

## Organizer activity of the polar cells during *Drosophila* oogenesis

Muriel Grammont\* and Kenneth D. Irvine†

Howard Hughes Medical Institute, Waksman Institute and Department of Molecular Biology and Biochemistry, Rutgers The State University of New Jersey, Piscataway NJ 08854, USA

\*Present address: INSERM UMR 384, Laboratoire de Biochimie, 28 place Henri Dunant, 63001 Clermont-Ferrand, France

†Author for correspondence (e-mail: irvine@mbcl.rutgers.edu)

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### SUMMARY

Patterning of the *Drosophila* egg requires the establishment of several distinct types of somatic follicle cells, as well as interactions between these follicle cells and the oocyte. The polar cells occupy the termini of the follicle and are specified by the activation of Notch. We have investigated their role in follicle patterning by creating clones of cells mutant for the Notch modulator *fringe*. This genetic ablation of polar cells results in cell fate defects within surrounding follicle cells. At the anterior, the border cells, the immediately adjacent follicle cell fate, are absent, as are the more distant stretched and centripetal follicle cells. Conversely, increasing the number of polar cells by expressing an activated form of the Notch receptor increases the number of border cells. At the posterior,

elimination of polar cells results in abnormal oocyte localization. Moreover, when polar cells are mislocalized laterally, the surrounding follicle cells adopt a posterior fate, the oocyte is located adjacent to them, and the anteroposterior axis of the oocyte is re-oriented with respect to the ectopic polar cells. Our observations demonstrate that the polar cells act as an organizer that patterns surrounding follicle cells and establishes the anteroposterior axis of the oocyte. The origin of asymmetry during *Drosophila* development can thus be traced back to the specification of the polar cells during early oogenesis.

Key words: *fringe*, *Notch*, Polar cells, Oogenesis, Polarity

### INTRODUCTION

A recurring theme for tissue patterning during development is the initial specification of a small group of cells, which then serve as the source of signals that influence the patterning of surrounding cells. The ability of a group of cells to direct the patterning of surrounding cells was investigated by Spemann, who coined the term organizer to describe this behavior (reviewed by De Robertis et al., 2000). A series of landmark studies have demonstrated that cells along the borders between compartments in the imaginal discs of *Drosophila* organize growth and patterning by virtue of their expression of secreted signaling molecules (reviewed by Irvine and Rauskolb, 2001; Vincent and Briscoe, 2001). We describe our analysis of the basis of patterning in the somatic follicle cells that surround clusters of germline cells during oogenesis. Until now, it has not been established whether the follicular cells are patterned by an organizer or by other mechanisms.

A *Drosophila* egg chamber, or follicle, is formed from a cyst of 16 inter-connected germline cells, and a similar number of somatic cells, which surround them. One of the germline cells becomes the oocyte and adopts a posterior localization inside the follicle, while the remainder become nurse cells (Fig. 1) (King, 1970; Spradling, 1993). Soon after follicle formation, the somatic cells can be divided into three populations: two pairs of polar cells at each end of the follicle; four to six interfollicular stalk cells that separate one follicle from the

next; and the other follicular cells that surround the follicle. The polar and stalk cells come from a common precursor, and they stop dividing in region IIB of the germarium (Margolis and Spradling, 1995; Tworoger et al., 1999). The other follicle cells continue to proliferate through stage 6 of oogenesis, when they form a population of about 1000 cells. They also become further subdivided into distinct groups that have specialized roles during oogenesis, including border cells, stretched cells and centripetal cells at the anterior of the follicle, posterior terminal cells at the posterior of the follicle, and what we will refer to as central follicle cells in the middle of the follicle (Fig. 1).

The polar cells are located at the center of the distinct anterior and posterior terminal follicle cell types (Fig. 1), raising the possibility that they could be involved in the process of follicle cell patterning. Support for this possibility has begun to emerge from recent studies on EGFR signaling (Gonzalez-Reyes and St Johnston, 1998b), which promotes posterior follicle cell fate; Hedgehog signaling, which is thought to exert general effects on follicle cell proliferation and differentiation (Forbes et al., 1996; Liu and Montell, 1999; Zhang and Kalderon, 2000); and JAK-STAT signaling, which influences border cell fate (Beccari et al., 2002; Silver and Montell, 2001). Although some results from these studies suggest a role for polar cells in patterning surrounding follicle cells, a clear understanding of polar cell function is still lacking, as none of these studies involved a direct manipulation of polar cell fate.

Recent studies have revealed that the polar cells are specified within the polar-stalk lineage by the activation of the Notch signaling pathway (Grammont and Irvine, 2001; Lopez-Schier and St Johnston, 2001). Notch is a transmembrane receptor protein that is involved in a wide range of cell fate decisions during animal development (reviewed by Artavanis-Tsakonas et al., 1999). Activation of Notch in the polar cells is dependent upon the Notch pathway modulator *fringe* (*fng*), which encodes a glycosyltransferase that can modify carbohydrates on EGF repeats of Notch (Bruckner et al., 2000; Moloney et al., 2000). In the absence of *fng* or *Notch* function, polar cells do not form, and the requirement for these genes in polar cell fate is strictly cell autonomous. In most cases, the absence of polar cells during follicle formation leads to compound follicles, in which multiple germline cysts are enclosed within a single follicle cell epithelium. Even though the polar cells cannot be distinguished by specific molecular markers until stage 3, these observations established a function for polar cells much earlier, in region IIb of the germarium. Although polar cells are required at this stage for the separation of germline cysts into distinct follicles, in rare cases a follicle lacking anterior or posterior polar cells can form and enclose a single germline cyst.

The realization that *fng* is autonomously required for polar cell fate and the existence of rare late stage follicles without polar cells in *fng* genetic mosaics has presented for the first time the possibility of determining the role of polar cells in follicle patterning. We report that polar cells are required for the specification of both anterior and posterior terminal follicle cell fates. Polar cells are also sufficient to induce at least the immediately adjacent anterior cell fate, the border cells. Our results indicate that the polar cells function as an organizer of terminal follicle cell patterning. At the posterior, the terminal cells localize the oocyte, and then participate in a reciprocal signaling process with the oocyte that re-organizes the oocyte cytoskeleton and thereby establishes the anteroposterior and dorsoventral axes of the oocyte (Gonzalez-Reyes et al., 1995; Roth et al., 1995). Through their influence on the posterior terminal cells, the polar cells are thus ultimately responsible for the establishment of asymmetry during *Drosophila* development.

## MATERIALS AND METHODS

### *Drosophila* stocks and crosses

Clones of cells mutant for *fng*<sup>13</sup> or *upd*<sup>YM55</sup> or ectopically expressing activated Notch, were generated as described previously (Grammont and Irvine, 2001). *fng*<sup>13</sup> and *upd*<sup>YM55</sup> are null alleles (Harrison et al., 1998; Irvine and Wieschaus, 1994). Oregon-R was used as wild type, and reporter lines used were *neur-lacZ*<sup>A101</sup> (Bier et al., 1989), *slbo-lacZ*<sup>1310</sup> (Montell et al., 1992), *MA33* and *BB127* (Roth et al., 1995), *nod-lacZ*<sup>143.2</sup> and *kin-lacZ*<sup>503</sup> (Clark et al., 1997), and *pnt-lacZ*<sup>S99812</sup> (Gonzalez-Reyes and St Johnston, 1998b).

### Follicle staining

Histochemical and immunofluorescent staining of follicles was carried out as described previously (Grammont and Irvine, 2001). For Fig. 6G-J, X-gal and peroxidase staining were employed and the *fng* clones are unmarked because the fixation protocol required to detect cytoskeletal  $\beta$ -galactosidase in the germline and MYC staining in the soma by immunofluorescence was not consistent enough to reliably

determine the orientation of the cytoskeleton in those rare follicles with ectopic polar cells. We used the same method to analyze posterior follicle fate (Fig. 6E,F).

Follicles were staged according to King (King, 1970) and Spradling (Spradling, 1993). Because *fng* mosaic follicles can be abnormally shaped, and lack border, stretched and centripetal cells, we relied upon the following criteria: the size of the follicle, the ratio of volume of the oocyte to the volume of the nurse cells, the migration of the central follicle cells towards the oocyte, and the stage of neighboring follicles within the same ovariole.

## RESULTS

The primary strategy we pursued to investigate the role of the polar cells in terminal follicle cell patterning was to genetically ablate the polar cells by mutation of *fng*, and then to use molecular and morphological criteria to assay the fates of the surrounding cells in these follicles. Somatic follicle cells derive from two stem cells that are located in the germarium (Margolis and Spradling, 1995). Follicles genetically mosaic for *fng* were created by inducing mitotic recombination in animals heterozygous for a *fng* null allele, and marked by the absence of expression of a synthetic Myc-tagged protein in *fng* mutant cells. When this recombination event occurs in one of the two follicle stem cells, many of the follicles that develop in the ovariole will be a mosaic of wild-type cells derived from one stem cell and *fng* mutant cells derived from the other stem cell. When the cells that would ordinarily have formed polar cells are mutant for *fng*, no polar cells form (Grammont and Irvine, 2001). The absence of polar cells in these follicles can be monitored by staining with antibodies that recognize the FasIII protein, which is expressed specifically in the polar cells from stage 4 through the end of oogenesis (Ruohola et al., 1991). Both *Notch* and *fng* are autonomously required within polar cells, but we chose to focus our analysis on *fng* because *fng* is only required in the polar cells during follicle formation (Grammont and Irvine, 2001). Even in the case of *fng*, the majority of mosaic follicles lacking polar cells either form compound egg chambers or fail to develop to stages late enough for the patterning of terminal follicle cells to be assessed, and several thousand follicles had to be examined in order to identify the examples described below.

### Anterior polar cells are required for border cell formation

The border cells are a cluster of six to ten cells that are specified at the extreme anterior of the follicle. They include the two anterior polar cells, plus an additional four to eight adjacent terminal cells, the outer border cells. The border cells leave the follicle epithelium at the beginning of stage 9 of oogenesis, and then migrate between the nurse cells to the oocyte. They can be recognized by their unique morphology and behavior during stages 9 and 10 of oogenesis, when the small diploid border cells can be found migrating through the large polyploid nurse cells (Fig. 1B), and by the expression of the *slow border cells* (*slbo*) gene, which starts at stage 8 of oogenesis in the border cells during oogenesis, and is required for their migration (Montell et al., 1992; Rorth et al., 2000). We have thus also used a *lacZ* enhancer trap insertion in *slbo*, *slbo-lacZ*, to aid in the identification of border cells.

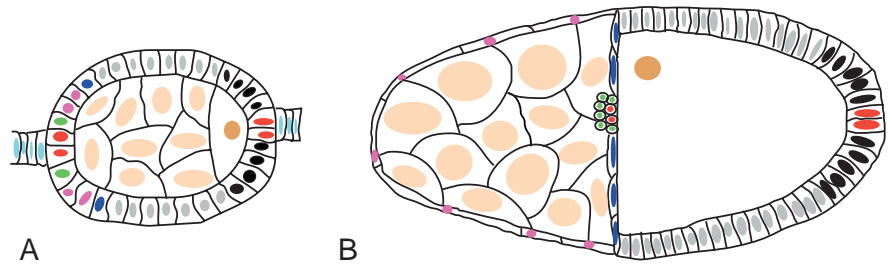
Altogether, we identified nine *fng* mosaic follicles at early

stage 9 to late stage 10B of oogenesis that lacked anterior polar cells. All nine of these follicles lacked detectable border cells, as visualized by DNA staining (Fig. 2A). In addition, five of the nine carried the *slbo-lacZ* enhancer trap, which was not detectably expressed in any of these mosaic follicles (Fig. 2A). These observations indicate that *fng* is required for border cell fate, but because both border cells and polar cells are mutant for *fng* in these mosaics, they do not strictly localize the requirement for *fng*. Importantly, however, we also identified ten counter examples in which all of the most anterior terminal cells, including the outer border cells, were *fng*<sup>-</sup>, except for the two polar cells, which were *fng*<sup>+</sup>. In all cases, *slbo-lacZ* was expressed normally, the number of border cells appeared normal, and they migrated away from the anterior of the follicle (Fig. 2B). Together, these observations demonstrate that *fng* is not required within the outer border cells themselves, and instead that polar cells are essential for the specification of border cell fate.

#### Anterior polar cells are required for stretched cell formation

The stretched cells comprise a population of about 40 to 50 cells that undergo a transition from a columnar to a squamous epithelium during stage 9 of oogenesis (Fig. 1). This morphological transition allows them to expand and cover the nurse cells when the central follicle cells migrate towards and over the oocyte. The stretched cells can be recognized morphologically by the middle of stage 9. At this time, they also begin to express an enhancer trap insertion in an unknown gene, *MA33*.

We identified six *fng* mosaic follicles from late stage 9 to late stage 10 of oogenesis without anterior polar cells. All six lacked detectable stretched cells as visualized by DNA staining (Fig. 3A). In addition, four out of the six also carried the *MA33* enhancer trap line, but it was not detectably expressed in any of them (Fig. 3A). Interestingly, despite the absence of the stretched cells, the anterior follicular cells continue to migrate over the oocyte, such that many nurse cells are no longer covered by a follicular epithelium (Fig. 3A, Fig. 4A). These data demonstrate that *fng* is required for stretched cell determination. The requirement for *fng* was localized



**Fig. 1.** Schematic of stage 6 (A) and 10 (B) follicles. In all figures, follicles are arranged with anterior towards the left. The arrangement and fates of different cells are indicated by the colors of their nuclei: oocyte (dark brown), nurse cells (light brown), central follicle cells (gray), polar cells (red), stalk cells (light blue), outer border cells (green), stretched cells (pink), centripetal cells (dark blue) and posterior terminal cells (black). Although it is thought that the border, stretched and centripetal cells are specified by stage 6, no markers are available to differentiate them until later.

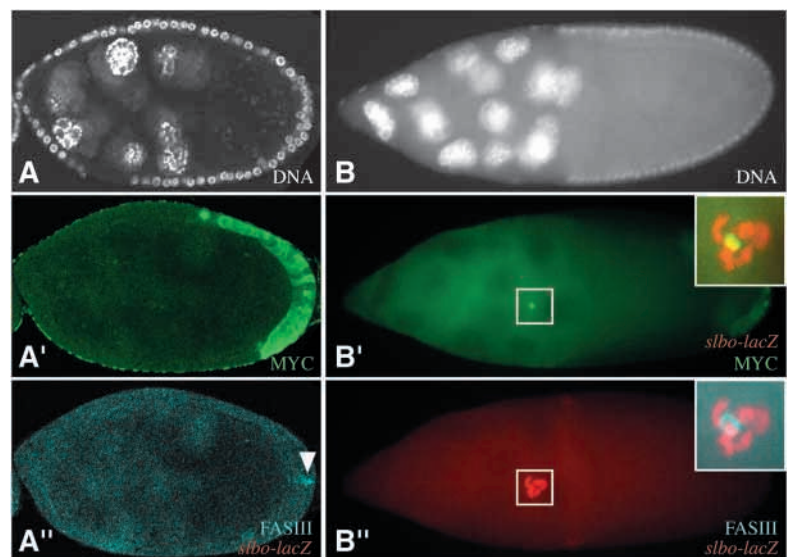
to the polar cells by the identification of 12 counter examples in which most anterior cells were *fng*<sup>-</sup> except the two anterior polar cells, which were *fng*<sup>+</sup>. In all 12 cases, *MA33* was expressed and the stretched cells appeared morphologically normal (Fig. 3B). Thus, the polar cells are required for the specification of stretched cell fate.

#### Anterior polar cells are required for centripetal cell formation

The centripetal cells consist of 30 to 40 follicle cells that migrate during stage 10B of oogenesis in between the nurse cells and the anterior of the oocyte. They are morphologically recognizable from stage 10B of oogenesis, and can also be distinguished at this time by their expression of an enhancer trap insertion in an unknown gene, *BB127*, and by expression of *slbo*.

We identified three late stage 10B *fng* mosaic follicles without anterior polar cells. All three lacked centripetal cells, as visualized by DNA staining (Fig. 4A). In addition, one carried the *slbo-lacZ* enhancer trap, but it was not detectably expressed. Thus, *fng* is required for centripetal cell formation. The anterior of the oocyte is abnormally shaped in these

**Fig. 2.** Polar cells are required to specify border cells. *fng* mosaic follicles stained with Hoechst (DNA, white) and antibodies recognizing  $\pi$ -MYC (*fng*<sup>+</sup> cells, green),  $\beta$ -galactosidase (*slbo-lacZ*, border cells, red) and FasIII (polar cells, cyan). In all figures, panels marked ' or ' ' show separate channels of the same follicle. (A) Stage 9 follicle with no *fng*<sup>+</sup> anterior cells. No anterior polar cells are formed and no border cells are specified. Arrowhead indicates posterior polar cells. (B) Stage 10A follicle with only the two polar cells *fng*<sup>+</sup> at the anterior. The polar and outer border cells are specified and properly migrate, even though all outer border cells are *fng*<sup>-</sup> (insets).

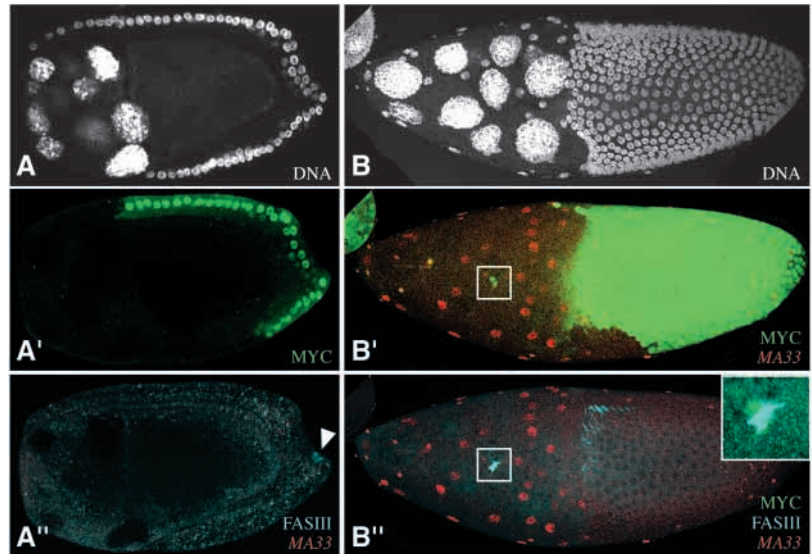


follicles, which we attribute to the lack of centripetal cells (Fig. 4A). The requirement for *fng* was localized to the polar cells by the identification of seven counter examples in which all centripetal cells were *fng*<sup>-</sup> and the two anterior polar cells were *fng*<sup>+</sup>. In all cases, centripetal cells in the process of migrating between the nurse cells and the oocyte could be identified (Fig. 4B). Three of the follicles were from animals that carried the *BB127* enhancer trap, and one was from an animal that carried *slbo-lacZ*. In all four cases, these markers of centripetal cell fate were expressed normally (Fig. 4B). Thus, the polar cells are required for the correct specification of centripetal cells.

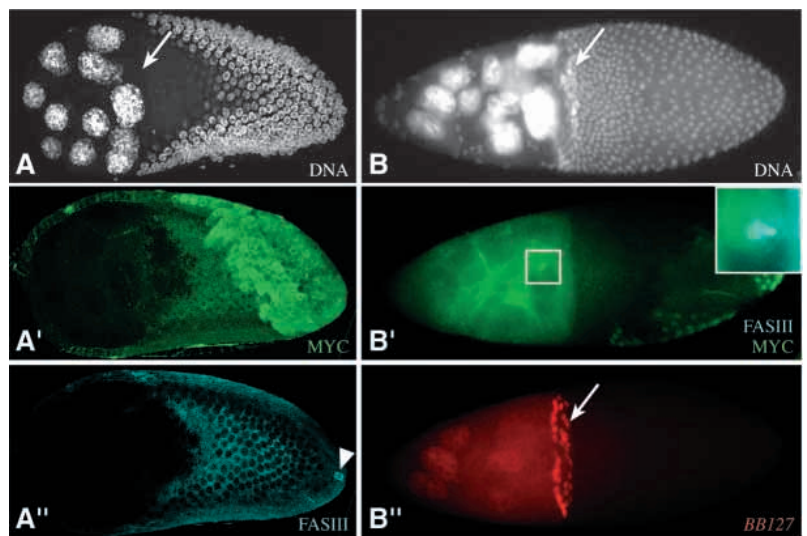
### Extra Polar cells are sufficient to induce additional border cells

The observations described above demonstrate that anterior polar cells are required for the specification of other anterior terminal cell fates. In order to investigate whether polar cells are sufficient to induce these fates, we assayed the consequences of increasing the number of polar cells by inducing the production of clones of cells expressing a constitutively-activated form of the Notch receptor. Expression of activated-Notch can induce additional polar cells (Grammont and Irvine, 2001). However, only cells at the extreme termini of the follicle (presumably cells of the polar/stalk lineage) are competent to respond to Notch activation by becoming polar cells. Thus, expression of activated-Notch can increase the number of polar cells at their normal location at the end of the follicle, but can not induce polar cell formation at ectopic locations.

Sixteen examples of stage 9 or 10 follicles with additional polar cells, as assayed by expression of *FasIII* or *neuralized-lacZ*<sup>A101</sup>, were identified and characterized. All sixteen were associated with an increase in the number of outer border cells. In eleven of these cases, all cells in the follicle expressed activated-Notch (Fig. 5A,C), yet aside from the formation of extra polar and border cells, no morphological defects were observed. In the other five examples, activated-Notch was only expressed in two or three cells (Fig. 5B). These cells were polar cells and were surrounded by border cells. Thus, the formation of additional border cells does not require the expression of the activated Notch in any follicular cells besides the polar cells. In three of the examples, the wild-type polar cells could be clearly identified, as they did not express activated-Notch. The presence of extra polar cells thus does not inhibit the formation of the normal polar cells, nor does it inhibit their function as these wild-type polar cells were surrounded by outer border cells and they migrated normally (Fig. 5B). In all cases, the additional polar cells are also surrounded by outer border cells, and they migrate normally through the nurse cells. In six examples, all

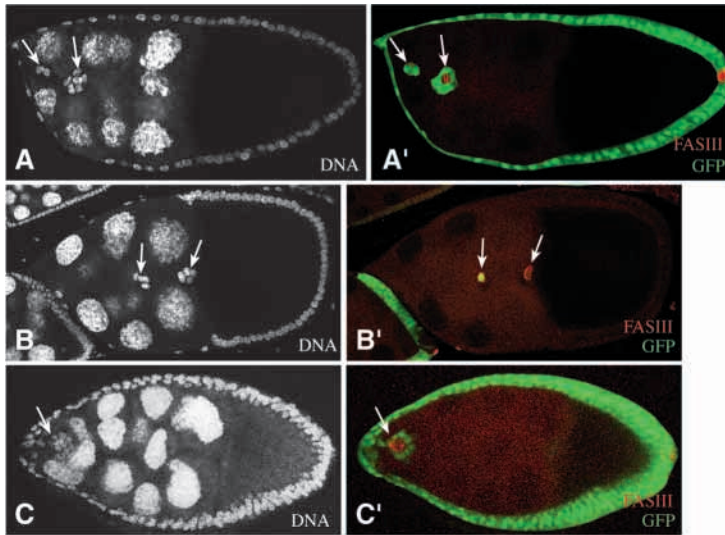


**Fig. 3.** Polar cells are required to specify stretched cells. (A,B) *fng* mosaic follicles stained with Hoechst (DNA, white) and antibodies recognizing  $\pi$ -MYC (*fng*<sup>+</sup> cells, green),  $\beta$ -galactosidase (*MA33*, stretched cells, red) and *FasIII* (polar cells, cyan). (A) Stage 9 follicle with no *fng*<sup>+</sup> anterior cells. No anterior polar cells form and no stretched cells are specified. Arrowhead indicates posterior polar cells. (B) Stage 10B follicle with only a few *fng*<sup>+</sup> cells at the anterior, including the polar cells (inset). The polar and stretched cells are specified properly, even though almost all stretched cells are *fng*<sup>-</sup>.



**Fig. 4.** Polar cells are required to specify centripetal cells. (A,B) *fng* mosaic follicles stained with Hoechst (DNA, white) and antibodies recognizing  $\pi$ -MYC (*fng*<sup>+</sup> cells, green),  $\beta$ -galactosidase (*BB127*, centripetal cells, red) and *FasIII* (polar cells, cyan). (A) Stage 9 follicle with no *fng*<sup>+</sup> anterior cells. No anterior polar cells form and no centripetal cells are specified (arrow in A). Arrowhead marks posterior polar cells. (B) Stage 10B follicle with only the two polar cells *fng*<sup>+</sup> at the anterior (inset). The polar and centripetal cells are specified and properly migrate (arrow), even though all centripetal cells are *fng*<sup>-</sup>. Faint MYC stain in the anterior is germline expression.

of the border cells were together in one large cluster (Fig. 5C), while in ten examples the border cells were split into up to four independently migrating groups (Fig. 5A,B). These additional



**Fig. 5.** Polar cells are sufficient to specify border cells. Follicles with flip-out clones (marked with GFP, green) expressing constitutively activated Notch (ActN) and stained with Hoechst (DNA, white) and antibodies recognizing FasIII (polar cells, red). The arrows indicate the position of border cell groups. (A) Stage 9 follicle with an anterior group containing one polar cell and two outer border cells, and a posterior group containing two polar cells and six outer border cells. All cells express ActN. (B) Stage 10A follicle with an anterior group containing the only ActN cell, which behaves as a polar cell and migrates with three outer border cells. The posterior group includes the wild-type polar cells and five outer border cells. (C) Stage 9 follicle in which all cells express ActN and one group of border cells forms from four polar cells and 11 outer border cells.

clusters of migrating border cells always include at least one polar cell. In eight of these cases, the migrating border cells included only a single polar cell, demonstrating that one polar cell is sufficient for the formation and the migration of a border cell cluster (Fig. 5A,B).

Notably, the number of outer border cells in each migrating cluster correlates with the number of polar cells. When only a single polar cell was detected at the center of a cluster, an average of three outer border cells was observed (Fig. 5A,B). When two polar cells are observed, they were surrounded by approximately six outer border cells, as in wild-type follicles (12 examples, Fig. 5A,B). In the six examples where four polar cells were identified at the center of a cluster of border cells, they were surrounded by approximately twelve outer border cells (Fig. 5C). Thus, the number of outer border cells that are specified and migrate with each cluster is proportional to the number of polar cells.

### Posterior polar cells control posterior localization of the oocyte

Posterior terminal cells have two crucial functions during oogenesis: to localize the oocyte to the posterior of the follicle at stage 1, and to establish the developmental axes of the oocyte at stage 6 (see below). The posterior localization of the oocyte is achieved through differential adhesion: the oocyte expresses higher levels of E-cadherin than do other germline cells, and the posterior follicular cells express higher levels of E-cadherin than do central follicular cells (Godt and Tepass, 1998; Gonzalez-Reyes and St Johnston, 1998a). Homophilic

adhesion mediated by E-cadherin then maintains the oocyte at the posterior of the follicle. Mosaic analysis has demonstrated that the requirement for E-cadherin maps to posterior terminal cells, but not specifically to the polar cells. To examine the role of the polar cells in oocyte localization, we searched for *fng* mosaic follicles that lacked posterior polar cells.

Four *fng* mosaic follicles without posterior polar cells were identified, and in all cases the oocyte was abnormally localized to the anterior of the follicle, in contact with the anterior polar cells (Fig. 6A). The anterior terminal cells also express elevated levels of E-cadherin, and posterior clones mutant for E-cadherin similarly result in mislocalization of the oocyte to the anterior. Importantly, *fng* mosaics in which only the two polar cells are *fng*<sup>+</sup>, and all other posterior cells are mutant, form wild-type follicles with a posteriorly located oocyte (four examples), as described previously (Grammont and Irvine, 2001). Together, these observations localize the requirement for *fng* in oocyte positioning to the polar cells, and demonstrate that posterior follicle cells are unable to localize the oocyte in the absence of polar cells.

To determine whether a polar cell-dependent terminal cell fate is required for the initial asymmetric localization of the oocyte within the follicle, or only for its maintenance, we examined *fng* mosaics at stage 1, when the oocyte first becomes localized to the posterior. As no markers are available for polar cells at this stage, we looked for stage 1 follicles surrounded entirely by *fng*<sup>-</sup> cells, which can not form polar cells. We identified ten *fng*<sup>-</sup> stage 1 follicles, and the oocyte was mislocalized in all ten (Fig. 6D, compare with 6C). Thus, the dependence of oocyte positioning on *fng* begins during follicle formation and not later, during follicle maturation. Because *fng* acts cell-autonomously to specify polar cell fate, and is later not required in any other follicle cells for oocyte localization, these observations imply that the polar cells have an essential role during early oogenesis in oocyte localization, well before they become recognizable through their expression of specific molecular markers.

In 33 cases, *fng* mosaic follicles were recovered that lacked posterior polar cells, but instead had two polar cells along the lateral side of the follicle (Fig. 6B,F,H,J). Although we do not understand how these abnormally constructed follicles arise, they presented an opportunity to investigate the ability of ectopic polar cells to control the position of the oocyte. In all cases, the oocyte was in contact with these mislocalized polar cells rather than at the posterior or anterior termini of the follicle. As prior studies have ruled out any role for the oocyte in the induction of polar cell fate (Oh and Steward, 2001) and for the polar cells in the specification of the oocyte (Grammont and Irvine, 2001; Lopez-Schier and St Johnston, 2001), we conclude from the co-localization of the oocyte and the polar cells that the polar cells are not only necessary but also sufficient to direct the localization of the oocyte within the follicle.

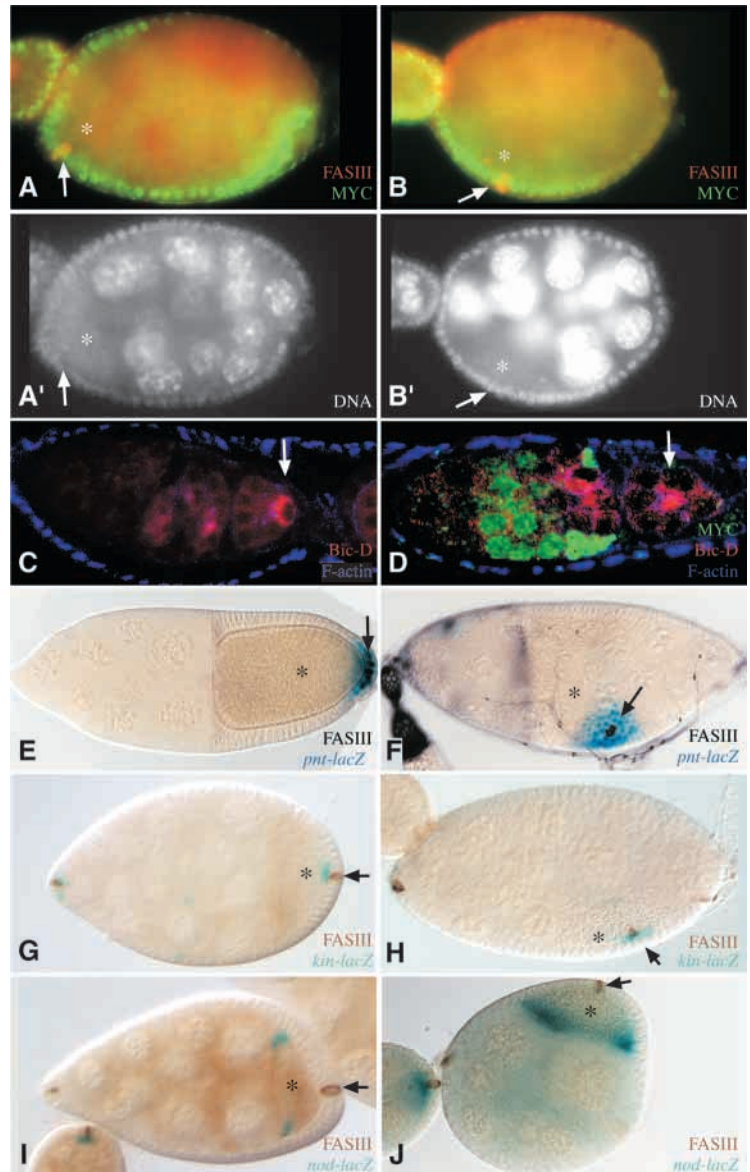
We note also that when these follicles lack normal anterior as well as posterior polar cells, they tend to be round, or even elongated perpendicular to the axis of the ovariole (Fig. 6J). This phenotype is reminiscent of that of *Leucocyte antigen*

**Fig. 6.** Polar cells localize the oocyte and establish the AP axis. (A,B) *fng* mosaic follicles stained with Hoechst (DNA, white) and antibodies recognizing  $\pi$ -MYC (wild-type cells, green) and FasIII (polar cells, red). (A) Stage 6 follicle with no posterior polar cells; the oocyte (asterisk) is at the anterior, in contact with the anterior polar cells (arrow). (B) Stage 5 follicle with ectopic lateral polar cells and no posterior polar cells. The oocyte (asterisk) is ectopically localized, in contact with the lateral polar cells (arrow). (C,D) Germaria stained with phalloidin (F-actin, blue) and antibodies recognizing Bic-D (oocyte, red). (C) Wild type. At stage 1, the oocyte (arrow) is located at the posterior of the cyst (D) *fng* mosaic marked with  $\pi$ -MYC (*fng*<sup>+</sup> cells, green). In a stage 1 mutant follicle, the oocyte (arrow) is in the center of the cyst. (E,G,I) Wild-type follicles and (F,H,J) *fng* mosaic follicles stained with X-gal (blue/turquoise, *pnt*<sup>S99812</sup> in E,F; *kin*:*lacZ* in G,H; *nod*:*lacZ* in I,J) and antibodies recognizing FasIII (polar cells, black/brown). In F,H,J, the polar cells (arrow) are ectopically localized to the side of the follicle, but (F) the follicle cells surrounding them adopt a posterior identity, and (H,J) the AP axis of the oocyte (asterisk) is correctly oriented with respect to the polar cells.

*related* (*Lar*) mutations (Frydman and Spradling, 2001). *Lar* encodes for a receptor-like tyrosine phosphatase, which is required for epithelial planar polarity during oogenesis. Our observations are thus consistent with a hypothesized role for the polar cells in a *Lar*-dependent reorganization of actin filaments that influences follicle elongation (Frydman and Spradling, 2001).

### The posterior polar cells control responsiveness to the EGFR signal that establishes the anteroposterior and dorsoventral axes of the oocyte

Posterior terminal cells also have a second crucial function during oogenesis, in establishing the anteroposterior and dorsoventral axes of the egg through reciprocal signaling with the oocyte. Prior to stage 6, a microtubule organizing center (MTOC) is located at the posterior of the oocyte, resulting in a network of microtubules with their minus ends at the posterior of the oocyte and their plus ends at the anterior of the follicle (Theurkauf et al., 1992). At stage 6, the oocyte signals to the follicle cells through the EGFR ligand Gurken (Gonzalez-Reyes et al., 1995; Roth et al., 1995). This signal represses anterior terminal follicle fate and establishes posterior terminal follicle fate (Gonzalez-Reyes and St Johnston, 1998b). The posterior follicular cells then send back an unknown signal to the oocyte that inactivates the existing MTOC (Deng and Ruohola-Baker, 2000; Gonzalez-Reyes et al., 1995; MacDougall et al., 2001; Roth et al., 1995). In parallel, a new microtubule network is established with the minus ends of the microtubules at the anterior of the oocyte and the plus ends at the posterior (Theurkauf et al., 1992). This new microtubule network is essential for the correct localization of the anterior, posterior and dorsal determinants within the oocyte, and consequently for the later establishment of embryo polarity (Gonzalez-Reyes et al., 1995; Neuman-Silberberg and Schupbach, 1993; Roth et al., 1995). The posterior terminal cells also express specific genes or markers of posterior identity, such as the



*pointed* gene (Fig. 6E) (Gonzalez-Reyes and St Johnston, 1998b).

Prior studies have established that only posterior terminal cells, and not central follicle cells, are competent to express these genes and to signal back and inactivate the first MTOC in response to the Gurken signal (Gonzalez-Reyes et al., 1997; Gonzalez-Reyes and St Johnston, 1998b). These studies also show that this competence does not depend on signaling from the oocyte (Gonzalez-Reyes and St Johnston, 1998b). Although this competence maps to posterior terminal cells and not specifically to polar cells, we hypothesized that the distinct behavior of the posterior terminal cells could nonetheless be established by signaling from the polar cells. It is not possible to examine the fate of posterior cells or the microtubule network in oocytes without posterior polar cells, as in such mosaics the oocyte simply relocates to the anterior. Thus, to address the question of whether the polar cells can induce a terminal fate in neighboring follicular cells, we analyzed *fng* mosaic follicles that lacked posterior polar cells and instead

possessed two lateral polar cells. As described above, the oocyte localizes to the side of the follicle in these cases.

In 7/7 *fng* follicles with lateral polar cells that carried an enhancer trap in the *pointed* gene, *pnt-lacZ*<sup>S99812</sup>,  $\beta$ -galactosidase staining was observed in follicle cells surrounding the ectopic polar cells (Fig. 6F). Thus, ectopic polar cells induce a competence in neighboring cells to respond to the Gurken signal by expressing a posterior follicle marker. If these cells are fully functional posterior cells, they should also be able to signal back to the oocyte to inactivate the first MTOC. To examine the polarity of the microtubule cytoskeleton, we took advantage of previously established *nod:lacZ* and *kin:lacZ* reporter constructs (Clark et al., 1997). These constructs fuse the minus end directed motor Nod or the plus-end-directed motor kinesin to  $\beta$ -galactosidase, and consequently they serve as reporters of the minus and plus ends of microtubules (Fig. 6G,I). In seven out of seven *fng* follicles with lateral polar cells that carried the *kin:lacZ* marker,  $\beta$ -galactosidase staining was observed where the oocyte contacts follicle, near the polar cells (Fig. 6H). In seven out of seven *fng* follicles with lateral polar cells that carried the *nod:lacZ* marker,  $\beta$ -galactosidase staining was observed where the oocyte contacts the nurse cells, far from the polar cells (Fig. 6J). Thus, in these lateral oocytes the microtubule cytoskeleton is oriented perpendicular to the normal AP axis of the follicle, but is nonetheless correctly established with respect to the polar cells. We conclude from this that the follicle cells surrounding these lateral polar cells have been instructed by the polar cells to adopt a terminal follicular fate that renders them competent to adopt a posterior fate in response to Gurken signaling from the oocyte.

#### *upd* is not required for terminal follicle cell fates

The results of the experiments described above indicate that polar cells signal to neighboring anterior and posterior terminal cells to influence their fate. The *upd* gene encodes a ligand for the JAK-STAT pathway that is expressed specifically in the polar cells and is required for normal follicle formation (Baksa et al., 2002; Harrison et al., 1998; McGregor et al., 2002; Sefton et al., 2000). In order to investigate whether *upd* could account for some or all of the signaling capabilities of the polar cells, we initiated experiments to analyze the consequences of removal of *upd* on polar cell-dependent terminal fates. Recently, two other laboratories have published the results of investigations into the role of the JAK-STAT pathway in follicle patterning (Beccari et al., 2002; Silver and Montell, 2001). These studies demonstrated that *upd* is required for the normal recruitment and migration of the border cells, but did not directly address the potential role of *upd* in other terminal fates. Thus, we present here briefly the results of our own analysis of follicles that are mosaic for a null allele of *upd*.

We examined eight stage 9-10A mosaic follicles with *upd*<sup>-</sup> anterior polar cells. As judged by DNA staining, these follicles contain fewer than normal border cells, and these border cells fail to migrate (data not shown) (Silver and Montell, 2001). Although this indicates that *upd* influences the outer border cells, this phenotype is significantly milder than the complete absence of border

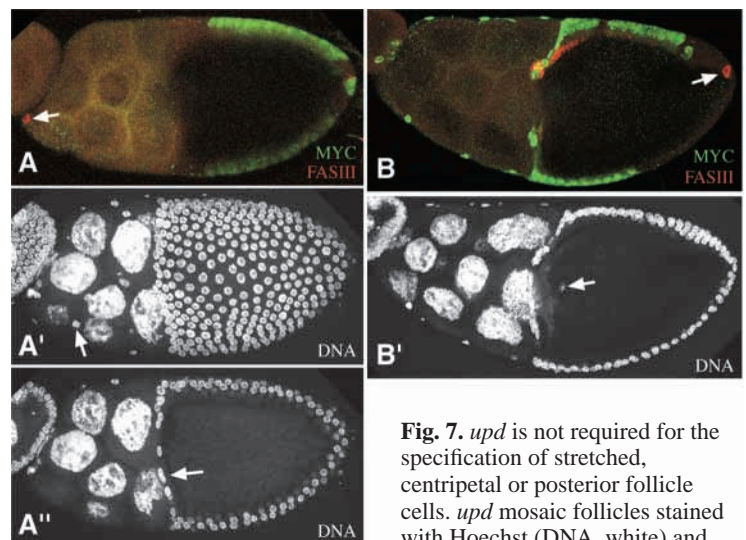
cells that is observed upon genetic ablation of the anterior polar cells. Examination of DNA staining in stage 9 to 10 follicles with *upd*<sup>-</sup> anterior polar cells revealed the presence of morphologically normal stretched cells (eight examples, Fig. 7A), while examination of DNA staining in stage 10B follicles with *upd*<sup>-</sup> anterior polar cells revealed the presence of morphologically normal centripetal cells migrating at the interface between the nurse cells and the oocyte (six examples, Fig. 7A). Thus, *upd* is not essential for the differentiation of stretched or centripetal cells.

We also identified 17 mosaic follicles with *upd*<sup>-</sup> posterior polar cells. In all cases, DNA staining and Nomarsky optics revealed the presence of a normally localized oocyte at the posterior part of the follicle (Fig. 7B). As a marker of the establishment of anteroposterior polarity, we determined the position of the oocyte nucleus in stage 9 or 10 follicles. In all cases (11 examples, Fig. 7B), the oocyte nucleus was correctly positioned in the anterior part of the ooplasm. Thus, *upd* is also not essential for the specification of surrounding terminal cells at the posterior of the follicle. The observation that *upd* mutant polar cells are able to assume most of the functions of polar cells in follicle patterning further implies that *upd* is not required for polar cell fate.

## DISCUSSION

### Polar cells organize terminal follicle cell patterning

The possibility that the polar cells influence the fate of surrounding cells has been suggested previously, however, it was not possible to establish a definitive link between the function of the polar cells and the fates of surrounding cells, because it was not possible to eliminate or specifically manipulate the polar cells. Recently, we and others established that activation of the Notch signaling pathway is essential for



**Fig. 7.** *upd* is not required for the specification of stretched, centripetal or posterior follicle cells. *upd* mosaic follicles stained with Hoechst (DNA, white) and antibodies recognizing  $\pi$ -MYC

(*upd*<sup>+</sup> cells, green) and FasIII (polar cells, red). (A) Stage 10B follicle with no *upd*<sup>+</sup> anterior cells. The polar cells form (A, arrow) and the stretched (A', surface section, arrow) and centripetal (A'', internal section, arrow) cells are specified. (B) Stage 10B follicle with no *upd*<sup>+</sup> cells at the posterior. The polar cells form (B, arrow), the oocyte is posteriorly localized, and the oocyte nucleus is at the anterior of the oocyte (B', arrow).

polar cell specification, and that activation of Notch in the polar cells requires the Notch modulator *fringe* (Grammont and Irvine, 2001; Lopez-Schier and St Johnston, 2001). We have taken advantage of this to genetically ablate the polar cells and demonstrate their essential functions in follicle patterning.

At the anterior of the follicle, each of the three distinct cell types that surround the polar cells, the border cells, the stretched cells, and the centripetal cells, fail to form in the absence of polar cells. Instead, these cells appear to adopt the fate of central follicle cells, which is to migrate over the nurse cells towards and around the oocyte, leaving the anterior germ cells uncovered. The conclusion that the polar cells serve as organizers of follicle patterning is also supported by the ability of additional or mispositioned polar cells to redirect the fates of neighboring cells. When the number of anterior polar cells is increased by expression of an activated form of the Notch receptor, the number of border cells is increased, and this increase occurs in proportion to the number of extra polar cells. Similarly, activation of the HH pathway can result in both extra polar cells and a corresponding increase in border cells (Forbes et al., 1996; Liu and Montell, 1999; Zhang and Kalderon, 2000).

In contrast to the anterior terminal cells, the posterior terminal cells do not exhibit obvious differences in morphology or behavior from central follicle cells. Nor are distinct molecular markers available, because all of the known posterior-specific genes are also targets of EGFR signaling from the oocyte, which cannot occur in the absence of polar cells due to the mislocalization of the oocyte. However, *fng* mosaic follicles are sometimes abnormally constructed such that polar cells are formed along the sides of the follicle, rather than at the posterior. These ectopic polar cells are sufficient to confer to the neighboring cells a posterior identity, which then directs the reorganization of the oocyte cytoskeleton.

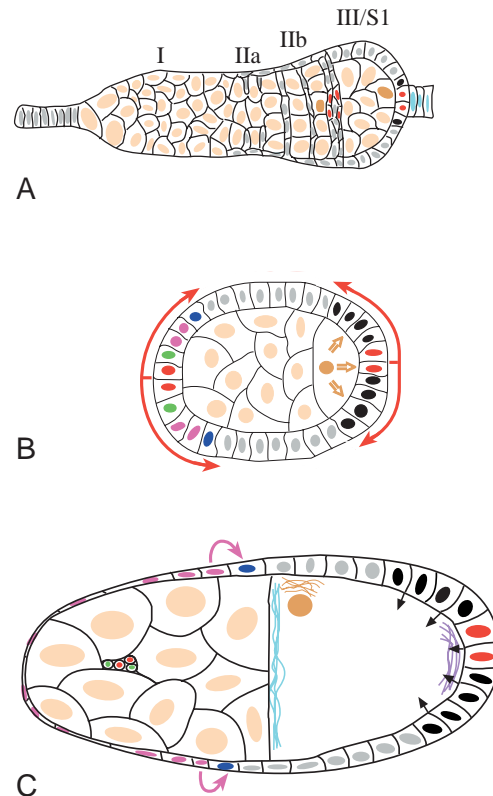
Thus, we conclude that polar cells are both necessary and sufficient to direct the fates of surrounding cells. The polar cells exhibit the hallmarks of an organizer because they not only influence the fates of surrounding cells, but they establish distinct cell fates at different distances, and they can redirect the fates of surrounding cells at ectopic locations. Although the induction of distinct fates at different distances normally only occurs at the anterior of the follicle, the capacity to establish these distinct fates also exists at the posterior, but is suppressed there by EGFR signaling (Gonzalez-Reyes and St Johnston, 1998b). The observations that distinct cell fates arise in rings at discrete distances from the polar cells at both poles in the absence of EGFR signaling pointed to the polar cells as a potential source of a signal that directs terminal fate (Gonzalez-Reyes and St Johnston, 1998b). Our analysis confirms this hypothesis and leads to an understanding of terminal patterning as a two-step process in which cells at each end of the follicle first receive identical polar-cell signals that distinguish terminal from central follicle cell fates, and then later GRK signaling from the oocyte represses anterior fates and promotes posterior fate (Fig. 8).

As is the case for boundary organizers in the *Drosophila* wing and eye, the polar cells are specified by Notch activation. Prior studies have revealed that the Notch pathway is required for the differentiation of the border and centripetal cells, and for the establishment of posterior terminal identity. These studies were conducted with a temperature sensitive allele of

*Notch*, and the location of the requirements for *Notch* was not mapped (Gonzalez-Reyes and St Johnston, 1998b; Keller Larkin et al., 1999). Our results raise the possibility that these previously established requirements for *Notch* in terminal cell patterning can be accounted for by its requirement for polar cell specification. However, *Notch* also has other roles in oogenesis, and the differentiation of all follicle cells, including the terminal cells, appears to be additionally influenced directly by Notch activation that is dependent upon Delta signaling from germline cells (Deng et al., 2001; Lopez-Schier and St Johnston, 2001).

### Nature of the polar cell signal

Although more complex models are possible, two basic mechanisms for organizer activity are a morphogen or a signal relay. In the morphogen model, a signal would be produced by polar cells and then spread to all of the cells whose fate is polar-



**Fig. 8.** Schematic representation of signals required for patterning the follicle. Cells are identified by the color of their nuclei, as in Fig. 1. (A) Germarium. In region IIb, the posterior polar cells contact the oocyte. In region III, the oocyte is at the posterior of the cyst, in contact with the polar cells. (B) Before stage 6, signal(s) from the polar cells (large red arrow) establish terminal fates in surrounding cells. In the anterior, they will become border, stretched or centripetal cells. In the posterior, they will become posterior after receiving the germline Grk signal from the oocyte at stage 6. (C) After stage 6, the posterior cells send an unknown signal to the oocyte (black arrows) to reorganize the cytoskeleton, which establishes the later localization of positional determinants, including mRNAs for *bcd* (light blue lines), *nanos* (violet lines) and *gurken* (brown lines). Grk signaling from the germline to central follicle cells establishes dorsal follicle fate. DPP signaling from the stretched cells (pink arrow) is required to establish centripetal cell fate.

cell dependent. The signal would exist in a concentration gradient, with different amounts of the signal being required to induce the distinct border, stretched and centripetal cell fates. In the signal relay model, the polar cells would produce a short range signal that induced border cells, which would then produce a second signal that induced stretched cells, which would then in turn express a third signal that induced centripetal cells. Although these models can not be definitively distinguished until the polar cell signal(s) are identified, several observations together suggest that a combination of the morphogen and signal relay mechanisms are actually employed (Fig. 8).

In support of a single long-range signal, reduction in the number of border cells by mutation of components of the JAK-STAT pathway does not result in any obvious reduction of the number of stretched or centripetal cells. Nor does ablation of the border cells by expression of a toxic protein exert obvious effects on stretched or centripetal cell fate (Han et al., 2000). Thus, the establishment of more distant polar-cell dependent cell fates does not require the establishment of intervening cell fates. Conversely, however, the determination that the specification of the centripetal cells depends in part upon DPP signaling from the stretched cells supports the idea of a signal relay from the stretched to the centripetal cells (Deng and Bownes, 1997; Dobens et al., 2000; Peri and Roth, 2000).

Further insight into the nature of the polar cell signal may be gleaned from the time and distance over which it acts. Polar cell signaling must occur at the anterior and at the posterior of the follicle prior to stage six, when terminal cells are required to confer a distinct responsiveness to EGF-R signaling from the oocyte (Gonzalez-Reyes and St Johnston, 1998b). Additionally, recent observations suggest that the polar cells behave as polarization centers with respect to the organization of F-actin within the follicular cells, which gradually aligns from the poles to the center of the follicle during stages 5-7 (Frydman and Spradling, 2001). This phenomenon suggests that a signal should exist from the polar cells even before the end of follicle cell proliferation at stage 5.

Only one gene, *upd*, is known that encodes for a signaling molecule that is expressed by polar cells (Baksa et al., 2002; Harrison et al., 1998; McGregor et al., 2002; Sefton et al., 2000). Although loss of *upd*, or other components of the JAK-STAT pathway, reduces the number of border cells (Beccari et al., 2002; Silver and Montell, 2001), this contrasts markedly with the complete elimination of border cells observed in the absence of polar cells. Moreover, loss of *upd* does not have obvious effects on any of the other terminal cell fates that are polar-cell dependent. Thus, the existence of additional signaling molecules must be invoked to account for the organizing activity of the polar cells.

### Establishment of asymmetry in *Drosophila* development

The anteroposterior and dorsoventral axes of *Drosophila* are established during oogenesis by localized determinants. These consist of mRNAs for *bcd* and *nanos* localized, respectively, at the anterior and the posterior pole for the AP axis, and mRNAs for *grk* around the oocyte nucleus for the DV axis (Neuman-Silberberg and Schupbach, 1996; Riechmann and Ephrussi, 2001) (Fig. 8). The localization of these mRNAs is dependent upon the establishment of the correct polarity of the microtubule cytoskeleton. Thus, prior work has made it

possible to trace the establishment of both the AP and the DV axes of *Drosophila* back to the signaling process between oocyte and terminal follicle cells that regulates the oocyte cytoskeleton (Gonzalez-Reyes et al., 1995; Roth et al., 1995; Theurkauf et al., 1992). This signaling further requires that the oocyte be correctly localized to the end of the follicle, which is dependent upon differential cadherin expression in the germarium (Godt and Tepass, 1998; Gonzalez-Reyes and St Johnston, 1998a).

Our observations now allow us to trace the origin of asymmetry back further, to the specification of the polar cells in the germarium, and their initial contact with the oocyte (Fig. 8). As the germline cysts move from region IIb to region III of the germarium, the oocyte localizes to the posterior of the cyst. This localization is dependent upon the polar cells, presumably because of their ability to upregulate the expression of E-cadherin. Although we cannot distinguish between the possibilities that the autonomous upregulation of E-cadherin effects oocyte localization, or that the non-autonomous upregulation induced by polar cell signaling also contributes to oocyte localization, in either case, oocyte localization and hence the initial AP asymmetry of the follicle, is established by the polar cells. All available evidence indicates that the anterior and posterior polar cells are equivalent, and the posterior localization of the oocyte is likely a consequence of the more advanced development of the posterior follicle cells surrounding region IIb cysts, which thus have the first opportunity to localize the oocyte (Fig. 8).

The localization of the oocyte at the posterior is then a necessary precondition for the second essential role of the polar cells in establishing oocyte polarity, which is to promote terminal follicle cell fate. Terminal follicle cell fate then confers a distinct responsiveness to EGFR signaling from the oocyte, which is manifest in their ability to signal back to the oocyte to destroy the initial posterior MTOC. Destruction of the initial MTOC in turn allows establishment of the correctly polarized microtubule cytoskeleton that is necessary for the ultimate establishment of the AP and DV axes. Notably, the dual roles of the polar cells in initiating the establishment of the axes of the oocyte thus work in concert, as the localization of the oocyte to the posterior of the follicle by the polar cells places the oocyte in the correct position to participate in the later reciprocal signaling process with terminal follicle cells.

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