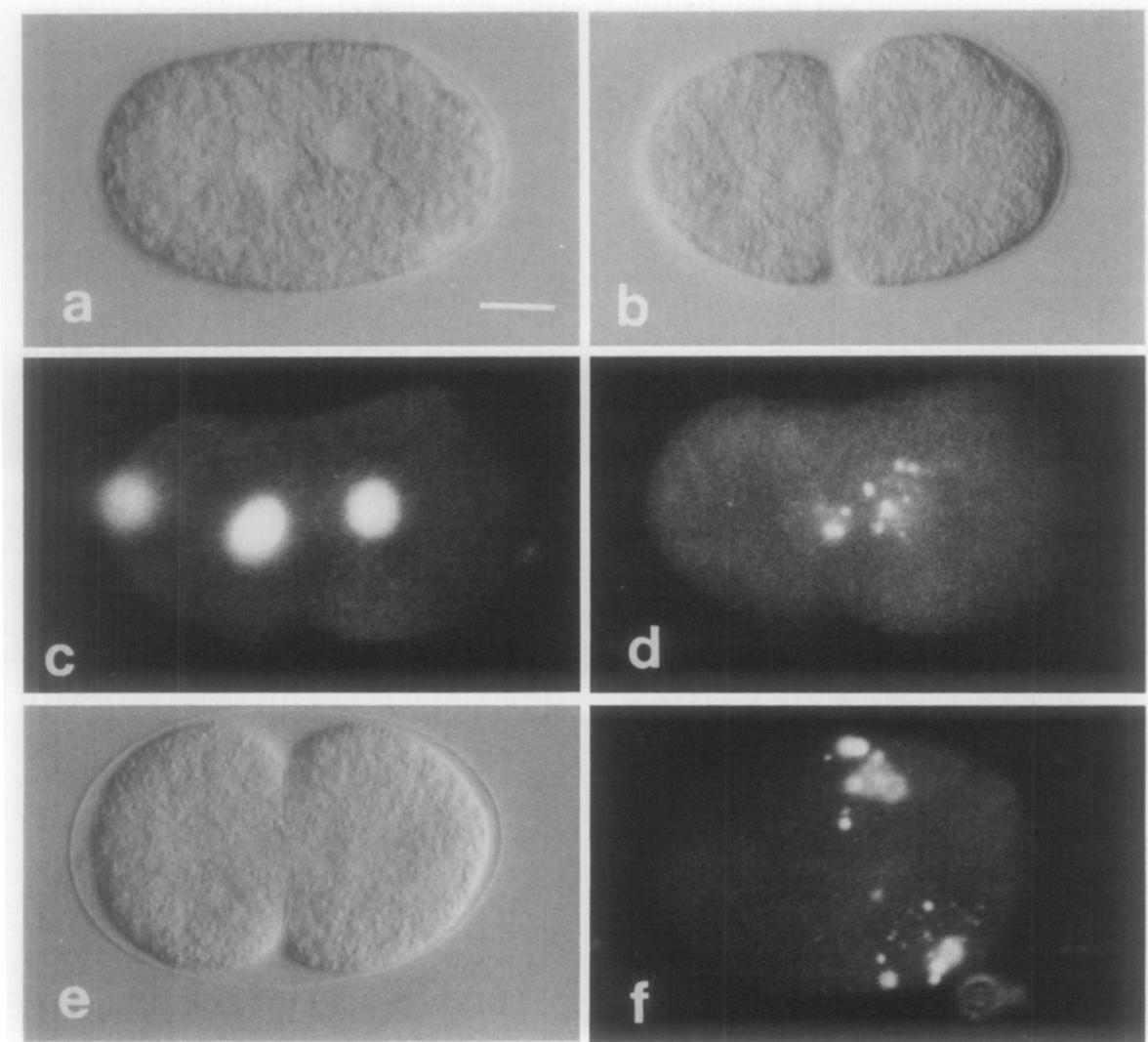


FIG. 5. Embryos pulsed with CD in the middle time interval, during late pseudocleavage and pronuclear migration. (a) CD treatment causes the disappearance of pseudocleavage and a central meeting of the pronuclei. (b) The embryo in (a) was washed with CD-free medium after the pronuclei had met (5 min after introducing CD). The embryo underwent an uneven cleavage to generate a two-cell embryo with a small anterior cell and a larger posterior cell (just opposite to the normal cell arrangement). (c, d) The embryo shown in (b) was fixed and stained with DAPI (c) and anti-P-granule antibodies (d). P granules are located in the center of the embryo in both daughter blastomeres. (e) This embryo, pulsed with CD as described in (a, b), underwent an even first cleavage. (f) The embryo in (e) was fixed and stained for P granules. Granules were located in both cells near the cell junctions. Bar = 10  $\mu$ m.

*Proper microfilament structure is not required to anchor P granules at the posterior cortex once they have been segregated.* The results described in the previous section suggest that microfilaments are not necessary to anchor P granules at the embryo's posterior end once they have been segregated. To further test this, we treated embryos with CD for longer periods of time (10–15 min) after P-granule segregation. P granules have been segregated to the posterior end of the embryo by the time of pronuclear meeting. When embryos are treated with CD at this time, without subsequent washing, the embryos are not able to undergo cytokinesis but do undergo karyokinesis. In these treated embryos P granules remain in the posterior cortex of the embryo (data not shown). Even in embryos treated with

CD and mechanically agitated by exerting gentle pressure on the overlying coverslip, granules remain posterior and cortical. When the embryos are left in CD for 3 to 5 min after nuclear formation, the P granules become distributed more internally, as is normally observed in a newly formed P1 cell. These results suggest that P granules do not require the presence of a normal microfilament network to remain posterior after segregation has taken place.

#### DISCUSSION

We have used rhodamine-phalloidin to characterize the distribution of microfilaments in normal (untreated) and CD-pulsed embryos. As observed in other

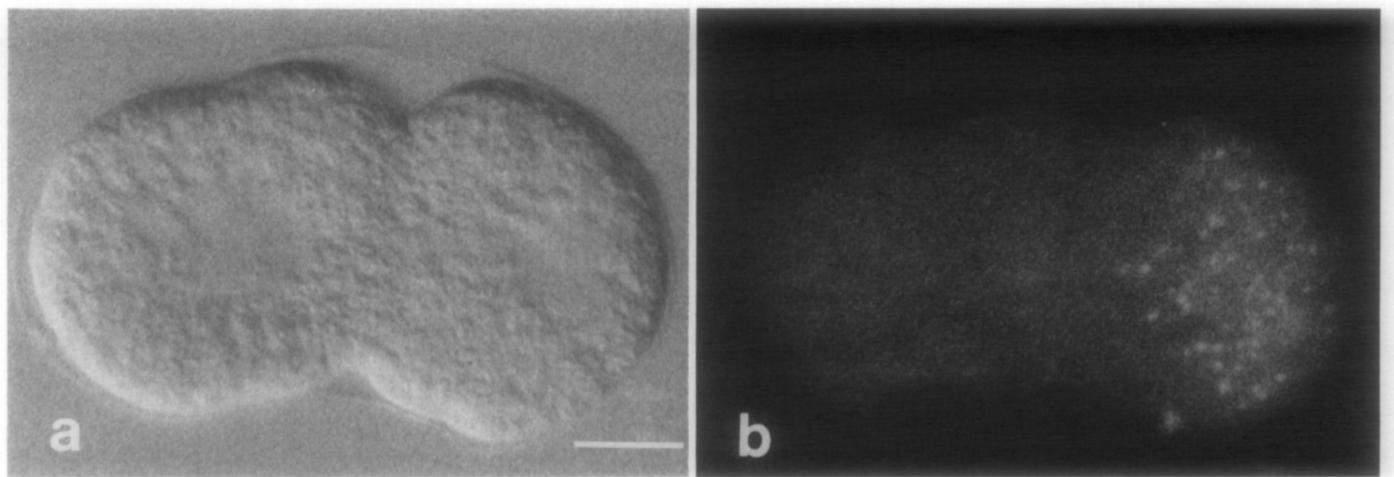


FIG. 6. Embryo pulsed with CD during the late time interval, from the time of pronuclear meeting until just before cytokinesis. (a) This zygote was exposed to CD after the pronuclei had met and migrated to the center of the zygote. It was washed 2 min after exposure to CD, after which it underwent a normal asymmetric cleavage. (b) The embryo shown in (a) was fixed and stained with anti-P-granule antibodies. P granules are localized in the posterior cortex of P1 after the first cell division. Bar = 10  $\mu$ m.

cell types (Schliwa, 1986), CD treatment leads to the disappearance of the normal cortical array of F-actin

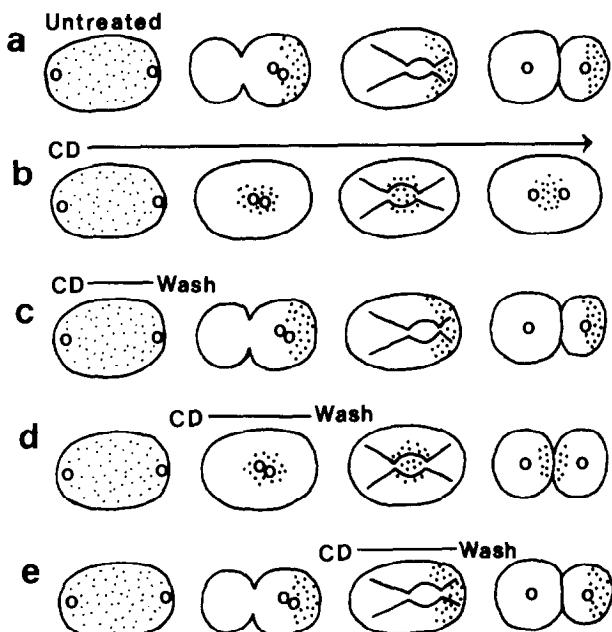


FIG. 7. Schematic summary of events in zygotes treated or pulsed with CD. Each row shows four progressively later stages: pronuclear stage after the completion of meiosis, pseudocleavage and pronuclear meeting, formation and asymmetric placement of the spindle, and first cleavage. Anterior is left; posterior is right. The small dots represent P granules, and the  $\times$  symbols are spindles. (a) Normal zygote for reference. (b) Zygote treated with CD during or after meiosis and allowed to develop in the presence of inhibitor. (c) Zygote treated briefly with CD during the early time interval, from meiosis until the commencement of pronuclear migration. (d) Zygote treated briefly with CD during the middle time interval, during pronuclear migration until pronuclear meeting. (e) Zygote treated briefly with CD during the late time interval, after pseudocleavage, pronuclear migration, and P-granule segregation until just before cytokinesis.

and the appearance of spikes and aggregates throughout the cytoplasm. In *C. elegans* embryos, this occurs within 1 min of exposure to CD (Strome, 1987). In each of the experimental CD pulses, the embryo was treated with inhibitor for at least 2 min, guaranteeing disruption of microfilaments. However, upon removal of CD, the microfilament distributions recovered nearly completely. This implies either that the correct microfilament array can assemble spontaneously in the embryo, or that there exists some type of "organizer" in the zygote to which microfilaments respond. Recovery of microfilaments and the normal occurrence of subsequent cellular events after inhibitor treatment indicates that treatment does not interfere with general cell viability.

Based on the analysis of CD-pulsed embryos, correct microfilament structure appears to be required only during a narrow time interval in the first cell cycle. The observation that a CD pulse during this middle time interval causes as severe a disruption of events as continuous treatment with CD, and a CD pulse outside this time interval has no effect, suggests that it is only during this middle interval that actin filaments play an active role in establishing the embryonic asymmetries. The finding that the actin fibers and foci can recover after a pulse in the middle time interval but P granules do not become properly localized suggests that a critical event(s) may be occurring during this interval; missing the event or undergoing the event in an abnormal fashion cannot be compensated for later in the first cell cycle.

Perhaps the most surprising finding was that the middle time period is critical not only for events occurring during the interval but also for the future asymmetric placement of the mitotic spindle. This suggests

that during the middle time interval, actin filaments are providing spatial cues or are participating in an event(s) that will ultimately determine the position of the mitotic spindle; once microfilaments have functioned, subsequent perturbation does not interfere with spindle placement.

By performing cytoplasmic extrusion experiments, Schierenberg has previously shown that material responsible for the early asymmetric cleavages that are characteristic of the P-cell lineage are already localized in the posterior of the embryo during the early time interval (Schierenberg, 1985). Our results taken together with those of Schierenberg suggest that microfilaments are part of the architecture or machinery that responds to or carries out the instructions of the posteriorly localized "asymmetry determinants." For example, microfilaments may participate in establishing "coordinates" in the embryo during the critical time interval by interacting with some substance that is prelocalized in the posterior end of the zygote. This hypothesis predicts the existence of a class of actin-binding proteins that would be found in the posterior of the zygote during at least the first two time intervals we have defined.

In an earlier study, Strome suggested that the foci of F-actin that become concentrated in the anterior of the zygote during late pseudocleavage and pronuclear migration may play an important role in the generation of asymmetries taking place during that time interval (Strome, 1986). We explored this possibility by determining whether foci reappear and become concentrated in embryos whose microfilament structure is disrupted very early in the cell cycle. If our results had shown that the foci did not reappear or become concentrated after a CD pulse, but that zygotic asymmetries were nevertheless generated, we would have ruled out the concentrated actin foci as a critical component of the microfilament array involved in the generation of asymmetry. We observed that the actin foci reappeared and became more concentrated in the anterior half of the zygote in 36 out of 42 cases, consistent with an involvement of the anteriorly concentrated actin foci in the events we are monitoring. However, the observation that approximately 10% of control and CD-pulsed embryos display more posteriorly concentrated actin foci implies that an anterior concentration of actin foci may not be necessary for the events we are monitoring to occur. Furthermore, at least one of the asymmetric events we monitor, P-granule segregation, is not accompanied by a concentration of actin foci in the later stage germ-line blastomeres P1, P2, and P3 (S. Strome and D. Hill, unpublished results). This suggests that the asymmetric reorganization of microfilaments we've described is specific to the zygote and that concentrated

microfilaments may not be required for P-granule segregation.

Once P granules have been segregated posteriorly, they remain in the posterior cortex of the zygote even if microfilaments are disrupted. After they become localized, P granules must be held in position by some means other than microfilaments. At least two possibilities exist: (1) P granules may be anchored at the posterior cortex by some as yet unidentified structure in the posterior cell membrane. (2) The viscosity of the cytoplasm may increase during the first cell cycle as it does in *Xenopus laevis* embryos (Elinson, 1983); the increased viscosity could hold the granules at the posterior cortex. We have been unable to disperse granules from the posterior end either by pumping on the embryo or by incubating embryos longer in CD to allow P-granule diffusion, favoring the anchoring hypothesis.

We thank E. Raff, M. MacMorris, T. Blumenthal, and G. Radice for critical reading of the manuscript. This work was supported by Research Grant GM34059 from the U.S. Public Health Service. D.P.H. is a predoctoral trainee in the Molecular, Cellular, and Developmental Program and is supported by Training Grant GM07227-12.

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