Development 128, 1201-1209 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
DEV7870

PAR-1 is required for the maintenance of oocyte fate in *Drosophila*

Jean-René Huynh, Joshua M. Shulman*, Richard Benton and Daniel St Johnston‡

The Wellcome/CRC Institute & The Department of Genetics, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QR, UK
*Present address: Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, 221 Longwood Avenue, Boston, MA 02115, USA
‡Author for correspondence (e-mail: ds139@mole.bio.cam.ac.uk)

Accepted 18 January; published on WWW 13 March 2001

SUMMARY

The PAR-1 kinase is required for the posterior localisation of the germline determinants in *C. elegans* and *Drosophila*, and localises to the posterior of the zygote and the oocyte in each case. We show that *Drosophila* PAR-1 is also required much earlier in oogenesis for the selection of one cell in a germline cyst to become the oocyte. Although the initial steps in oocyte determination are delayed, three markers for oocyte identity, the synaptonemal complex, the centrosomes and Orb protein, still become restricted to one cell in mutant clones. However, the centrosomes and Orb protein fail to translocate from the anterior to the posterior cortex of the presumptive oocyte in region 3 of the germarium, and the cell exits meiosis and becomes a nurse cell. Furthermore, markers for the minus ends of the microtubules also fail to move from the anterior to the posterior of the oocyte in mutant clones. Thus, PAR-1 is required for the maintenance of oocyte identity, and plays a role in microtubule-dependent localisation within the oocyte at two stages of oogenesis. Finally, we show that PAR-1 localises on the fusome, and provides a link between the asymmetry of the fusome and the selection of the oocyte.

Key words: Oogenesis, Anterior-posterior polarity, Fusome, Meiosis, Microtubules, *Drosophila*

INTRODUCTION

The anterior-posterior (A-P) axes of both *Caenorhabditis elegans* and *Drosophila* are defined at the one-cell stage through the localisation of cytoplasmic determinants, but the upstream events that generate A-P polarity seem to be very different in each case. The A-P axis of *C. elegans* only becomes polarised at fertilisation, when the site of sperm entry defines the posterior pole (Goldstein and Hird, 1996; Rose and Kemphues, 1998). This triggers a reorganisation of the cortical actin cytoskeleton to generate cytoplasmic flows in the interior of the cell that localise determinants. For example, these flows direct the posterior accumulation of the P granules, which contain factors that specify the germline lineage (Hird and White, 1993; Hird et al., 1996). In contrast, the A-P axis of *Drosophila* is specified before fertilisation, when an unknown signal from the posterior somatic follicle cells induces the polarisation of the microtubule cytoskeleton of the oocyte at stage 7 of oogenesis (Ruohola et al., 1991; Theurkauf et al., 1992; Clark et al., 1994; van Eeden and Johnston, 1999). This polarised microtubule network then directs the posterior localisation of *oskar* mRNA to define the site of formation of the polar granules, which contain the abdominal and germline determinants (Ephrussi et al., 1991; Kim-Ha et al., 1991; Ephrussi and Lehmann, 1992). At the same time, the microtubule-dependent localisation of *bicoid* mRNA to the anterior of the oocytes specifies where the head and thorax of the embryo will develop (Frohnhöfer and Nüsslein-Volhard, 1986; Pokrywka and Stephenson, 1995; Clark et al., 1997).

Despite these differences in axis formation in *C. elegans* and *Drosophila*, it has recently been shown that the PAR-1 serine/threonine kinase is required for the generation of A-P polarity in both organisms, suggesting that these processes may be related. In *C. elegans*, PAR-1 is recruited to the posterior cortex after sperm entry, and mutations in the gene disrupt the A-P asymmetry of the first cell division and the localisation of the P granules (Guo and Kemphues, 1995b). Similarly, *Drosophila* PAR-1 localises to the posterior of the oocyte at stage 9, while *par-1* mutations cause *oskar* mRNA to localise to the centre of the oocyte rather than the posterior pole (Shulman et al., 2000; Tomancak et al., 2000). This mislocalisation of *oskar* mRNA results from a defect in the organisation of the microtubule cytoskeleton, in which the plus ends are focussed on the middle of the oocyte instead of the posterior. The function of *Drosophila* PAR-1 may therefore resemble that of its mammalian homologues, the MARKs, which have been shown to regulate microtubule organisation by phosphorylating microtubule binding proteins (Drewes et al., 1995; Illenberger et al., 1996; Drewes et al., 1997; Ebneth et al., 1999). This is unlikely to be the case in *C. elegans*, however, as microtubules do not appear to be required for P granule localisation (Strome and Wood, 1983; Hird et al., 1996).

Although PAR-1 is required only maternally in *C. elegans* for the A-P polarity of the first few divisions of the embryo, it seems to play multiple roles during *Drosophila* development, as the complete removal of zygotic PAR-1 activity results in larval lethality (Guo and Kemphues, 1995a; Shulman et al.,

2000). Furthermore, the defect in *oskar* mRNA localisation can only be observed in hypomorphic mutant combinations, because germline clones of the null allele of *par-1* block oogenesis at around stage 5 (Shulman et al., 2000). In addition to its role in polarising the oocyte at stage 9, PAR-1 must therefore have another earlier function during oogenesis.

The first stages of oogenesis take place in a specialised structure at the tip of each ovariole called the germarium (Fig. 1). In region 1 of the germarium, a germline stem cell divides asymmetrically to produce a cystoblast, which then divides four times with incomplete cytokinesis to give rise to a cyst of 16 germ cells interconnected by ring canals (Spradling, 1993). The fusome, a membranous-rich cytoplasmic structure, anchors one pole of each mitotic spindle at every mitosis. This ensures that the cyst undergoes an invariant pattern of divisions to generate two cells with four ring canals, two with three ring canals, two with two and eight with one (Lin et al., 1994; Lin and Spradling, 1995a). The cyst then enters region 2a, where the two cells with four ring canals start to differentiate as pro-oocytes. One of these cells always becomes the oocyte, while the other will eventually become a nurse cell like the other 14 cells of the cyst. The restriction of the oocyte fate to one cell is a stepwise process that can be followed by three different kinds of markers. In early region 2a, cytoplasmic proteins like Orb and BicaudalD (BicD) show a uniform distribution within the cyst, but they progressively localise first to the two pro-oocytes and then to the oocyte by late region 2a (Suter et al., 1989; Wharton and Struhl, 1989; Lantz et al., 1994). The centrosomes also migrate through the ring canals towards the oocyte and follow the same pattern of restriction to one cell (Mahowald and Strassheim, 1970; Grieder et al., 2000). As the oocyte is the only cell to remain in meiosis, the third marker of the oocyte identity is the synatonemal complex (SC), which forms along the synapsed chromosomes during prophase I of meiosis. The SC appears in the two pro-oocytes in early region 2a, then spreads to the two cells with three ring canals, before it is restricted to two cells and then finally to the oocyte in region 2b (Carpenter, 1975; Huynh and St Johnston, 2000).

It is not known how one of the pro-oocytes is chosen to become the oocyte, but it has been suggested that the fusome provides the initial cue (Lin et al., 1994; Lin and Spradling, 1995b). The fusome segregates asymmetrically at each division of the cyst, so that one of the two cells with four ring canals always inherits more fusome material than the other germ cells (de Cuevas and Spradling, 1998). The fusome disintegrates before the oocyte can be identified with any other markers, however, and it has therefore not yet been possible to correlate fusome asymmetry with oocyte fate. Two proteins that are likely to play an essential role in translating this asymmetry into the determination of the oocyte are BicD and Egalitarian (Egl), which are part of the same protein complex (Schupbach and Wieschaus, 1991; Suter and Steward, 1991; Carpenter, 1994; Ran et al., 1994; Mach and Lehmann, 1997). The fusome is normal in *BicD* and *egl* mutant cysts, but all other asymmetries are absent: neither oocyte-specific cytoplasmic proteins nor the SC localise to one cell, and egg chambers develop no oocyte and 16 nurse cells (de Cuevas and Spradling, 1998; Huynh and St Johnston, 2000). Finally, treatments with microtubule depolymerising drugs also produce cysts with 16 nurse cells and no oocyte (Koch and Spitzer, 1983; Theurkauf et al., 1993). In such egg chambers, all cytoplasmic proteins fail to localise to the oocyte, but the SC still becomes restricted to one cell in region 2b (Huynh and St Johnston, 2000). However, the SC has disappeared from this cell by the time the cyst reaches the most posterior part of the germarium (called region 3 of the germarium or stage 1 of oogenesis), and it develops as a nurse cell. Thus, microtubules seem to be required for oocyte determination, but not for all aspects of the initial selection of this cell.

We show that germline clones of a null allele of *par-1* also give rise to 16 nurse cell cysts, and we have analysed this phenotype using a variety of oocyte markers to investigate the role of PAR-1 in oocyte determination.

MATERIALS AND METHODS

Fly stocks

The *par-1*^{W3} allele (Shulman et al., 2000) was recombined onto the FRT G13 (Chou and Perrimon, 1996). UASp-nod-GFP is a kind gift of Acaimo Gonzalez-Reyes. A nanos-Gal4:VP16 driver was used to express transgenes in the germarium (Van Doren et al., 1998). Rescue experiments were performed by heat-shocking flies of the following genotypes:

y,w,hs-Flp; UASp-par-1(N1S)-GFP, FRTG13-*par-1*^{W3}/FRTG13-GFPnls; nosGal4-VP16/+

y,w,hs-Flp, UASp-par-1(N1L)-GFP, FRTG13-*par-1*^{W3}/FRTG13-GFPnls; nosGal4-VP16/+.

par-1 clones that express nod-GFP were obtained by heat-shocking flies of the following genotype:

y,w,hs-Flp; FRTG13-*par-1*^{W3}/FRTG13-GFPnls; nosGal4-VP16, UASp-nodGFP/+.

Germline clones

Germline clones were generated by the FLP/FRT technique (Chou et al., 1993), using either the FRTG13-ovoD1 chromosome or a FRTG13 GFPnls chromosome (gift from Stephan Lushnig, Tübingen). Clones were induced by heat-shocking third instar larvae at 37°C for 2 hours on 2 consecutive days. Adult flies were dissected up to 12 days after heat shock to avoid any perdurance of the wild-type protein.

GFP:PAR-1 rescue constructs

A cDNA encoding the GFP variant, mGFP6, was subcloned from the modified P-element vector D277mGFP6 (Schuldt et al., 1998) into Bluescript KS (Stratagene), and from there as a *Kpn*I/*Bam*HI fragment into pUASp (Rorth, 1998). A *Bam*HI/*Xho*I fragment encoding the PAR-1 N1S isoform was subcloned into the cloning vector pIC20R, and from there as a *Bam*HI/*Xba*I fragment downstream of mGFP6 in pUASp. As no full-length PAR-1 N1L cDNAs have been identified, the GFP:PAR-1 N1L construct was made by replacing a *Mlu*I/*Xho*I fragment from pICR20R PAR-1 N1S (containing all N1S sequence from exon 5-exon S2), with an equivalent fragment from the BDGP cDNA LD34276 incorporating all known N1L-specific coding sequence, and subcloned as before into pUASp mGFP6. Transgenic lines were generated by standard procedures (Spradling and Rubin, 1982).

Staining procedures

Antibody staining was performed according to standard protocols (González-Reyes and St Johnston, 1994). The antibodies used were mouse anti-Orb at 1/20 (Lantz et al., 1994), mouse anti-γ-Tubulin (Sigma) at 1/100, rabbit and mouse anti-α-Spectrin (at 1/1000 and 1/1, respectively; Byers et al., 1987), rabbit anti-Anillin at 1/2000 (Field and Alberts, 1995; de Cuevas and Spradling, 1998), mouse anti-Hts (DSHB, Iowa University; Zaccai and Lipshitz, 1996) at 1/10, mouse anti-α-Tubulin (Sigma) at 1/500 and anti-PAR-1 at 1/5000

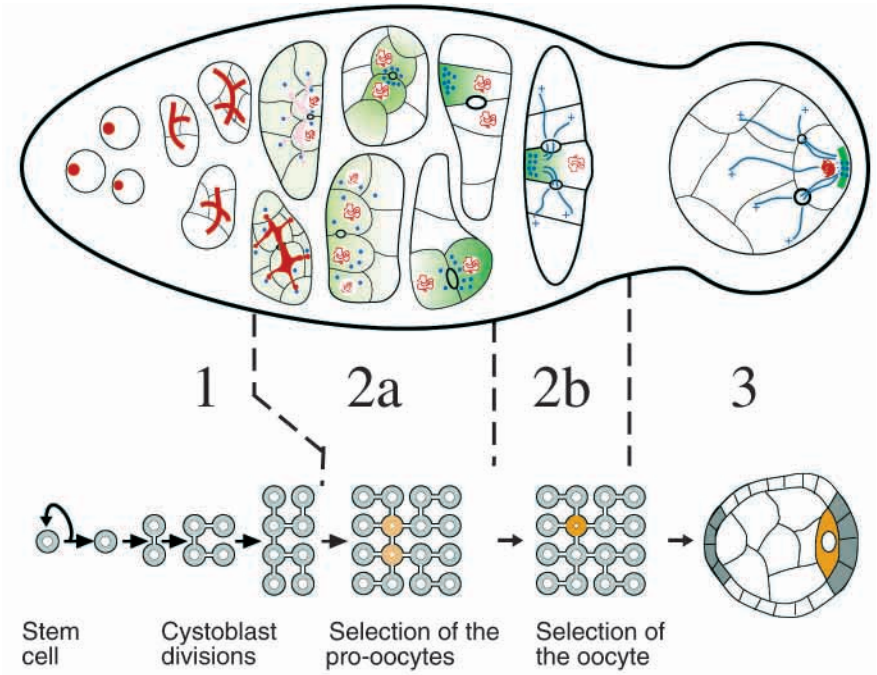

Fig. 1. The steps in oocyte selection in the germarium. The top panel shows the development of germline cysts in the germarium; the bottom panel diagrams the divisions of the cysts and the progressive restriction of oocyte fate to one cell. The germline stem cells reside at the at the anterior tip of the germarium (left) and divide to produce cytoblasts, which divide four more times in region 1 to produce 16 cell germline cysts that are connected by ring canals. The stem cells and cystoblasts contain a spectrosome (red circles), which develops into a branched structure called the fusome, that orients each division of the cyst. In early region 2a, the synaptonemal complex (SC, red lines) forms along the chromosomes of the two cells with four ring canals (the pro-oocytes) as they enter meiosis. The SC then appears transiently in the two cells with three ring canals, before becoming restricted to the pro-oocytes in late region 2a. By region 2b, the oocyte has been selected, and is the only cell to remain in meiosis. In region 2a, cytoplasmic proteins like Orb (green), and the centrosomes (blue circles) progressively accumulate in the oocyte. In region 2b, the minus ends of the microtubules are focused in the oocyte, and the plus ends extend through the ring canals into the nurse cells.

(Shulman et al., 2000). The synaptonemal complex was stained using a rabbit anti-Inscuteable antiserum at 1/1000 (Kraut et al., 1996; Huynh and St Johnston, 2000). For DNA staining, ovaries were pre-treated with 1 μg/ml RNAase for 1 hour, stained with propidium iodide (Molecular Probes) at 5 μg/ml for 2 hours and then washed extensively in PBT.

RESULTS

PAR-1 is required for the oocyte determination

To examine the phenotype caused by the complete lack of PAR-1 activity in the germline, we used the ovo^{D} technique to generate clones of *par-1*^{W3}, a null mutation that deletes most of the *par-1*-coding region (Chou et al., 1993; Shulman et al., 2000). This technique provides a powerful selection for homozygous mutant clones in the germline, because the ovo^{D} transgene blocks oogenesis at an early stage, so only the mutant clones that have lost the transgene survive. We failed to recover any late stage egg chambers, however, suggesting that cysts that lack PAR-1 arrest their development before stage 6. We therefore induced germline clones that are marked by the loss of nuclear GFP, so that the mutant egg chambers could be identified at all stages of oogenesis. Egg chambers with homozygous mutant germlines do develop, but they remain small, and never reach the stage where the oocyte is larger than the nurse cells. The oocyte can be distinguished from the nurse cells early in oogenesis, because it is arrested in meiosis and condenses its chromosomes into a hollow sphere called the karyosome, whereas the nurse cells endoreplicate their DNA to become polyploid (Fig. 2A). In the mutant egg chambers, none of the cells forms a karyosome, and instead all 16 germ cells become polyploid (Fig. 2B). The oocyte can also be identified by staining for Orb protein, which localises in a crescent at the posterior of the cell (Fig. 2C). Orb does not localise to any of the cells in *par-1* mutant egg chambers after stage 3, indicating that mutant cysts develop 16 nurse cells and no oocyte (Fig. 2D).

par-1 mutant oocytes exit meiosis prematurely

The 16 nurse cell phenotype of *par-1* null germline clones is

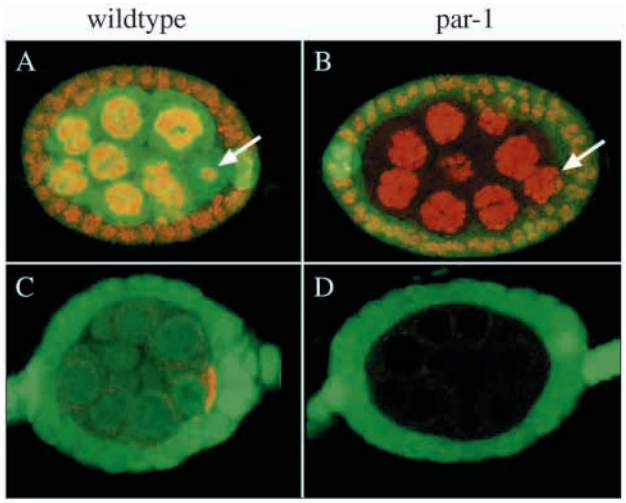

Fig. 2. PAR-1 is required for the oocyte determination. *par-1* null mutant germline clones marked by the loss of nuclear-GFP (green). (A) Wild-type stage 4 egg chamber stained for DNA (red). The DNA of the oocyte is compacted into a karyosome (arrow), whereas the nurse cells are polyploid. (B) *par-1* mutant egg chamber at stage 4. All 16 cells of the cyst appear polyploid, including the posterior one, which should be the oocyte (arrow). (C) Wild type stage four egg chamber stained for Orb protein (red), which localises at the posterior of the oocyte. (D) *par-1* mutant egg chamber. Orb is not localised to any cell of the cyst.

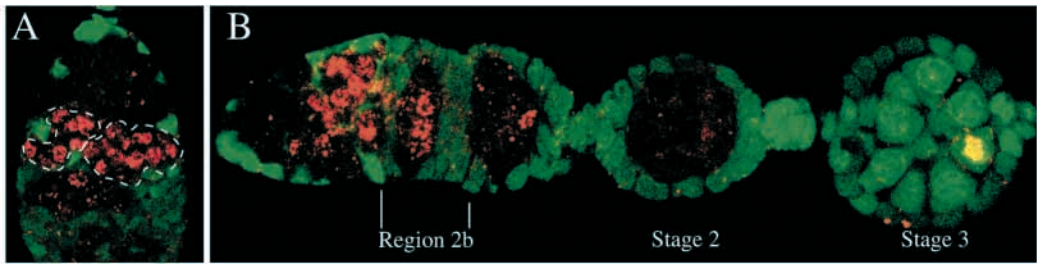

Fig. 3. PAR-1 is required to maintain the oocyte into meiosis (A) *par-1* mutant cysts in early region 2a, stained for the SC (red). Many more cells enter meiosis than in wild type. (B) The SC (red) in a germarium containing *par-1* germline clones, which are marked by the absence of nuclear GFP (green). In mutant cysts, the SC becomes restricted to one cell in region 3, but disappears prematurely from the oocyte at stage 2. The oldest egg chamber in the cyst (far right) is not mutant, and still contains the SC at stage 3.

very similar to that produced by mutations in *egl* and *BicD* (Suter et al., 1989; Wharton and Struhl, 1989; Schüpbach and Wieschaus, 1991). These mutants disrupt the earliest known step in the selection the oocyte, which is the formation of the SC in the two pro-oocytes (Carpenter, 1975; Carpenter, 1994; Huynh and St Johnston, 2000). In *egl* mutants, all cells in the cyst enter meiosis and reach the pachytene stage, but then lose the SC and revert to the nurse cell pathway of development. In contrast, none of the cells enters meiosis and forms any SC in *BicD* null germline clones (Huynh and St Johnston, 2000). To see whether PAR-1 is also required for these early steps, we examined the behaviour of the SC in *par-1* mutant clones. In contrast to wild type, mutant cysts contain up to 16 cells with SC in early region 2a, indicating that all of the cells have entered meiosis (Fig. 3A). Unlike *egl* mutant cysts, however, the SC does not disappear from all cells (Fig. 3B). Instead, the SC becomes progressively restricted to two cells, and then to one cell in region 3 (also called stage 1). This restriction is delayed compared with wild-type cysts, however, where the SC is always restricted to the oocyte by region 2b. In addition, the SC disappears prematurely from mutant oocytes. Wild-type oocytes retain a compacted SC until stage 3-4, whereas the SC can no longer be detected in mutant stage 2 egg chambers that have just left the germarium.

This behaviour of the SC suggests that PAR-1 acts at two stages in the germarium: first, it plays a role in the selection of the pro-oocytes and the oocyte, as too many cells enter meiosis in early region 2a, and the choice of the oocyte delayed. Second, PAR-1 is required to keep the oocyte in meiosis after stage 1, as the SC is not maintained.

PAR-1 defines a novel step in oocyte determination

The restriction of the SC to one cell suggests that the at least some of the initial steps in oocyte determination still occur in *par-1* clones. We therefore examined the behaviour of other early oocyte markers: the oocyte-specific cytoplasmic protein Orb and the centrosomes. In wild-type cysts, Orb protein accumulates in the pro-oocytes in region 2a of the germarium, and then concentrates at the anterior of the oocyte in late region 2a (Fig. 4A). As the cyst enters region 3, Orb moves from the anterior to the posterior of the oocyte, and forms a crescent at the posterior pole. The same movement have been reported for

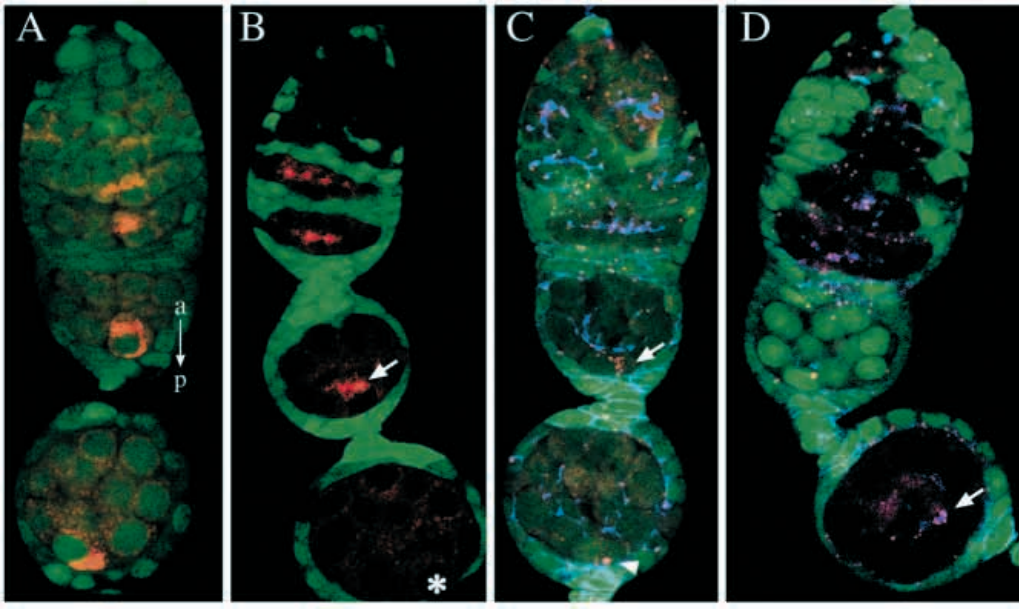

Fig. 4. PAR-1 is required for the maintenance of the oocyte fate and for the translocation of Orb and the centrosomes to the posterior of the oocyte. (A) Wild-type germarium expressing nuclear GFP (green) stained for Orb protein (red). Orb protein moves from the anterior to the posterior of the oocyte in region 3. (B) Germarium in which germline is mutant for *par-1*. Orb is restricted to one cell (arrow) but fails to localise to the posterior and is then lost from the oocyte by the next stage (asterisk). There is also a slight delay in the restriction of Orb to one cell, as the protein is still present in both pro-oocytes in region 2b cysts. (C) Wild-type germarium, stained for γ-Tubulin (red) to mark the centrosomes and α-spectrin (blue). The centrosomes first localise to the anterior of the oocyte, before switching to posterior at stage 2 (the arrow indicates centrosomes in transit to the posterior). They then coalesce to form a bright spot (arrowhead). (D) Chimaeric germarium containing *par-1* mutant germline clones marked by the loss of nuclear GFP (green). The centrosomes localise to the oocyte, but remain at the anterior of the cell (arrow).

a BicD-GFP fusion protein (Pare and Suter, 2000). In *par-1* mutant cysts, Orb protein still accumulates in one cell, but this localisation is delayed until region 3 (stage 1) like the restriction of the SC. Furthermore, Orb never translocates to the posterior of the oocyte, and the protein disappears from the oocyte by stage 2 (Fig. 4B).

The migration of the centrosomes from the nurse cell to the oocyte can be followed by staining for γ-tubulin (Grieder et al., 2000). Like Orb, the centrosomes localise to the anterior of the oocyte in region 2b. They then move from the anterior to the posterior of the oocyte in region 3, and coalesce to form a bright dot at the posterior pole (Fig. 4C). In *par-1* mutant clones, the centrosomes accumulate at the anterior of the oocyte in late region 2b or early region 3, indicating that the first phase of centrosome migration occurs normally (Fig. 4D). The centrosomes remain at the anterior, however, and never translocate to the posterior.

These results indicate that the loss of *par-1* blocks oocyte determination at a novel step. The initial selection of the oocyte occurs normally, as three independent oocyte markers, the SC, Orb and the centrosomes, still accumulate in one cell, but the identity of this cell is not fixed, and it soon reverts to the nurse cell pathway of development. This reversion to the nurse cell fate correlates with a defect in the migration of Orb and the centrosomes from the anterior to the posterior of the oocyte, suggesting that this PAR-1-dependent A-P movement is required for the maintenance of oocyte identity.

PAR-1 is required for the posterior translocation of microtubules minus-end markers

PAR-1 is required for the correct organisation of microtubules in the oocyte at stage 9, and we therefore examined whether this early phenotype in oocyte determination is also a consequence of a defect in the microtubule cytoskeleton. In a wild-type germarium, the microtubule cytoskeleton becomes progressively polarised in region 2 and is clearly focused on one cell in late region 2b (Fig. 5A; Grieder et al., 2000). *par-1* mutant clones stained for α-tubulin show a slight delay in the focusing of the microtubules to one cell in region 2b, which is similar to the delay in Orb restriction. However, the overall organisation of the microtubules in region 2 appears essentially normal (Fig. 5B).

As it is difficult to interpret the organisation of the microtubules within the oocyte in anti-tubulin stainings, we used markers for the minus-ends of the microtubules to examine the polarity of these microtubules in wild type and *par-1* mutant cysts. Although the centrosomes lie at the minus ends of the microtubules in most cells, this is not necessarily the case in the germline, as they appear to be inactivated when the cyst leaves region 1, and we therefore used a Nod-GFP transgene as an alternative marker for the minus ends (J. Bolivar, J.-R. H., H. Lopez-Schier, C. González, D. St J. and A. González-Reyes, unpublished). Nod-GFP localisation in wild-type cysts reveals that the minus ends are first focussed at the anterior of the oocyte, before switching to the posterior as the cyst buds off the germarium (Fig. 5D; Grieder et al., 2000). In *par-1* mutant cysts, Nod-GFP still becomes restricted to the anterior of one cell, but never re-localises to the posterior. Furthermore, the minus end-directed motor, dynein, shows an identical behaviour to Nod-GFP: it localises to the anterior of one cell in mutant cysts, but fails to switch to the posterior in region 3 (data not shown). Thus, PAR-1 is required for a reorganisation of the microtubule cytoskeleton of the oocyte in region 3, and this may account for the failure of Orb and the centrosomes to move from the anterior to the posterior of the cell.

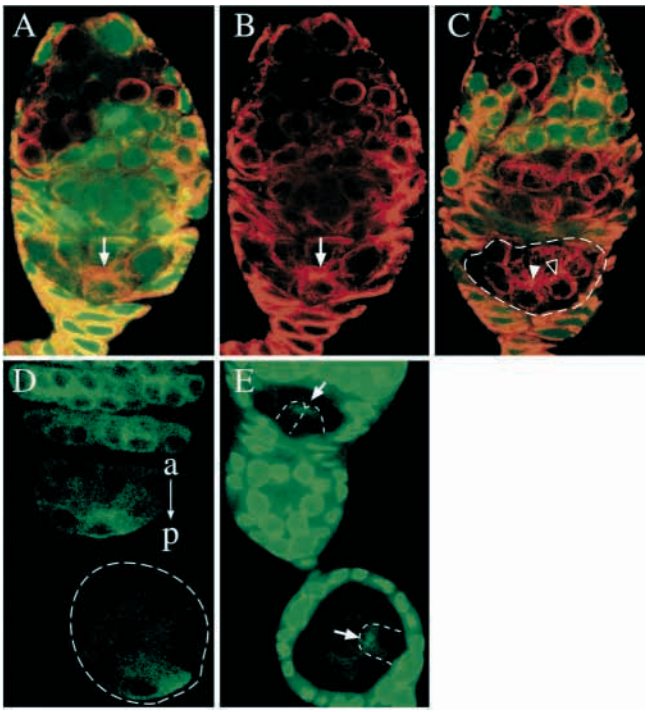

Fig. 5. Microtubule organisation in *par-1* mutant cysts. (A,B) Microtubule staining (red) of a germarium showing a wild type cyst in region 2b/3 marked by the presence of nuclear GFP (green).. The microtubules are concentrated in the oocyte. (arrow). (C) A *par-1* mutant cyst in region 2b/3 (circled by the broken line). Most microtubules are focussed on one cell (arrowhead), but the adjacent pro-oocyte still contains a large number of microtubules (open arrowhead). (D) A wild-type germarium showing the localisation of Nod-GFP, a marker for the minus ends of the microtubules. Nod-GFP concentrates in the oocyte in region 2b, and then re-localises to the posterior of the cell at stage 2. (E) Chimaeric germarium containing *par-1* germline clones that express Nod-GFP. Nod-GFP becomes restricted to one cell, but does not switch to the posterior (arrow). The pro-oocytes and the oocyte are outlined by the broken line.

The N1S isoform of PAR-1 rescues the early *par-1* function

The *par-1* null allele is also mutant for *mei-W68*, which shares a promoter with *par-1* and lies entirely within its first intron (Shulman et al., 2000). As Mei-W68 is required for the dsDNA breaks that initiate meiotic recombination, some or even all of the phenotypes of *par-1* clones could therefore be due to this meiotic defect rather than the loss of PAR-1 itself (McKim and Hayashi-Hagihara, 1998). To distinguish between these possibilities, we used the Gal4/UAS system and a Nanos-Gal4-VP16 driver to express the N1S isoform of PAR-1 in the germarium as a GFP-fusion protein, to determine whether it could rescue the phenotype of *par-1*^{W3} germline clones (Brand and Perrimon, 1993; Rorth, 1998; Van Doren et al., 1998). The fusion protein localises to a branched structure in regions 1 and

2 of the germarium, which is presumably the fusome, as the endogenous protein is a component of this structure, as described below (Fig. 6). More importantly, PAR-1N1S completely rescues the delay in Orb localisation to the oocyte in *par-1*^{W3} homozygous cysts, and also restores the anterior-posterior movement of Orb within the oocyte in region 3 (Fig. 6A). Furthermore, this construct rescues the posterior movement of the centrosomes, and the maintenance of oocyte identity (Fig. 6B, and data not shown). Thus, these phenotypes are specific to *par-1*, and are not due to the reduction in Mei-W68 activity or any other mutations on the *par-1*^{W3} chromosome.

We also expressed the N1L isoform of PAR-1, which differs only from N1S by the exclusion of five amino acids in an alternative exon in the linker region of the protein and the presence of the conserved C-terminal PAR-1 domain. The PAR-1N1L fusion protein also localises to the fusome, but is unable to rescue any of the early defects in *par-1*^{W3} germline clones, although it is functional because it rescues the *par-1* phenotype at other stages of development (Fig. 6C). This suggests that the PAR-1 domain inhibits the early function of the kinase, and provides the first example where different PAR-1 isoforms have different activities.

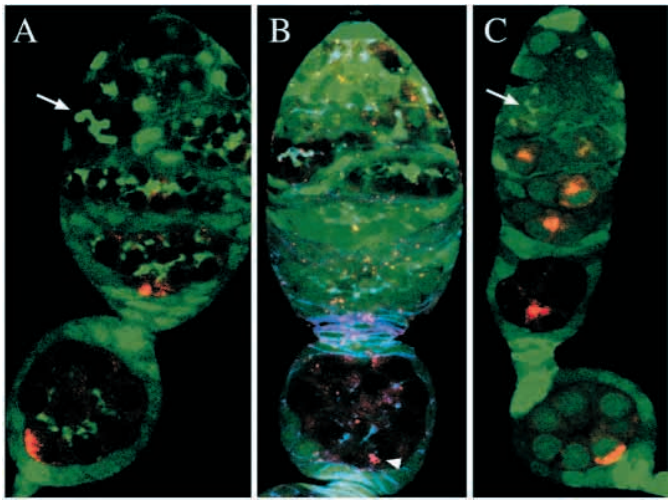

Fig. 6. A PAR1-N1S-GFP transgene rescues the early par1 defects. (A) A germarium in which all of the cysts are mutant for *par-1* (lack of nuclear GFP), expressing PAR1-N1S-GFP (green) from a transgene and stained for Orb protein (red). PAR1-N1S-GFP is recruited to the fusome (arrow), and rescues the *par-1* defect in Orb localisation. Orb localises to the oocyte, and the moves to the posterior of the cell in region 3. (B) A chimaeric germarium containing *par-1* germlines clones (lack of nuclear GFP) that express the PAR1-N1S-GFP transgene stained for γ-Tubulin (red) and α-Spectrin (blue). The transgene rescues the translocation of the centrosomes, which form a bright spot (arrowhead) at the posterior of the oocyte. (C) A chimaeric germarium expressing PAR1-N1L-GFP (green staining of the fusome, arrow) stained for Orb protein (red). Orb protein has failed to move to the posterior of the *par-1* mutant cyst in region 3, indicating that the PAR1-N1L-GFP transgene is not able to rescue the early *par-1* phenotype.

PAR-1 is a component of the fusome, but is not required for fusome asymmetry

The GFP-PAR-1 localisation in the germarium is intriguing, as it has been suggested that the fusome provides the initial cue for the determination of the oocyte. To confirm that PAR-1 is recruited to the fusome, we stained wild-type germaria for PAR-1, using an antibody that recognises all isoforms of the protein, and Hu li tai shao (Hts), an integral component of the fusome (Yue and Spradling, 1992; Zaccai and Lipshitz, 1996). PAR-1 and Hts co-localise on the spectrosome, which is the precursor of the fusome in the germline stem cells and cystoblasts, and on the fusome itself (Fig. 7A-C). This co-localisation is most apparent in region 1, but some PAR-1 persists on the fusome in region 2. In addition, the PAR-1 antibody labels small particles that do not contain Hts, but this is due to a cross-reacting antigen, because these are not seen with the GFP-PAR-1 fusions, and do not disappear in *par-1* null germline clones (data not shown).

Mutants in other components of the fusome, such as Hts and α-Spectrin, produce cysts with fewer than 16 nurse cells,

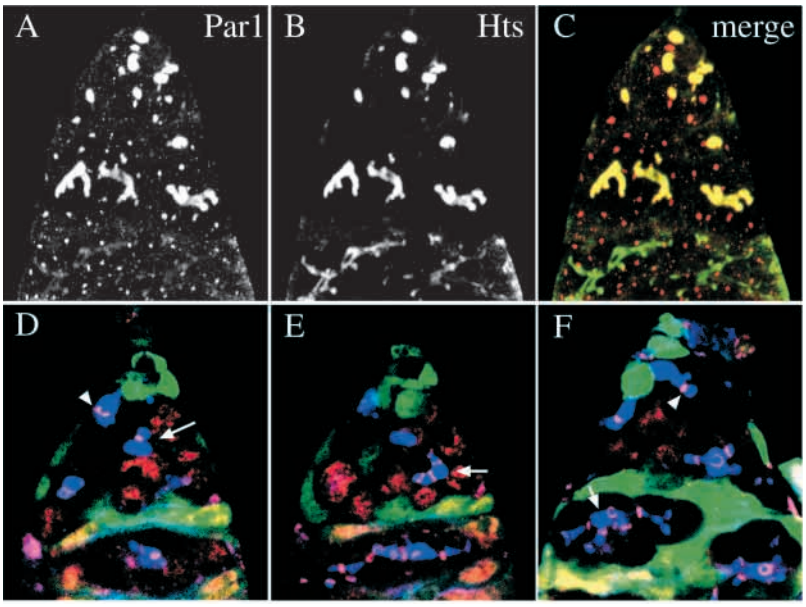

Fig. 7. PAR-1 localises on the fusome but is not required for its asymmetry. (A-C) Wild-type germarium, stained for PAR-1 (red) and Hts (green). PAR-1 co-localises with HTS on the fusome. PAR-1 expression is stronger in region 1, but we can still detect it on the fusome later on. (D-F) Chimaeric germaria containing *par-1* mutant germline clones marked by the loss of nuclear GFP (green) stained for the ring canal component, Anillin (red) and α-Spectrin (blue) to label the fusome. The morphogenesis of the spectrosome and fusome is normal in mutant clones. (D) Asymmetric partitioning of the fusome during a cystoblast division to form a two cell cyst (arrow). Cystoblast dividing to give a two-cell cyst (arrow). (E) A four-cell cyst in division, showing one cell with more fusome than the rest (arrow). (F) Fusome asymmetry is conserved in a post-mitotic cyst (arrow). The arrowheads in D and F indicate the asymmetric segregation of the spectrosome during stem cell divisions.

because the precise pattern of cyst divisions is disrupted (Lin et al., 1994; de Cuevas et al., 1996). All *par-1*^{W3} mutant egg chambers contain 16 cells, however, indicating that PAR-1 is not required for this function of the fusome. To rule out the possibility that the lack of an effect on the pattern of divisions in the cyst was due to the perdurance of wild-type PAR-1 protein in mutant clones, we also analysed mutant egg chambers derived from persistent stem cell clones that were induced 10 days earlier, and obtained the same result.

To test if *par-1* affects the formation or morphogenesis of the fusome, we stained mutant cysts for α-Spectrin, which is a component of the spectrosome and the fusome at all stages of its development (Deng and Lin, 1997; de Cuevas and Spradling, 1998). The spectrosome is asymmetrically partitioned during stem cell division, so that two thirds remain in the daughter stem cell, and 1/3 in the cystoblast, and this asymmetry is unaffected in *par-1*^{W3} clones (arrowheads in Fig. 7D,F). Furthermore, the fusome is asymmetrically partitioned at each subsequent division, as in wild type: when a mutant cystoblast divides, one cell still inherits more fusome than the other; this asymmetry is maintained throughout the next three mitoses, and can be seen in four-cell cysts and in 16-cell cysts that have stopped dividing (arrows in Fig. 7D-F). Thus, PAR-1 localises on the fusome, but is not required for its formation or its asymmetric segregation.

DISCUSSION

One cell in a *Drosophila* germline cyst inherits more fusome material than the others, leading to the proposal that this asymmetry provides the initial cue that selects the oocyte (Lin et al., 1994; Theurkauf, 1994; Lin and Spradling, 1995a). This hypothesis has been difficult to prove for two reasons. First, the fusome disintegrates before the oocyte can be unambiguously identified. Second, mutations that affect the formation of the fusome alter cyst development at an earlier stage. The fusome orients the cell divisions in region 1 of the germarium, and mutants in integral fusome components, such as Hts or α-Spectrin, disrupt the invariant pattern of cell divisions, and give rise to cysts with a variable number of cells (Lin et al., 1994; de Cuevas et al., 1996). Although these cysts very rarely form an oocyte, this may be an indirect consequence of the altered cell number. In contrast, PAR-1 is the first example of a fusome component that is required for oocyte determination, but not for the formation of the fusome itself. *par-1* null germline clones always give rise to egg chambers with 16 cells, but all of these cells ultimately develop as nurse cells. This phenotype provides strong support for the model in which the oocyte is selected by the asymmetric inheritance of the fusome, and suggests that one way that this is accomplished is by segregating a larger amount of fusome-associated factors such as PAR-1 to one cell.

The role of PAR-1 in oocyte determination is unclear, but it seems to be required at two distinct stages in this process. The *par-1* null mutant disrupts the earliest step in this pathway, the initial selection of the two pro-oocytes, as all 16 cells in the cyst enter meiosis in region 2a. This defect is transient, however, and meiosis eventually become restricted to one cell. Indeed, the oocyte seems to be selected normally, although slightly later than in wild type, because three independent markers for oocyte identity, the SC, the centrosomes and Orb protein, localise to one cell. Furthermore, the oocyte also starts to behave differently from the nurse cells, as it always moves to the posterior of the cyst in region 3, as in wild type (Godt and Tepass, 1998; González-Reyes and St Johnston, 1998). Despite this normal behaviour of the oocyte in region 3 (stage 1), all three oocyte markers start to disappear during stage 2, and the cell becomes indistinguishable from the other the nurse cells by stage 4. Thus, PAR-1 is also required for the maintenance of oocyte fate after region 3 of the germarium. This later defect could be a consequence of the earlier delay in oocyte selection. However, cysts that are mutant for *orb*^{mel} or any of the *spn* genes show a much more dramatic delay in the restriction of oocyte markers to one cell, but still form an oocyte that is maintained throughout oogenesis (González-Reyes et al., 1997; Huynh and St Johnston, 2000). These results indicate that it is not the delay itself that is responsible for the de-differentiation of the oocyte, and suggest that PAR-1 has a second specific function in the maintenance of oocyte fate.

PAR-1 is also required much later in oogenesis for the formation of the polarised microtubule (MT) cytoskeleton that directs the localisation of *oskar* mRNA to the posterior pole of the oocyte (Shulman et al., 2000; Tomancak et al., 2000). In contrast, several lines of evidence indicate that the early germarial phenotype of PAR-1 is not due to an effect on microtubules:

(1) Although there is a slight delay in the focussing of the MT in the oocyte, *par-1* null germline clones do not grossly disrupt the overall organisation of the microtubule cytoskeleton in region 2.

(2) Orb always localises to one cell in *par-1* clones, even though this localisation is microtubule-dependent, and is abolished by low concentrations of the microtubule-depolymerising drug, colcemid (Huynh and St Johnston, 2000).

(3) The restriction of meiosis to the two pro-oocytes in early region 2a does not require microtubules, as this is not disrupted by high concentrations of colcemid, and occurs before a polarised microtubule cytoskeleton has formed (Huynh and St Johnston, 2000).

Nevertheless, this process is strongly affected in *par-1* clones, as all 16 cells enter meiosis rather than the usual two. Thus, the function of PAR-1 in region 2a appears to be microtubule independent. Although the best characterised biochemical activity of the PAR-1 family of kinases is to modify microtubule-associated proteins, *C. elegans* PAR-1 provides a precedent for a microtubule-independent function of these kinases in vivo, as the localisation of the P-granules requires the actin cytoskeleton but not microtubules (Strome and Wood, 1983; Guo and Kemphues, 1995a; Drewes et al., 1997).

In contrast to its role early in the germarium, the later function of PAR-1 in the maintenance of oocyte fate could be mediated through an effect on the microtubule cytoskeleton. Although depolymerisation of the microtubules does not disrupt the initial restriction of the SC to one cell, it induces a precocious loss of the SC in region 3 (Huynh and St Johnston, 2000). Thus, microtubules are required to maintain the oocyte in meiosis, and their disruption has an identical effect on the behaviour of the SC to the *par-1* null at this stage. Furthermore, *par-1* mutant clones show a clear defect in the microtubule organisation in region 3. In wild-type cysts, Nod-GFP, which

is thought to be a marker for the minus ends of the microtubules, and the minus end-directed motor, dynein, accumulate at the anterior of the oocyte in region 2b (Li et al., 1994; Clark et al., 1997). They then re-localise to the posterior in region 3, suggesting that there is a switch in the site of microtubule nucleation from anterior to posterior at this stage. This switch does not occur in mutant cysts, however, as Nod-GFP and dynein still localise to the anterior, but fail to translocate to the posterior pole. This reorganisation of the oocyte cytoskeleton in wild type probably accounts for the anterior to posterior movement of Orb protein and the centrosomes at this stage, and these are also abolished in *par-1* mutant clones. Thus, the *par-1* null disrupts a novel step in the determination of the oocyte, during which the minus ends of the microtubules, the centrosomes and cytoplasmic proteins move from the anterior to the posterior of the cell. It is unclear, however, whether this anterior-posterior movement is required for the maintenance of oocyte identity or vice versa.

The re-localisation of cytoplasmic markers in region 3 reveals the earliest known A-P polarity within the oocyte (Pare and Suter, 2000). It is very intriguing that PAR-1 is required both for this polarisation, and the later polarisation of the oocyte at stage 9. This raises the question of whether the two are linked. For example, it is possible that the altered organisation of the microtubules and mislocalisation of *oskar* mRNA at stage 9 in *par-1* hypomorphs is a consequence of earlier problems in the polarisation of the oocyte in the germarium. This seems unlikely, however, for several reasons. First, the localisation of all known markers for oocyte polarity changes during stage 7, suggesting that the early polarisation of the oocyte is erased when it re-polarises in response to a signal from the posterior follicle cells. Second, in the vast majority of egg chambers that are mutant for *par-1* hypomorphs, Orb protein moves normally from the anterior to the posterior of the oocyte, and is maintained there as it is in wild type. Furthermore, the *oskar* mRNA localisation defect of these mutants can be rescued by a *par-1* transgene that is only expressed after the cysts have left the germarium. We therefore favour the view that PAR-1 plays a role in the anterior-posterior polarisation of the oocyte at two stages of oogenesis. It will be interesting to determine whether these processes are related in other ways, and whether PAR-1 serves a common function in oocyte determination and *oskar* mRNA localisation.

We are grateful to Acaimo Gonzalez-Reyes for the Nod-GFP line and to Christine Field, Bill Chia and the DSHB (University of Iowa) for antibodies. This work was supported by the European Community (J.-R. H.), Harvard University (J. M. S.) and the Wellcome Trust (R. B. and D. St J.).

REFERENCES

Brand, A. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Byers, T. J., Dubreuil, R., Branton, D., Kiehart, D. P. and Goldstein, L. S. (1987). *Drosophila* spectrin. II. Conserved features of the alpha-subunit are revealed by analysis of cDNA clones and fusion proteins. *J. Cell Biol.* **105**, 2103-2110.

Carpenter, A. T. (1975). Electron microscopy of meiosis in *Drosophila* melanogaster females. I. Structure, arrangement, and temporal change of the synaptonemal complex in wild-type. *Chromosoma* **51**, 157-182.

Carpenter, A. (1994). Egalitarian and the choice of cell fates in *Drosophila* melanogaster oogenesis. *Ciba Found. Symp.* **182**, 223-246.

Chou, T., Noll, E. and Perrimon, N. (1993). Autosomal P[ovoD1] dominant female-sterile insertions in *Drosophila* and their use in generating germ-line chimeras. *Development* **119**, 1359-1369.

Chou, T.-B. and Perrimon, N. (1996). The autosomal FLP-DFS technique for generating germline mosaics in *Drosophila melanogaster*. *Genetics* **144**, 1673-1679.

Clark, I., Giniger, E., Ruohola-Baker, H., Jan, L. and Jan, Y. (1994). Transient posterior localization of a kinesin fusion protein reflects anteroposterior polarity of the *Drosophila* oocyte. *Curr. Biol.* **4**, 289-300.

Clark, I., Jan, L. and Jan, Y. (1997). Reciprocal localization of Nod and kinesin fusion proteins indicates microtubule polarity in the *Drosophila* oocyte, epithelium, neuron and muscle. *Development* **124**, 461-470.

de Cuevas, M., Lee, J. and Spradling, A. (1996). alpha-spectrin is required for germline cell division and differentiation in the *Drosophila* ovary. *Development* **122**, 3959-3968.

de Cuevas, M. and Spradling, A. C. (1998). Morphogenesis of the *Drosophila* fusome and its implications for oocyte specification. *Development* **125**, 2781-2789.

Deng, W. and Lin, H. (1997). Spectrosomes and fusomes anchor mitotic spindles during asymmetric germ cell divisions and facilitate the formation of a polarized microtubule array for oocyte specification in *Drosophila*. *Dev. Biol.* **189**, 79-94.

Drewes, G., Ebneth, A., Preuss, U., Mandelkow, E. M. and Mandelkow, E. (1997). MARK, a novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption. *Cell* **89**, 297-308.

Drewes, G., Trinczek, B., Illenberger, S., Biernat, J., Schmitt-Ulms, G., Meyer, H. E., Mandelkow, E. M. and Mandelkow, E. (1995). Microtubule-associated protein/microtubule affinity-regulating kinase (p110mark). A novel protein kinase that regulates tau-microtubule interactions and dynamic instability by phosphorylation at the Alzheimer-specific site serine 262. *J. Biol. Chem.* **270**, 7679-7688.

Ebneth, A., Drewes, G. and Mandelkow, E. (1999). Phosphorylation of MAP2c and MAP4 by MARK kinases leads to the destabilization of microtubules in cells. *Cell Motil. Cytoskeleton* **44**, 209-224.

Ephrussi, A., Dickinson, L. and Lehmann, R. (1991). Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. *Cell* **66**, 37-50.

Ephrussi, A. and Lehmann, R. (1992). Induction of germ cell formation by oskar. *Nature* **358**, 387-392.

Field, C. and Alberts, B. (1995). Anillin, a contractile ring protein that cycles from the nucleus to the cell cortex. *J Cell Biol.* **131**, 165-178.

Frohnhöfer, H. G. and Nüsslein-Volhard, C. (1986). Organization of anterior pattern in the *Drosophila* embryo by the maternal gene *bicoid*. *Nature* **324**, 120-125.

Godt, D. and Tepass, U. (1998). *Drosophila* oocyte localisation is mediated by differential cadherin-based adhesion. *Nature* **395**, 387-391.

Goldstein, B. and Hird, S. N. (1996). Specification of the anteroposterior axis in Caenorhabditis elegans. *Development* **122**, 1467-1474.

González-Reyes, A., Elliott, H. and St Johnston, D. (1997). Oocyte determination and the origin of polarity in *Drosophila*: the role of the spindle genes. *Development* **124**, 4927-4937.

González-Reyes, A. and St Johnston, D. (1994). Role of oocyte position in establishment of anterior-posterior polarity in *Drosophila*. *Science* **266**, 639-642.

González-Reyes, A. and St Johnston, D. (1998). The *Drosophila* AP axis is polarised by the cadherin-mediated positioning of the oocyte. *Development* **125**, 3635-3644.

Grieder, N., De Cuevas, M. and Spradling, A. (2000). The fusome organizes the microtubules network during oocyte differentiation in *Drosophila*. *Development* **127**, 4253-4264.

Guo, S. and Kemphues, K. (1995a). par-1, a gene required for establishing polarity in C. elegans embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* **81**, 611-620.

Guo, S. and Kemphues, K. J. (1995b). par-1, a gene required for establishing polarity in C. elegans embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* **81**, 611-620.

Hird, S. N. and White, J. G. (1993). Cortical and cytoplasmic flow polarity in early embryonic cells of Caenorhabditis elegans. *J. Cell Biol.* **121**, 1343-1355.

Hird, S. N., Paulsen, J. E. and Strome, S. (1996). Segregation of germ granules in living Caenorhabditis elegans embryos: cell-type-

specific mechanisms for cytoplasmic localisation. *Development* **122**, 1303-1312.

Huynh, J. and St Johnston, D. (2000). The role of BicD, egl, orb and the microtubules in the restriction of meiosis to the *Drosophila* oocyte. *Development* **127**, 2785-2794.

Illenberger, S., Drewes, G., Trinczek, B., Biernat, J., Meyer, H. E., Olmsted, J. B., Mandelkow, E. M. and Mandelkow, E. (1996). Phosphorylation of microtubule-associated proteins MAP2 and MAP4 by the protein kinase p110mark. Phosphorylation sites and regulation of microtubule dynamics. *J. Biol. Chem.* **271**, 10834-10843.

Kim-Ha, J., Smith, J. L. and Macdonald, P. M. (1991). oskar mRNA is localized to the posterior pole of the *Drosophila* oocyte. *Cell* **66**, 23-35.

Koch, E. A. and Spitzer, R. H. (1983). Multiple effects of colchicine on oogenesis in *Drosophila*: induced sterility and switch of potential oocyte to nurse-cell developmental pathway. *Cell Tiss. Res.* **228**, 21-32.

Kraut, R., Chia, W., Jan, L., Jan, Y. and Knoblich, J. (1996). Role of inscuteable in orienting asymmetric cell divisions in *Drosophila*. *Nature* **383**, 50-55.

Lantz, V., Chang, J., Horabin, J., Bopp, D. and Schedl, P. (1994). The *Drosophila* orb RNA-binding protein is required for the formation of the egg chamber and establishment of polarity. *Genes Dev.* **8**, 598-613.

Li, M., McGrail, M., Serr, M. and Hays, T. S. (1994). *Drosophila* cytoplasmic dynein, a microtubule motor that is asymmetrically localized in the oocyte. *J. Cell Biol.* **126**, 1475-1494.

Lin, H., Yue, L. and Spradling, A. (1994). The *Drosophila* fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation. *Development* **120**, 947-956.

Lin, H. and Spradling, A. (1995a). Fusome asymmetry and oocyte determination in *Drosophila*. *Dev. Genet.* **16**, 6-12.

Lin, H. and Spradling, A. C. (1995b). Fusome asymmetry and oocyte determination in *Drosophila*. *Dev. Genet.* **16**, 6-12.

Mach, J. and Lehmann, R. (1997). An Egalitarian-BicaudalD complex is essential for oocyte specification and axis determination in *Drosophila*. *Genes Dev.* **11**, 423-435.

Mahowald, A. and Strassheim, J. M. (1970). Intercellular migration of the centrioles in the germarium of *Drosophila melanogaster*. *J. Cell Biol.* **45**, 306-320.

McKim, K. S. and Hayashi-Hagihara, A. (1998). mei-W68 in *Drosophila* melanogaster encodes a Spo11 homolog: evidence that the mechanism for initiating meiotic recombination is conserved. *Genes Dev.* **12**, 2932-2942.

Pare, C. and Suter, B. (2000). Subcellular localization of Bic-D::GFP is linked to an asymmetric oocyte nucleus. *J. Cell Sci.* **113**, 2119-2127.

Pokrywka, N. and Stephenson, E. (1995). Microtubules are a general component of mRNA localization systems in *Drosophila* oocytes. *Dev. Biol.* **167**, 363-370.

Ran, B., Bopp, R. and Suter, B. (1994). Null alleles reveal novel requirements for Bic-D during *Drosophila* oogenesis and zygotic development. *Development* **120**, 1233-1242.

Rorth, P. (1998). Gal4 in the *Drosophila* female germline. *Mech. Dev.* **78**, 113-118.

Rose, L. S. and Kemphues, K. J. (1998). Early patterning of the C. elegans embryo. *Annu. Rev. Genet.* **32**, 521-545.

Ruohola, H., Bremer, K., Baker, D., Swedlow, J., Jan, L. and Jan, Y. (1991). Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in *Drosophila*. *Cell* **66**, 433-449.

Schuldt, A. J., Adams, J. H. J., Davidson, C. M., Micklem, D. R., St Johnston, D. and Brand, A. (1998). Miranda mediates the asymmetric protein and RNA localisation in the developing nervous system. *Genes Dev.* **12**, 1847-1857.

Schupbach, T. and Wieschaus, E. (1991). Female sterile mutations on the second chromosome of *Drosophila* melanogaster. II. Mutations blocking oogenesis or altering egg morphology. *Genetics* **129**, 1119-1136.

Schüpbach, T. and Wieschaus, E. (1991). Female sterile mutations on the second chromosome of *Drosophila* melanogaster. II. Mutations blocking oogenesis or altering egg morphology. *Genetics* **129**, 1119-1136.

Shulman, J. M., Benton, R. and St Johnston, D. (2000). The *Drosophila* homolog of C. elegans PAR-1 organizes the oocyte cytoskeleton and directs oskar mRNA localization to the posterior pole. *Cell* **101**, 377-388.

Spradling, A. (1993). Developmental genetics of oogenesis. In *The Development of* Drosophila melanogaster (ed. M. Bate and A. Martinez-Arias), pp. 1-70. New-York: Cold Spring Harbor Laboratory Press.

Spradling, A. C. and Rubin, G. M. (1982). Transposition of cloned P elements into *Drosophila* germ line chromosomes. *Science* **218**, 341-347.

Strome, S. and Wood, W. B. (1983). Generation of asymmetry and segregation of germ-line granules in early C. elegans embryos. *Cell* **35**, 15-25.

Suter, B., Romberg, L. M. and Steward, R. (1989). Bicaudal-D, a *Drosophila* gene involved in developmental asymmetry: localized transcript accumulation in ovaries and sequence similarity to myosin heavy chain tail domains. *Genes Dev.* **3**, 1957-1968.

Suter, B. and Steward, R. (1991). Requirement for phosphorylation and localization of the Bicaudal-D protein in *Drosophila* oocyte differentiation. *Cell* **67**, 917-926.

Theurkauf, W., Smiley, S., Wong, M. and Alberts, B. (1992). Reorganization of the cytoskeleton during *Drosophila* oogenesis: implications for axis specification and intercellular transport. *Development* **115**, 923-936.

Theurkauf, W. E., Alberts, B. M., Jan, Y. N. and Jongens, T. A. (1993). A central role for microtubules in the differentiation of *Drosophila* oocytes. *Development* **118**, 1169-1180.

Theurkauf, W. (1994). Microtubules and cytoplasm organization during Drosophila oogenesis. *Dev. Biol.* **165**, 352-360.

Tomancak, P., Piano, F., Riechmann, V., Gunsalus, K. C., Kemphues, K. J. and Ephrussi, A. (2000). A *Drosophila* melanogaster homologue of Caenorhabditis elegans par-1 acts at an early step in embryonic-axis formation. *Nat. Cell Biol* **2**, 458-460.

Van Doren, M., Williamson, A. L. and Lehmann, R. (1998). Regulation of zygotic gene expression in *Drosophila* primordial germ cells. *Curr. Biol.* **8**, 243-246.

van Eeden, F. and Johnston, D. S. (1999). The polarisation of the anterior-posterior and dorsal-ventral axes during *Drosophila* oogenesis. *Curr. Opin. Genet. Dev.* **9**, 396-404.

Wharton, R. P. and Struhl, G. (1989). Structure of the *Drosophila* BicaudalD protein and its role in localizing the the posterior determinant nanos. *Cell* **59**, 881-892.

Yue, L. and Spradling, A. (1992). hu-li tai shao, a gene required for ring canal formation during *Drosophila* oogenesis, encodes a homolog of adducin. *Genes Dev.* **6**, 2443-2454.

Zaccai, M. and Lipshitz, H. (1996). Role of Adducin-like (hu-li tai shao) mRNA and protein localization in regulating cytoskeletal structure and function during *Drosophila* Oogenesis and early embryogenesis. *Dev. Genet.* **19**, 249-257.