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Cortical actin movements during the first cell cycle of the *Caenorhabditis elegans* embryo

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SUMMARY

The first division of the *Caenorhabditis elegans* embryo is unequal, generating daughter cells with distinct fates. The differences between the cells are believed to result from the partitioning of cytoplasmic determinants during the first cell cycle. Actin microfilaments play a critical, but poorly defined, role in this event. In this paper, the actin cortex in live embryos is studied during cytoplasmic localisation by fluorescently labelling microfilaments in oocytes and then using in vivo fluorescence microscopy to observe their behaviour. This reveals that there is a concerted movement of cortical actin to the anterior of the embryo at the time cytoplasmic localisation takes place. Furthermore, it is demonstrated that endogenous foci of F-actin are asymmetrically distributed following this event; these structures have previously been seen in fixed cortices. A model for the participation of the actin cytoskeleton in cytoplasmic localisation is presented based on these results.

Key words: *Caenorhabditis elegans*, Cytoplasmic localisation, Microfilament, Cytokinesis

INTRODUCTION

The embryos of many organisms, both vertebrates and invertebrates, undergo pronounced cytoplasmic and cortical reorganisations following fertilisation. These movements serve to localise cytoplasmic and cortical factors, including cell fate determinants, to particular regions of the uncleaved zygote (reviewed by Sardet et al., 1994). Such factors can then be differentially partitioned to distinct daughter cells during the early divisions, thereby contributing to the generation of cell diversity. Both microfilaments and microtubules have been demonstrated to participate in these events in a variety of embryos (see for examples: Houliston and Elinson, 1991; Jeffery and Meier, 1983; Sawada and Osanai, 1985; Sawada and Schatten, 1988).

A segregation of cytoplasmic factors takes place in the fertilised egg of the nematode *Caenorhabditis elegans*. For example, germ-line specific factors termed P granules are initially uniformly distributed in the egg, but become localised to the posterior pole during the first cell cycle (Strome and Wood, 1982, 1983). In this way they are inherited by the germline precursor cell, P_1, which is the posterior daughter of the first division. It is believed that other factors specifying cell fate are similarly partitioned along the anteroposterior axis during the first cell cycle (Bowerman et al., 1993; Cowan and McIntosh, 1985; Edgar and McGhee, 1986; Laufer et al., 1980; Schierenberg, 1985; Strome, 1989).

The cytoplasmic localisation events in *C. elegans* occur during a process known as pseudocleavage (Hill and Strome, 1988, 1990; Strome, 1989; Strome and Wood, 1983). Pseudocleavage begins just as the oocyte and sperm pronuclei appear at the anterior and posterior poles, respectively, of the elongated embryo (Hirsh et al., 1976; Nigon et al., 1960) (Fig. 1). During this process, waves of cortical contraction sweep from posterior to anterior, and eventually become confined to the anterior membrane. A single large constriction, known as the pseudocleavage furrow, forms and subsequently regresses, but is dispensable for normal development (Rose et al., 1995). At the same time as pseudocleavage, cortical yolk granules flow anteriorly, whilst deeper cytoplasm streams posteriorly. These events, and the localisation of cytoplasmic factors described above, are all dependent on actin microfilaments but do not require intact microtubules (Strome and Wood, 1983). It has therefore been suggested that the posteriorly-directed cytoplasmic streaming is responsible for the localisation of cytoplasmic factors to the posterior daughter of the first division, namely the P_1 cell (Hill and Strome, 1988; Strome, 1986, 1989; Strome and Wood, 1983).

Previous work has shown that there is a reorganisation of the actin cytoskeleton during the interval when pseudocleavage and cytoplasmic localisation take place (Strome, 1986). When embryos are fixed and stained for actin prior to this interval, the cortex contains a large number of small foci of actin which are uniformly distributed. During pseudocleavage, the foci become concentrated in the cortex anterior to the pseudocleavage furrow, forming an actin 'cap'. This redistribution of cortical actin-containing structures may result from an anteriorly directed flow of cortical actin; this may in turn drive the actin-dependent cortical granule flow that occurs during this period (Hird and White, 1993). This view is supported by the observation that the cortical flow velocity (4-7 μm/minute) is similar to previously determined surface particle movements

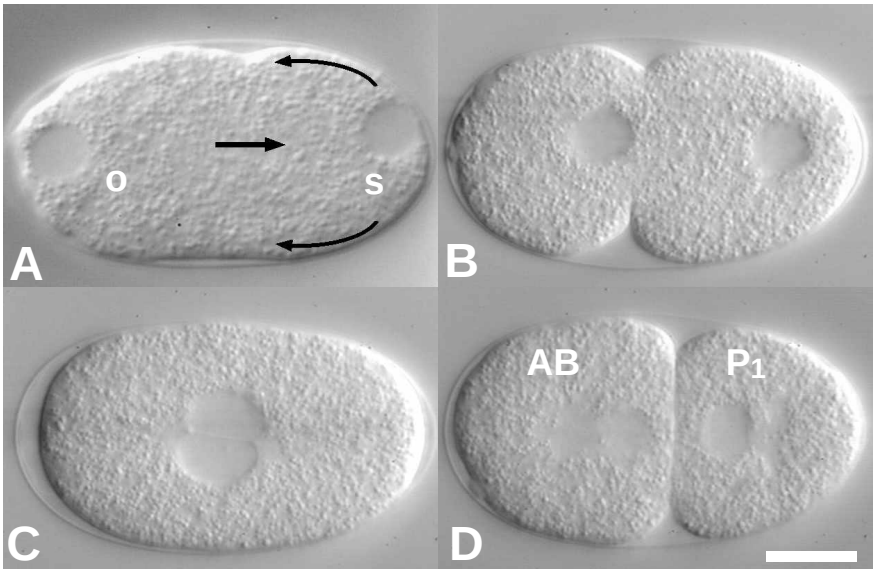

Fig. 1. Pseudocleavage and first cleavage in the *C. elegans* embryo. (A) An embryo early in pseudocleavage is shown. The positions of the oocyte (o) and sperm (s) pronuclei are indicated. Contractions in the cortex move anteriorly, carrying along cortical granules (curved arrows). Deeper cytoplasm streams posteriorly (straight arrow). (B) Later in pseudocleavage, a single large constriction forms at 50% embryo length. This is termed the pseudocleavage furrow. The cortical and cytoplasmic flows continue, and pronuclear migration occurs. (C) The pseudocleavage furrow regresses and the flows cease as the pronuclei meet, forming the zygote P_0. (D) The mitotic spindle is set up along the anteroposterior axis, and the embryo divides forming the anterior daughter cell AB and the posterior daughter cell P_1. These cells differ in both size and fate. Anterior, left. Bar, 10 μm.

driven by cortical actin flows in motile cells (Abercrombie et al., 1970; Forscher and Smith, 1990). An alternative possibility is that the rearrangement of the actin foci is a result of their destabilisation at the posterior of the embryo.

Studying the behaviour of cortical actin during pseudocleavage might indicate how microfilaments bring about cytoplasmic localisation. In order to do this, the actin cytoskeleton of living *C. elegans* oocytes was fluorescently labelled and then monitored by time-lapse in vivo fluorescence microscopy. The results indicate that there is a movement of cortical actin to the anterior of the embryo at the same time as actin-dependent cytoplasmic localisation takes place.

MATERIALS AND METHODS

Nematode culture

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

Microinjection of oocytes

Oocyte injections were performed using a modification of the transformation protocol of Fire (1986). Microneedles (Clarke Electromedical GC120 F-15, pulled on a Sutter puller) were backfilled by capillary action and mounted on a MPM-10/ DC3001 piezo-translator micromanipulator (World Precision Instruments) attached to a Zeiss Axiovert inverted microscope equipped with DIC optics. The two oocytes nearest the spermatheca in both arms of the bi-lobed gonad were injected using compressed air with <5% cell volume.

For injection of fluorescent phalloidin, the solutions used were 30 μM fluorescein-phalloidin (fl-pha) or rhodamine-phalloidin (rh-pha) (Molecular Probes, Eugene, Oregon), either in methanol or in 5 mM Tris-acetate, pH 6.95. Identical results were achieved with both solvents, but smaller volumes could be injected in the methanol. The resulting intracellular phalloidin concentration (approximately 1 μM) has been shown in tissue culture cells to label actin effectively without detrimental effects to the cell (Cao and Wang, 1990a; Wang, 1985). Following injection, the worms were rehydrated in M9 buffer until they regained movement, and then placed on bacteria-seeded NGM plates for between 20-40 minutes. During this time, at least one of the oocytes in each oviduct was fertilised and passed into the uterus. Due to the variation in time from injection to fertilisation and the briefness of the period of pseudocleavage (approximately 10 minutes at 20°C), less than 10% of injected worms yielded embryos of the desired stage.

The worms were dissected in egg salts, and the labelled embryos gathered using the tip of the scalpel blade. They were mouth-pipetted in a capillary tube to a coverslip coated with 0.1% poly L-lysine (Sigma), and the coverslip was inverted over a depression slide containing egg salts (118 mM NaCl, 40 mM KCl, 3.4 mM $MgCl_2$, 3.4 mM $CaCl_2$, 5 mM HEPES, pH 7.4; Edgar and McGhee, 1986). The coverslip was sealed to the slide with Vaseline to prevent desiccation, and the slide mounted on a Zeiss Axiovert equipped with a laser-scanning confocal scanhead (MRC-500 prototype built in the MRC Laboratory of Molecular Biology Mechanical Workshop) and DIC optics. Injected embryos were identified by very brief exposure (approximately 0.5 seconds) to light from a 50 W Hg arc lamp attenuated with neutral density filters and viewed with the appropriate epi-fluorescence filter. For time lapse studies, a single confocal section was collected at the slowest scan speed (3 seconds/scan) every 30 seconds at 20°C using a 63× oil immersion lens. The Argon laser light was attenuated by neutral density filters (Oriel) to give the lowest amount of illumination necessary for a satisfactory fluorescent signal. A transmission detector allowed the collection of simultaneous fluorescence and DIC images of the embryo. Fluorescence images were normalised using Bio-Rad MRC-600 software. The displacement of actin-containing structures was measured using the length function supplied in the software.

Microinjection of rhodamine-labelled actin

Rabbit skeletal muscle actin labelled with iodoacetamidotetramethyl-rhodamine was a kind gift of Drs Yu-Li Wang and Douglas Fishkind, Worcester Foundation for Biomedical Research, Shrewsbury, MA. The derivatised actin was stored in 40 μl aliquots in liquid nitrogen. To prepare the actin for injection, an aliquot was transferred to a microdialysis chamber, and dialysed for 4-6 hours against fresh actin monomer buffer (2 mM Tris-HCl, pH 8.0, 0.2 mM $CaCl_2$, 0.2 mM ATP, 0.25 mM DTT) at 4°C. The actin was then dialysed against actin injection buffer (2 mM Tris-acetate, pH 7.0, 0.2 mM ATP, 0.05 mM $MgCl_2$) for 4-6 hours at 4°C. Immediately prior to injection, the actin was spun at 100,000 ***g***, 4°C in a Beckman TLA-100 microcentrifuge for 30 minutes. The actin was then injected into oocytes at a concentration of approximately 5 mg/ml. The injection procedure and observation of the labelled embryos was carried out as described above.

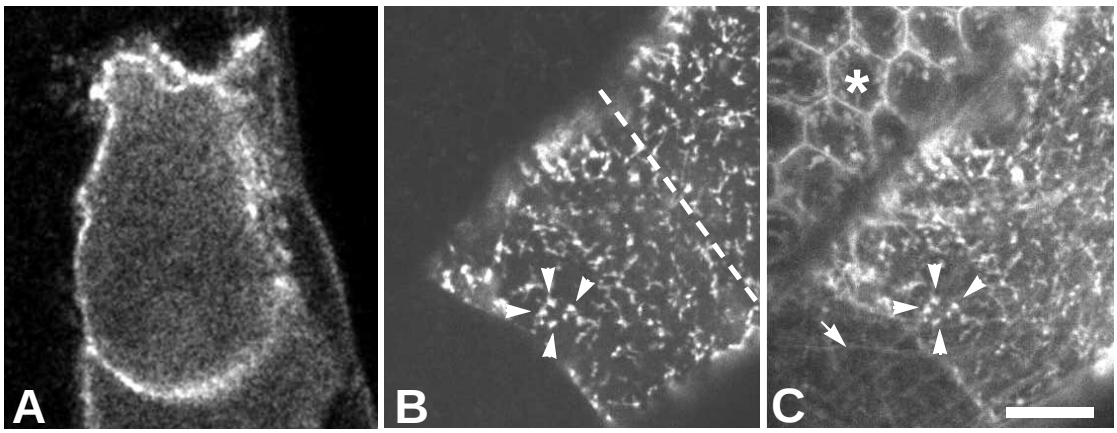

Fig. 2. Oocytes injected with fluorescent phalloidin in the gonad. (A) An oocyte injected with fl-pha, and imaged 2 minutes later with laser-scanning confocal microscopy in a plane through the centre of the oocyte. The phalloidin rapidly associates with the cortex. (B) Two oocytes (dashed white line indicates boundary between them) fixed 5 minutes after injection of fl-pha within the gonad and viewed in the plane of the cortex. The injected phalloidin has stained numerous foci and fibres: this is the same type of pattern seen when uninjected oocytes are fixed and stained for actin (Strome, 1986). Four actin foci are marked by arrowheads in one of the oocytes. (C) The same oocytes as in B, but showing the rhodamine-phalloidin staining of total F-actin. Note that as all F-actin is revealed by postfixation staining, microfilaments in the gonad (within which the oocytes are contained) are visible. The cortices of proliferating germ cells can clearly be seen as the honeycomb structure at the top of the panel (star). The fibrous structures (arrow) are in the layer of somatic cells that surround the oocytes (Strome, 1986). The pattern of staining in the actual oocytes is identical to B, confirming that the injected phalloidin has bound actin. For example, the four foci shown by arrowheads in B are also seen in C (arrowheads). Bar, 10 μm.

Fixation of selected embryos for phalloidin staining

For fluorescent phalloidin visualisation of the total actin cytoskeleton in phalloidin-injected embryos, dissected embryos were placed in a drop of egg salts on a slide coated with 0.1% poly-L-lysine. The embryos were then fixed and stained with fluorescein (fl-pha) or rhodamine phalloidin (rh-pha), as described previously (Hird and White, 1993; Strome, 1986). If the embryo was injected with fl-pha, then it was fixed and stained with rh-pha; conversely if the injectant was rh-pha then fl-pha was used for staining. Doubly-labelled embryos were imaged with a Bio-Rad MRC-600 laser-scanning confocal microscope equipped with a Ar/Kr laser, using separate excitation filters for each channel.

RESULTS

Fluorescent phalloidin rapidly associates with cortical actin when injected into oocytes

In order to label F-actin, oocytes contained within the oviduct were injected with either rhodamine-phalloidin (rh-pha) or fluorescein-phalloidin (fl-pha). The phalloidin injected into oocytes became associated with the cortex within less than 1 minute (Fig. 2A). Cytoplasmic staining was low, suggesting that most of the phalloidin was bound to cortical actin and not freely diffusible within the cytoplasm. Oocytes that were fixed immediately after injection, and stained with phalloidin that was labelled with a different fluorescent molecule, confirmed that the injected phalloidin had bound to cortical actin (Fig. 2B,C). Labelled oocytes gave rise to normal embryos and worms, showing that neither the concentration of phalloidin used nor the solvent was harmful to the oocyte.

Injected oocytes develop discrete cortical actin aggregates that are reorganised during pseudocleavage

Oocytes were dissected from hermaphrodites 40 minutes after

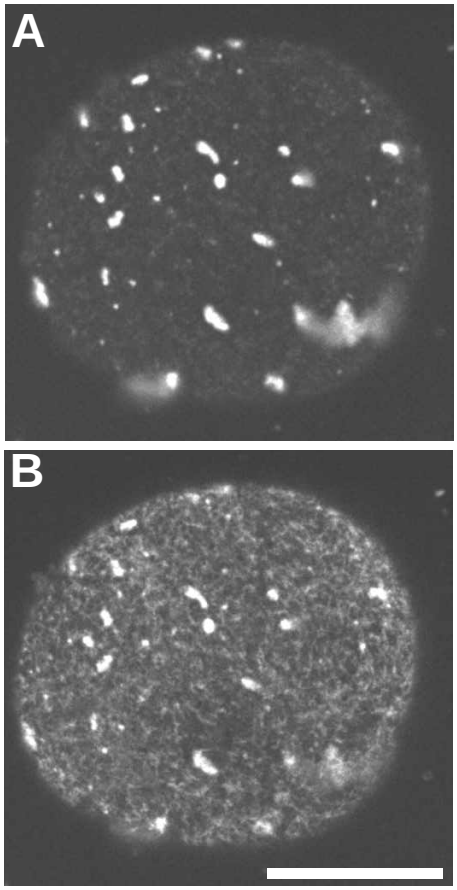

Fig. 3. The phalloidin signal later becomes localised to aggregates of cortical actin. An oocyte was injected with fl-pha, then dissected out of the gonad and fixed after 30 minutes (cf. 5 minutes in Fig. 2) and stained with rh-pha to visualise total F-actin. In this particular case, this was still prior to fertilisation. In A, the fixed oocyte is shown in the fluorescein channel. The injected phalloidin is now localised to many large foci (compare to Fig. 2B). In B, the same oocyte is viewed in the rhodamine channel. The foci in A all stain with the rh-pha, verifying that they contain F-actin. Note that fibres of actin are also visible in this image. Bar, 10 μm.

injection. Depending on their proximity to the spermatheca (the site of fertilisation) at the time of injection, such dissected oocytes were often not yet fertilised. In both oocytes that were not yet fertilised after 40 minutes and in newly fertilised eggs, the fluorescent phalloidin signal was localised to bright cortical foci of approximately 0.5 μm diameter. Fixation followed by staining with phalloidin coupled to a different fluorochrome revealed that all the foci were aggregates of F-actin associated with the cortex. Fig. 3A shows a fixed late stage oocyte with the foci that arose from injection of fl-pha. Fig. 3B shows the foci in the same fixed oocyte stained with rh-pha to visualise total F-actin. The foci are surrounded by a meshwork of cortical actin, suggesting that they are part of the actin cortex.

In fertilised eggs, the aggregates were initially uniformly distributed over all regions of the cortex (Fig. 4A,B), but after pseudocleavage they were present only in the anterior cortex (Fig. 4C-E). They remained anteriorly localised during the first cytokinesis so that in two-cell embryos they were located in the cortex of the anterior daughter cell, AB, but not in its sister, P_1 (Fig. 4F-H). The aggregates remained in the cortex of the progeny of AB. In over 100 phalloidin-containing embryos and late oocytes that were examined, actin aggregates were never observed in the cytoplasm.

It can be concluded that the phalloidin injection results in the formation of F-actin aggregates that are embedded in the cortical actin network. The aggregates become localised to the anterior cortex of the embryo during pseudocleavage.

Cortical actin moves anteriorly during pseudocleavage

The reorganisation of the cortical actin aggregates during pseudocleavage could result from either: (a) movement of the aggregates to the anterior of the embryo; or (b) degradation of the aggregates at the posterior of the embryo. In order to investigate this, labelled embryos were followed by time-lapse confocal fluorescence microscopy. It was found that the aggregates all moved anteriorly along the cortex during the course of pseudocleavage (Fig. 5). This coincides with the period of anteriorly directed cortical granule movement (Hird and White, 1993). The aggregates moved at 4.0±0.8 μm/minute (n=17, 3 embryos) in the posterior 50% of the embryo, and at 1.8±0.2 μm/minute (n=8, 3 embryos) in the anterior 50%. These rates are similar to that of the cortical granule flow (Hird and White,

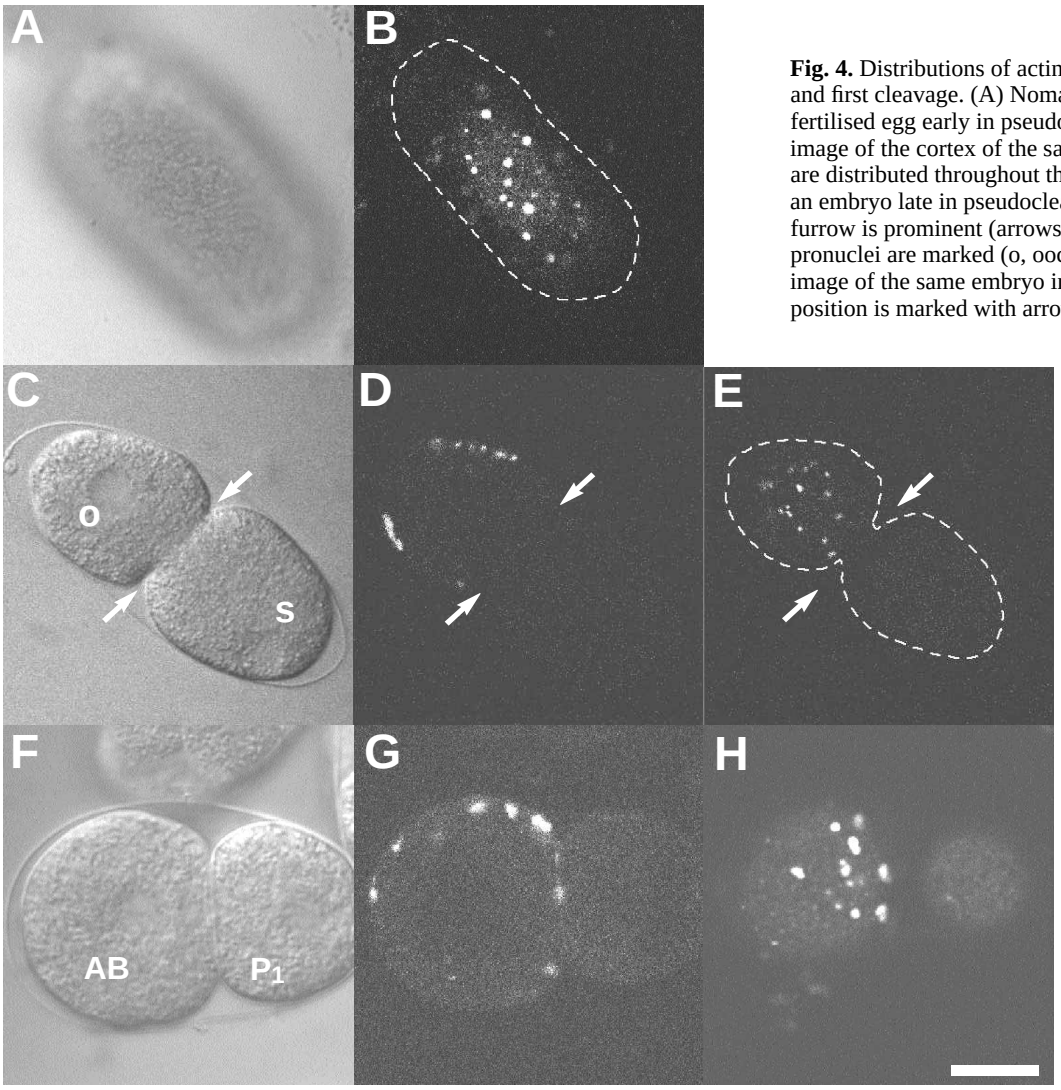

Fig. 4. Distributions of actin aggregates at pseudocleavage and first cleavage. (A) Nomarski image of the cortex of a fertilised egg early in pseudocleavage. (B) Live confocal image of the cortex of the same embryo. The actin aggregates are distributed throughout the cortex. (C) Nomarski image of an embryo late in pseudocleavage. The pseudocleavage furrow is prominent (arrows), and the positions of the pronuclei are marked (o, oocyte; s, sperm). (D) Live confocal image of the same embryo in a medial plane (the furrow position is marked with arrows). The aggregates are located on the cortex (seen as the cell periphery in this plane) anterior to the pseudocleavage furrow. (E) Live confocal image of the same embryo in a cortical plane. The actin aggregates are anterior to the furrow (the furrow position is marked with arrows). (F) Nomarski image of a two-cell embryo in medial plane. The cells AB and P_1 are indicated. (G) Live confocal image in a medial plane. The actin aggregates can be seen in the cortex (seen as the cell periphery in this plane) of only AB. (H) Live confocal image in a cortical plane. The actin aggregates are present in AB. Anterior, left. Bar, 10 μm.

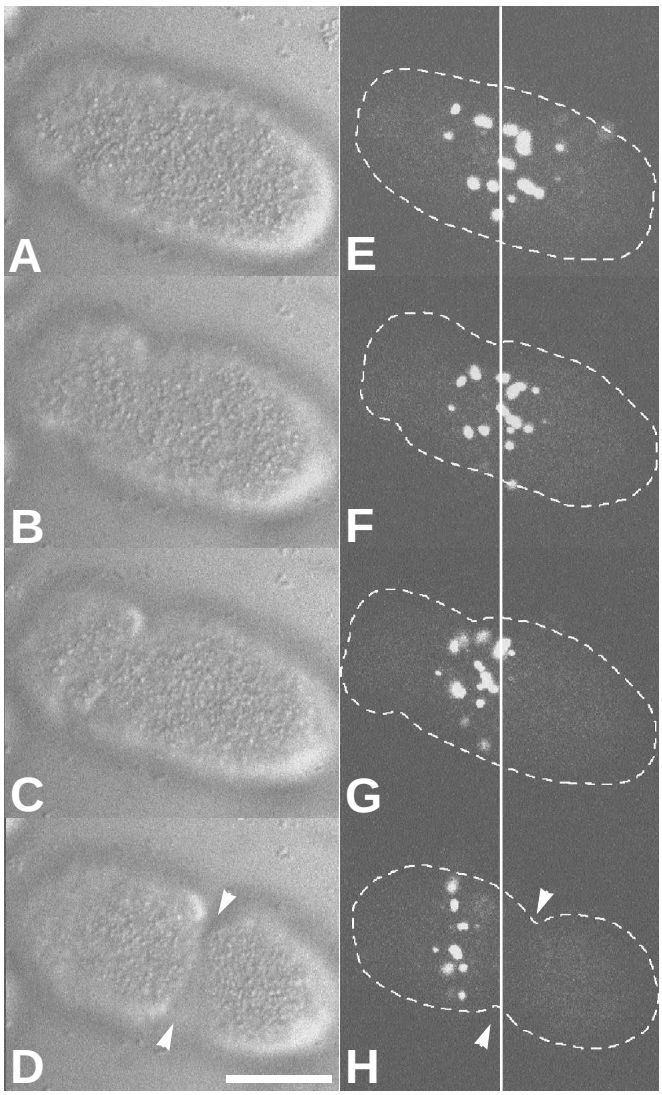

Fig. 5. Redistribution of actin aggregates at pseudocleavage. Nomarski images (A-D) are displayed next to single-scan confocal images of the fluorescein-phalloidin signal (E-H) which were recorded at the same time and in the same focal plane. The actin aggregates move anteriorly during the period of observation, such that they are all on the anterior side of the pseudocleavage furrow as it forms (arrowheads in D and H). The time between images is approximately 2.5 minutes. The fluorescein images were recorded with the confocal aperture fully open and the photomultiplier gain at maximum to optimise signal detection. As a result, the phalloidin foci falsely appear larger in the first image than in later ones. Some of the foci seem to merge during the recording, so that there seem to be fewer foci in the last panel than in the first. The vertical white line in E-H is a stationary reference marker. Anterior, left. Bar, 10 μm.

1993) (Fig. 6). Aggregates that were posterior to the developing pseudocleavage furrow disappeared out of the plane of focus as they moved anteriorly into the furrow, whilst those that were anterior to the furrow continued moving anteriorly. Movement of the aggregates ceased as the maternal pronucleus migrated through the pseudocleavage furrow. This is the same time as the cortical and cytoplasmic streaming ceases. By this time, all of the aggregates had passed into the anterior half of

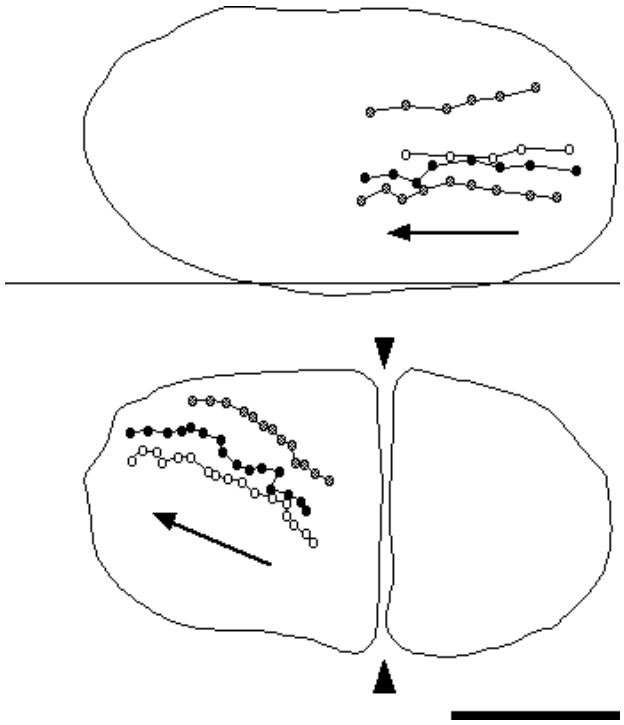

Fig. 6. Tracing of movement of actin aggregates during pseudocleavage. (A) Tracing of 4 aggregates every 30 seconds in the posterior half during the early part of pseudocleavage. The cortical phalloidin foci move in the direction of the arrow (anteriorly). (B) Tracing of 3 aggregates in the anterior half of a second embryo at a later stage of pseudocleavage, after the formation of the furrow (arrowheads). The interval between points is also 30 seconds. Note that the foci in the anterior move at approximately half the rate of the foci in the posterior. Anterior, left. Bar, 10 μm.

the cortex, as delimited by the pseudocleavage furrow, and none remained posteriorly. As the aggregates appear to be part of the actin network, this result strongly suggests that cortical actin moves from posterior to anterior over the course of pseudocleavage.

Cortical actin moves towards the cytokinetic furrow at the first cleavage

There is evidence that actin microfilaments flow along the cortex during cytokinesis towards the furrow in NRK epithelial cells (Cao and Wang, 1990b). This movement is believed to be important for the formation of the contractile ring (White and Borisy, 1983). In order to see whether a similar flow occurs in dividing *C. elegans* cells, the movement of the cortical actin aggregates was followed in four embryos during the first cell division. The aggregates, now present only in the anterior cortex, began to move posteriorly about 1 minute before the first indication of furrowing (Fig. 7). As the furrow appeared, they continued moving towards it. As the nearest foci reached the furrow, they dropped out of the plane of focus. Other aggregates did not reach the furrow in this time, and remained visible in the cortical plane. In the embryo shown in Fig. 7, cortical actin foci could be seen to move at approximately 3.3 μm/minute during this period. These results suggest that an actin flow occurs in *C. elegans* cytokinesis.

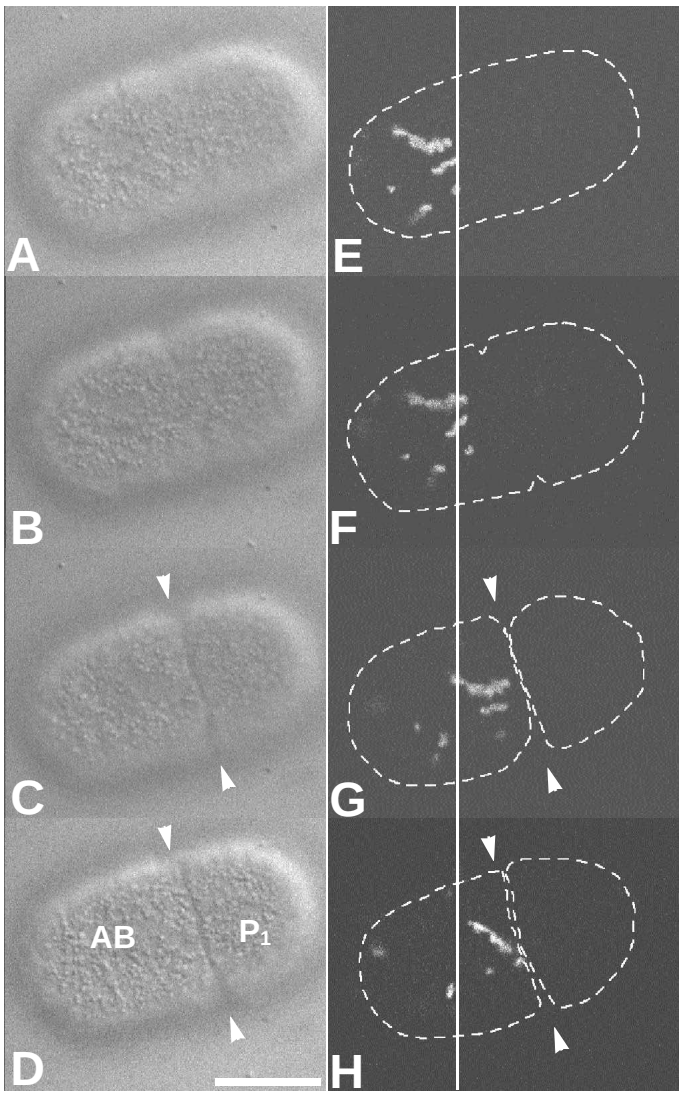

Fig. 7. Movement of cortical actin aggregates at cytokinesis. Nomarski images (A-D) are displayed next to single scan confocal images of the fluorescein-phalloidin signal (E-H) which were recorded at the same time and in the same focal plane. Fluorescent actin aggregates are shown in the anterior cortical surface of an embryo prior to cytokinesis in E, and at 2, 4 and 5 minutes after this time in F-H, respectively. The foci in E-H move towards the developing furrow (arrowheads), and when they reach the furrow, they move out of focus. The newly formed blastomeres AB and P_1 are indicated. The vertical white line in E-H is a stationary reference marker. Anterior, left. Bar, 10 μm.

Injection of rhodamine-labelled G-actin into oocytes

Rabbit skeletal muscle actin labelled with iodoacetamido-tetramethyl-rhodamine was injected into oocytes (Wang, 1985). *C. elegans* oocytes incorporated the injected rhodamine-actin into their cortices within 1 minute of microinjection (Fig. 8A,B). This rapid assembly suggests that the cortical actin cytoskeleton in oocytes is dynamic.

In newly fertilised eggs prior to pseudocleavage, little specific rhodamine-actin signal was detectable, possibly due to degradation of the derivatised actin. However, after the completion of pseudocleavage faint cortical foci were visible (Fig. 8C). The foci were present only in the anterior 50% of the cortex, with a strikingly sharp boundary separating this region from the remainder of the cortex. This boundary coincided with the position of the pseudocleavage furrow. The rhodamine-actin foci, unlike the actin aggregates described above, appeared identical in both morphology and size to the foci previously described in fixed embryos (Strome, 1986). This result indicates that the actin foci are present in living embryos. The actin foci remained visible on the anterior cortex during the first cleavage, but the fluorescence intensity rapidly decreased until no specific cortical structure was visible.

DISCUSSION

The actin cytoskeleton of live *C. elegans* embryos was labelled with either fluorescent-phalloidins or rhodamine-actin to investigate how the cortical actin network is reorganised during pseudocleavage. The intention was to reveal how microfilaments participate in cytoplasmic localisation in the early embryo (Hill and Strome, 1988, 1990; Strome and Wood, 1983).

Labelling cortical actin with phalloidin

The injection of phalloidin into oocytes induces the formation of cortically-associated aggregates of F-actin. These aggregates could correspond to the actin foci described in fixed embryos by Strome (1986). These foci are also initially spread out over the entire cortex, but become concentrated anteriorly during pseudocleavage. However, the aggregates seen in phalloidin-injected embryos are much larger and less numerous than these fixed foci. It seems more likely that the aggregates described here form as a result of the phalloidin injection. How are these aggregates formed? Phalloidin binds very tightly to F-actin, and in doing so prevents depolymerisation (Wieland and Faulstich, 1978). Therefore, one possibility is that the aggregates represent bundles of stabilised F-actin.

High concentrations of microinjected phalloidin drive polymerisation of cytoplasmic actin in tissue culture cells (Wehland et al., 1977). The resulting aggregates of stabilised actin remain in the cytoplasm and recruit actin-binding proteins that are usually associated with cytoplasmic actin, such as fimbrin. It is possible that the cortical actin aggregates described in this paper could initially form in this way in the cytoplasm of *C. elegans* oocytes. They might then become associated with the cortex, perhaps by interaction with cortical actin-binding proteins, rather than remaining in the cytoplasm. This explanation is less likely for a number of reasons. Firstly, the intracellular concentration of phalloidin injected into oocytes (approximately 1 μM) does not promote cytoplasmic actin polymerisation in tissue culture cells (Cao and Wang, 1990a; Wang, 1987; Wehland et al., 1977). Secondly, the aggregates seen in oocytes are exclusively cortical: they are never seen in the cytoplasm. Therefore, it is more likely that the cortical actin aggregates are formed from pre-existing cortical actin rather than from phalloidin-induced de novo polymerisation in the cytoplasm.

The movement of cortical foci

The cortical actin aggregates move anteriorly during pseudocleavage, and towards the cytokinetic furrow during cytokin-

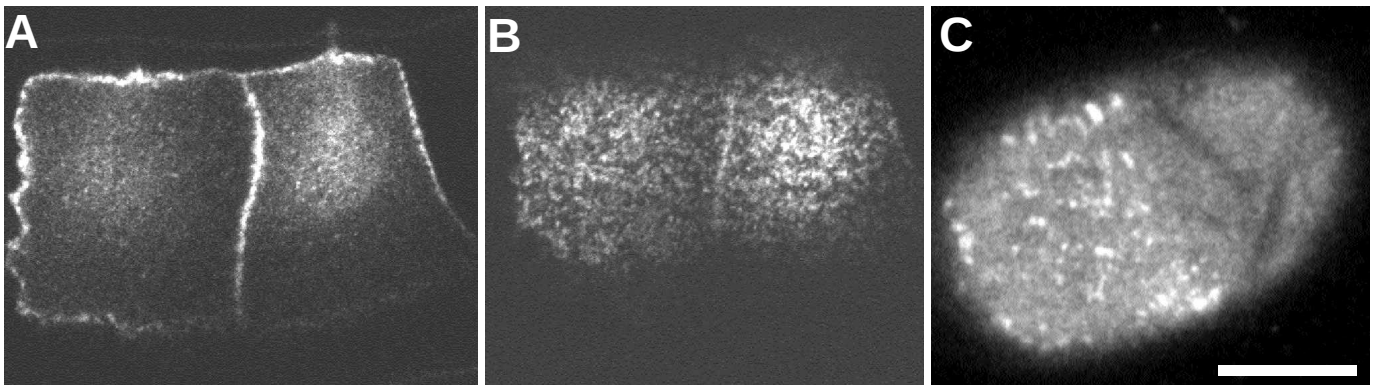

Fig. 8. Injection of rhodamine-actin into oocytes and embryos. (A) Two oocytes viewed in a medial plane. The incorporation of labelled actin into the cortex is visible around the periphery of the oocyte. (B) The same oocytes are viewed in a cortical plane. The cortical labelling appears punctate. (C) Confocal image in a cortical plane of an embryo following pseudocleavage. The rhodamine-actin foci are in the cortex anterior to position of the pseudocleavage furrow, which has now regressed. Anterior is to the left in this image. Bar, 10 μm.

esis. These movements could occur because the actin foci are incorporated within a moving actin network. A flow of actin from posterior to anterior during pseudocleavage was suggested by previous work (Hird and White, 1993; Strome, 1986). Actin flows with similar velocities have been described previously in a number of motile cell types (Heath, 1983; Forscher and Smith, 1988, 1990; Okabe and Hirokawa, 1991; Theriot and Mitchison, 1991, 1992; Wang, 1985).

Cao and Wang (1990b) have also shown that microinjected phalloidin-labelled actin-aggregates can serve as useful markers for the behaviour of the actin cortex in tissue culture cells. Rhodamine-phalloidin labelled filaments were injected into normal rat kidney cells, where they formed aggregates associated with the actin cortex. At cytokinesis they moved along the cortex towards the equatorial region of the cell (the site of the cleavage furrow) (Cao and Wang, 1990b). This movement is believed to indicate that there is a flow of cortical actin (to which the aggregates are attached) from the poles of the cell towards the furrow during cytokinesis. A similar movement is described in this paper during cytokinesis in a *C. elegans* cell. These flows may deposit microfilaments in the equatorial region of dividing cells for incorporation into the contractile ring (Cao and Wang, 1990a,b; White and Borisy, 1983).

The incorporation of labelled G-actin into the oocyte cytoskeleton

The injection of rhodamine-labelled actin reveals that microfilament foci are present in the anterior cortex of live embryos following pseudocleavage. These foci are distinct from the aggregates described above in phalloidin-injected oocytes, both in terms of their size and number. Anteriorly restricted actin foci were observed in the cortex of fixed embryos of the same stage by Strome (1986). These foci were present in embryos fixed by a number of fixation protocols, and were stained with both fluorescent phalloidin and antibodies raised against F-actin (Strome, 1986). Foci of F-actin have also been seen following fixation in mouse embryos (Lehtonen and Badley, 1980), in transformed cells (Carley et al., 1981), in the fungus *Uromyces phaseoli* (Hoch and Staples, 1983), in fission yeast (Marks and Hyams, 1985) and in budding yeast (Kilmartin and Adams, 1984; Mulholland et al., 1994). However, it has been suspected that the foci in *C. elegans* are fixation artefacts (Strome, 1986). The size, number and cortical location of the rhodamine-actin labelled structures described here indicates that the foci exist in living embryos. The anterior concentration of the rhodamine-actin foci supports the idea that endogenous cortical actin-containing structures are asymmetrically distributed following pseudocleavage (Strome, 1986). Although no specific rhodamine-actin containing foci were visible prior to pseudocleavage, it is conceivable that they are simply too dim at this time to be detected.

The role of the actin movement

Cytoplasmic localisation of P granules and cell-fate determinants takes place at the same time as the cortical actin flow, and is dependent on actin microfilaments (Hill and Strome, 1988; Strome and Wood, 1983). How might the cortical actin flow be involved in the segregation of these factors? One idea is that the cortical actin flow drives cytoplasmic streaming, propelling cytoplasmic factors to the posterior of the embryo. This could work in the following way. Cortical actin flows away from the posterior of the embryo; continual actin polymerisation at the posterior would perpetuate this flow. The actin flow would carry material from the posterior and move it anteriorly along the cortex (Hird and White, 1993). Central cytoplasm would continually move towards the posterior to compensate volumetrically for this depletion of material. Cytoplasmic factors (such as P granules) would thus be swept to the posterior of the uncleaved embryo, where they may become anchored and inherited by the posterior daughter cell (Strome, 1989; Strome and Wood, 1983). It has been proposed that the asters of the sperm pronucleus polarise these cortical and cytoplasmic movements by directing the movement of cortical actin away from their vicinity (Hird and White, 1993). This mechanism for generating a cyclic ‘fountain’ flow of cortical and cytoplasmic components is analogous to that proposed to operate in locomoting amoeboid cells (Bray and White, 1988; Spudich, 1989).

The cortical actin aggregates observed following phalloidin injection are translocated to the anterior of the embryo during pseudocleavage. Upon first cleavage, they are therefore inherited exclusively by the anterior daughter cell AB. This segregation of the aggregates reveals that the embryo can

localise factors anteriorly during pseudocleavage. In principle, the cortical actin flow during pseudocleavage could be harnessed to segregate factors necessary for the specification of the AB lineage. This mechanism would require that such factors be associated with the actin cortex. The endogenous cortical actin foci described by Strome (1986) and in this report could be complexes that include such cortically-located determinants.

Cytoplasmic reorganisation in the ascidian embryo bears some similarities to this model for pseudocleavage in *C. elegans*. Shortly after fertilisation of this egg, myoplasm (a subcortical layer with muscle-determining activity) moves along the cortex towards the vegetal pole of the embryo. This is coincident with a vegetally-directed collapse of the cortical actin 'basket' (Sardet et al., 1994; Speksnijder et al., 1990). It has been proposed that the myoplasm moves because it is attached to the actin network. The regression of the actin network creates a contraction wave that forces cytoplasm in the opposite direction. The exact tilt of the axis of myoplasmic segregation is believed to be specified by the site of sperm entry, possibly via a calcium wave which emanates from this point at fertilisation (Speksnijder et al., 1990). A similar series of events takes place during ooplasmic segregation in the embryo of the freshwater oligochaete *Tubifex*. In this embryo, cortical actin and the associated subcortical ooplasm (which contains membranous organelles) segregate to both the animal and vegetal poles (Shimizu, 1984, 1986).

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