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Specification of the anteroposterior axis in *Caenorhabditis elegans*

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SUMMARY

Anteroposterior asymmetries are apparent in *C. elegans* development before the first cell division. Here we identify the cue that specifies the anteroposterior axis, and investigate how this cue is interpreted to generate initial asymmetry. In *C. elegans*, the sperm normally enters the egg in an invariant position. We have found that causing fertilisation to occur in the abnormal end of the egg completely reverses the orientation of the anteroposterior axis, but gives otherwise normal development. This result suggests that a component of the sperm normally specifies the anteroposterior axis. We have found that a cytoplasmic rearrangement in the uncleaved zygote is directed by the sperm, suggesting a mechanism by which the sperm may specify the axis. The results additionally reveal that the *C. elegans* oocyte is constructed with no axis prespecified in the form of asymmetrically localised cytoplasmic determinants.

Key words: *Caenorhabditis elegans*, axis specification, Rhabditidae, sperm, fertilisation

INTRODUCTION

How the diverse cell types and organisation of an adult animal are generated from a single cell is a central question of developmental biology. *Caenorhabditis elegans* is used as a model system for studying development, because of its potential for combining descriptive and experimental embryology, genetics, molecular biology and biochemistry. Although much is being learned about development in *C. elegans*, the source of initial asymmetry in its development has remained unknown.

C. elegans has an obvious anteroposterior (AP) axis, with distinct structures at distinct positions along the body. The axis appears to be determined very early in development, before first cleavage, as asymmetries of critical regulatory proteins arise by this time. A cytoplasmic rearrangement during the first cell cycle has been implicated in establishing some asymmetries. In a 5-10 minute interval found to be critical for generating certain cellular and developmental asymmetries (Hill and Strome, 1988, 1990), central cytoplasm flows toward the future posterior pole and cortical material flows in the opposite direction (Nigon et al., 1960; Hird and White, 1993). In concert with these movements, cytoplasmic P granules are segregated to the posterior (Strome and Wood, 1982, 1983) and foci of F-actin in the cortex become concentrated in the anterior (Strome, 1986b). Imaging live embryos has revealed that the P granules segregate to the posterior primarily by moving, rather than by being degraded en masse in the anterior (Hird et al., 1996), and there is evidence that cortical actin moves anteriorly at this time (Hird, 1996).

Recent work has identified several gene products that become asymmetrically localised in the first cell cycle. PAR-1 and PAR-2 proteins become localised to the posterior cortex, and PAR-3 and CABP11 become localised to the anterior cortex (Guo and Kemphues, 1995; Etemad-Moghadam et al., 1995; L. Boyd and K. Kemphues, personal communication; R. Aroian, personal communication). At least some GLH-1 and MEX-3 appear associated with P granules and become asymmetrically segregated with P granules (Roussell and Bennett, 1993; M. Gruidl and K. Bennett, personal communication; B. Draper, C. Mello, B. Bowerman and J. Priess, personal communication). The *par* genes and *mex-3* are believed to play roles in the generation of developmental asymmetry, as mutations in each gene can prevent certain asymmetries from arising (Kemphues et al., 1988; Kirby et al., 1990; B. Draper, C. Mello, B. Bowerman and J. Priess, personal communication). Hence even before first cleavage, AP asymmetry appears to be established in the embryo.

Several asymmetries can be seen even earlier than those cited above (Honda, 1925; Nigon et al., 1960; Ward and Carrel, 1979; White, 1988); each is a candidate cue which may specify the AP axis. Minutes before fertilisation, the oocyte nucleus moves to the presumptive anterior pole (Fig. 1). The oocyte pinches off a cytoplasmic bridge to the syncytial germline at approximately the same time, also at the presumptive anterior pole. The oocyte then enters the spermatheca and is fertilised at its leading edge, the presumptive posterior pole. After fertilisation, the oocyte completes meiosis, releasing polar bodies at the presumptive anterior pole. Although no asymmetries in determinants or in cytoskeletal organisation have been identified in the oocyte, such asymmetries play a role in axis specification in most other animals (for review see Wall, 1990).

In this paper, we describe experiments aimed at identifying the cue used for AP axis specification. Previous work by Albertson (1984) had shown that the oocyte nucleus' position at the time of meiosis can occasionally vary, with no apparent effect on development. This suggested that the oocyte nucleus'

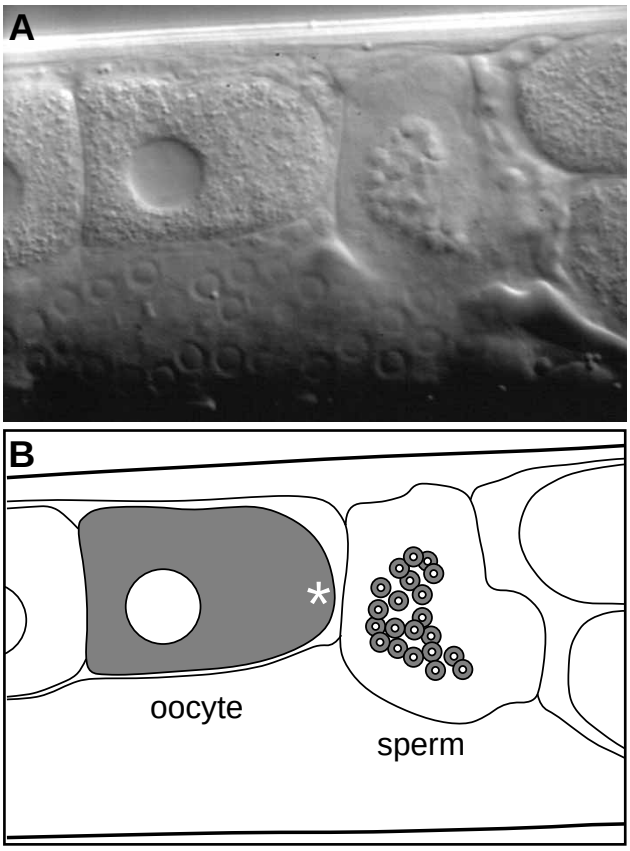

Fig. 1. Fertilisation in *C. elegans*, Nomarski micrograph (A) and diagram (B). Oocyte is shown in oviduct, sperm in spermatheca. Oocytes are normally fertilised at their leading edge (asterisk) as they pass into the spermatheca, which is a coiled tube. Fertilised eggs then pass into the uterus (far right). For a full description of fertilisation see Honda (1925), Hirsh et al. (1976) and Ward and Carrel (1979). The oocyte shown is approximately 45 μm long.

position does not specify the AP axis, at least at the time of meiosis. We attempted to alter the position of sperm entry at fertilisation and follow resulting development, in order to determine whether this is the cue used to specify the axis. We found that the site of sperm entry could be altered using animals in which fertilisations occurred more quickly than normal. This allowed us to test whether the sperm specifies the AP axis. Additional experiments addressed how the cue for axis specification is interpreted to generate the initial asymmetries in development.

MATERIALS AND METHODS

C. elegans strains used in this paper were N2 wild-type, *fem-1*(*hc17*) and *fer-1*(*hc1*). Other nematodes used were PS1191 (an undescribed isolate in the Family Rhabditidae, in a genus related to *Oscheius*; L. Carta, personal communication), and those listed below (with their Caenorhabditis Genetics Center strain numbers and taxonomic nomenclature):
Caenorhabditis briggsae (G16) (Dougherty and Nigon, 1949) Dougherty, 1953
Caenorhabditis vulgaris (EM464) Baird, Fitch and Emmons, 1993
Pellioditis typica (DF5025) Stefanski, 1922
Rhabditella axei (DF5006) (Cobbold, 1884)
Teratorhabditis palmarum (DF5019) Gerber and Giblin-Davis, 1990
Oscheius myriophila (DF5020) (Poinar, 1986) Sudhaus, 1994
Dolichorhabditis dolichura (DF0202) (Schneider, 1866) Andrassy, 1983
Dolichorhabditis dolichuroides (DF5018) (Anderson and Sudhaus, 1985) Andrassy, 1983
Pelodera strongyloides (Schneider, 1860) ssp. *dermatitica* (DF5022) Sudhaus and Schulte, 1988

In *C. elegans*, temperature-sensitive *fem-1* and *fer-1* mutants were used to alter the sperm entry position. These worms were mated to wild-type males within the first twelve hours of oocyte accumulation. Older *fem-1* and *fer-1* mutants when mated produced many embryos which appeared sick or lysed in the uterus, presumably because the oocytes had piled up for too long (Nelson et al., 1978; Doniach and Hodgkin, 1984; Kimble et al., 1984; Ward et al., 1981).

Embryos were dissected from adult worms in 'egg salts' (118 mM NaCl, 40 mM KCl, 3 mM $CaCl_2$, 3 mM $MgCl_2$, 5 mM Hepes, pH 7.2; Edgar and McGhee, 1986). The first polar body appears to form in *C. elegans* before the vitelline membrane is completely formed. Polar bodies were visible by Nomarski optics, but were sometimes difficult to see when they formed away from the poles. In order to score for polar body positions efficiently in large numbers of embryos, embryos were incubated in DAPI (2 μg/ml in egg salts) without permeabilising the vitelline membrane. One polar body was labelled by this method.

For immunofluorescence, embryos were mounted on slides, freeze-cracked and fixed in –20°C methanol, –20°C acetone as in Albertson (1984), except that an acetone series was used to rehydrate embryos after fixation. Antibodies were used as in Albertson (1984).

Worms were anaesthetised by the method of Kirby et al. (1990) in 0.1% tricaine, 0.01% tetramisole in water, for 20-30 minutes. To facilitate infusion of the anaesthetic without hypotonic shock, worms were sometimes placed instead in 0.1% tricaine, 0.01% tetramisole in egg salts, and the solution was then titrated with the water-based anaesthetic until worms became sluggish. Worms were then mounted on agar pads (Wood, 1988) for observation. Some hermaphrodites continued fertilising oocytes for 1-4 hours, without moving considerably during observation. Embryos observed from fertilisation inside

Table 1. Position of polar bodies in embryos from wild-type hermaphrodites and mated *fem-1* worms

	n	Anterior	Lateral	Posterior
Wild type				
Exp. 1	70	97%	3%	0
Exp. 2	74	97%	3%	0
Exp. 3	105	94%	5%	1%
Exp. 4	81	98%	2%	0
Mated *fem-1*				
Exp. 1	65	74%	15%	11%
Exp. 2	103	72%	17%	11%

Mating *fem-1* worms with wild-type males often altered polar body position relative to the AP axis. The effect shown is not a peculiarity of the *fem-1* genetic background, as results using mated *fer-1* worms (data not shown) were similar to the *fem-1* results. The effect is not produced by mating alone, as mating wild-type hermaphrodites (data not shown) gave similar results to that shown for unmated wild-type hermaphrodites. Anterior refers to roughly the anterior 7% of embryo length, posterior to roughly the posterior 7% and lateral to roughly the middle 86%. '*n*' is number of embryos scored in each experiment. Altered polar body positions are seen occasionally in wild-type embryos (see for example Albertson, 1984). This may indicate that either the position of sperm entry or the position of the oocyte nucleus varies in a small proportion of normal fertilisations; the latter is more likely, as unusual oocyte nucleus positions occasionally can be seen in normal fertilisations, whereas reversal of sperm entry position has not been seen in wild-type *C. elegans* fertilisations (B. G., unpublished).

an anaesthetised mother could be followed through gastrulation, whereas embryos dissected from the mothers (in these, fertilisation could not be observed) could be followed through a complete generation.

Cytochalasin D (Sigma) was used at 2 μg/ml in EGM (Edgar's Growth Medium; Edgar, 1995). *Oscheius myriophila* embryos were permeable to cytochalasin before the pronuclei appeared, presumably because the eggshell had not completely formed, as later stages were impermeable to the drug.

Time-lapse recordings were carried out on a multiplane recording system as described in Hird and White (1993). In most cases, single-plane time-lapse recordings were used. To follow cortical and cytoplasmic streaming, exposures for the time-lapse recording were taken a minimum of 5 seconds apart. Methods for live observations of P granules are described in Hird et al. (1996).

Other attempts to alter the sperm entry position included in vitro fertilisation using isolated oocytes placed in contact with either isolated sperm or loaded spermathecae in EGM. No sign of fertilisation was seen. Also, reproductive tracts of hermaphrodites were dissected out in EGM and a glass needle was used to try pushing an oocyte into the spermatheca prematurely. In each case, the oocyte lysed without entering the spermatheca.

RESULTS

Altering the sperm entry site alters the orientation of the AP axis

To alter the position of sperm entry, we mated wild-type males with worms that had accumulated some unfertilised oocytes (*fem-1* and *fer-1* mutants). This caused oocytes to enter the spermatheca at approximately twice the normal rate (5-6 per hour, compared to 2-3 per hour in wild-type hermaphrodites). We hoped that speeding up fertilisations might cause an oocyte to either pass by some sperm before being fertilised, or to enter the spermatheca before becoming fertilisable; either event might cause the oocyte to be fertilised at a random site by one of the surrounding sperm. Although we have not conclusively determined why the position of sperm entry is altered, we show below that it is sometimes altered in these matings.

After mating worms as above, embryos were examined for the position of their polar bodies, used as a marker for the former position of the oocyte nucleus (at meiosis). An initial sign that the sperm entry site and the AP axis both might have been altered was that the position of the polar bodies relative to the AP axis was changed in many of the embryos (Table 1). A sperm-specific marker (Strome, 1986a; Ward et al., 1986) was then used to confirm that the sperm entered in various locations relative to the position of the oocyte nucleus after the matings (Fig. 2A,E).

These results suggested that the position of sperm entry might have been altered in some fertilisations and that the AP axis orientation was altered as a consequence. Alternatively, simply altering the oocyte nucleus position could have produced the same results. In order to determine directly which was the case, worms were mated as above, and individual oocytes were followed inside anaesthetised mothers through fertilisation and development, both in real time and in time-lapse recordings. In this way, the position of the oocyte nucleus and the sperm could be seen; the position of sperm entry was inferred by noting where the sperm pronucleus formed, after the oocyte had undergone meiosis. When a sperm entered an oocyte in the abnormal end, development proceeded normally, but with one important difference: the AP axis was reversed. Among 29 oocytes, three were fertilised at the abnormal end and each of these developed with a reversed AP axis. The other 26, fertilised at the normal end, developed normally. In the reversed embryos, the region of the oocyte that normally would have become the anterior end instead became the posterior end (Figs 2, 3). This result suggests that the *C. elegans* oocyte has no fixed polarity and that the sperm specifies the AP axis. Cases were also found where the sperm entered the oocyte in a lateral position rather than at a pole; these cases are discussed further below.

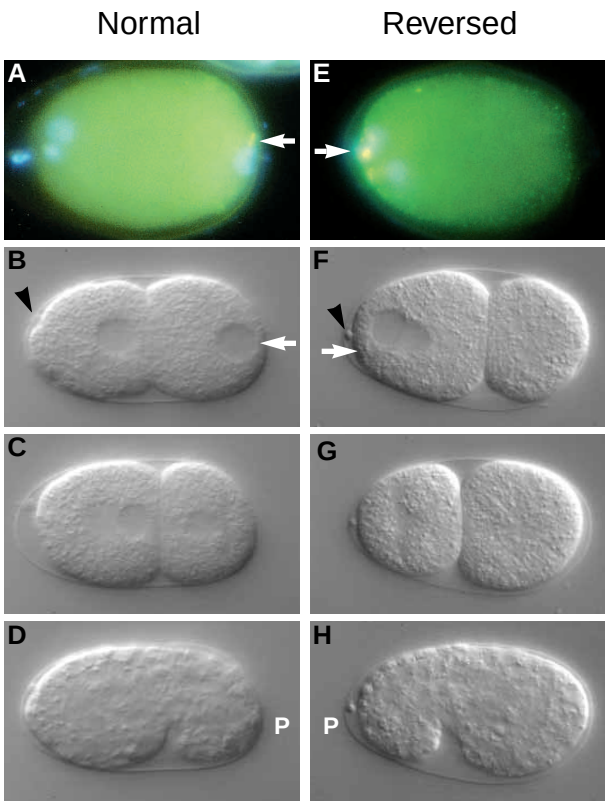

Fig. 2. The AP axis is specified by the sperm; normal (A-D) and reversed (E-H) development are shown. (A,E) Fixed embryos stained with SP56, a monoclonal antibody that recognises sperm-specific antigens and marks the egg cortex at the site of sperm entry (Strome, 1986a; Ward et al., 1986) (bright green, arrows), and DAPI (blue) to show nuclei. (A) The sperm has entered in its normal position. (E) The sperm has entered near the oocyte pronucleus. (B-D, F-H) Nomarski micrographs of two developing embryos. (B,F) One-cell embryos undergoing cytoplasmic rearrangement. The transient constriction in the middle of each embryo is the pseudocleavage furrow. (B) The oocyte pronucleus (left) has undergone meiosis, releasing a polar body (arrowhead) at the future anterior end, and has begun migrating toward the sperm pronucleus (arrow). (F) The sperm (arrow) has entered near the oocyte pronucleus; the oocyte pronucleus stays next to the sperm pronucleus rather than migrating to the opposite end. The polar body is marked by an arrowhead. (C,G) 2-cell stages; the smaller cell (P_1) forms on the side in which the sperm entered. (D,H) Comma stages, P marks the posterior. Similar results were found in oocytes followed through fertilisation and development (see Fig. 3). The *C. elegans* embryo is approximately 55 μm long.

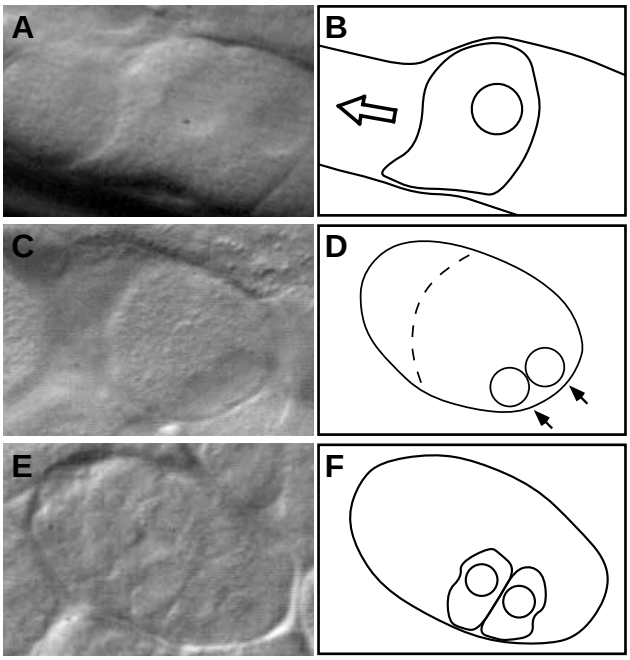

Fig. 3. A single case of reversal followed from before fertilisation; Nomarski micrographs are shown on the left, corresponding diagrams on the right. (A,B) The oocyte before fertilisation: the oocyte nucleus has moved within the oocyte to the end away from the spermatheca as normal. The arrow in B indicates the direction in which the oocyte will progress, through the spermatheca. (C,D) After fertilisation and meiosis, both pronuclei (arrows) appeared at the end of the embryo where only the oocyte pronucleus would have appeared normally; hence it is inferred that fertilisation has occurred at the abnormal end. The pseudocleavage furrow is indicated by a dashed line in D. (E,F) The same embryo beginning gastrulation. The two gut precursor cells (called Ea and Ep, drawn in diagram) are the first to gastrulate and are one sign that this end of the embryo is the developing posterior end. Following subsequent development confirmed that the pole of sperm entry become the posterior end.

The sperm's role is confirmed using other species with naturally varying sperm entry sites

We have searched for close relatives of *C. elegans* in which the site of sperm entry might vary naturally. Time-lapse videomicrography was used to follow fertilisation and early development in ten other species of the family Rhabditidae: *Caenorhabditis briggsae*, *Caenorhabditis vulgaris*, *Dolichorhabditis dolichura*, *Dolichorhabditis dolichuroides*, *Pellioditis typica*, *Pelodera strongyloides* ssp. *dermatitica*, *Rhabditella axei*, *Teratorhabditis palmarum*, *Oscheius myriophila* and one as yet undescribed species (PS1191; see Materials and Methods for other strain numbers). The position of sperm entry was again inferred by the position where the sperm pronucleus formed, after the oocyte had undergone meiosis.

Two species were found with naturally variable sperm entry sites. In time-lapse recordings of individual fertilisations in *Oscheius myriophila* and PS1191 (undescribed species), the sperm entered the egg in various positions. The AP axis was found to develop in either orientation relative to apparent oocyte polarity, depending on the site of sperm entry. These results essentially replicate the results from artificially altering the sperm entry position in *C. elegans*, but in an unmanipulated situation.

The recordings of the ten species also determined that the pole of sperm entry predicts the posterior pole in all ten species.

The sperm's effect on the cytoplasmic rearrangement

In order to understand how the sperm specifies the AP axis in *C. elegans*, we have used time-lapse videomicrography to observe the cytoplasmic rearrangement that occurs during the first cell cycle, as it has been implicated in establishing some embryonic asymmetries (Strome, 1983, 1986b; Hill and Strome, 1988, 1990; Hird and White, 1993; Hird et al., 1996; Hird, 1996; for review see Goldstein et al., 1993; Kemphues and Strome, 1996). The cytoplasmic rearrangement can be observed as a streaming of cytoplasmic and cortical granules in time-lapse recordings of developing embryos. Our simplest hypothesis was that a component of the sperm might direct this rearrangement. This appears to be the case: we found that the cytoplasmic rearrangement was always directed toward the sperm pronucleus, regardless of its position. The cytoplasmic rearrangement also followed the sperm pronucleus in cases where the pronucleus moved, as described below.

The *C. elegans* embryo is elongated along the AP axis by the eggshell, which forms soon after fertilisation, before the cytoplasmic rearrangement begins. Sixteen cases were observed where the sperm entered the oocyte laterally rather than at a pole. In all sixteen cases, the cytoplasmic rearrangement initially was directed across the short axis of the egg toward the sperm pronucleus, and then continually followed the sperm pronucleus as it moved to the closer pole, which became the posterior end of the developing embryo (Fig. 4). These results suggest that a component of the sperm directs the cytoplasmic rearrangement.

The movement of the sperm pronucleus to a pole after a lateral entry appears to be a result of the sperm driving itself to the closer pole by directing the cytoplasmic rearrangement: first, the sperm pronucleus always moved to the pole to which it was closer at the time the rearrangement began (Fig. 4D), as predicted if its movement is a simple consequence of it driving the rearrangement in the confined space of the elongate eggshell. Second, as the cytoplasmic rearrangement is known to depend on intact microfilaments (Hird and White, 1993), the sperm pronuclear movement (following a lateral entry) should also depend on intact microfilaments; this was found to be the case. 2 μg/ml Cytochalasin D inhibited the cytoplasmic reorganisation and sperm pronuclear movement following lateral entries in *Oscheius myriophila* embryos (17/17 cases). This species was used instead of *C. elegans* because the hermaphrodites of this species produce many lateral entries (as the sperm entry point varies naturally) and because the embryos are permeable to cytochalasin at this stage.

Results from time-lapse recordings of the ten species cited above were in concordance with the *C. elegans* results: a similar cytoplasmic rearrangement was observed in all ten species and was directed toward the sperm pronucleus in all cases. In *Oscheius myriophila*, in which the site of sperm entry varies naturally, the streaming followed the sperm pronucleus as it moved to the closer pole (26/26 cases), as described above for *C. elegans*. The results suggest that, although the site of

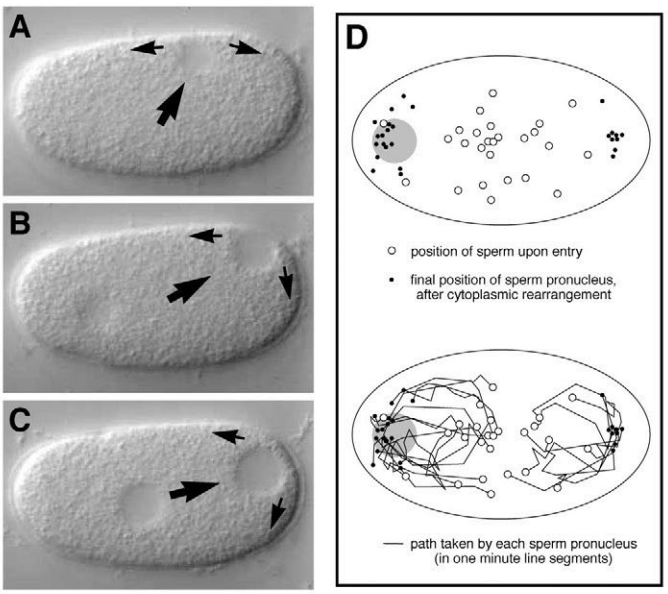

Fig. 4. The cytoplasmic rearrangement is directed by a sperm-associated component. (A-C) Orientation of the cytoplasmic rearrangement in cases where the sperm entered the oocyte laterally, Nomarski micrographs from time-lapse recordings. Arrows represent the direction of cytoplasmic streaming observed in time-lapse recordings. (A) The sperm pronucleus is shown soon after forming. Central cytoplasm flows toward the sperm pronucleus (large arrow), cortical material away from the sperm pronucleus (pair of small arrows). (B,C) The cytoplasmic rearrangement follows the sperm pronucleus as it moves. (D) Following a lateral entry, the sperm pronucleus appears to drive itself to the closer of the two poles. Sperm nuclear movement following lateral entries were traced. Results are superimposed for 26 cases from *O. myriophila*; 16 cases from *C. elegans* yielded similar results. The beginning and ending positions are depicted in the upper drawing. Lines showing the path of each sperm pronucleus are added in the lower drawing, showing that each sperm pronucleus moved to the closer of the two poles. Each line segment represents the displacement that took place over 1 minute. The drawings are two-dimensional tracings of the embryos, hence positions drawn as in the centre of the embryo may have been at the centre or at the upper or lower surfaces. The pole with the oocyte nucleus is represented by the grey disk. Lateral entries were also found in recordings of fertilisations inside anaesthetised mothers, with similar results.

sperm entry is not tightly controlled in all species, each of these species probably shares a similar mechanism for generating embryonic asymmetry.

In summary, these results demonstrate that the cytoplasmic rearrangement that occurs in the uncleaved zygote is directed by the sperm pronucleus, or a component associated with it.

The sperm's effect on P granule localisation

We were interested in determining whether a component of the sperm directs the redistribution of P granules. P granule segregation can be followed in confocal time-lapse recordings of embryos containing a fluorescently tagged antibody recognising P granules (Hird et al., 1996). In normal embryos, P granules stream to the future posterior pole in concert with the cytoplasmic rearrangement (Hird et al., 1996). Cases were examined in which the sperm entered the oocyte laterally, rather than at a pole. Three such cases were found; in all three

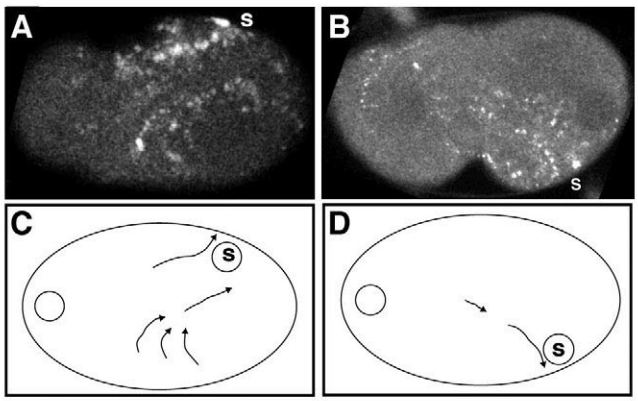

Fig. 5. The segregation of P granules is directed by a sperm-associated component. P granules normally progress along the AP axis to the presumptive posterior pole in concert with the cytoplasmic rearrangement (Hird et al., 1996). P granule movements are shown from two cases where the sperm entered the oocyte laterally; P granules moved toward the sperm pronucleus rather than toward a pole. (A,B) Trails of P granules, produced by overlaying successive confocal exposures of a live embryo containing fluorescently tagged antibodies recognising P granules. 'S' marks just outside the position where the sperm pronucleus formed. (C,D) Tracings of some P granules from the cases shown in A and B, respectively. Each arrow represents the movement of a single P granule; many more P granules could be seen (moving in the same general direction) in recordings. 'S' marks the position of the sperm pronucleus at the beginning of the recording. Each sperm pronucleus moved to the closer of the two poles during the recording, as in Fig. 4.

cases, the P granules streamed toward the sperm pronucleus, across the short axis of the egg (Fig. 5). This resulted in the P granules becoming effectively segregated to the posterior, but at least initially at an oblique angle rather than along the long axis of the embryo. The result indicates that P granule segregation is directed by the sperm.

DISCUSSION

These results identify the sperm as the cue for AP axis specification in *C. elegans* (Fig. 6): the normal site of sperm entry becomes the posterior and, when the sperm enters at the opposite pole, the AP axis is reversed. The sperm pronucleus, or a component associated with it, directs a cytoplasmic rearrangement that has been implicated in establishing asymmetries (Hill and Strome, 1988, 1990). The cytoplasmic rearrangement might segregate factors present in the oocyte, to generate the initial AP asymmetries in the embryo (see model, Fig. 7).

When the sperm enters the egg laterally instead of at a pole, it moves to the closer pole, which then becomes the posterior. The dynamics of sperm pronucleus movement during the cytoplasmic rearrangement, together with the physical constraint of eggshell shape, appear to make AP axis specification into a binary choice, in which either end of the oocyte can be specified as posterior depending on the position of sperm entry.

The results reveal that the *C. elegans* oocyte is constructed with no axis prespecified in the form of asymmetrically localised determinants. The oocyte is clearly a polarised cell,

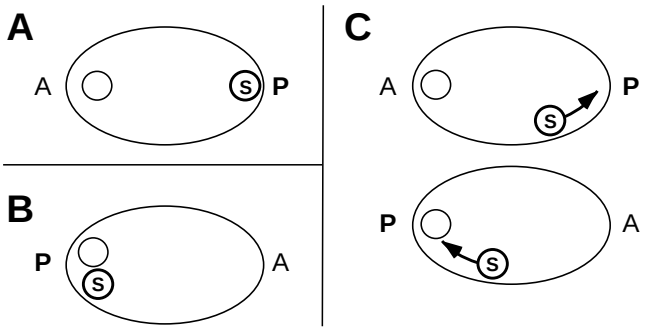

Fig. 6. Summary of the sperm's effect on the AP axis. (A) Normal *C. elegans* development: the sperm (s) enters at the pole opposite the oocyte nucleus; the pole of sperm entry develops as the posterior end. (B,C) Experimental alteration of the sperm entry position in *C. elegans* and natural variation of sperm entry position in two other species. (B) The sperm enters the 'abnormal' end: this end now becomes posterior instead of anterior. (C) The sperm enters in other locations (lateral entries): during the cytoplasmic rearrangement, the sperm moves to the closer pole, which then becomes the posterior. The sperm appears to drive itself to this pole as a consequence of directing the cytoplasmic rearrangement; see text for details.

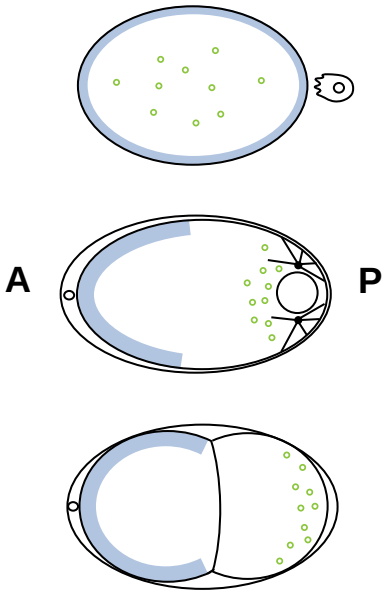

Fig. 7. Model for AP axis specification: the sperm generates AP asymmetry by directing a rearrangement of cortical and central cytoplasmic contents. (Top) The sperm enters an oocyte which has no developmentally important asymmetries. The elongated shape of the oocyte becomes fixed when the eggshell forms, soon after fertilisation. (Middle) At the time of sperm aster growth, cortical material is directed away from the sperm, and central cytoplasm and P granules are directed toward the sperm, establishing AP asymmetries. (Bottom) The asymmetries are partitioned into cells by cleavage. Cortical material is represented in blue, cytoplasmic P granules in green.

as the oocyte nucleus generally moves to the end away from the spermatheca before fertilisation (Ward and Carrel, 1979), but either end of the oocyte can become the anterior or the posterior end of the developing worm. *C. elegans* is unusual in this respect, as the oocytes of most other animals are constructed with inherent developmental asymmetry. Evidence for this derives largely from two types of findings: first, the unfertilised eggs of several animals have been cut in two and both halves fertilised (Boveri, 1901; Wilson, 1904; Yatsu, 1904; Freeman, 1978, 1993, 1995). In the animals tested, animal and vegetal halves developed differently, and they developed to some extent as they would during normal development, suggesting that regional specification occurs during oocyte construction. Second, some regulatory gene products have been identified that are localised in the egg before fertilisation, and need to be localised for normal development, such as *bicoid* in *Drosophila* and *Vg1* in *Xenopus* (Rebagliati et al., 1985; Driever et al., 1990; Thomsen and Melton, 1993; Nüsslein-Volhard, 1991). Although most animals construct oocytes with inherent developmental asymmetry, *C. elegans* is not the sole exception; mammals and ctenophores are also believed to have axially naïve oocytes (for review see Wall, 1990).

The sperm specifies a subsequent axis in some animals (Newport, 1854; Ancel and Vintemberger, 1948; Sawada and Schatten, 1988). In ascidians, the sperm specifies the second (anteroposterior) axis by affecting the direction of a cytoplasmic reorganisation (Sawada and Schatten, 1988). In *Xenopus*, the second (dorsoventral) axis is specified by the sperm. The sperm asters in *Xenopus* control the direction of a 30° rotation of the cortex relative to the central yolk mass. This rotation has been implicated in axis specification, although rotation can occur in the absence of sperm and the sperm's effect can be overridden by gravity (Newport, 1854; Ancel and Vintemberger, 1948; Vincent and Gerhart, 1987). No gravitational effect was seen here in *C. elegans*, as eggs in all orientations, including those with one pole or the other facing up, developed as described with respect to the orientation of both the cytoplasmic rearrangement and the AP axis.

How might the sperm direct the cytoplasmic rearrangement in *C. elegans*? The sperm brings the centrosome into the oocyte; the poles of the oocyte meiotic spindle lack centrioles and apparently do not function in mitosis (Ward and Carrell, 1979; Albertson, 1984; Albertson and Thomson, 1993). The sperm centrosome duplicates and then, near the time that the cytoplasmic rearrangement begins, both centrosomes nucleate astral arrays of microtubules, which remain associated with the sperm pronucleus (Albertson, 1984). The sperm asters might direct the cytoplasmic rearrangement by causing a movement of cortical actin away from their vicinity. Actin flow in the cortex might carry cortical material away from the sperm pronucleus, driving deeper cytoplasmic material in the opposite direction, redistributing cytoplasmic determinants and hence establishing AP asymmetries (Fig. 7). Alternatively, it remains possible that the rearrangement might generate only some asymmetries, and others might be caused by the sperm via another mechanism, for example by bringing in a determinant.

The hypothesis that the sperm asters could cause a movement of cortical actin was raised by an experiment in which the cytoplasmic rearrangement was mimicked in other cells during cytokinesis, by displacing the mitotic apparatus with the microtubule polymerisation inhibitor nocodazole. The polarity of the induced rearrangement was found to depend on the position of the attenuated mitotic apparatus, suggesting that components of

the mitotic apparatus can direct a cytoplasmic rearrangement (Hird and White, 1993). A similar role for astral microtubules in driving movements of the actin cortex has been proposed in cytokinesis (Bray and White, 1988; Cao and Wang, 1990). The finding that microtubule depolymerisation does not prevent the rearrangement in *C. elegans* (Strome and Wood, 1983) appears to be at odds with the proposed role for the sperm asters. However, the drugs used may not depolymerise microtubules completely (Strome and Wood, 1983; Hyman and White, 1987; Hird and White, 1993) and microtubules remaining associated with the centrosomes might still interact with the cortex.

Several gene products may become segregated by the sperm-directed movements. Gene products that might be moved by cortical streaming include PAR-3, which becomes localised to the anterior cortex during the first cell cycle. PAR-3 is required for many aspects of AP polarity and is a novel protein (Etemad-Moghadam and Kemphues, 1995). Gene products that may be moved on the P granules include MEX-3 and GLH-1, both of which are found partly on P granules by immunofluorescence. Mutations in *mex-3* affect some AP asymmetries; *mex-3* encodes a protein with putative RNA-binding KH domains (B. Draper, C. Mello, B. Bowerman and J. Priess, personal communication). *glh-1* encodes a putative germline-specific RNA helicase which is similar in sequence to *vasa*, a *Drosophila* polar granule component (Roussell and Bennett, 1993; M. Gruidl and K. Bennett, personal communication).

The other species of Rhabditidae were useful in generating natural replicates of the *C. elegans* experiments involving altered sperm entry positions. Other species that might be interesting to examine include a gynogenetic species in the family Rhabditidae observed by Nigon et al. (1960), in which the sperm contributes centrosomes but its nucleus usually degenerates and does not participate in development. Nigon described a visible cytoplasmic rearrangement in this species, similar to that in *C. elegans*, but did not report on whether it was directed toward the sperm asters. Some nematodes lack sperm altogether and develop as parthenogens; how these nematodes specify their AP axes is not clear.

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