Segregation of germ granules in living Caenorhabditis elegans embryos: cell-type-specific mechanisms for cytoplasmic localisation

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SUMMARY

Germ granules are ribonucleoprotein particles that are thought to function in germline specification in invertebrates and possibly in vertebrates. In Caenorhabditis elegans, these structures, termed P granules, are partitioned to the germline P cells during the early embryonic divisions. By injecting a fluorescently labelled anti-P-granule antibody into the C. elegans germ line syncytium, we followed P-granule segregation in live embryos using laser-scanning confocal microscopy. We show that, in early P cells (P0 and P1), P-granule partitioning is achieved primarily by their migration through the cytoplasm towards the site of formation of the germline daughter cell.

A different mechanism appears to operate in later P cells (P2 and P3): P granules associate with the nucleus and move with it toward the site of formation of the germline daughter cell, where they are then deposited. At each division, there is also disassembly or degradation of those P granules that remain in the cytoplasm destined for the somatic daughter cell. Microfilaments, microtubules and the product of the gene mes-1 are required for the normal pattern of P-granule segregation in P2.

Key words: Caenorhabditis elegans, P granules, cytoplasmic localisation, in vivo confocal fluorescence microscopy, germ granule

INTRODUCTION

The eggs of many organisms display asymmetric distributions of determinants. These molecules are differentially partitioned to different daughter cells during the early embryonic cleavages and then specify the fates of the cells that contain them. The mechanisms by which determinants become localised are poorly understood at present. We are investigating localisation mechanisms using Caenorhabditis elegans P granules as a model system.

P granules are cytoplasmic factors that contain poly(A)+ mRNA and are visible both by electron microscopy and by indirect immunofluorescence (Strome and Wood, 1982, 1983; Wolf et al., 1983; Seydoux and Fire, 1994). They are synthesised in the germline of hermaphrodites and males and passed on to offspring via the oocyte. The cell that gives rise to the germline in C. elegans, P0, is formed by a series of four asymmetric, stem-cell-like divisions of the fertilised egg; each division produces a somatic precursor cell and a germline P cell (Fig. 1) (Deppe et al., 1978; Sulston et al., 1983). P0, P1, P2, and P3 partition P granules to the cortical and cytoplasmic domains of the mother cell that are destined for the germline-cell daughter, resulting in inheritance of the P granules by P1. P1 divides once during embryonic development, distributing P granules to its daughters, Z2 and Z3. During postembryonic development, Z2 and Z3 proliferate to give rise to the germline. P granules are associated with the nuclei of all undifferentiated germ cells throughout larval and adult development (Strome and Wood, 1982). Structures that are morphologically similar to P granules are involved in determination of the germline in Drosophila melanogaster (reviewed in Lehmann, 1992); P granules in C. elegans might serve the same function.

By visualising P granules in fixed embryos, it has been shown that their localisation in the fertilised egg occurs during a specific interval in the first cell cycle and is disrupted by the microfilament inhibitor cytochalasin D (Strome and Wood, 1983; Hill and Strome, 1988). In addition, treatment with cytochalasin D during this interval often causes the first division to be asymmetric, producing daughters with altered division axes and cell-cycle timing (Hill and Strome, 1990). Mutations in the maternal-effect par genes (for partitioning-defective) also disrupt P-granule localisation in the fertilised egg, as well as altering the axes of the early cleavages and the fates of blastomeres (Kemphues et al., 1988; Kirby et al., 1990; Morton et al., 1992; Levitan et al., 1994; Cheng et al., 1995). Considered together, these results have led to the proposal that actin microfilaments and the par gene products mediate the localisation of P granules and developmental determinants during a critical interval in the first cell cycle. Furthermore, some mutations that disrupt P-granule segregation also disrupt the localisation of the putative determinant SKN-1 (Bowerman et al., 1993). This also suggests that common mechanisms are used to localise a variety of cytoplasmic factors in C. elegans embryos.
In this paper, we address how P granules are localised by asking the following. (1) Is the localisation of P granules due to granule migration toward the cytoplasm/cortex inherited by the next P cell, or due to stabilisation of granules in this region and destabilisation of granules in the region inherited by the somatic cell? (2) Do all P cells use the same mechanism of P-granule segregation? This seemed especially important to investigate in P2 and P3, which switch the polarity of their segregation axis relative to P0 and P1 (Fig. 1) (Schierenberg, 1987). (3) In mutant embryos that display mispartitioned P granules, which aspects of P-granule localisation are affected?

To answer these questions, we developed a means of directly visualising P granules in live embryos. This was done by fluorescently labelling an anti-P-granule monoclonal antibody and injecting the fluorescent antibody into the germline syncytium of adult hermaphrodites. Embryos produced by injected hermaphrodites contain fluorescent P granules and these were examined by laser scanning confocal microscopy during the early cleavages. Our results show that, in P0 and P1, P-granule localisation is achieved by a combination of polarised migration and localised degradation. P2 and P3 utilise a different strategy to localise P granules; this involves transport of many of the granules attached to the nucleus and also localised cytoplasmic degradation. Embryos mutant for the maternal-effect sterile gene mes-1 (Capowski et al., 1991; Strome et al., 1995) show defects in nuclear behavior in P2, which results in mispartitioning of P granules.

**MATERIALS AND METHODS**

**Strain maintenance**

*C. elegans* was cultured as described in Brenner (1974). The strains used were the wild-type N2 variety Bristol, *mes-1(bn7ts)* and *pgl-1(ct131)*. *mes-1(bn7ts)* was maintained at the permissive temperature of 15°C, while the other strains were maintained at 20°C.

**Preparation of fluorescein-conjugated anti-P-granule antibody**

The anti-P-granule monoclonal antibody OIC1D4 (Strome and Wood, 1983), an IgG, was purified by affinity chromatography on Protein A-Sepharose CL-4B (Pharmacia) according to Hudson and Hay (1980). Purified OIC1D4 was conjugated to fluorescein according to Goding (1976), with the following modifications. Purified OIC1D4 at 2-4 mg/ml was dialysed against 0.05 M sodium phosphate pH 8.0 for 1-2 hours. An appropriate volume of 5(6)-carboxyfluorescein succinimidyl ester (Molecular Probes, Eugene, OR) at 5 mg/ml in DMSO or DMF was added dropwise to the antibody solution, to achieve a fluorochrome to antibody ratio of between 40 and 60 µg fluorochrome.
to 1 mg antibody. The reaction was incubated for 2 hours at room temperature with continuous stirring. Unreacted fluorochrome was separated from fluorochrome-conjugated antibody on Sephadex G-25 (Pharmacia) that had been equilibrated with PBS, pH 7.4 (150 mM NaCl, 3 mM KCl, 0.1 mM KH₂PO₄, 1.5 mM KH₂PO₄). The conjugated antibody, which was easily identified by its color, was collected in 1-2 ml of the void volume of the column.

The antibody conjugate was analysed for the presence of unreacted fluorochrome according to Kreis and Birchmeier (1980). The approximate molar ratio of fluorochrome to protein was calculated as described by The and Feltkamp (1969). A conjugate with an F/P ratio of 7.3 was used in all experiments.

Microinjection
Purified conjugated OIC1D4 was dialysed against injection buffer (5 mM KCl, 0.1 mM KH₂PO₄) overnight at 4°C. Microneedles (Clarke Electromedical GC120 F-15, pulled on a Sutter puller) were backfilled by capillary action with the dialysed conjugate at 1-2 mg/ml. The filled needles were mounted on a MPM-10/ DC3001 piezo-translator micro-manipulator (World Precision Instruments) attached to a Zeiss Axiovert inverted microscope equipped with DIC optics. The germline syncytium of gravid hermaphrodites was then injected using the protocol developed as described in Albertson (1984).

RESULTS
Background information
The aspects of early embryogenesis relevant to this paper are briefly reviewed here and shown in Fig. 1 (Nigon et al., 1960; Hirsh et al., 1976; Albertson, 1984; Kirby et al., 1990; Hird and White, 1993). The fertilised C. elegans embryo is contained within an elongated eggshell. The oocyte and sperm pronuclei form at the future anterior and posterior end, respectively, of the embryo (Fig. 1B), after which a period of cytoplasmic reorganisation takes place. During this period internal cytoplasm streams posteriorly, while cortical cytoplasm streams anteriorly, and the oocyte pronucleus migrates toward the sperm pronucleus. P granules become localised in the posterior half of the embryo during this interval, where they are concentrated around the cortex. These events occur at the same time as a process called pseudocleavage in which a furrow forms and regresses (Fig. 1B). After cytoplasmic streaming ceases, the sperm-contributed centrosomes of the pronuclear complex rotate 90 degrees so that they are aligned along the anteroposterior axis (Fig. 1C). Centrosome alignment along the anteroposterior axis sets the mitotic spindle up along the axis of P-granule segregation and is a feature of each P-cell division (Albertson, 1984; Hyman and White, 1987; Hyman, 1989). The first division is unequal, producing the somatic founder cell AB at the anterior, and the smaller germline cell P₁ at the posterior (Fig. 1D). P₁ inherits all of the P granules.

P granules also become localised to the posterior of P₁, P₁ then divides unequally to produce the somatic daughter EMS on the ventral-anterior side and the germline daughter P₂ at the posterior pole of the embryo (Fig. 1E,F). P₂ inherits all of the P granules.

Unlike P₀ and P₁, P₂ segregates P granules anteriorly, and generates its germline daughter P₃ anteriorly and its larger somatic daughter C posteriorly (Fig. 1A). This reversal in segregation and division polarity is most easily seen in "partial embryos", which have been released from the constraints of the eggshell. In the intact embryo, the constraints of the eggshell and neighbouring blastomers force the P₂ division to occur along the dorsal-ventral axis (Fig. 1G,H). During alignment of the P₂ centrosomes onto this axis, the ventral-most centrosome is pulled via its astral microtubules toward a specific site in the ventral cortex of P₂. This causes a ventrally directed movement of the nucleus-centrosome complex (Fig. 1F,G) (Hyman and White, 1987). P granules are segregated to the ventral side of P₂, where P₂ is born (Fig. 1H,1). P₂ reiterates this polarity-reversed segregation and cleavage pattern, passing the P granules ventrally to the primordial germ cell P₃ (Fig. 1J).

Visualisation of P granules in living embryos
We have investigated the timing and mechanism(s) of P-granule segregation in living embryos. The anti-P-granule mouse monoclonal antibody OIC1D4 (Strome, 1986) was conjugated to fluorescein and injected into the syncitial gonads of adult hermaphrodites. Fluorescent structures that looked identical in size to P granules and displayed the same localisation pattern were seen in the germline of injected hermaphrodites (data not shown) and in their embryos (see below).

In order to prove that the fluorescent structures were indeed P granules, rather than, for example, aggregates of unbound...
antibody, we repeated the injections using hermaphrodites homozygous for the maternal-effect mutation \textit{pgl-1(ct131)}. Fixed P granules in such hermaphrodites and their embryos lack OIC1D4 immunoreactivity, but do stain with other anti-P-granule antibodies (Wood et al., 1984; I. Kawasaki and S.S., unpublished data). When we injected fluorescent OIC1D4 antibody into \textit{pgl-1(ct131)} mothers, no granular staining was visible in their embryos (data not shown). This shows that the fluorescent granules in wild-type embryos result from the in vivo binding of the injected OIC1D4 antibody to P granules.

\section*{Localisation of P granules in P\textsubscript{0}}
We followed fluorescently tagged P granules in 22 one-cell embryos (Fig. 2). Prior to pseudocleavage, the P granules were dispersed throughout the cytoplasm, as described previously (Strome and Wood, 1982, 1983). During pseudocleavage, almost all the P granules in the cytoplasm moved toward the posterior pole, at an average rate of 3.97±0.8 \textmu m/min ($n=19$ granules; 6 embryos) (Fig. 2E). Similar rates were observed when labelled embryos were observed using a silicon-intensified camera and continuous illumination (data not shown). Upon reaching the posterior, they often appeared to move anteriorly for some distance along the cortex. These movements occurred coincident with the posteriorly directed streaming of central cytoplasm and anteriorly directed streaming of cortical material described previously (Nigon et al., 1960; Hird and White, 1993).

As pseudocleavage and pronuclear migration ended, in 16/22 cases all of the P granules were associated with the cortex of the posterior half of the embryo and were inherited by the posterior daughter cell P\textsubscript{1}. In the remaining 6/22 cases, a few P granules at the extreme anterior of the pre-pseudocleavage embryo did not move posteriorly during pseudocleavage, but instead remained anterior (Fig. 2C). There is little cytoplasmic streaming visible in this region of the embryo during pseudocleavage (Hird and White, 1993). The anterior P granules progressively disappeared during mitosis, so that none were detectable in the anterior cell AB at the time of its birth (Fig. 2D). We suggest that this depletion of anterior P granules resulted from disassembly or degradation of P granules in the anterior, non-germline cytoplasm of the embryo. However, since the P granules at this stage were rather small and poorly visible (compared to later stages; see below), we were unable to follow individual granules without photobleaching their fluorescence significantly. Therefore, we cannot rule out the possibility that the few granules that remained after pseudocleavage dropped out of the plane of focus and then moved posteriorly between the end of the normal localisation period and the commencement of cytokinesis.

We conclude that the localisation of P granules in the one-cell embryo is achieved primarily by their movement through the cytoplasm toward the posterior cortex. There may also be disassembly or degradation of any stray P granules that remain in the anterior of the embryo prior to division. The localisation of P granules in living embryos, as described here, occurs during the same interval as defined by previous immunofluorescence studies of fixed embryos (Strome and Wood, 1983; Rose et al., 1995).

\section*{Localisation in P\textsubscript{1}}
We monitored P-granule segregation in 24 two-cell embryos (Fig. 3). Following the first division, most of the tagged P granules were located around the periphery of P\textsubscript{1}, except at the boundary with its sister cell AB (Fig. 3A). Therefore, the majority of P granules were already localised to the cortex, many to the cortex destined for P\textsubscript{2}.
Two patterns of P-granule movement were evident in P1 immediately after its birth: the few cytoplasmic P granules moved posteriorly through the cytoplasm and eventually became associated with the cortex, and P granules located close to the cortex often moved anteriorly along the cortex. The eventual result was that all the P granules became located close to the cortex of P1, except for the region of contact with AB (Fig. 3B). The P-granule movements in P1 occurred at the same time as yolk granules flow posteriorly through the cytoplasm and anteriorly along the cortex (Hird and White, 1993). The pattern of P-granule movement in P1 was similar to that in P0, although the extent of segregation was not as dramatic in P1 as in P0.

P1 becomes somewhat extended along the anteroposterior axis as a result of skewing of the anaphase spindle in the dividing AB cell (Fig. 3c). At the same time, P granules progressively disappeared from the most anterior region of P1 (Fig. 3C). Again, this may have resulted from disassembly or degradation of the P granules in this vicinity. The extension of the P1 spindle along the anteroposterior axis appeared to push this depleted region further anteriorly and, as a result, the P granules eventually remained only around the posterior region of the cell, which is inherited by P2 at cytokinesis (Fig. 3D).

In summary, P-granule localisation in P1 appears superficially similar to this process in P0. However, at the time of P1’s birth, most of the P granules are already localised to the site of formation of P2. In P1, there is a limited amount of P-granule movement visible in the cytoplasm and cortex, and an apparent depletion of P granules in the anteriormost region of the cell.

**Localisation in P2 and P3**

We followed tagged P granules in 20 four-cell embryos. Initially, the P granules were present throughout the cytoplasm of P2. However, they assumed a striking perinuclear location immediately before the rotation of the P2 centrosomes. As a result, confocal sections through the centre of the nucleus revealed a bright ring of P granules encircling it (Fig. 4A). This is also visible when embryos at this stage are fixed and then stained with OIC1D4 (Fig. 4E). Only P granules that were in the immediate vicinity of the nucleus became associated with it in this way; those distant from the nucleus remained in the cytoplasm.

As one of the P2 centrosomes migrated onto the division axis, the nucleus moved toward the ventral side of P2 next to EMS (Fig. 4B). This brought the perinuclear P granules into the cytoplasm that would be inherited by P3. P granules were then deposited near the ventral pole of the P granules at nuclear membrane breakdown (NMB) (Fig. 4C). This process of perinuclear association of P granules, followed by ventrally directed nuclear migration, was repeated in P3, partitioning the majority of P granules to P4 (12 embryos followed; data not shown). In P4, P granules remained associated with the nucleus and were eventually distributed to both its daughters, the primordial germ cells Z2 and Z3, later in embryogenesis (Strome and Wood, 1982).

Disassembly/degradation of P granules also seems to take place in both P2 and P3. Immediately before NMB in P2 and P3, the more dorsal side of the nucleus became depleted of P granules (Fig. 4B). This could be due to disassembly or degradation of granules on the dorsal side of the nucleus, disassociation of granules from the dorsal side of the nucleus, or movement of perinuclear P granules to the ventral side of the nucleus. Depletion of P granules from the dorsal side of the P2 nucleus was accompanied in 16/20 cases by the progressive loss of the non-perinuclear P granules that remained in the more dorsal cytoplasm destined for the somatic precursor cell C. In the remaining 4/20 cases, a few P granules remained in the dorsal cytoplasm inherited by C. Similarly, in P3, stray P granules were sometimes seen in the cytoplasm inherited by D (3/12 cases).

To summarise, it appears that a different mechanism is used...
mutations in the maternal-effect gene *mes-1* develop into sterile adults (Capowski et al., 1991). This is due to transformation of the primordial germ cell P4 into a muscle precursor, like its somatic sister cell D (Strome et al., 1995). This transformation in cell fate is preceded in P2 and P3 by defects in both P-granule segregation and in the orientation of division axes. P2 (in some embryos) and P3 (in most embryos) mispartition P granules to both their P-cell daughter and their somatic daughter cell (Strome et al., 1995). We examined the basis of this segregation defect by monitoring tagged P granules in embryos from *mes-1(bn7ts)* mothers raised at the restrictive temperature (referred to here as Mes-1 embryos).

In Mes-1 embryos, P granules in P2 assumed a perinuclear localisation, as in wild-type embryos (Fig. 5A). However, centrosome alignment and the concomitant ventral migration of the nucleus did not take place. As a result, the nucleus remained in the centre of the cell. Furthermore, there was no depletion of P granules from the dorsal side of the nucleus prior to NMB. Instead, either the posterior face of the nucleus became depleted of P granules (Fig. 5B) or, less frequently, neither face became depleted. As NMB was initiated and the spindle formed, the P granules were released to the anterior (13/18 cases) (Fig. 5C) or both sides (5/18 cases) of the spindle, instead of at one pole of the spindle. Although there was no centrosome rotation, neighbouring dividing cells physically constrained the P2 cell in such a way that the spindle was established along the dorsal-ventral axis, as in wild-type embryos. The cytokinetic furrow that bisected the spindle passed through the mass of P granules, distributing them to both P3 and C (Fig. 5D). P granules often assumed a perinuclear localisation in both P3 and C (Fig. 5E), and were distributed in turn to their daughters, namely P4/D and Ca/Cp, where they again became perinuclear, forming four rings of P-granule staining (Fig. 5F).

To conclude, Mes-1 embryos fail to partition P granules to the ventral region of P2 due to the absence of nuclear migration and the subsequent failure of the dorsal face of the nucleus to become depleted of P granules. This results in the P granules being deposited on one or both sides of the spindle, instead of at one spindle pole after NMB.

**Inhibitor studies**

The posterior segregation of P granules in P0 was previously found to depend on the microfilament cytoskeleton, but not on the microtubule cytoskeleton (Strome and Wood, 1983; Hill and Strome, 1988). Our analysis of P granules in living embryos suggests that, in P2 and P3, centrosome alignment and the accompanying migration of the nucleus play a major role.
in P-granule segregation. Centrosome alignment in the P cells is dependent on both microfilament and microtubule function (Hyman and White, 1987). Therefore, we investigated whether P-granule segregation in P2 requires microfilament function, microtubule function, or both.

Microfilament inhibition
Four-cell embryos were laser-permeabilised in a solution of 10 μg/ml of the microfilament inhibitor cytochalasin D. This concentration blocks P-granule segregation in one-cell embryos and also blocks centrosome rotation and cytokinesis (Strome and Wood, 1983; Hyman and White, 1987). In all of the 15 cases followed, P granules became perinuclear, as in untreated embryos (Fig. 6A). Due to the inhibition of centrosome alignment, the nucleus did not move ventrally, but remained central, as in Mes-1 embryos. The P granules reproducibly became depleted from the posterior side of the nucleus prior to NMB (Fig. 6B), rather than from the dorsal side as in untreated embryos. Again, this is reminiscent of the situation described above in Mes-1 embryos. As NMB occurred and the spindle formed along the dorsal-ventral axis (again presumably due to constraints from the eggshell and neighbouring blastomeres), the P granules were deposited along the anterior periphery of P2 and thus along the side of the spindle (Fig. 6C). Other regions of the cell were devoid of P granules; as in untreated embryos, this may result at least in part from disassembly or degradation of the granules. Although cytokinesis did not occur in the presence of cytochalasin D, it was nevertheless clear that the P granules had been mislocalised relative to the spindle. Therefore, microfilaments are required for proper P-granule localisation in P2. Moreover, cytochalasin D appears to phenocopy the P-granule localisation defect seen in Mes-1 embryos at this stage.

Microtubule inhibition
To inhibit microtubule function, 15 four-cell stage embryos were permeabilised in a solution of 10 μg/ml of nocodazole, at 10 μg/ml. This concentration is sufficient to block P2 centrosome rotation and attenuate the mitotic spindle; no long astral microtubules are visible, but short microtubules may remain associated with the centrosomes (Strome and Wood, 1983; Hyman and White, 1987). As in untreated embryos, P granules formed a perinuclear ring (Fig. 7A), but there was no ventral movement of the nucleus due to the inhibition of centrosome rotation. P granules then became depleted from the dorsal side of the nucleus prior to NMB (Fig. 7B). The depletion often appeared more pronounced than in untreated embryos, and seemed to involve the progressive reduction in size (over a two-minute interval) of individual granules on the dorsal side of the nucleus. Granules in the dorsal cytoplasm also shrank gradually and eventually disappeared. The observation that the whole dorsal population of P granules progressively and simultaneously disappeared from the same plane of focus strongly suggests that a disassembly/degradation mechanism is operating in this cell.

As NMB was initiated and the attenuated spindle formed in nocodazole-treated P2 cells, there was a general flow of cytoplasmic material toward the ventral side of the cell. Similar
cytoplasmic streaming is seen in other embryonic blastomeres treated with nocodazole, and is thought to result from the polarised activation of the cytoskeletal machinery that normally brings about cytokinesis (Hird and White, 1993). The P granules present in the cytoplasm at NMB appeared to be swept by this flow toward the ventral side of P2, and therefore were artificially localised to the cytoplasm that would be inherited by P3 in a normal cleavage (Fig. 7C). This streaming was clearly an effect of drug treatment and, therefore, not a normal component of the localisation mechanism in untreated embryos. However, the observation that the depletion of P granules from the dorsal face of the P2 nucleus proceeded normally in nocodazole-treated embryos shows that this process does not require intact microtubules.

To summarise, the ventral movement of the nucleus and associated shell of P granules in P2 requires both microfilaments and microtubules, whilst the subsequent depletion of P granules specifically from the dorsal face of the nucleus requires microfilaments but not microtubules.

**DISCUSSION**

We have investigated the mechanism of segregation of the germline-specific P granules in the early *C. elegans* embryo. These factors have been tagged in living embryos by injecting a fluorescently labelled anti-P-granule monoclonal antibody into the germline of adult hermaphrodites. The fluorescent granules are segregated to P cells during the early cleavages, and are absent in a mutant in which the P granules are not recognised by the monoclonal antibody. This confirms that the tagged structures are indeed P granules, and that binding of the antibody does not disrupt their segregation. Our results show that P-granule localisation is achieved primarily through the directed movement of P granules toward the site of formation of the germline daughter cell. However, the mechanism of movement in P0 and P1 is different from that in P2 and P3. We have also presented evidence that disassembly or degradation of P granules takes place in the cortical and cytoplasmic domains that will be inherited by the somatic daughter. These results illustrate how determinants can be localised to specific cells.

**Cell-type-specific mechanisms for localising P granules**

One surprising result of this study is that different P cells use...
different mechanisms to localise P granules to the site of formation of the next P cell. In this section, we compare these mechanisms, and propose why they may differ.

In the one-cell embryo, localisation results primarily from P granules in the cytoplasm migrating toward the posterior cortex at approximately 4 μm/min. At the same time as the P granules move, general cytoplasmic material streams posteriorly at approximately the same rate (4-7 μm/min) (Nigon et al., 1960; Hird and White, 1993). Both streaming and P-granule localisation are disrupted by microfilament inhibitors (Strome and Wood, 1983; Hird and White, 1993). These observations suggest that P granules are swept posteriorly by the cytoplasmic streaming in the fertilised egg. The trapping of P granules at the posterior in this model could result from the presence of specific P granule attachment sites in the posterior cortex. Alternatively, the P granules may move posteriorly along an array of polarised cytoplasmic actin cables. However, we do not believe this to be the case as there is no evidence for such an array in the embryo at this time.

P granules do not display dramatic migration in P1. This may be because the vast majority of P granules are already located around the periphery of the cell when P1 is born and many are therefore located in the region of the cell that will be inherited by P2. Further restriction of P granules to the cortex destined for P2 is accomplished primarily by depletion of P granules from the regions of the cortex destined for EMS. This depletion takes place just prior to mitosis in P1 and may result from disassembly/degradation of P granules (see next section).

P-granule localisation in P2 and P3 is achieved by the apparent attachment of the P granules to the nucleus, followed by movement of the nucleus toward the ventral side of P2; this movement is a result of centrosome rotation. As far as we are aware, this is a novel mechanism for the localisation of cytoplasmic factors during embryogenesis. Why do P2 and P3 use a different P-granule localisation mechanism than P0 and P1? P0 and P1 segregate P granules posteriorly, while P2 and P3 segregate them anteriorly in partial embryos, which corresponds to the ventral side of intact embryos. We propose that the nuclear migration mechanism may have been adopted to bring about this reversal of P-granule segregation polarity. This hypothesis can be tested by following P-granule segregation in other nematode species that do not undergo a reversal of polarity in P2 and P3, such as Cephalobus (Skiba and Schierenberg, 1992). This experiment was not included in the present study, because the monoclonal antibody used here does not cross-react with Cephalobus P granules (B. Goldstein, personal communication).

Evidence for localised disassembly or degradation of P granules

Each P-cell division is immediately preceded by the disappearance of P granules that remain in the cytoplasm or cortex destined for the somatic daughter. In P2 and P3, there is also a depletion of P granules from the dorsal face (the ‘somatic’ side) of the nucleus. We believe that in all cases this results from the progressive disassembly or degradation of individual P granules. The proposal that P granules are disassembled or degraded in somatic cytoplasm is not without precedent. In fixed embryos, P granules are sometimes seen in C and D, the somatic sisters of P2 and P3, respectively (Strome and Wood, 1982). However, P granules are not seen in the daughters of C and D (Strome and Wood, 1982). Analysis of fixed embryos also revealed that stray P granules are occasionally seen in the anterior of late one-cell embryos (Rose et al., 1995). However, they are not observed in the somatic daughter cell, AB (Strome and Wood, 1982).

These observations suggest that, prior to division, the cytoplasm of each P cell is polarised with respect to its ability to maintain P granules. Specifically, there could be a gradient of P-granule-stabilising activity, or an oppositely oriented gradient of destabilising activity, along the segregation axis of each P cell. This would allow properly localised P granules to be maintained and would cause stray P granules in future somatic cytoplasm to be destroyed prior to division. Interestingly, P granules are not visible until the four-cell stage in par-1 (Guo and Kemphues, 1995), par-4 and some par-3 mutant embryos (S. N. H. and K. J. Kemphues, unpublished observations) and then are visible in multiple cells. Thus, these par gene products may be involved in the normal regulation or localisation of the stabilising/destabilising activity. PAR-1 (a serine/threonine kinase) is localised to the periphery of each P cell in the region where P granules become localised (Guo and Kemphues, 1995), while PAR-3 (a novel protein) shows a complementary distribution (Etemad-Moghadam et al., 1995). Thus, these two PAR proteins are located in appropriate positions to localise or regulate a P-granule stabilising or destabilising activity.

Cellular asymmetry in P2

Microfilaments are required for the one-cell embryo to generate asymmetries, both in the distribution of P granules and in the placement of the mitotic spindle (Strome and Wood, 1983). In contrast, microfilaments are not required for P2 to display asymmetry, but are required to orient the segregation axis correctly: in cytochalasin-treated embryos, P granules in P2 become aberrantly localised to the anterior region of the cell, apparently as a consequence of depletion of granules from the posterior side of the nucleus and the posterior cytoplasm. Thus, microfilaments are needed for the normal pattern of ventral localisation and dorsal depletion of P granules. Microfilaments are also required for the rotation of the P2 centrosomes onto the segregation axis (although in their absence the spindle is constrained in such a way that it forms approximately along the normal axis) (Hyman and White, 1987). The microfilament-dependent rotation of the P2 centrosomes may be responsible for shifting the P-granule segregation axis to the correct orientation.

Insights into mes-1 defects

Our results show that the mes-1 product is required to bring about centrosome rotation exclusively in the P cells that display polarity reversal, namely P2 and P3. Furthermore, the striking similarity between the effects of mutations in mes-1 and the effects of cytochalasin treatment on P2 suggests that the mes-1 product may interact with the microfilament cytoskeleton. As discussed above, the failure in centrosome rotation could account for the altered pattern of P-granule segregation in Mes-1 embryos (Strome et al., 1995) and should also lead to an altered pattern of division. In fact, Mes-1 embryos that have been freed from the constraints of the eggshell display defects in the pattern of the P2 division (E. Schierenberg and S.S., unpublished results). However, in intact...
Mes-1 embryos, constraints from the eggshell and surrounding cells cause the P2 mitotic spindle to assume an approximately normal dorsal-ventral orientation in the absence of centrosome rotation, just as in cytochalasin-treated P2. This results in the incorrectly localised P granules being distributed to both daughter cells.

It is worth noting that, in living Mes-1 embryos, P-granule mis-segregation was observed in all 18 P2 divisions that were monitored, whereas only 29% of fixed Mes-1 embryos contained mis-segregated P granules in C. This difference may reflect a difference in the timing of observation, living embryos being monitored through the cell cycle and division of P2 and fixed embryos being scored at later stages. This observation may indicate that in most Mes-1 embryos, C still retains the ability to disassemble/degrade P granules.

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