J. Embryol. exp. Morph. **97 Supplement**, 15–29 (1986)
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Asymmetric movements of cytoplasmic components in *Caenorhabditis elegans* zygotes

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INTRODUCTION

One of the central problems facing developmental biologists is understanding how the unicellular zygote develops into a multicellular embryo composed of different tissue types. It is now clear that differentiated cell types differ because they express different sets of genes. However, how cells become instructed to express different sets of genes remains a mystery.

One popular model for how cell fates are determined invokes the existence and asymmetric distribution of cytoplasmic 'determinants' of cell fates (for reviews see Wilson, 1925; Davidson, 1976). According to this model, the developmental programmes of embryonic blastomeres are specified by internal factors that are differentially segregated to different blastomeres during the early cleavages of the zygote. Alternatively, cells may be instructed by extrinsic signals, in which case the positions of cells in the embryo and cell–cell interactions would be important. Observation and manipulation of embryos that show 'mosaic' development provide indirect support for the cell determinant theory. Individual blastomeres isolated from these embryos divide and express the differentiated phenotypes that they would have expressed in the intact embryo (Wilson, 1904; Whittaker, Ortolani & Farinella-Ferruzza, 1977). Conversely removal of individual blastomeres from these embryos leads to juveniles or adults missing the tissues derived from the removed blastomeres (Conklin, 1905). The autonomy of blastomere development suggests that, at least in some embryos, instructions for development are internal and that external cues are not required.

Despite many suggestive experiments like those described above, a determinant of cell fate has yet to be identified. Perhaps the strongest candidates are germ granules. These electron-dense structures are found uniquely in germ cells, in almost every metazoan that has been examined (for reviews see Beams & Kessel, 1974; Eddy, 1975). The experiments of Illmensee & Mahowald (1974) demonstrated that the cytoplasm containing these germ or polar granules is determinative for the germline in *Drosophila*, although the structures themselves have not been proven to be the determinants. The questions that need to be answered are: (1) are germ granules required for germ cell development?; (2) do determinants of

Key words: *Caenorhabditis elegans*, zygotes, asymmetric movement, cell fates, cytoskeleton, cleavage, microfilament.

somatic cell fates exist?; (3) if such determinants exist, what are they composed of?; and (4) how are cytoplasmic components differentially partitioned to different embryonic blastomeres? Basically, how are cell differences generated?

AN APPROACH TO STUDYING HOW CELL DIFFERENCES ARE GENERATED AND CELL FATES SPECIFIED

My laboratory's approach to investigating how cell differences are generated and how cells are instructed to follow different developmental pathways is (1) to generate immunologic probes to molecules present in the zygote, (2) to use these probes to characterize the distribution and any rearrangement of components that occurs during early development, and (3) to analyse the effects that mutations and various manipulations have on component distributions and rearrangements. We are especially interested in components that are asymmetrically distributed, components that are lineage-specific, and components of the cytoskeleton. Asymmetrically segregated components may lead to differences in cell behaviour and development. Lineage-specific molecules are candidates for determinants of cell fates. The cytoskeleton may be the mediator of movement and reorganization of cell components.

The organism we are studying is the free-living soil nematode *Caenorhabditis elegans*. The *C. elegans* embryo is well suited for studies on how different cells are generated. The embryo is visibly asymmetric immediately after fertilization, and early development appears to be 'mosaic'. The early embryo undergoes an invariant pattern of asymmetric cleavages, giving rise to cells that differ in size, cell cycle timing, cleavage pattern and, most importantly, developmental fate (Nigon, Guerrier & Minin, 1960; Hirsh, Oppenheim & Klass, 1976; Deppe *et al.* 1978; Sulston, Schierenberg, White & Thomson, 1983). The results of cleavage-inhibition experiments (Laufer, Bazzicalupo & Wood, 1980; Cowan & McIntosh, 1985) suggest that the potentials for gut, muscle and hypodermal development are specified by intracellular cues that are progressively segregated to the appropriate precursor cells during the early cleavages. Furthermore, cell and cytoplasm fusion experiments (Schierenberg, 1985) provide evidence that the intracellular cues reside in the cytoplasm, rather than the nucleus. Therefore, the *C. elegans* embryo provides an opportunity to investigate the molecular basis for asymmetry and to carry out a search for cytoplasmic determinants.

The aim of this review is to assemble microscopic and immunologic analyses of *C. elegans* zygote development and present an overview of the movements and rearrangements of zygotic components that accompany the generation of different blastomeres. Included in this overview is a description of the asymmetric segregation of germ granules (termed P granules in *C. elegans*). It is not known whether these granules are determinative for the germline in worms, but they provide a graphic example of how cytoplasmic determinants might be partitioned to specific cells. After a description of intracellular movements in normal zygotes has been presented, a description of the events in mutant and manipulated embryos will be

given to illustrate the roles that different elements of the cytoskeleton appear to play in zygotic rearrangements.

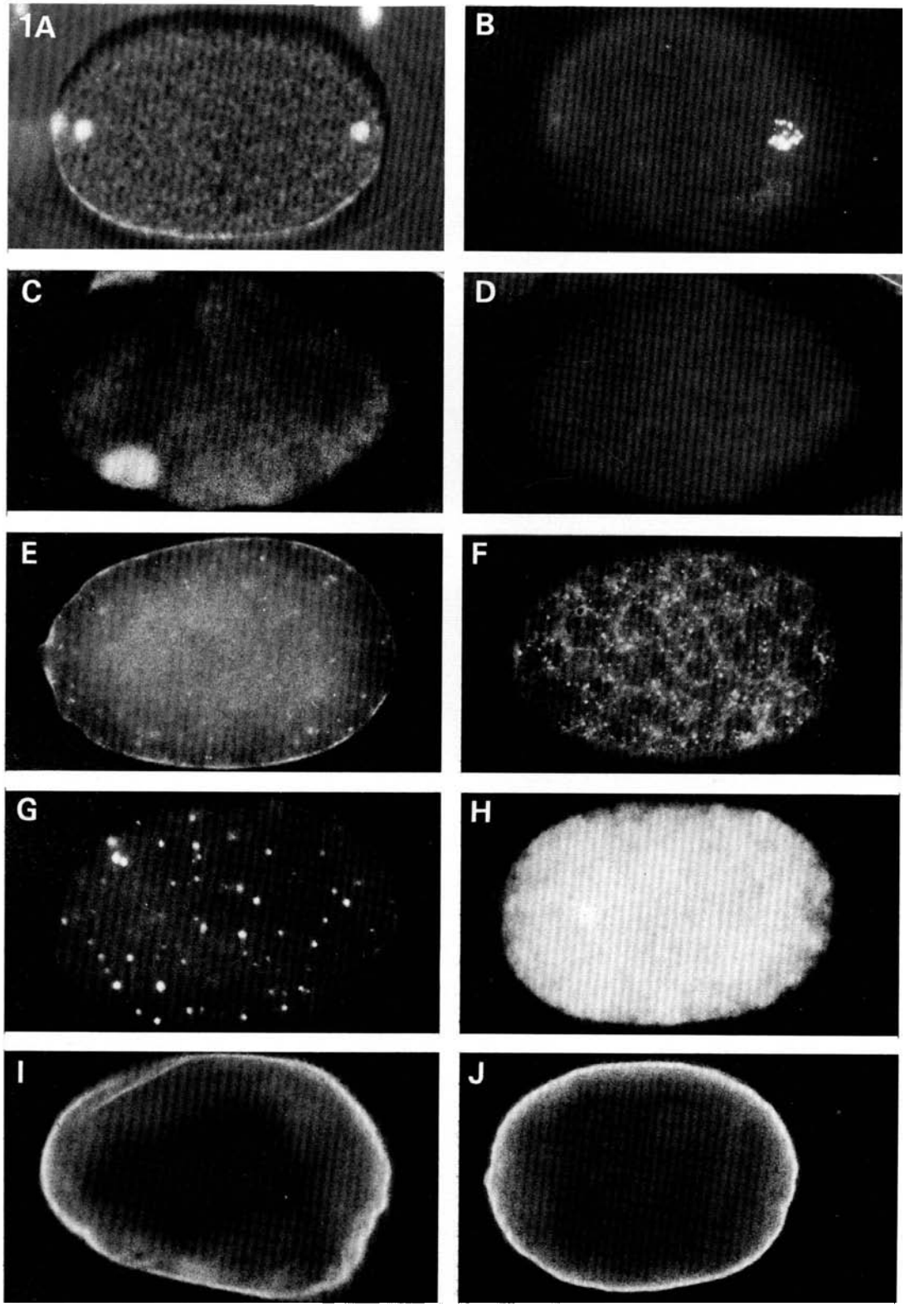
THE NEWLY FERTILIZED ZYGOTE

The oblong *C. elegans* zygote is asymmetric immediately after fertilization. The end where the sperm entered is posterior, and the end where the egg nucleus completes meiosis and extrudes two polar bodies is anterior (Fig. 1A). It is not known whether the oocyte becomes polarized prior to fertilization or whether the site of sperm entry specifies posterior. The uniform distribution of cell surface and cytoskeletal components that have been monitored in the oocyte (Albertson, 1984; Strome, 1986*a*,*b*) suggest that the oocyte is not polarized prior to fertilization.

The sperm, because of its small size (5–6 μm in diameter), does not make a substantial contribution to the overall cytoplasmic volume of the zygote (approximately 40×60 μm; Hirsh *et al.* 1976). *C. elegans* sperm contain almost no actin or tubulin (Nelson, Roberts & Ward, 1982; Ward, 1986). What they do contain and contribute to the zygote are a haploid nucleus, a microtubule-organizing centre (MTOC) and an extensive system of membranes (Ward & Carrel, 1979). These components can be visualized by fluorescence microscopy near the site of sperm entry (Fig. 1A,B). The sperm and egg nuclei are visualized by staining the DNA with the DNA intercalator DAPI (Fig. 1A). MTOCs are visualized using a rabbit serum that stains centrosomes, the clouds of amorphous material that surround centrioles (Fig. 2D). The sperm membrane is seen using monoclonal antibodies that stain the sperm surface and intracellular membrane organelles (Fig. 1B; Strome, 1986*a*).

The existence of a variety of maternal lethal mutations suggests that, as in many other organisms, the *C. elegans* oocyte provides the zygote with many of the components required for early embryogenesis (Wood *et al.* 1980; Schierenberg, Miwa & von Ehrenstein, 1980; Kemphues, Wolf, Wood & Hirsh, 1986). Molecular analysis confirms that the oocyte provides microtubules (MTs), microfilaments (MFs), myosin, yolk proteins, P granules, mitochondria and surface components, in addition to the egg nucleus (Albertson, 1984; Strome & Wood, 1982, 1983; Strome, 1986*a*,*b*; Wolf, Priess & Hirsh, 1983). As shown in Fig. 1, MTs, yolk proteins, P granules, and surface molecules can be visualized using monoclonal antibodies directed against each component (Strome & Wood, 1983; Strome, 1986*a*). MFs are seen using the F-actin-specific probe rhodamine-phalloidin (R-ph; Wieland & Faulstich, 1978; Wulf *et al.* 1979) and anti-actin antibodies (Strome, 1986*b*). As described below and illustrated in Fig. 1, the oocyte-contributed components described above are homogeneously distributed in the newly fertilized zygote.

MTs appear as a random array of fibres throughout the zygote (Albertson, 1984). During the completion of meiosis, the meiotic spindle at the anterior end of the zygote is brightly stained by anti-MT antibodies (Fig. 1C). The meiotic spindle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lacks astral arrays of MTs, and centrioles have not been seen at the spindle poles (Albertson, 1984).

Anti-centrosome antibodies do not stain the meiotic spindle (Fig. 1D), in contrast to the results seen in mouse zygotes where anti-centrosome antibodies stain multiple acentriolar bodies at each pole of the meiotic spindle and throughout the cytoplasm (Maro, Howlett & Johnson, 1986; Schatten *et al.* 1986). In *C. elegans* zygotes anti-centrosome antibodies only stain the material surrounding the centrioles that come into the zygote *via* the sperm. These centrosomes are not yet detectable in newly fertilized zygotes (Fig. 1D) but, as described below, the centrosomes become visible and grow in size during mitosis. These paternally derived MTOCs nucleate the MTs of the first mitotic spindle, as in sea-urchin embryos (Schatten *et al.* 1986).

Rhodamine-phalloidin and anti-actin antibodies stain an even cortical array of fine fibres and dots or foci that are located among the fibres and somewhat deeper in the cytoplasm as well (Fig. 1E,F; Strome, 1986*a*,*b*). There is no concentration of MFs at the site of sperm entry or the site of polar body extrusion, again in contrast to the results seen in mouse zygotes (Maro, Johnson, Pickering & Flach, 1984).

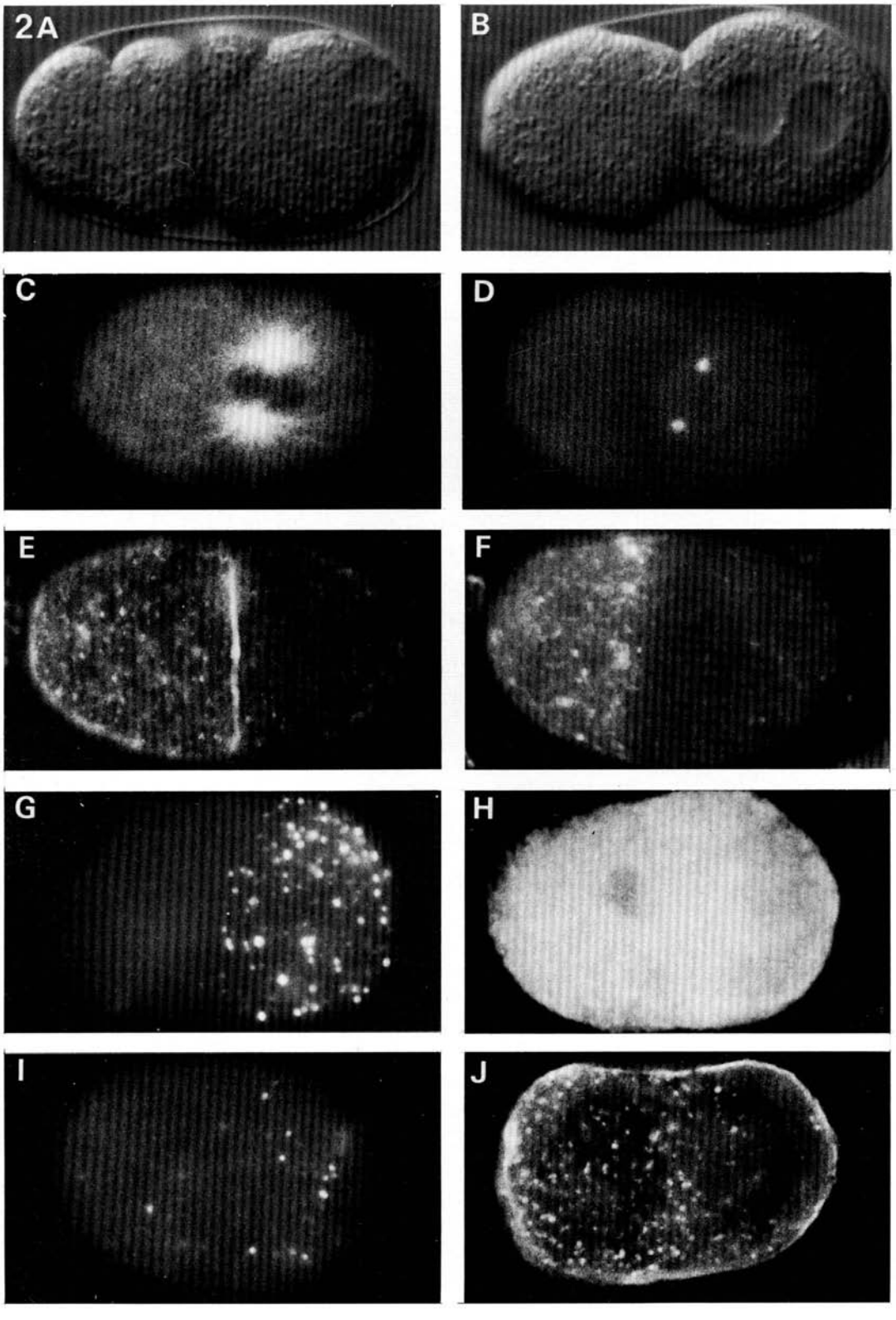
In a newly fertilized zygote, P granules are small (0·5–1 μm), numerous and are distributed apparently randomly throughout the cytoplasm (Fig. 1G; Strome & Wood, 1982, 1983).

The zygote is densely packed with yolk granules that are evenly distributed throughout the cytoplasm (Fig. 1H).

Sperm-surface-specific antibodies stain components that form a tight cluster around the sperm nucleus in the newly fertilized zygote (Fig. 1B), consistent with the observation (Ward & Carrel, 1979) that the sperm is engulfed by the oocyte at fertilization.

Several antibodies directed against oocyte-surface-specific components stain the surface of newly fertilized zygotes in an even pattern (Fig. 1J). These antigens start disappearing from the zygote surface soon after fertilization (Strome, 1986*a*).

Fig. 1. The newly fertilized zygote. Embryos were cut out of adult hermaphrodite worms, fixed and stained with antibodies and DAPI, as described in detail in Strome & Wood (1983) and Strome (1986*a*,*b*). Antibody staining was visualized by indirect immunofluorescence microscopy. The panels illustrate different zygotes at approximately the same stage of development (completion of meiosis) stained with different antibodies. Embryos measure approximately 40–60 μm. All embryos are oriented anterior-left, posterior-right. (A) Nomarski image of a fixed zygote with DAPI-stained DNA. (B) Immunofluorescence image of a zygote stained with monoclonal antibodies directed against sperm membrane components. (C,D) Immunofluorescence images of a zygote stained with monoclonal antibodies against tubulin (C) and rabbit antiserum against centrosomes (D). (E,F) Fluorescence images showing staining of microfilaments by rhodamine-phalloidin; central focal plane (E) and top surface (F). (G) Immunofluorescence image of a zygote stained with monoclonal antibodies against P granules. (H) Immunofluorescence image showing staining with monoclonal anti-yolk antibodies. (I) Immunofluorescence image of staining of the vitelline membrane/eggshell by a monoclonal antibody directed against oocyte surface components. (J) Immunofluorescence image showing uniform staining of the zygote surface by a different monoclonal antibody against oocyte surface components.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Their appearance on the surface of newly forming oocytes suggests that they may be involved in oocyte maturation or fertilization, while their modes of disappearance from the surface following fertilization may indicate zygotic functions. One antigen disappears from the zygote surface and seems to be transiently incorporated into the vitelline membrane or eggshell (Fig. 1I). Another surface antigen (Fig. 1J) is cleared from the surface by internalization, as described in the next section.

Although the anterior–posterior (A–P) axis seems to have been established at least by the time of fertilization, the only asymmetries seen at the molecular level in the newly fertilized zygote are the posterior location of sperm components and the anterior location of the meiotic spindle. However, once the pronuclei have formed, a series of dramatic and directed movements of many zygotic components occurs. These movements appear to be guided by mechanisms that sense the A–P axis, creating an asymmetric zygote that will give rise to different daughter cells.

DIRECTED MOVEMENTS OF CELL COMPONENTS AND THE GENERATION OF ASYMMETRY

When meiosis is complete, the haploid nuclei at the ends of the zygote expand into *bona fide* pronuclei. After this, the anterior membrane of the zygote begins to contract (Fig. 2A), and a pseudocleavage furrow is formed (Fig. 2B). Concomitant with pseudocleavage, the egg pronucleus begins its migration posterior, moving slowly until it reaches the centre of the zygote and then quickly migrating toward the sperm pronucleus. During egg pronuclear migration, the sperm pronucleus moves very slowly away from the posterior membrane. Meeting of the pronuclei occurs in the posterior half of the zygote (Fig. 2B). By this time the pseudocleavage furrow is being resorbed. During this period of pseudocleavage and pronuclear migration, some of the intracellular components that were described in the previous section undergo dramatic movements and reorganization.

The MTOCs on each side of the sperm pronucleus nucleate the MTs that will form the first mitotic spindle (Albertson, 1984). Elongation of these MTs pushes the growing spindle toward the centre of the zygote (Fig. 2C), probably explaining the movement of the sperm pronucleus towards the centre. During this time the centrosomes enlarge, as judged by antibody staining (Fig. 2D). Once in the centre

Fig. 2. The generation of zygotic asymmetry. All panels show zygotes at similar stages of development, after the completion of meiosis, when reorganization of cytoplasmic components is occurring. Anterior is left, posterior is right. (A,B) Nomarski images of a living zygote undergoing contractions of the anterior membrane, formation of a pseudocleavage furrow, and pronuclear migration. The egg pronucleus is not visible at the anterior end of the zygote in (A). (C,D) Anti-tubulin (C) and anti-centrosome (D) staining. (E,F) Rhodamine-phalloidin staining of MFs during pseudocleavage and pronuclear migration (E) and at pronuclear meeting (F). (G) Staining of P granules. (H) Anti-yolk staining. (I) Staining of sperm membrane components. (J) Staining with the antibody to oocyte surface components that uniformly stains the surface of newly fertilized zygotes.

of the zygote, the growing spindle rotates from its initial orientation perpendicular to the A–P axis to its final orientation parallel to the A–P axis.

MFs also undergo unusual reorganization during this period (Strome, 1986*a*,*b*). Although the cortical meshwork of fine fibres of actin persists around the periphery of the zygote, the foci of actin become progressively more concentrated in the anterior half of the embryo (Fig. 2E,F). In addition to staining fibres and foci, R-ph and anti-actin antibodies stain the pseudocleavage furrow during its transient appearance (Fig. 2E), suggesting that the pseudocleavage furrow resembles a normal cleavage furrow in containing F-actin.

P granules undergo two processes, coalescence and asymmetric segregation, during the time period of pseudocleavage and pronuclear migration (Strome & Wood, 1982, 1983). Coalescence leads to fewer, larger P granules than were present immediately after fertilization. Asymmetric segregation causes the P granules to become localized at the posterior cortex of the zygote (Fig. 2G), that region destined for the small germline cell (P1) of the 2-cell embryo.

Not all components become asymmetrically rearranged. Yolk granules remain homogeneously distributed throughout the cytoplasm (Fig. 2H).

Sperm-surface components disperse apparently randomly throughout the cytoplasm of the zygote (Fig. 2F; Strome, 1986*a*). They do not remain as a discrete patch, in contrast to results seen in mouse and sea urchin embryos (Gabel, Eddy & Shapiro, 1979). The dispersal of sperm surface components argues that they do not serve to mark posterior (or do so only transiently) and that they do not mark a particular lineage.

The oocyte surface component that is internalized appears as intracellular granules that are somewhat more concentrated in the anterior hemisphere of the zygote (Fig. 2J; Strome, 1986*a*).

The period just described, although short (5–10 min), seems to be a critical period for subsequent normal development. Major redistributions of intracellular components occur, most notably the asymmetric localization of P granules and MFs. During the next period that leads up to cleavage of the zygote, the spindle also becomes asymmetric.

THE ASYMMETRIC SPINDLE AND UNEQUAL FIRST CLEAVAGE

The mitotic spindle is initially located in the centre of the zygote, but becomes asymmetrically located along the A–P axis (Fig. 3A–D). This occurs during spindle elongation; the posterior pole of the spindle moves closer to the posterior end of the zygote, while the anterior pole remains relatively immobile (Albertson, 1984). The spindle also appears asymmetric in the movement and shape of the two spindle poles. The posterior pole swings from side to side, while the anterior pole appears fixed, and the posterior pole takes on a flattened disk shape, while the anterior pole remains round (Fig. 3B).

The centrosomes, visualized by antibody staining, grow larger as mitosis proceeds, starting as small bright dots on each side of the nucleus during prophase and

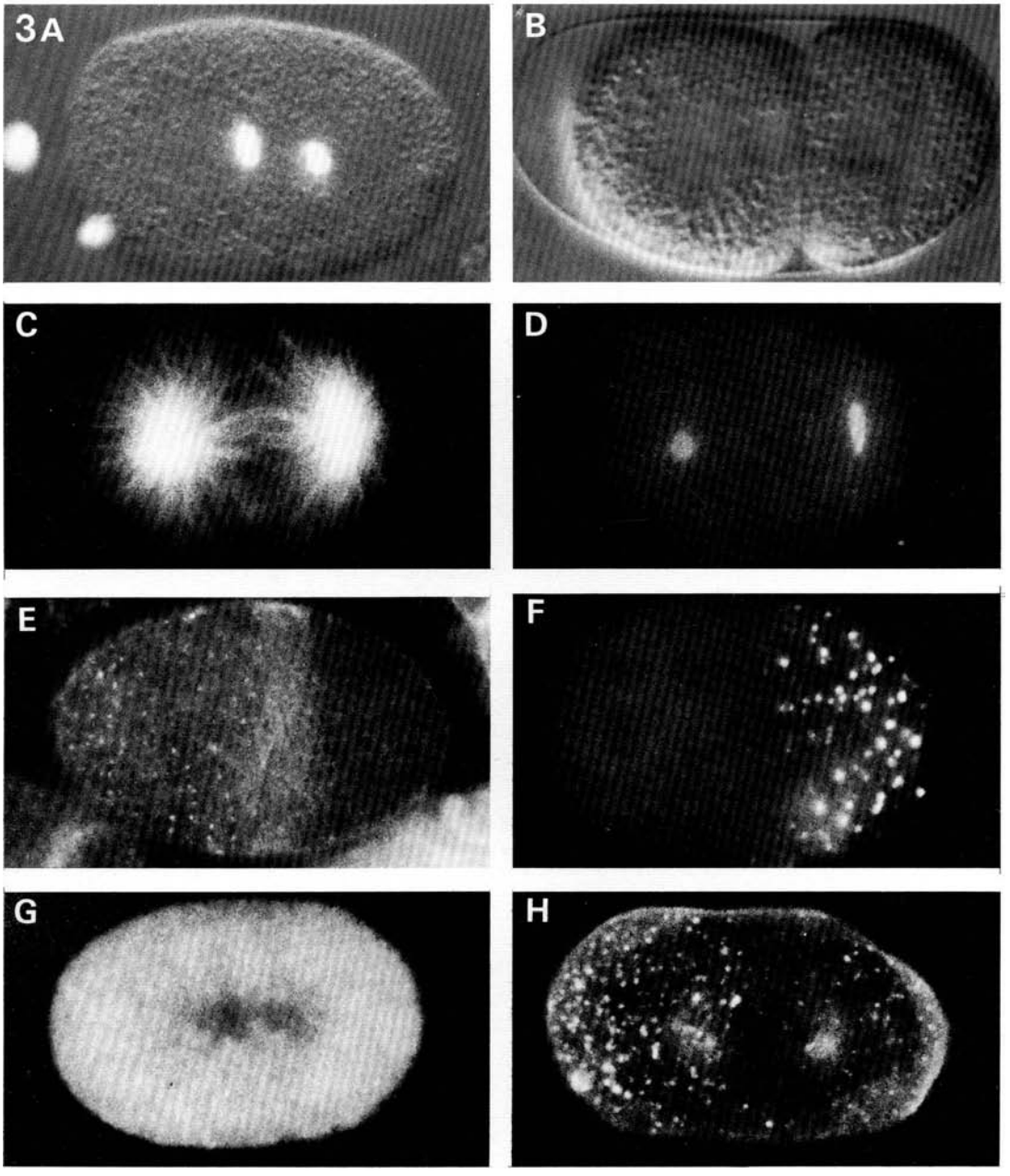

Fig. 3. First mitosis. All panels show zygotes at anaphase/telophase. Anterior is left, posterior is right. (A) Nomarski/DAPI image of a fixed zygote. (B) Nomarski image of a living zygote during cytokinesis, showing the different appearances of the two poles of the mitotic spindle and the sizes of the blastomeres generated by the first division. (C,D) Anti-tubulin (C) and anti-centrosome (D) staining. (E) Rhodamine-phalloidin staining of MFs. (F) Staining of P granules. (G) Anti-yolk staining. (H) Staining with the antibody to oocyte surface components that uniformly stains the surface of newly fertilized zygotes.

growing larger and more diffuse as mitosis progresses (Fig. 3D). The flat disk shape of the posterior pole seen by Nomarski microscopy is also seen by antibody staining (Fig. 3D). The centrosomes disappear altogether after mitosis is complete. In 2-cell and later embryos, centrosomal staining is not seen during interphase, but centrosomes appear, grow and disappear during mitosis.

The foci of actin remain anterior as the zygote generates an asymmetric mitotic spindle and undergoes mitosis. As mitosis is being completed, actin fibres align circumferentially around the zygote where the cleavage furrow will form (Fig. 3E; Strome, 1986*b*). During cytokinesis the cleavage furrow is very brightly stained by R-ph and anti-actin antibodies, consistent with the role MFs are thought to play in closing the cleavage furrow (Schroeder, 1976). Because the mitotic spindle is asymmetrically positioned in the zygote, the cleavage furrow is also asymmetric, leading to an unequal first division.

The localization of P granules to the posterior end of the zygote (Fig. 3F) assures their passage to the posterior cell (P1) of the 2-cell embryo (Strome & Wood, 1982). The cleavage-inhibition experiments of Laufer *et al.* (1980) and Cowan & McIntosh (1985) suggest that the potentials for gut, muscle and hypodermal differentiation are also asymmetrically partitioned during this first cleavage.

The oocyte-surface antigens that are internalized as granules are associated mainly with the poles of the mitotic spindle, with more granules at the anterior pole than the posterior pole (Fig. 3H).

Components such as yolk granules that are homogeneously distributed in the zygote do not show any apparent localization in the 2-cell embryo (Fig. 3G).

Thus, as a result of dramatic reorganization of cytoplasmic components (summarized in Fig. 4) and then an uneven first cleavage, the *C. elegans* zygote gives

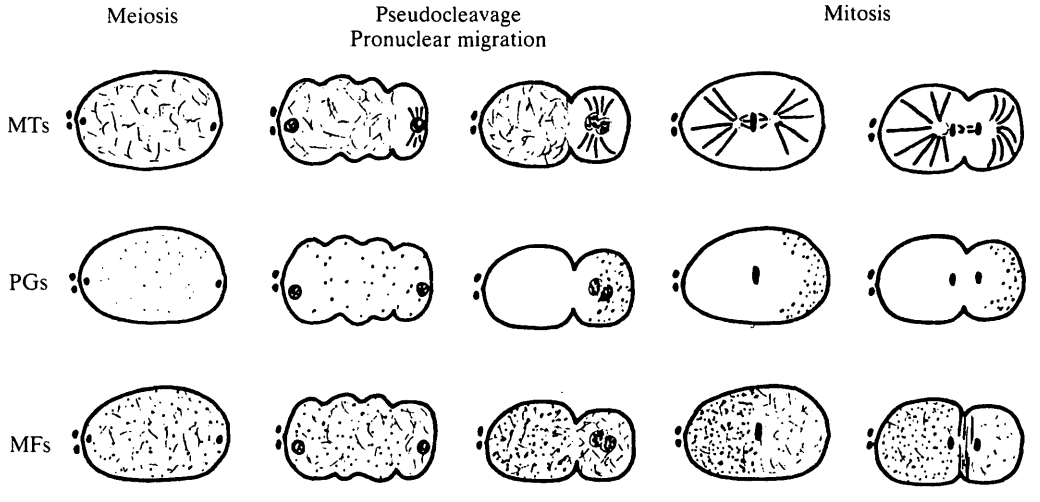

Fig. 4. Summary of the reorganization of microtubules (MTs), P granules (PGs), and microfilaments (MFs) that occurs between fertilization and first cleavage of the zygote. Anterior is left, posterior is right.

rise to different daughter blastomeres. The large anterior cell (AB) is a somatic blast cell. It has a characteristically fast cell cycle time, divides symmetrically and gives rise to several somatic cell types. The small posterior cell (P1) is a germline cell. It divides more slowly and undergoes a series of three more asymmetric cleavages similar to the asymmetric cleavage of the zygote, each time generating a larger somatic blast cell and a smaller germline (P) cell (Nigon *et al.* 1960; Hirsh *et al.* 1976; Sulston *et al.* 1983). At each asymmetric cleavage, P granules are segregated to the smaller germline P cell (Strome & Wood, 1982). By the 16-cell stage, five somatic lineages and the germline have been established. These follow invariant patterns of cell division and differentiation, described in detail by Sulston *et al.* (1983).

ANALYSIS OF THE DEPENDENCE OF ZYGOTIC MOVEMENTS AND THE GENERATION OF ASYMMETRY ON ELEMENTS OF THE CYTOSKELETON

Two approaches have been taken to analyse the interrelationships of different zygotic movements and their dependence on different elements of the cytoskeleton: (1) analysis of mutants defective in early embryogenesis, and (2) investigation of the effects of MT and MF inhibitors on zygotic motility events. Based on the results described below (from Strome & Wood, 1983), MFs appear to play a critical role in several zygotic movements and in the ability of the zygote to manifest asymmetry. MTs, although required for certain movements, do not appear to be required for zygotic asymmetry.

P-granule segregation is one of the most striking movements and manifestations of asymmetry seen in the zygote. In order to ask whether fertilization is required for the asymmetric localization of P granules, oocytes from the mutant *fem-2(b245)* were analysed. This mutant is defective in spermatogenesis and so produces unfertilized oocytes (Kimble, Edgar & Hirsh, 1984). Although P granules coalesce somewhat in unfertilized oocytes, they do not become asymmetrically localized (not shown; see Strome & Wood, 1983). This observation suggests that either fertilization is required for polarity or fertilization provides or triggers the motility system involved in granule segregation.

To ask whether P-granule segregation is dependent on the spindle, zygotes treated with low concentrations of the MT inhibitor nocodazole and zygotes from the mutant *zyg-9(b244)* (Kemphues, Wolf, Wood & Hirsh, 1986) were analysed. These zygotes show similar early defects in spindle placement: the spindle remains posterior and is oriented perpendicular to the A–P axis, leading to abnormal (longitudinal) first cleavage. P granules show their normal localization to the posterior end of such zygotes, resulting in their distribution to both daughter blastomeres (not shown; see Strome & Wood, 1983). Therefore P-granule segregation does not require the correct positioning or orientation of the mitotic spindle.

The effects of eliminating MTs altogether, by treating zygotes with MT inhibitors (colcemid, vinblastine or griseofulvin), reveal that MTs do not seem to be required for zygotes to manifest asymmetry. MTs are required for pronuclear migration, as in sea-urchin embryos (Schatten & Schatten, 1981), but pseudocleavage and P-granule segregation occur apparently normally in embryos that lack cytoplasmic MT arrays and spindles (not shown; see Strome & Wood, 1983). The asymmetric localization of actin foci has not yet been examined in zygotes treated with MT inhibitors.

Disruption of MFs by treatment of zygotes with MF inhibitors (cytochalasin D or B) prevents the zygotes from manifesting the aspects of asymmetry that we can monitor (Fig. 5). Contractions of the anterior membrane and pseudocleavage do not occur (Fig. 5A,B). Pronuclear migration occurs, but the two pronuclei meet in the centre instead of the posterior hemisphere (Fig. 5B,C). P granules are not segregated to the posterior cortex but instead coalesce into a tight cluster in the centre of the inhibited zygote (Fig. 5I). The spindle does not become asymmetric in its position along the A–P axis, and the two poles of the spindle do not behave differently (Fig. 5D–F), as they do in normal zygotes. As expected, cytochalasin treatment of zygotes dramatically alters the distribution of MFs seen using R-ph or anti-actin antibodies; the cortical meshwork of fibres and foci disappears, and large aggregates and spikes of staining material appear throughout the cytoplasm (Strome, 1986*b*). The effects of disrupting normal MF arrays suggest that the ability of *C. elegans* zygotes to manifest asymmetry requires cytoskeletal functions that depend on MFs.

A MODEL FOR MICROFILAMENT PARTICIPATION IN ASYMMETRIC MOVEMENTS

Based on the distribution of MFs seen in the zygote and the effects of MF inhibitors, a model of how MFs participate in early zygotic development has been proposed (Strome, 1986*b*). The foci of actin may represent a contractile network of actin. Contractions of the anterior membrane are seen as the foci become concentrated in the anterior cortex of the zygote. These contractions may push cytoplasmic components such as the egg pronucleus and P granules posterior. P granules become anchored in the posterior cortex. (Based on recent results (Hill & Strome, unpublished data), once P granules are segregated they do not depend on MFs to remain posterior.) The egg pronucleus meets the sperm pronucleus in the posterior hemisphere and both are pushed to the centre of the zygote by the growing spindle. The anterior network of actin foci may participate in spindle asymmetry by holding the anterior aster immobile while the posterior aster moves closer to the posterior end of the zygote and swings from side to side.

The above model depends upon an active involvement of the foci of actin in generation of zygotic asymmetry. An alternative possibility is that the foci do not actively participate in generating asymmetry but instead respond to the asymmetry of the zygote and become localized anterior. In addition, the fact that P granules

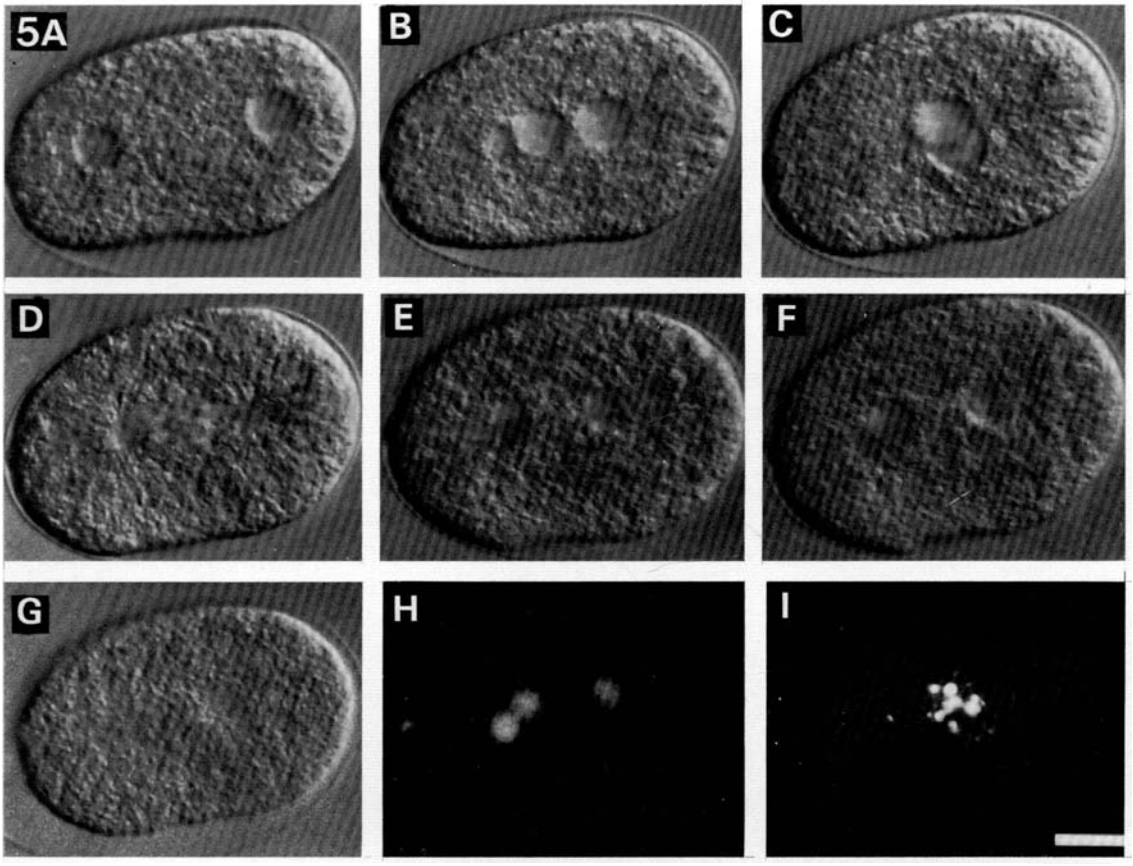

Fig. 5. Treatment of zygotes with cytochalasin D interferes with P-granule segregation and other manifestations of asymmetry. Anterior is left, posterior is right. (A–F) Nomarski images of a living zygote rendered permeable to $1\,\mu g\,ml^{-1}$ cytochalasin D, as described in Strome & Wood (1983). (A,B) Pronuclear migration in the absence of pseudocleavage and contractions of the anterior membrane. The pronuclei meet in the centre of the zygote. (C) Rotation of the pronuclei. (D) Formation of the first mitotic spindle, approximately equidistant from the two ends of the zygote. (E) Anaphase. (F) Telophase. The reforming nuclei are symmetrically positioned with respect to the ends of the zygote, and the asters distal to each nucleus appear similar. (G,H) Nomarski and DAPI images of the same zygote, which was observed for 26 min and then fixed. This zygote was treated early enough that cytochalasin D prevented extrusion of the second polar body, and the extra DNA is seen next to one of the telophase nuclei. (I) Immunofluorescence staining showing P granules in the centre of the zygote. Bar, 10 μm. Reproduced from Strome & Wood (1983) (MIT Press).

form a tight cluster in the centre of cytochalasin-treated zygotes may suggest the existence of an as yet unidentified contractile network to which P granules are attached. If such a network exists, then the posterior segregation of P granules in normal zygotes may be due to release of the network from anterior attachment points, perhaps involving the anterior actin foci and contraction of the network posterior; cytochalasin treatment would cause release of the network from the entire periphery and allow contraction to the centre of the zygote.

FUTURE PROSPECTS

The approach we have taken has allowed us to visualize some of the molecular events underlying the generation of asymmetry and cellular differences in the *C. elegans* zygote. We have come to a better understanding of which zygotic movements depend on MTs and which on MFs and which motility events may be related to each other. Thus far we have only one lineage-specific marker, P granules. The asymmetric segregation of P granules to the germline during the early cleavages is consistent with their participation in development of germ cells, but that remains to be proven. My laboratory and others continue to search for zygotic components that are specifically segregated to other lineages.

Ultimately, it is expected that we will gain further insights into early development by being able (1) mutationally to alter early events and (2) to analyse the resulting mutants both phenotypically and molecularly. Some very interesting mutants already exist (Wood *et al.* 1980; Schierenberg *et al.* 1980; Kemphues *et al.* 1986) and more are being generated. With immunologic probes to serve as landmarks of normal development and indicators of abnormal development, we may be able to analyse the molecular defects underlying mutant phenotypes and in so doing come to a better understanding of how normal zygotes are able to develop successfully into healthy adult organisms.

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