Patterning of the follicle cell epithelium along the anterior-posterior axis during Drosophila oogenesis

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SUMMARY

Gurken signals from the oocyte to the adjacent follicle cells twice during Drosophila oogenesis; first to induce posterior fate, thereby polarising the anterior-posterior axis of the future embryo and then to induce dorsal fate and polarise the dorsal-ventral axis. Here we show that Gurken induces two different follicle cell fates because the follicle cells at the termini of the egg chamber differ in their competence to respond to Gurken from the main-body follicle cells in between. By removing the putative Gurken receptor, Egfr, in clones of cells, we show that Gurken signals directly to induce posterior fate in about 200 cells, defining a terminal competence domain that extends 10-11 cell diameters from the pole. Furthermore, small clones of Egfr mutant cells at the posterior interpret their position with respect to the pole and differentiate as the appropriate anterior cell type. Thus, the two terminal follicle cell populations contain a symmetric prepattern that is independent of Gurken signalling. These results suggest a three-step model for the anterior-posterior patterning of the follicular epithelium that subdivides this axis into at least five distinct cell types. Finally, we show that Notch plays a role in both the specification and patterning of the terminal follicle cells, providing a possible explanation for the defect in anterior-posterior axis formation caused by Notch and Delta mutants.

Key words: Drosophila, Oogenesis, Axis formation, Follicle cell, Egfr, Notch

INTRODUCTION

The somatic follicle cells of Drosophila egg chambers provide a simple system for studying epithelial morphogenesis. These cells initially form a monolayer around each germline cyst, but they become progressively subdivided into multiple cell types that undergo a variety of morphogenetic movements, before they secrete the elaborate structure of the egg shell. Both the anterior-posterior and dorsal-ventral axes of the embryo become polarised by inductive signals from specific follicle cell populations. Thus, an understanding of the patterning of the follicle cell layer is an essential step in determining how the two main-body axes of Drosophila are defined.

Oogenesis commences when a germline cystoblast divides four times to give rise to a cyst of 15 nurse cells and a posteriorly placed oocyte. In region 2b of the germarium, about 16 somatic follicle cells migrate to form a uniform monolayer of cells around each cyst (King, 1970). The first differences between the follicle cells that surround a cyst become visible soon after the resulting egg chamber leaves the germarium, when two follicle cells at each end of the egg chamber start to express higher levels of certain proteins such as Fasciclin III and Neuralised (Brower et al., 1981; Ruohola et al., 1991). These polar follicle cells constitute a separate lineage and do not divide again (Margolis and Spradling, 1995). In contrast, the rest of the follicle cells undergo five to six mitoses before they cease dividing at stage 6 (King, 1970; Mahowald and Kambsellis, 1980; Spradling, 1993; Margolis and Spradling, 1995).

The follicle cell layer becomes polarised along the anterior-posterior axis when the oocyte induces the follicle cells at one end of the egg chamber to adopt a posterior rather than an anterior fate (González-Reyes and St Johnston, 1994). This induction requires gurken in the germline and the Drosophila homologue of the epidermal growth factor receptor, Egfr, in the soma (González-Reyes et al., 1995; Roth et al., 1995). Since Gurken protein is a TGFα-like molecule and is expressed at the posterior of the oocyte, it is thought that it binds directly to the Egfr to activate a typical receptor tyrosine kinase signal transduction cascade that specifies posterior fate (Neuman-Silberberg and Schüpbach, 1993, 1996). Although it is not known when Gurken induces posterior fate, this must occur before stage 7 when the anterior and posterior follicle cell populations start to behave differently.

The anterior follicle cells become subdivided into three distinct follicle cell types along the anterior-posterior axis: border cells, stretched follicle cells and centripetal follicle cells. The border cells are a group of 6-10 cells that delaminate from the follicular epithelium at the anterior tip of the egg chamber and migrate between the nurse cells to the anterior of
the oocyte. At the same time, the adjacent stretched follicle cells spread to cover the nurse cells as the rest of the follicular epithelium moves posteriorly to envelop the oocyte. The centripetal follicle cells just posterior to the stretched follicle cells come to lie over the anterior of the oocyte after these movements are complete, and these cells then migrate between the oocyte and the nurse cells towards the centre of the egg chamber during stage 10b.

The posterior follicle cells do not undergo any obvious subdivisions or migrations, but they do perform a specialised function, since these cells send an unknown signal back to the germline during stages 6-9 of oogenesis to induce a reorganisation of the oocyte microtubule cytoskeleton (Ruohola et al., 1991; Theurkauf et al., 1992; Clark et al., 1994; Lane and Kalderon, 1994; Micklem et al., 1997). This results in the formation of a polarised microtubule array that directs the localisation of bicoid mRNA to the anterior of the oocyte and oskar mRNA to the posterior, thereby defining the anterior-posterior axis of the embryo (Pokrywka and Stephenson, 1991, 1995; Clark et al., 1994). The polarisation of the microtubule cytoskeleton also triggers the movement of the germinal vesicle from the posterior to the dorsal/anterior corner of the oocyte (Koch and Spitzer, 1983). Since Gurken mRNA is localised on one side of the germinal vesicle, this migration changes the site of synthesis of Gurken protein, which then signals to the Egfr for a second time to induce the adjacent follicle cells to adopt a dorsal, rather than a ventral fate (Schüpbach, 1987; Neuman-Silberberg and Schüpbach, 1993, 1996). The resulting polarisation of the follicular epithelium along the dorsal/ventral axis defines this axis in the embryo, since the ventral follicle cells eventually secrete the asymmetric signal that leads to the formation of the gradient of dorsal protein in the blastoderm nuclei (Chasan and Anderson, 1993). Thus, two sequential inductions from the germline to the follicle cell layer play a crucial role in the subdivision of the follicular epithelium along each axis and the specification of the distinct follicle cell types that polarise the two main axes of the embryo.

Although many of the events that pattern the follicle cell epithelium are now well-characterised, there are still a number of important gaps in our understanding of this process. For example, the discovery that Gurken signals twice during oogenesis to induce first posterior and then dorsal follicle cell fates raises the question of how the competence to respond to this signal is controlled. It has previously been suggested that the follicular epithelium is divided into two cell types early in oogenesis, a terminal population that responds to Gurken by becoming posterior rather than anterior, and a second population, which we will refer to as the main-body follicle cells, that responds by becoming dorsal rather than ventral (González-Reyes et al., 1995, Roth et al., 1995; Ray and Schüpbach, 1996). Although there are a number of enhancer trap lines that label groups of cells at each end of the egg chamber, it is not known whether any of these distinguish a terminal population of follicle cells that corresponds to the large number of cells that will eventually adopt either an anterior or posterior fate (Fasano and Kerridge, 1988; Grossniklaus et al, 1989; Spradling, 1993). Indeed, the only distinct cell type that can be identified at each end of the egg chamber is the pair of polar follicle cells. This discrepancy raises the question of how the larger terminal follicle cell population is specified, or if it exists at all. For example, it has been proposed that the competence to become posterior in response to Gurken is restricted to just the polar follicle cells (Lehmann, 1995). These cells might then send a secondary signal later in oogenesis to induce a similar fate in a larger population of surrounding cells. Alternatively, it is possible that this competence is not spatially restricted at all, but is instead determined by the time at which the signal is received, since the posterior follicle cells are the only cells that contact the oocyte during the previtellogenic stages of oogenesis, and which are therefore in a position to receive the Gurken signal at this stage.

A second unresolved question concerns the relationship between the subdivision of the terminal follicle cells into most terminal, subterminal and more lateral populations and the decision between anterior and posterior fate. Although the anterior follicle cells show a clear division into three cell types that differ in both their gene expression and behaviour, no such differences are visible at the posterior. When the induction of posterior fate is disrupted, however, either by mutations in the Gurken/Egfr signalling pathway or by misplacement of the oocyte, the population of follicle cells at the posterior of the egg chamber becomes subdivided into border, stretched and centripetal cells in exactly the same way as at the anterior (González-Reyes and St Johnston, 1994; González-Reyes et al., 1995; Roth et al., 1995). This observation can be explained by two alternative models. In the first, the subdivision of the terminal follicle cells into three cell types depends on the acquisition of anterior fate. In this case, Gurken must signal to induce posterior fate before the subdivision occurs, thereby suppressing this process in the posterior terminal cells. It is also possible that there is a symmetric prepattern that subdivides both terminal follicle cell populations before the decision between anterior and posterior takes place. Although this subdivision is only visible at the anterior, the failure to observe distinct populations of posterior cells may be due to the lack of appropriate markers, since very few genes that are expressed at the posterior of the egg chamber have been identified so far.

A third outstanding issue concerns the role of the Notch pathway in the patterning of the follicle cell layer. If the activity of the Notch receptor or its ligand Delta is removed in the gerarium, the follicle cells fail to surround each germline cyst or to form the stalk that separates adjacent cysts (Ruohola et al., 1991; Xu et al., 1992). When the activity of either gene is removed after this stage, the overall organisation of the egg chamber appears normal, but the oocyte develops a symmetric microtubule cytoskeleton which directs the localisation of bicoid mRNA to both poles of the oocyte and oskar mRNA to the centre. This phenotype is very similar to that produced by mutants that disrupt the induction of posterior follicle cell fate by the oocyte, or that block the reciprocal signal from the posterior follicle cells that polarises the oocyte cytoskeleton (González-Reyes et al., 1995; Roth et al., 1995; Micklem et al., 1997). The anterior-posterior axis forms normally, however, when either Notch or Delta activity is removed from the germline (Ruohola et al., 1991). Since this result indicates that both genes are required in the somatic follicle cell layer, the exact function of Delta/Notch signalling in anterior-posterior axis formation remains unclear.

In this paper, we have taken a variety of different approaches
to address the three questions mentioned above, and our results have allowed us to draw up a simple outline for the steps in the patterning of the follicular epithelium and the relationships between them.

MATERIALS AND METHODS

Fly stocks

The enhancer trap lines used in this paper are the following: L53b (Fasano and Kerridge, 1988), slow border cells (sbc) (Montell et al., 1992), 5A7, MA33 and BB127 (Roth et al., 1995). As a marker of posterior follicle cells, we used the lethal P-element line 998/12, which is expressed in posterior follicle cells from stage 6 onwards (P. Deak, D. Glover, M. Bownes, W-M Deng, S. Pathirana, A. G-R and D. St J., unpublished data). The reporter protein produced by the different lines is a nuclear β-galactosidase (β-gal).

To study the effect of removing Notch (N) function during oogenesis, we used the temperature-sensitive allele Nts1 and followed the protocol described previously to produce Notch mutant egg chambers (Shellenbarger and Mohler, 1978; Ruohola et al., 1991). The other mutant used in this study is dicephalic (Lohs-Schardin, 1982).

Staining procedures

Antibody stainings were performed according to the protocol described elsewhere (St Johnston et al., 1991). The α-myc and α-β-gal antibodies are commercially available from Oncogene Science and Cappel, respectively. The concentrations used are 1/500 for α-myc (1/100 when a fluorescently labelled secondary antibody was used), and 1/1000 for α-β-gal with a fluorescent secondary. The FITC and Texas Red-labelled secondary antibodies (from Cappel) were diluted 1/250. X-gal and Hoechst stainings were performed according to the protocols described previously (González-Reyes and St Johnston, 1994). Ovaries were mounted in 80% glycerol in PBS, with the addition of an anti-bleaching agent for fluorescent stainings.

Generation of clones

We used the FRT/FLP technique to generate clones of follicle cells that were homozygous for a null mutation in the Egfr gene, top (Clifford and Schüpbach, 1989; Xu and Rubin, 1993). hs-FLP 12; FRT 42D top+/flk; FRT 42D hs-myc; enhancer trap/ + females were yeasted for 24 hours, heat shocked for 1 hour in a 37°C incubator and put back in yeasted vials at 25°C for 36, 60 or 72 hours, depending on the experiment. Females were then heat shocked again for 1 hour at 37°C to induce expression of the myc tag, and were dissected and fixed 1½-2 hours later. Thus, the egg chambers were analysed 39, 63 or 75 hours after heat shock (hours AHS). Considering the length of time required to go from stage 6 to 10b, clones present in stage 10b egg chambers fixed 39 hours AHS were produced around stage 5 of oogenesis, whereas stage 10b egg chamber clones fixed either at 63 hours or 76 hours AHS were induced at, or before stage 2 (see Fig. 2C for a description of the timing of relevant stages during oogenesis; Lin and Spradling, 1993).

Cell lineage analysis

In the lineage analysis experiment, females of the genotype hs-FLP12; FRT-42D/ FRT-42D hs-myc were treated under the same conditions as above, and their ovaries fixed 51 or 63 hours AHS. In this case, myc+ cells carry the two wild-type copies of Egfr.

RESULTS

The terminal follicle cell populations are equivalent prior to gurken signalling

To explain how Gurken can induce two different follicle cell fates, it has been proposed that the follicle cell layer is divided into two cell types during early oogenesis: the terminal follicle cells at each end of the egg chamber, which become posterior if they receive the Gurken signal and anterior if they do not, and the main-body follicle cells, which are induced to become dorsal rather than ventral (González-Reyes et al., 1995; Ray and Schüpbach, 1996). To determine whether the main-body follicle cells can adopt a posterior fate in response to Gurken, we have analysed the original dicephalic mutation, which, unlike the spin mutants, alters the position of the oocyte without affecting Gurken signalling (Lohs-Schardin, 1982; González-Reyes et al., 1997; R. McCaffrey, A. G-R. and D. St J., unpublished results). We followed the induction of posterior follicle cell fate directly, using a recently identified enhancer trap line that specifically labels these cells (Micklem et al., 1997). When the oocyte is correctly positioned at the posterior of dicephalic mutant egg chambers, the enhancer trap line is strongly expressed in the posterior follicle cells just as it is in wild type (Fig. 1A). In contrast, no expression is observed

![Fig. 1. Competence to adopt a posterior fate is restricted to the terminal follicle cells at both poles of the egg chamber. (A) A dicephalic egg chamber in which the oocyte is correctly positioned showing the normal expression of 988/12 in the posterior terminal cells. (B) Bipolar egg chamber from a dicephalic mutant mother, which does not express the posterior follicle cell marker 988/12. (C) A dicephalic egg chamber in which the oocyte is positioned at the anterior, as determined by the overall AP axis of the ovariole (anterior to the left, posterior to the right). The 998/12 line is expressed in the anterior terminal cells. (D) Hoechst staining of a wild-type ovariole to reveal the normal position of the border cells, and the stretched and centripetal follicle cells. (E) dicephalic ovariole with a stage 10 egg chamber in which the oocyte is placed at the anterior. The follicle cell layer develops a reversed polarity: the terminal follicle cells at the anterior become posterior columnar follicle cells (pfc), and the terminal follicle cells at the posterior pole now acquire anterior fates. sfc, stretched follicle cells; arrowheads, centripetal follicle cells; bc, border cells.](https://example.com/image-url)
when the oocyte lies in the middle of the mutant egg chamber (Fig. 1B). Thus, misplaced oocytes cannot induce main-body follicle cells to adopt a posterior fate.

Our model also predicts that the two terminal follicle cell populations should be equivalent prior to Gurken signalling. Although it has been clearly demonstrated that the posterior follicle cells adopt an anterior fate if they do not receive the signal, it has not been shown that the anterior terminal cells are competent to adopt a posterior fate (González-Reyes and St Johnston, 1994; González-Reyes et al., 1995; Roth et al., 1995). We therefore examined the dicephalic mutant egg chambers where the oocyte lies at the anterior of the cyst. As predicted, the posterior marker is expressed in the anterior follicle cells of these egg chambers, which confirms that misplaced oocytes can still signal to induce posterior fate (Fig. 1C). Furthermore, these egg chambers develop completely normally although they have a reversed polarity with respect to the anterior-posterior axis of the whole ovariole (Fig. 1D,E). The terminal follicle cells at the opposite end to the oocyte become subdivided into the three anterior follicle cell types which undergo their normal migrations, whereas the ‘anterior’ cells in contact with the oocyte not only express the posterior marker, but also signal to polarise the oocyte cytoskeleton, since all of the maternal mRNAs that we have tested localise to the appropriate position (data not shown). These results demonstrate that the main-body follicle cells and the terminal follicle cells do indeed constitute two distinct populations that differ in their competence to respond to Gurken and prove that the terminal follicle cells at each end of the egg chamber are equivalent prior to Gurken signalling.

The *Egfr* is required cell autonomously to repress anterior fate in all posterior follicle cells

Although the expression of several markers at the termini of developing egg chambers suggests the existence of populations of terminal follicle cells, it is not clear how many cells respond to Gurken directly by adopting a posterior rather than an anterior fate. To define this population, we have mapped which cells revert to the default anterior fate when they cannot respond to Gurken because they lack its putative receptor. Using the scheme diagrammed in Fig. 2, we generated small marked clones of cells that are homozygous for *top* (a null allele of the *Egfr*, and followed their fate by staining for the β-gal activity of the L53b enhancer trap line, which labels all three subpopulations of anterior follicle cells from stage 9 onwards (Fig. 2B) (Fasano and Kerridge, 1988; Clifford and Schüpbach, 1989). When the clones were generated at approximately stage 2 of oogenesis and scored at stage 10 (63 hours AHS), mutant cells that lie near the posterior of the oocyte always express L53b, whereas clones over the middle of the oocyte do not (Fig. 3A,B). Furthermore, there is no overlap between the expression of L53b and the myc epitope which labels the non-mutant cells, as can be seen most clearly in the confocal micrograph shown in Fig. 3C. Thus, removal of the *Egfr* causes a cell-autonomous transformation from posterior to anterior fate, indicating that Gurken signals directly to induce posterior fate in the whole terminal follicle cell population. To measure the size of the terminal follicle cell population more accurately, we plotted the position along the anterior-posterior axis of a random sample of 65 *Egfr*− clones, and determined which cells express the anterior marker L53b.
layer performed by Margolis and Spradling (1995) to determine whether the terminal and main-body follicle cells arise from different progenitors. Ovaries containing wild-type myc<sup>-</sup> and 2x myc twin spot follicle cell clones were fixed either 51 or 63 hours AHS, and were double stained with the DNA dye Hoechst and α-myc antibody. When examined at stage 10b, both 32- and 16-cell clones can be seen to cross the boundary between the posterior terminal cells and the main-body follicle cells that falls 10/11 cell diameters from the posterior pole (Fig. 3E and data not shown). Thus, the progeny of a single cell can contribute to both populations and there is no lineage restriction between these two cell types 4 to 5 divisions before the follicle cells cease mitosis. The terminal follicle cell must therefore be determined after stage 1 of oogenesis, when the clones were induced.

**Egfr<sup>-</sup> clones reveal a symmetric prepattern in the terminal follicle cell population**

In mutants such as gurken in which the induction of posterior follicle cell fate is blocked, the terminal follicle cells at the posterior develop like their anterior counterparts by forming border, stretched and centripetal follicle cells. This raises the question of whether the anterior follicle cells are subdivided into three cell types after the decision between anterior and posterior is taken, or whether there is a symmetric prepattern in the terminal follicle cells at both ends of the egg chamber.

**Fig. 3.** The *Egfr* is required cell autonomously to repress anterior fate in all posterior cells. (A) Stage 10b egg chamber double stained with X-gal and α-myc to reveal two *Egfr<sup>-</sup>* clones. The clone that falls three cell diameters from the posterior end of the egg chamber (black line) expresses L53b whereas the clone that lies 13 cells from the posterior does not (asterisk). Cell number 11 is marked with a white arrowhead. (B,B<sup>'</sup>) An L53b-expressing clone that falls 5/6 cell diameters from the posterior pole. (C) Confocal image of an egg chamber double stained to visualised myc (red) and β-gal (green) proteins. Only the *Egfr<sup>-</sup>* cells express L53b, demonstrating the cell autonomy of the transformation to anterior fate. (D) Plot of 65 clones scored in experimental stage 10 egg chambers. The diagram shows 37 L53b-expressing clones (blue outline) and 28 myc<sup>-</sup> clones, which do not express L53b (brown outline), and reveals that the boundary between positive and negative clones falls 10/11 cells from the posterior pole. In total, we scored 421 L53b-expressing clones, all of which fall within this posterior region (111 clones in stage 9 egg chambers, 246 in stage 10 and 64 in stage 11-14). (E) An egg chamber containing wild-type myc<sup>-</sup> and 2x myc twin spot clones that cross the 10/11-cell boundary between the terminal cells and the main-body cells. The egg chamber was fixed 63 hours AHS, and double stained for α-myc and the DNA dye Hoechst. The white dot indicates cell 11, as counted from the posterior pole (black, white outlined dots). The dotted line represents the 10/11-cell boundary. The cells that do not express myc do not quench the Hoechst signal (bright nuclei), whereas the 2x myc cells appear black.

**Fig. 4.** *Egfr<sup>-</sup>* cells that fall between 8 and 11 cell diameters from the posterior pole express the centripetal cell marker BB127. (A) A wild-type egg chamber showing the expression of the BB127 enhancer trap line in the centripetal follicle cells (arrowheads) and the nurse cell nuclei. (B,B<sup>'</sup>) Egg chamber containing *Egfr<sup>-</sup>* cells that lie 9-11 cell diameters from the posterior pole and express BB127. The white arrowhead indicates cell number 11 from the posterior end (black line). (B<sup>'</sup>) Top view of the clone in B. The black arrow points to a more posterior cell that does not express BB127. (C) *Egfr<sup>-</sup>* clone anterior to cell 11 (arrowhead) which does not show X-gal staining. (D) *Egfr<sup>-</sup>* clone at the posterior pole of the egg chamber. In spite of the lack of *Egfr* function (white cells; asterisks), the cells of the clone do not express BB127. (A-D) Stage 10b egg chambers stained with X-gal (A-D) and α-myc (B-D).
The ability to generate small clones of anterior cells at the posterior by removing the Egfr makes it possible to distinguish between these possibilities. If the latter model is correct, isolated patches of anterior cells should still respond to the symmetric prepattern correctly and form the appropriate type of anterior cell even though they are surrounded by posterior cells, whereas the former model predicts that these cells should be unable to interpret their position.

To follow the fate of small patches of anterior cells at the posterior of the egg chamber, we again generated small Egfr clones, but in this case, in the presence of enhancer trap lines that are expressed specifically in each of the three anterior follicle cell types (Roth et al., 1995). In the first experiment, stage 10 egg chambers were fixed 75 hours AHS and stained for β-galactosidase activity to detect the expression of BB127, an enhancer trap line that labels only the centripetal follicle cells. Egfr cells that fall within a region 8-11 cell diameters from the posterior pole show strong blue staining, whereas clones that fall either proximal or distal to this 3-cell-wide belt do not turn on BB127 (Fig. 4). Thus, anterior cells at the posterior express BB127 cell autonomously in a region that is the exact posterior counterpart of the anterior centripetal follicle cell domain. Furthermore, clones of as few as 4 cells express BB127 if they fall within this region, indicating that anterior cells can correctly interpret their position with respect to the posterior pole, although all of the surrounding cells are posterior (Fig. 4B’).

We next analysed the pattern of expression of the border cell marker 5A7 in mosaic follicle cell epithelia (Fig. 5A). In this case, the Egfr cells that are situated at the very posterior of the egg chamber express 5A7, whereas mutant cells that lie a few cell diameters away do not (Fig. 5B,C). Furthermore, the 5A7-positive cells at the posterior pole occasionally appear to delaminate from the follicular epithelium in a similar fashion to the anterior border cells (Fig. 5B’). Finally, we induced Egfr clones in flies that carry one copy of the enhancer trap line MA33, which labels the stretched follicle cells (Fig. 5D). As expected, this enhancer trap is only expressed in Egfr cells that lie between the domains defined by the centripetal and border cell markers, in a region that extends 7/8 cell diameters from the posterior pole (Fig. 5E).

The results obtained with the BB127, 5A7 and MA33 lines demonstrate that small posterior clones of anterior cells can interpret their position with respect to the posterior pole by adopting the appropriate anterior follicle cell fate: the most terminal Egfr cells behave like border cells, the subterminal Egfr cells behave like stretched follicle cells, and the least terminal like centripetal cells. Thus, the positional information that specifies the positions of these distinct cell types at the anterior pole is also present at the posterior, strongly suggesting that there is a symmetric prepattern within the terminal follicle cell population that is independent of the decision between anterior and posterior fate.

**Notch is required for the correct subdivision of the terminal follicle cells**

Although the phenotype of Notch and Delta mutants provided the first evidence that the posterior follicle cells play a role in the polarisation of the oocyte, it is still not known at which step in anterior-posterior axis formation Delta/Notch signalling is required (Ruohola et al., 1991). To address this question, we asked whether the Nts mutant disrupts this pathway before or after the induction of the posterior follicle cells by the oocyte. Since the most-sensitive assay for a failure in posterior follicle cell determination is transformation to anterior fate, we examined the expression of the border cell enhancer trap line slbo in stage 10 Nts egg chambers that had been maintained at the restrictive temperature of 32°C. These conditions produce a penetrant oocyte polarisation phenotype in which the germinal vesicle often remains at the posterior of the oocyte (Fig. 6B). Surprisingly, slbo is expressed in neither the anterior nor posterior follicle cells of these egg chambers. In addition, the anterior most follicle cells in Nts mutant egg chambers never round up or migrate between the nurse cells towards the oocyte, indicating that Notch activity is required for border cell development (Fig. 6A, B).

To determine whether Notch activity is required for the

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**Fig. 5.** Expression of border cell and stretched cell markers in posterior Egfr clones. (A) Wild-type egg chamber showing the expression of 5A7 in the border cells. (B,B’) Egg chamber showing an Egfr clone at the posterior pole that expresses 5A7. (C,C’) Egfr clones that lie a few cell diameters from the posterior pole do not express 5A7. (D) Wild-type egg chamber showing the expression of the MA33 line in the stretched follicle cells. (E) Egg chamber displaying an Egfr clone in which some cells express MA33; the border between the expressing and non-expressing Egfr cells falls 7-8 cell diameters from the posterior pole. (A-E) Stage 10b egg chambers stained with X-gal (A,D) and α-myc (B,C,E).
specification of other anterior follicle cell types, we repeated this experiment using the L53b enhancer trap line to label all anterior follicle cells. In experimental egg chambers, L53b is expressed normally in the stretched follicle cells, but there are no detectable centripetal follicle cells (Fig. 6C,D). Although it is difficult to count the number of stretched follicle cells accurately, there do not appear to be significantly more cells than usual in these egg chambers. Thus, Nts egg chambers lack both border and centripetal follicle cells, but these missing cells do not appear to be transformed into stretched follicle cells. Notch therefore seems to play a role in determining the size of the anterior terminal follicle cell population, as well as its subdivision into distinct cell types. Because neither slbo nor L53b are expressed at the posterior of Notch mutant egg chambers, this mutant does not disrupt the polarisation of the oocyte by causing a transformation of the posterior follicle cells to an anterior fate.

Since our previous results show that both terminal follicle cell populations contain the same positional information, we next asked whether the Nts mutant disrupts the patterning of the posterior terminal follicle cells. The enhancer trap line that specifically labels these cells is still expressed at the posterior of mutant egg chambers, confirming that Notch is not required for the determination of posterior identity (Fig. 6E,F). However, many fewer cells express this marker than in the control egg chambers. This reduction in posterior follicle cell number indicates that Notch plays a role in specifying the size of the terminal follicle cell population that is competent to respond to Gurken and is consistent with the decrease in terminal follicle cell number observed at the anterior of these egg chambers.

**DISCUSSION**

According to the current model, the formation of the anterior-posterior axis in *Drosophila* is a two-step process: (1) The determination of the terminal follicle cell populations at each end of the egg chamber defines the orientation of the axis, and (2) the positioning of the oocyte posterior to the nurse cells

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**Fig. 6.** Reduction of Notch function disrupts both the specification and the subdivision of the terminal follicle cells. (A) The expression of the slbo1 enhancer trap line in a Nts1 egg chamber grown at the permissive temperature showing the labelling of the border cells. (B) slbo is not expressed in Nts1 egg chambers grown at the restrictive temperature. The arrow indicates the position of the oocyte nucleus, which has not migrated to the anterior. (C) The expression of L53b in the stretched and centripetal cells (arrowhead) in Nts1 egg chambers grown at the permissive temperature. (D) Nts1 egg chambers grown at the restrictive temperature lack centripetal cells but show normal L53b expression in the stretched follicle cells. This egg chamber has been overstained to show the absence of L53b expression at the posterior, indicating that Nts1 does not transform the posterior cells into an anterior fate. (E) The 988/12 enhancer trap line is expressed in all posterior follicle cells in Nts1 egg chambers grown at the permissive temperature. (F) At the restrictive temperature, 988/12 is expressed in many fewer posterior follicle cells.
then imposes polarity on this axis when the oocyte signals to induce one of these populations to adopt a posterior rather than an anterior fate (González-Reyes et al., 1995; Roth et al., 1995; Ray and Schüpbach, 1996). Despite the important role attributed to the terminal follicle cells in this model, the nature and origin of this cell type have remained obscure. Our results allow us to draw several conclusions about the patterning of these cells.

Most importantly, our data prove that the terminal follicle cells exist as distinct populations of cells at both poles of the egg chamber. By using the dicerphal mutant to alter the position of the oocyte without affecting detectably Gurken signalling, we have shown that the anterior terminal cells can be induced to adopt a posterior fate when they contact the oocyte, whereas the cells in the middle of the egg chamber cannot. Furthermore, only the terminal cells at both poles of the egg chamber adopt an anterior fate when Gurken, signalling is impaired (González-Reyes et al., 1995; Roth et al., 1995). Thus, the two populations of terminal follicle cells are equivalent, and become posterior when they receive the Gurken signal and anterior when they do not, in contrast to the main-body follicle cells which cannot adopt either fate. The follicular epithelium is therefore partitioned into terminal and main-body follicle cell populations before Gurken signalling takes place, ruling out the alternative model in which the response of the follicle cells to Gurken is controlled by the time at which they receive the signal. These results provide clear confirmation of both parts of the original model for anterior-posterior axis formation. Firstly, only the terminal follicle cells are competent to respond to Gurken and to become posterior. This competence is independent of the position of the oocyte and demonstrates that the direction, but not the polarity, of the anterior-posterior axis is defined by the initial patterning of the follicle cell layer. Secondly, the observation that egg chambers develop a normal but reversed anterior-posterior axis when the oocyte comes to lie anterior to the nurse cells demonstrates that the polarisation of the anterior-posterior axis depends solely on the positioning of the oocyte.

Since there are no markers that label the terminal follicle cells, we have measured the size of this population indirectly, by determining how many cells at the posterior of the egg chamber need to respond to the Gurken signal directly to prevent them from adopting the default anterior fate. This clonal analysis reveals that the Egfr is required cell autonomously in approximately 200 posterior cells to repress anterior fate, ruling out the model in which the response of the follicle cells contains many cells of the same type. An epithelium is therefore partitioned into terminal and main-body follicle cells before Gurken signalling takes place, ruling out the alternative model in which the response of the follicle cells to Gurken is controlled by the time at which they receive the signal. These results provide clear confirmation of both parts of the original model for anterior-posterior axis formation. Firstly, only the terminal follicle cells are competent to respond to Gurken and to become posterior. This competence is independent of the position of the oocyte and demonstrates that the direction, but not the polarity, of the anterior-posterior axis is defined by the initial patterning of the follicle cell layer. Secondly, the observation that egg chambers develop a normal but reversed anterior-posterior axis when the oocyte comes to lie anterior to the nurse cells demonstrates that the polarisation of the anterior-posterior axis depends solely on the positioning of the oocyte.

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Role of Notch in terminal follicle cell patterning

In addition to its early role in the germarium, our data show that Notch activity is required for two steps in the patterning of the terminal follicle cells. Firstly, $N^{su}$ mutant egg chambers show a large reduction in the size of the terminal follicle cell population, suggesting that Notch plays a role in the initial determination of this cell type. Indeed, it is possible that the complete absence of Notch would lead to the loss of all terminal cells, since the $N^{su}$ allele is not a null even at the restrictive temperature. Secondly, Notch is required for the subdivision of the anterior terminal cells into three cell types, since only the stretched cells differentiate in the mutant egg chambers. In many cases, Delta/Notch signalling has been shown to mediate a process of lateral inhibition that ensures that only a single cell from an equivalence group is selected to adopt a particular fate (Artavanis-Tsakonas et al., 1995; Simpson, 1997). However, it is hard to imagine how this process could play a role in terminal follicle cell patterning, since there are about 200 terminal follicle cells at each end of the egg chamber and even the subpopulations of anterior follicle cells contain many cells of the same type. An alternative possibility is suggested by the observation that the follicle cells lose their epithelial organisation in $N^{su}$ egg chambers that are kept at 32°C for 2 days (Goode et al., 1996). Although the shorter treatments at restrictive temperature used in our experiments do not lead to obvious abnormalities in the follicular epithelium, these conditions may cause a partial disruption of epithelial cell polarity and it is possible that this interferes with other signalling events that pattern the terminal follicle cells.

The reduced number of posterior follicle cells in $N^{su}$ egg chambers may account for their anterior-posterior axis defects, since these cells are presumed to be the source of the unknown signal that polarises the oocyte cytoskeleton. However, several mutants (the spindle genes, and weak gurken and Egfr alleles) cause a comparable reduction in the number of posterior follicle cells without producing a penetrant oocyte polarisation phenotype (González-Reyes et al., 1997; our unpublished results). We therefore favour an alternative model in which the polarising signal is only produced by a subset of the posterior follicle cells, and these cells are missing in $N^{su}$ egg chambers.
For example, the polarising signal could be produced by the posterior counterparts of the border cells, and these cells should be absent from Nth egg chambers if the posterior cells are subdivided in the same way as the anterior cells.

It has been previously shown that lack of Delta/Notch signalling in the follicle cell layer produces an hyperplasia of the posterior follicle cells (Ruohola et al., 1991), and we have also observed this phenotype, although it only becomes visible at stages 7-8 of oogenesis (data not shown). The late appearance of this phenotype, however, suggests that it may be a secondary consequence of earlier defects in terminal follicle cell patterning.

### Three steps in the AP patterning of the follicle cell epithelium

Altogether, these observations support a stepwise model for the patterning of the follicle cell layer along the AP axis (Fig. 7). In the first step, the follicle cell epithelium is divided into terminal and main-body follicle cell populations. There is no lineage restriction boundary between the posterior terminal follicle cells and the main-body follicle cells four cell divisions before stage 6, indicating that the distinction between these two cell types arises after stage 1. This result is consistent with the observation that the polar cells constitute the only distinct lineage in the early egg chamber (Margolis and Spradling, 1995). In addition, Gurken must signal to induce posterior fate before stage 6, when the expression of the posterior-specific enhancer trap line is first detectable. Because the terminal cells have to be specified before Gurken signalling occurs, this restricts the time at which this population is determined to between stages 2 and 5. This is consistent with the observation that the ectopic activation of the Egrf pathway is able to induce the transient expression of a posterior follicle cell marker in the anterior terminal cells when it is expressed in stage 2 to 6 egg chambers, but not at other stages (Lee and Montell, 1997).

The formation of the two symmetric terminal follicle cell populations is independent of the positioning of the oocyte, suggesting that the germline does not play an instructive role in the determination of these cells. Although our data do not suggest a mechanism for how these cells are specified, their position suggests a simple model in which they are induced by a ‘terminalising’ signal that spreads from the two poles of the egg chamber. The most-likely source for such a signal are the two polar follicle cells at each end of the egg chamber, as these cells lie in the centre of the terminal domain and adopt a terminal fate themselves (see also Brower et al., 1981).

The next step in the patterning of the follicular epithelium is the formation of a symmetric prepattern within each terminal follicle cell population. How this prepattern is established is unknown, but the geometry of the egg chamber again suggests that it might involve signals that emanate from the poles. Indeed, it is possible that the terminal follicle cells are specified and patterned by the same process, since both events require Notch activity. For example, the ‘terminalising’ signal could induce distinct terminal fates at different distances from the pole.

The third step in the patterning of the follicle cell layer occurs when the oocyte induces one population of terminal follicle cells to adopt a posterior fate, thereby breaking the symmetry of the follicle cell layer. As a consequence, the symmetric prepattern in the terminal follicle cells is interpreted differently in the anterior and posterior populations. The anterior cells become subdivided into border, stretched and centripetal follicle cells, while the posterior cells may undergo a similar subdivision into posterior cell types. In this way, the sequential patterning of the terminal follicle cells gives rise to at least five different cell types along the anterior-posterior axis.

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