The Drosophila AP axis is polarised by the cadherin-mediated positioning of the oocyte

Acaimo González-Reyes* and Daniel St Johnston

The Wellcome/CRC Institute and Department of Genetics, University of Cambridge, Cambridge CB2 1QR, UK
*Author for correspondence (e-mail: agr@mole.bio.cam.ac.uk)

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SUMMARY

The anterior-posterior axis of Drosophila originates from two symmetry-breaking steps during early oogenesis. First, one of the two pro-oocytes within the cyst of 16 germine cells is selected to become the oocyte. This cell then comes to lie posterior to the other germine cells of the cyst, thereby defining the polarity of the axis. Here we show that the oocyte reaches the posterior of the cyst in two steps. (1) The cyst flattens as it enters region 2 of the germarium to place the two pro-oocytes in the centre of the cyst, where they contact the posterior follicle cells. (2) One cell is selected to become the oocyte and protrudes into the posterior follicle cell layer when the cyst rounds up on entering region 3. During this germ cell rearrangement, the components of the homophilic cadherin adhesion complex, DE-cadherin, Armadillo and α-catenin, accumulate along the border between the oocyte and the posterior follicle cells. Furthermore, the positioning of the oocyte requires cadherin-dependent adhesion between these two cell types, since the oocyte is frequently misplaced when DE-cadherin is removed from either the germline or the posterior follicle cells. We conclude that the oocyte reaches the posterior of the germine cyst because it adheres more strongly to the posterior follicle cells than its neighbours during the germ cell rearrangement that occurs as the cyst moves into region 3. The Drosophila anterior-posterior axis therefore becomes polarised by an unusual cadherin-mediated adhesion between a germ cell and mesodermal follicle cells.

Key words: Drosophila, AP polarity, Cadherin adhesion, Axis, Oocyte

INTRODUCTION

The anterior-posterior axis of the Drosophila embryo is defined by the localisation of maternal determinants within the oocyte (St Johnston and Nüsslein-Volhard, 1992). bicoid mRNA localises to the anterior pole and is translated after fertilisation to give rise to a morphogen gradient of Bicoid protein that patterns the head and thorax (Berleth et al., 1988; Driever and Nüsslein-Volhard, 1989a,b; Driever, 1993). Similarly, oskar mRNA localises to the posterior pole of the oocyte where it directs the assembly of the pole plasm, which contains the posterior and gerline determinants (Ephrussi et al., 1991; Kim-Ha et al., 1991; Ephrussi and Lehmann, 1992). The polarity of the anterior-posterior axis therefore requires the correct targeting of these two mRNAs to opposite poles of the oocyte, a process that depends on a series of steps during early oogenesis that lead to the polarisation of the oocyte cytoskeleton.

Oogenesis begins when a germline cystoblast divides four times to produce a cyst of 16 germ cells that are connected by cytoplasmic bridges called ring canals (Mahowald and Kambsyellis, 1980). In region 2 of the germarium, the two cells with 4 ring canals become pro-oocytes and enter meiosis (Koch et al., 1967; Rasmussen, 1974; Carpenter, 1975). One of these cells is then selected to become the oocyte and accumulates oocyte-specific markers such as BicD, Orb, Cup and Egl proteins, while the losing pro-oocyte reverts to the nurse cell pathway of development along with the other 14 germ cells in the cyst (Lantz et al., 1994; Ran et al., 1994; Keyes and Spradling, 1997; Mach and Lehmann, 1997). As the cyst moves down the germarium, the oocyte comes to lie posterior to the other 15 germ cells to generate an anterior-posterior asymmetry that polarises this axis for the rest of development (Lehmann, 1995). At the same time, somatic follicle cells migrate to surround the cyst and to form a short stalk that separates it from the adjacent cysts (Fig. 1).

Once the cyst has left the germarium, the follicle cell layer becomes subdivided into symmetric populations of terminal follicle cells at each end of the egg chamber, separated by a population of main-body follicle cells in between (González-Reyes and St Johnston, 1998). The oocyte then signals through the Gurken/Egfr pathway to induce the adjacent terminal follicle cells to adopt a posterior rather than an anterior fate (González-Reyes and St Johnston, 1994; González-Reyes et al., 1995; Roth et al., 1995). This polarisation of the follicle cell epithelium is transmitted back to the germline during stage 7 of oogenesis, when the posterior follicle cells produce an unknown signal that induces the formation of a polarised microtubule network within the oocyte (Ruo-hola et al., 1991; Theurkauf et al., 1992; Clark et al., 1994, 1997). Because the localisation of bicoid and oskar mRNAs is microtubule-dependent, this cytoskeletal reorganisation directs bicoid
mRNA to the anterior of the oocyte and oskar mRNA to the posterior, thereby defining the polarity of the anterior-posterior axis of the embryo (Pokrywka and Stephenson, 1991; Clark et al., 1994).

Although anterior-posterior polarity arises from the positioning of the oocyte posterior to the nurse cells, very little is known about how the oocyte reaches this position. However, a number of genes have been shown to be required for this process. For example, mutants in dicephalic, vasa and five spindle loci give rise to egg chambers in which the oocyte often lies in the middle of the cyst with nurse cells on either side (Lohs-Schardin, 1982; Gillespie and Berg, 1995; González-Reyes et al., 1997; Styhler et al., 1998; Tomancak et al., 1998). In the case of the spindle loci, the misplacement of the oocyte has been suggested to be an indirect consequence of a defect earlier in oogenesis. In double mutant combinations, spindle mutants cause a long delay in the choice between the two pro-oocytes (González-Reyes et al., 1997). The bipolar phenotype in the single spindle mutants might therefore result from a shorter delay in oocyte determination, as it seems likely that the selection of the oocyte is a prerequisite for its posterior localisation.

The only other candidates for proteins involved in oocyte positioning are the components of the DE-cadherin adhesion complex. Cadherins are large transmembrane glycoproteins that mediate calcium-dependent homophilic adhesion between cells of the same type (Takeichi, 1995). In addition to cadherin itself, the formation of a functional adherens junction requires β-catenin, which binds to the cytoplasmic tail of cadherin, and α-catenin, which binds to β-catenin to link this complex to the actin cytoskeleton (McCrea et al., 1991; Rimm et al., 1995; Aberle et al., 1996). The Drosophila homologues of β-catenin and E-cadherin are encoded by the armadillo and shotgun genes and lethal mutations have been identified at both loci (Peifer and Wieschaus, 1990; Peifer, 1993; Tepass et al., 1996; Uemura et al., 1996). When the germline is mutant for armadillo, the oocyte is often found in the wrong position in late-stage egg chambers (Peifer et al., 1993). Furthermore, misplaced oocytes have also been observed in egg chambers that contain germline clones for a strong allele of shotgun, although this phenotype was not seen in clones for a different allele (Oda et al., 1997; White et al., 1998). Both armadillo and shotgun mutants cause a number of other defects in oogenesis, such as cell fusions, a disorganisation of the actin cytoskeleton and changes in cell shape, and these may result in a failure to maintain the oocyte at the posterior as the egg chamber grows. Alternatively, cadherin-dependent adhesion may play a direct role in the initial localisation of the oocyte to the posterior of the cyst early in oogenesis.

To distinguish between these possibilities, we have examined how the oocyte reaches the posterior of the germ line cyst and have analysed the role of cadherin-dependent adhesion in this process. Our results reveal that the positioning of the oocyte occurs in two steps, both of which require cadherin-dependent adhesion, but in different ways.

MATERIALS AND METHODS

Fly stocks

shotgun (shg) alleles used in this study are shg^G29, shg^H1, shg^P34.1 and shg^G119 (Nüsslein-Volhard et al., 1984; Tepass et al., 1996). shg^P34.1 and shg^G119 are hypomorphs, whereas shg^G29 and shg^H1 are putative nulls which produce no protein that can be detected with the DCAD1 and DCAD2 antibodies (Oda et al., 1994). In our hands, however, shg^G29 shows a stronger phenotype than shg^H1. The armadillo (arm) allele used is arm^K22 (Wieschaus et al., 1984). Other mutants used are eg^WUS^0, a putative null (Schüpbach and Wieschaus, 1991; Mach and Lehmann, 1997), and spn-C094 (Tearle and Nüsslein-Volhard, 1987).

Germline clones were generated using the DFS technique (Chou et al., 1993). shg^G29 or shg^H1 follicle cell clones of were generated using the FLP/FRT technique (Xu and Rubin, 1993).

Staining procedures

Antibody, daunomycin and rhodamine-phalloidin stainings were performed according to standard procedures. Antibodies were used at the following concentrations: anti-BicD (Suter and Stewart, 1991), 1/10; anti-orb (Lantz et al., 1994), 1/20; anti-cup (Keyes and Spradling, 1997), 1/200; anti-DE-cad (Oda et al., 1994), 1/10 (a 1:1 mixture of both DCAD antibodies 1/20 each); anti-te-catenin (Oda et al., 1993), 1/20; anti-arm (Riggelman et al., 1990), 1/100; anti-myc (Oncogene Science), 1/100. FITC- and Texas Red-Conjugated secondary antibodies (Jackson) were used 1/200.

RESULTS

To gain a better understanding of how the oocyte reaches the posterior, we first examined the arrangement of the germ cells in wild-type cysts as they move down the gerarium, in relation to the determination of the oocyte as revealed by Bic-D staining (Fig. 1A,B). In region 2a, the cysts already contain 16 germ cells and form clusters that are several cells thick and extend only part way across of the gerarium. At this stage, Bic-D protein is usually concentrated in the two adjacent pro-oocytes which occupy random positions within the cyst. These two cells each have 4 ring canals and are connected to each other by the oldest ring canal, which can be distinguished from the others because it stains more strongly with Rhodamine-Phalloidin. When the cyst enters region 2b, it spreads to form a 1-cell-thick disc that extends across the width of the gerarium. Bic-D protein has now accumulated in only one of the pro-oocytes, the presumptive oocyte, but the two cells with 4 ring canals occupy equivalent positions in the middle of the cyst. At the same time, the somatic follicle cells start to migrate to surround the cyst, and accumulate at its posterior to separate it from the preceding cyst.

As the cyst moves down the gerarium from region 2b to region 3, the germ cells rearrange to change the shape of the cyst from a flattened disc to a sphere. At the end of this reorganisation, the oocyte always lies at the posterior of the cyst and protrudes from the sphere of germ cells into the surrounding follicle cell layer (Figs 1, 3A). Thus, the oocyte reaches the posterior during the transition from region 2b to region 3, in a process that seems to involve an interaction between the oocyte and the adjacent follicle cells.

It has previously been reported that the arrangement of germ cells in region 2b is unaffected by egalitarian and Bic-D mutants that cause all 16 germ cells to adopt the same fate (Carpenter, 1994; Ran et al., 1994). As in wild type, egalitarian mutant cysts flatten to extend across the width of the gerarium in region 2b, and then round up to form a sphere as they move into region 3 (Fig. 2A-C). However, none of the
germ cells protrude into the follicle cell layer in region 3. Although Bic-D protein does not accumulate in either of the two cells with 4 ring canals in this mutant, the position of these cells can be determined by staining with Rhodamine-Phalloidin to detect the ring canal that connects them. These two cells always occupy their normal position in the centre of the cyst in region 2b. Thus, the invariant arrangement of germ cells at this stage is independent of pro-oocyte or oocyte determination, and seems to depend on the pattern of ring canals connecting the germ cells. During the transition to region 3, at least one of the two cells with 4 ring canals comes to lie at the posterior of the cyst in 86% of cases (n=71) (Fig. 2B). The initial organisation of the germ cells in region 2b therefore seems to bias the cell rearrangement that occurs during the transition to region 3, so that the two pro-oocytes are more likely to end up at the posterior of the cyst than the other 14 cells.

To further investigate the relationship between oocyte determination and positioning, we also examined germaria that were homozygous for spn-C094, the spindle allele that produces the most penetrant oocyte localisation defect (González-Reyes et al., 1997). In contrast to wild-type cysts, Bic-D protein is often still enriched in both pro-oocytes in region 2b, but the protein is usually restricted to a single cell by the time the cyst reaches region 3 (Fig. 2D). Thus, this single spindle mutant causes a similar but shorter delay in oocyte determination to that seen in the double mutants.

**Fig. 1.** Posterior positioning of the oocyte during germlarial stages. (A) Optical sections of wild-type germaria stained with anti-orb antibody (green) to show the arrangement of the oocyte and nurse cells, and rhodamine-phalloidin (red) to label F-actin. As the germline cyst moves from region 2a to region 2b, it flattens to form a 1-cell-thick disc that extends across the width of the germarium. At the same time, the somatic follicle cells migrate to surround the cyst. The oocyte becomes localised to the posterior during the transition from region 2b to region 3. During this process the nurse cells re-arrange to form a sphere, while the oocyte protrudes into the follicle cell layer at the posterior. This protrusion coincides with the formation of a stalk of follicle cells which interleaf and contract to separate region 3 egg chambers from the germarium (bracket). (B) Diagram of the steps in the positioning of the oocyte at the posterior and the events in oocyte determination. Oocyte-specific markers such as BicD (Suter and Steward, 1991), Orb (Lantz et al., 1994) or Cup (Keyes and Spradling, 1997) proteins accumulate in the two pro-oocytes in early region 2a cysts (shaded green) and in the presumptive oocyte in region 2b (green cells). The ring canal connecting the two 4-ring-canal cells is shown in red. The follicle cells are drawn in red.

**Fig. 2.** Oocyte determination is not required for the correct positioning of the oocyte in germlarial region 2b. (A,C) egl^{WU50} germaria stained with actin to label the cell outlines and the ring canals. As egl^{-} cysts do not develop an oocyte, we use the position of the largest and brightest ring canal of each cyst (arrowheads) to follow the positions of the two cells with 4 ring canals. (A) In all of the egl^{-} region 2b cysts, the two 4-ring-canal cells occupy a central position (top, open arrowhead). In the majority of region 2b cysts, one of the cells with 4 ring canals remains at the posterior of the cyst (middle and bottom open arrowheads, respectively). (B) Diagram of the germarium shown in (A). Only the positions of the two 4-ring-canal cells and their common ring canal are shown. (C) Germaria containing region 2b/3 and region 3 cysts, one of the cells with 4 ring canals remains at the posterior of the cyst (middle and bottom open arrowheads, respectively). (D) spn-C^{094} germaria stained with anti-Orb to show the delay in oocyte determination and the misplacement of the oocyte. The region 2b cyst shows the persistence of Orb protein in both pro-oocytes. In spite of this delay in oocyte determination, the two pro-oocytes still lie in the centre of the cyst. By region 3, Orb has accumulated in a single cell, the oocyte, but this has not been positioned at the posterior.
Despite the correct determination of the oocyte, this cell is not correctly positioned in 32% ($n=118$) of the cysts. These observations indicate that the oocyte needs to be specified by region 2b to guarantee that it is correctly positioned. This suggests that the oocyte plays an active role in its positioning during the germ cell rearrangement that occurs as the cyst enters region 3, and that this is essential to ensure that the oocyte rather than the losing pro-oocyte comes to lie at the posterior of the cyst.

**DE-cadherin is required at two stages of oocyte positioning**

The phenotypes produced by *armadillo* and *shotgun* mutant germline clones have suggested that DE-cadherin-mediated adhesion may be required either to localise the oocyte to the posterior of the cyst or to maintain it in this position as the oocyte enters region 3, and that this is essential to ensure that the oocyte rather than the losing pro-oocyte comes to lie at the posterior of the cyst.

**Table 1. Lack of cadherin-mediated adhesion in the germline produces misplaced oocytes**

<table>
<thead>
<tr>
<th>Frequency of misplaced oocytes in germline clones of ($%$ ($n$)):</th>
<th>$shg^{119}$</th>
<th>$shg^{P34-1}$</th>
<th>$shg^{HI}$</th>
<th>$shg^{G29}$</th>
<th>$arm^{X22}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 (76)</td>
<td>18 (44)</td>
<td>46 (76)</td>
<td>73 (103)</td>
<td>47.5 (196)</td>
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In addition to producing a very high frequency of misplaced oocytes in region 3, the mutants of the *shotgun* and *armadillo* disrupt the organisation of the germ cells earlier in the germarium (Fig. 3D). The mutant cysts do not flatten in region 2b to form the 1-cell-thick disc that extends across the width of the germarium, and remain the same shape as region 2a cysts.

**shotgun mutants do not affect oocyte determination**

Most known mutants that affect oocyte positioning seem to do so indirectly by disrupting the determination and differentiation of the oocyte. This does not appear to be case for *shotgun* and *armadillo* mutants, however, since all of the markers for oocyte differentiation that we have examined are expressed normally in the misplaced oocytes produced by germline clones. For example, all *spindle* mutants show a highly penetrant defect in the formation of the karyosome, which is the hollow sphere of chromatin that forms when the meiotic chromosomes of the oocyte condense. In contrast, the karyosome always appears normal in *shotgun* and *armadillo* mutant oocytes (Fig. 4A,B, and data not shown). Similarly, mutant oocytes show a wild-type subcortical enrichment of actin and cytoplasmic accumulation of Bic-D protein (Fig. 4C-F). Since these mutants disrupt the positioning of the oocyte without affecting its differentiation, cadherin-mediated adhesion seems to play a direct role in the localisation of the oocyte to the posterior of the cyst.
The components of the DE-cadherin adhesion complex become transiently enriched at the boundary between the oocyte and posterior follicle cells

Two models could explain the requirement for DE-cadherin and Armadillo during the positioning of the oocyte. Cadherin adhesion could be upregulated in all germ cells except the oocyte, causing these cells to undergo a process of compaction that extrudes the oocyte from the posterior. Alternatively, cadherin adhesion could be upregulated in the oocyte, allowing it to remain attached to the posterior follicle cells as the cyst changes shape. Although DE-cadherin becomes enriched in the most anterior follicle cells, the highest levels are seen along the boundary between the oocyte and the posterior follicle cells as the cyst moves from region 2b to region 3 (Fig. 5A). This accumulation disappears once the cyst has left the germarium, however, although the two polar follicle cells continue to express higher levels of DE-cadherin throughout oogenesis (Fig. 5B). Furthermore, Armadillo and α-catenin show an identical transient concentration at the junction between the oocyte and the posterior follicle cells (Fig. 5D-F). The co-localisation of three components of the cadherin adhesion complex to this boundary strongly supports the second model, in which the localisation of the oocyte is driven by an increase in cadherin-dependent adhesion between the oocyte and these specific somatic cells.

To determine the relative contributions of the oocyte and the follicle cells to this posterior enrichment, we also examined the distribution of DE-cadherin in egg chambers that contain shotgun mutant germline clones. Despite the lack of DE-cadherin in the germline and the mispositioning of the oocyte, the protein is still concentrated at the posterior of these cysts in the follicle cell membranes that face the germ cells (Fig. 5C). DE-cadherin therefore accumulates in these apical membranes, even when these cells do not contact the oocyte and there is no DE-cadherin in the adjacent germ cells. Furthermore, an identical enrichment is observed in egalitarian

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**Fig. 4.** DE-cadherin is not required for oocyte determination. (A,C,E) Wild-type egg chambers; (B,D,F) shgIG29 germline clones. The misplaced oocytes of the chimaeric egg chambers are properly determined, as shown by the formation of the karyosome (arrowhead) (A,B), by the subcortical enrichment of actin in the oocyte (C,D), and by the normal accumulation of BicD protein in a single cell, the misplaced oocyte (E,F).

**Fig. 5.** DE-cadherin, Armadillo and α-catenin proteins concentrate at the boundary between the oocyte and the posterior follicle cells. (A,B) Wild-type ovariole stained with anti-DE-cad antibodies. (A) DE-cadherin protein is expressed in the membranes of germline cells and in the adhesive junctions of the follicle cells from germarial region 2 onwards. Its levels of expression are higher in the area of contact between the oocyte and the follicle cells at the posterior pole in region 2b and region 3 cysts, and in the apical side of the follicle cells forming the stalk in region 3 (arrowheads). (B) This high level of DE-cadherin staining at the posterior is lost in older egg chambers. (C) shgIG29 germline clone stained with anti-DE-cad antibodies (red) and anti-BicD (green). Since the germ cells in this germarium lack DE-cadherin, this staining reveals the contribution of the follicle cells to the pattern of expression of DE-cadherin. Note that the enrichment of DE-cadherin in the posterior follicle cells of region 2b and region 3 cysts is still present. This enrichment is not induced directly by the oocyte, as shown in this particular case where the oocyte is misplaced. (D,E) Wild-type ovariole stained with anti-Arm antibody. Like DE-cadherin, the expression of Armadillo is higher in the area of contact between the oocyte and the polar follicle cells during germarial stages, but not in older egg chambers. (F) Wild-type germarium stained with anti-α-catenin antibody showing a similar pattern of expression to DE-cadherin. Asterisks, oocytes.
The positioning of the oocyte is disrupted when the posterior follicle cells lack DE-cadherin

If cadherin-dependent adhesion between the oocyte and the posterior follicle cells is responsible for the localisation of the oocyte, the position of the oocyte should also be disrupted when DE-cadherin is removed from these follicle cells, a prediction that can be tested by generating shotgun mutant clones in the follicle cell layer. Since DE-cadherin is required for the formation and maintenance of epithelia in the embryo (Tepass et al., 1996; Uemura et al., 1996), we first examined whether shotgun clones could participate in the formation of the follicular epithelium. Surprisingly, we recovered chimaeric egg chambers that contain large clones of the follicular epithelium. Thus, the loss of cadherin-dependent adhesion does not cause any obvious defects in the migration or epithelial organisation of the follicle cell layer. Interestingly, these clones do show two more subtle defects. Firstly, DE-cadherin is not localised to the lateral membranes of wild-type follicle cells where these cells contact mutant cells. Thus, the recruitment of DE-cadherin to the lateral membrane requires homophilic adhesion to DE-cadherin in the adjacent cells. Secondly, the mutant clones have straight boundaries, suggesting that the loss of cadherin-mediated adhesion causes these cells to sort out from their wild-type neighbours.

Since the formation of the follicular epithelium around the germline cysts is not disrupted by shotgun mutant clones in the follicle cell layer, we next examined whether shgIH and shgIG29 clones affect the arrangement of the germ cells within the cyst. In both cases, the oocyte is misplaced in 23% (n=35) of the cysts where the mutant clone includes the follicle cells at the posterior of the cyst, whereas the oocyte always reaches the correct position when the clone includes only lateral or anterior cells (Fig. 6C,D). These results are consistent with the model in which the oocyte is positioned at the posterior of the cyst by cadherin-dependent adhesion between the oocyte and the posterior follicle cells.

In addition, we noticed that the position of the misplaced oocytes is not random in cysts that are surrounded by both wild-type and mutant follicle cells. In 7/7 cases that we have examined, the oocyte lies adjacent to the wild-type follicle cells, even though these cells lie at some distance from the posterior pole (Fig. 6D,E). The strong bias in the position of the misplaced oocytes indicates that the oocyte has a higher affinity than the nurse cells for the cadherin-positive follicle cells, which suggests that cadherin-dependent adhesion is upregulated in the oocyte.

**DE-cadherin localises around the ring canals of region 2 germline cysts**

Germline clones of the strongest shotgun allele, shgIG29, disrupt the arrangement of the germ cells in region 2b cysts and produce a significantly higher frequency of misplaced oocytes than posterior follicle cell clones of the same allele. This suggests that cadherin-dependent adhesion is required for

![Fig. 6](image)
the flattening of the cyst as it moves from region 2a to 2b. Moreover, the difference between the phenotypes produced by germ line and follicle cell clones suggests that this requirement does not involve adhesion between the follicle cells and the germ cells, but between the germ cells themselves. We therefore examined the distribution of DE-cadherin at these stages in germaria that were also stained with Rhodamine-Phalloidin to reveal the positions of the ring canals. In all region 2 cysts, DE-cadherin can be detected around each ring canal in a circle that is about twice the diameter of the ring canal itself (Fig. 7). By the time that the cysts enter region 3, however, this localisation has almost completely disappeared. Because the ring canals connect the germ cells within a cyst, this transient concentration of DE-cadherin most probably reflects the accumulation of DE-cadherin at the sites of adhesion between these germ cells and is consistent with a model in which cadherin-dependent adhesion between the germ cells is required for the flattening of the cyst as it enters region 2b.

This second germline-dependent role for cadherin probably accounts for the very high frequency of misplaced oocytes produced by shg	extsuperscript{G29} germ line clones (Table 1). The flattening of the cyst is a prerequisite for the placement of the two pro-oocytes in the centre of the cyst where they contact the posterior follicle cells. Since the altered morphology of the mutant 2b cysts changes the position of these cells, this should significantly reduce their chance of reaching the posterior of the cyst in region 3.

DISCUSSION

The results presented in this paper lead us to propose a two-step model for the positioning of the oocyte at the posterior of the germ line cyst. In the first step, the 16-cell germ line cyst moves from region 2a of the germlarium into region 2b, and flattens to form a 1-cell-thick disc that extends across the whole width of the germarium. As a consequence of this change in the shape of the cyst, the germ cells become arranged in a stereotypic pattern in which the two pro-oocytes always lie in the centre of the cyst. This establishes the necessary preconditions for the second step in this process, by placing the two pro-oocytes in contact with the follicle cells that migrate to surround the posterior of the cyst.

Although the two cells with 4 ring canals occupy equivalent positions in the centre of the cyst in region 2b, one of these cells has already been selected to become the oocyte, and this triggers the second step in oocyte positioning. The determination of the oocyte appears to cause an increase in the cadherin-dependent adhesiveness of this cell, since the oocyte outcompetes the nurse cells for adhesion to cadherin-positive follicle cells. At the same time, the follicle cells that surround the posterior of the cyst also upregulate cadherin adhesion by expressing more DE-cadherin, Armadillo and α-catenin in their apical membranes, which face the germ cells. Because of these independent increases in adhesiveness, the posterior follicle cells adhere more strongly to the oocyte than to the other germ cells, and these two cell types therefore maximise their area of mutual contact, causing the oocyte to protrude into the follicle cell layer. Indeed, this increase in adhesion may account for the earlier observation that, in electron micrographs, the membrane between the oocyte and these follicle cells shows undulations, whereas that between the nurse cells and the follicle cells is smooth (Koch and King, 1969). This differential adhesion localises the oocyte to the posterior of the cyst and anchors it in this position as the germ cells rearrange during the transition to region 3. The initial asymmetry that defines the polarity of the anterior-posterior axis in Drosophila therefore originates from homophilic adhesion between the oocyte and posterior follicle cells.

In addition to its role in the second step of oocyte positioning, our results indicate that cadherin-dependent adhesion is also required for the flattening of the cyst in region 2b, since this does not occur in shg	extsuperscript{G29} germ line clones. The invariant arrangement of germ cells that is generated by this flattening is unaffected by Bic-D (Ran et al., 1994), egl and spn-C mutants, indicating that it is independent of pro-oocyte or oocyte determination. The position of the pro-oocytes must therefore depend on some other distinguishing feature, which is most probably that these are the two cells with 4 ring canals. The striking localisation of DE-cadherin around the ring canals as the cyst flattens suggests that the positions of the ring canals determine the site of adhesion between the germ cells. This connection between the adhesion that contributes to the flattening of the cyst and the ring canals may explain why the location of the pro-oocyte depends on the number of ring canals that they possess. The arrangement of the germ cells in region 2b is usually sufficient on its own to result in the positioning of one of the two cells with 4 ring canals at the

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**Fig. 7.** DE-cadherin is localised around the ring canals. Wild-type germarium double stained to visualise filamentous actin (A) and DE-cadherin (B). DE-cadherin is localised around the ring canals in region 2 cysts, but this staining is lost in region 3 egg chambers. (C) Merged image.
posterior of the cyst when it rounds up in region 3. It is the specific adhesion between the oocyte and the posterior follicle cells, however, that ensures that it is the oocyte rather than the losing pro-oocyte that reaches the posterior 100% of the time. These observations may explain why most mutants that disrupt oocyte positioning do so with a penetrance of less than 50%. Unlike the shg

 mutant, which disrupts both steps in oocyte positioning and produces a high frequency of misplaced oocytes, these mutants do not affect the flattening of the cyst and the oocyte should therefore reach the posterior by chance in about half of the egg chambers.

The cadherin family of adhesion molecules generally mediate homophilic adhesion between cells of the same type, and this is also the case for the DE-cadherin-dependent adhesion between the germ cells during the flattening of the cyst in region 2b. The differential adhesion between the oocyte and the posterior follicle cells that occurs at the next stage is quite different, however, because these two cell types are completely unrelated and arise from separate lineages that are set aside at the earliest stages of embryogenesis. The oocyte is descended from the pole cells, which are the primordial germ cells that form at the posterior of the embryo about one and a half hours after fertilisation, whereas the follicle cells arise from the gonadal mesoderm. This role for cadherin in heterotypic adhesion is very unusual, but not entirely without precedent. In mammals, E-cadherin has been shown to mediate adhesion between Langerhans cells and keratinocytes, while N-cadherin contributes to the attachment between developing spermatocytes and the Sertoli cells of the testis (Newton et al., 1993; Tang et al., 1993). It is interesting to note that the latter example also involves adhesion between germline and somatic cells.

A second interesting feature of the interaction between the oocyte and the posterior follicle cells concerns the regulation of the subcellular distribution of DE-cadherin. After the egg chambers leave the gerarium, DE-cadherin accumulates at the sites of contact between the follicle cells, where it participates in the formation of typical adherens junctions. When wild-type follicle cells contact shotgun mutant clones, however, the protein does not accumulate in the membrane facing the mutant cells, indicating that the recruitment of DE-cadherin requires homophilic adhesion to the cadherin in the neighbouring cell. In contrast, the accumulation of DE-cadherin in the apical membranes of the posterior follicle cells does not depend on homophilic adhesion to the adjacent germ cells, because it occurs normally when the germ line lacks DE-cadherin. Thus, the protein must be localised to this apical membrane domain by a cell-intrinsic process. Indeed, it is an essential feature of our model for oocyte positioning that both the oocyte and the posterior follicle cells increase their cadherin-dependent adhesiveness independently of each other, since this allows each cell type to select the appropriate partner when it is surrounded by a group of less adhesive cells.

Although cadherin-dependent adhesion is upregulated in the follicle cells by increasing the amount of protein in the apical membrane, it is less clear why the oocyte is more adhesive. Indeed, we only have indirect evidence for this increase in adhesiveness, based on the observation that the oocyte outcompetes the nurse cells for adhesion to the DE-cadherin-positive follicle cells. Because the follicle cells express more DE-cadherin than the germ cells, and it is not possible to distinguish in the light microscope between staining in the germ cell or follicle cell membranes, we have not been able to determine whether the oocyte expresses higher levels of DE-cadherin protein than the nurse cells. There is, however, no obvious enrichment of DE-cadherin, armadillo or a catenin mRNA in the oocyte (data not shown). This suggests that DE-cadherin-mediated adhesion is upregulated in the oocyte post-transcriptionally, but this could occur in a number of different ways. For example, the translation of the mRNA could be higher in the oocyte, more protein could be targeted to the correct membrane domain, or the activity of the adhesion complex could be increased through the modification of one of its components.

One important question raised by our results is how the posterior follicle cells that upregulate DE-cadherin are selected, since it is the position of these cells that determines where the oocyte becomes localised. The posterior cells that adhere to the oocyte do not seem to correspond to the polar follicle cells, a pair of distinct cells at each pole of the egg chamber that form a separate lineage from the rest of the follicle cell layer (Margolis and Spradling, 1995). Firstly, the pola r cells can only be distinguished from the other follicle cells after the cyst has left the gerarium, when the expression of polar follicle cell markers such as Fasciclin III first becomes restricted to these two cells (Brower et al., 1981; Ruohola et al., 1991). Secondly, more than two cells express higher levels of DE-cadherin in their apical membranes as the cyst moves from region 2b to region 3 of the gerarium. Indeed, in a reconstruction of a region 3 egg chamber from serial electron micrographs, Koch and King (1969) describe that ‘the oocyte has come to lie in a pocket formed by eight cuboidal follicle cells’. Thus, the posterior cells that adhere to the oocyte constitute a population that includes the precursors of the polar cells, but also contains several additional cells. At the moment, we can only speculate about how these cells are specified, but a possible model is suggested by their position. These are the only follicle cells that contact both the follicle cells that will form the stalk between adjacent cysts and the germline cells, and these two cues may act together to induce the upregulation of DE-cadherin.

All previous results have indicated that the follicle cells at the anterior and posterior of the egg chamber are equivalent during early oogenesis, and only become different when Gurken signals to induce the cells adjacent to the oocyte to adopt a posterior fate (González-Reyes and St Johnston, 1994; 1998; González-Reyes et al., 1995; Roth et al., 1995). Indeed, DE-cadherin, Armadillo and a catenin also accumulate in the follicle cells at the anterior of the cyst, although this enrichment is less striking than that seen at the posterior. This raises two important questions. Firstly, why does the oocyte always end up at the posterior of the cyst in wild-type germaria, and never at the anterior? Secondly, what generates the asymmetric expression of the components of the DE-cadherin adhesion complex if the anterior and posterior follicle cells are equivalent? One possible answer is suggested by the observation that the follicle cells migrate to surround the posterior of the cyst before they surround the anterior. This temporal difference might lead to earlier and higher expression of DE-cadherin in the posterior cells, which could result in the oocyte adhering preferentially to these cells. It is also possible to envisage a number of alternative mechanisms that direct the
oocyte to the posterior, and the resolution of this question will be essential to an understanding how anterior-posterior polarity arises in Drosophila.

CONCLUSIONS

We have shown that the anterior-posterior axis of Drosophila becomes polarised as a result of two DE-cadherin-dependent steps that lead to the positioning of the oocyte at the posterior of the germine cyst. Firstly, homotypic adhesion between the germ cells is required for the flattening of the germine cyst in region 2b that places the two pro-oocytes in contact with the posterior follicle cells. Secondly, DE-cadherin mediates an unusual heterotypic adhesion between the oocyte and these follicle cells, and this anchors the oocyte at posterior of the cyst as the germ cells rearrange during the transition to region 3.

It has been demonstrated in vitro that cells expressing different levels of the same cadherin molecule sort out from each other, leading to the proposal that morphogenesis can be driven by quantitative differences in the activity of a single adhesion molecule (Friedlander et al., 1989; Steinberg and Takeichi, 1994). Since the specificity of the adhesion between the oocyte and the posterior follicle cells seems to depend on the independent increase in the DE-cadherin-dependent adhesiveness of both cell types, our results suggest that this mechanism is responsible for the positioning of the oocyte, providing the first in vivo example of a cell-sorting event that is mediated by differential cadherin-dependent adhesion.

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