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

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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 (Frohnhofer 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.,
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 asymmetries 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,hs-Flp; UASp-par-1(N1S)-GFP, FRTG13-par-1\(^{W3}\)/FRTG13-GFPnls; nosG4a4-VP16/+;
- y,hs-Flp; UASp-par-1(N1L)-GFP, FRTG13-par-1\(^{W3}\)/FRTG13-GFPnls; nosG4a4-VP16/+;
- par-1 clones that express nod-GFP were obtained by heat-shocking flies of the following genotype:
- y,hs-Flp; FRTG13-par-1\(^{W3}\)/FRTG13-GFPnls; nosG4a4-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 KpnI/BamHI fragment into pUASp (Rorth, 1998). A BamHI/Xhol fragment encoding the PAR-1 N1S isoform was subcloned into the cloning vector pIC20R, and from there as a BamHI/Xhol 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 MluI/Xhol fragment from pICR20 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-\(\gamma\)-Tubulin (Sigma) at 1/100, rabbit and mouse anti-\(\alpha\)-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-Hits (DSHB, Iowa University; Zaccaci and Lipshitz, 1996) at 1/10, mouse anti-\(\alpha\)-Tubulin (Sigma) at 1/500 and anti-PAR-1 at 1/5000.
PAR-1 and oocyte determination

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 pretreated 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 ovoD technique to generate clones of par-1W3, 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 ovoD 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 germ lines 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
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 of the germarium (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 meiosis 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. 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.

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 shows that the minus ends are focussed on the anterior of the oocyte, before switching to the posterior as the cyst buds off the gerarium (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.

**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-1W3 germ line clones (Brand and Perrimon, 1993; Rorth, 1998; Van Doren et al., 1998). The fusion protein localises to a branched structure in regions 1 and
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-1W3 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-1W3 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-1W3 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.

**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,
because the precise pattern of cyst divisions is disrupted (Lin et al., 1994; de Cuevas et al., 1996). All par-1* null 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 spectosome and the fusome at all stages of its development (Deng and Lin, 1997; de Cuevas and Spradling, 1998). The spectosome 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* 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 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 germlarial 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 in region 3. 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 gerarium. 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 gerarium. 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.

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REFERENCES


specific mechanisms for cytoplasmic localization. Development 122, 1303-1312.


