The receptor-like tyrosine phosphatase Lar is required for epithelial planar polarity and for axis determination within *Drosophila* ovarian follicles

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SUMMARY

The follicle cell monolayer that encircles each developing *Drosophila* oocyte contributes actively to egg development and patterning, and also represents a model stem cell-derived epithelium. We have identified mutations in the receptor-like transmembrane tyrosine phosphatase *Lar* that disorganize follicle formation, block egg chamber elongation and disrupt Oskar localization, which is an indicator of oocyte anterior-posterior polarity. Alterations in actin filament organization correlate with these defects. Actin filaments in the basal follicle cell domain normally become polarized during stage 6 around the anterior-posterior axis defined by the polar cells, but mutations in *Lar* frequently disrupt polar cell differentiation and actin polarization. Lar function is only needed in somatic cells, and (for Oskar localization) its action is autonomous to posterior follicle cells. Polarity signals may be laid down by these cells within the extracellular matrix (ECM), possibly in the distribution of the candidate Lar ligand Laminin A, and read out at the time Oskar is localized in a Lar-dependent manner. *Lar* is not required autonomously to polarize somatic cell actin during stages 6. We show that *Lar* acts somatically early in oogenesis, during follicle formation, and postulate that it functions in germarium intercyst cells that are required for polar cell specification and differentiation. Our studies suggest that positional information can be stored transiently in the ECM. A major function of Lar may be to transduce such signals.

Key words: *Drosophila*, Oogenesis, Lar, Axis formation, Planar polarity, Follicle, Extracellular matrix, Actin

INTRODUCTION

Somatic follicle cells participate extensively in the production and patterning of *Drosophila* eggs (Fig. 1A; reviewed in Spradling, 1993; Nilson and Schupbach, 1999; Dobens and Raftery, 2000). After their birth from stem cells, follicle cell precursors proliferate in region 2b of the germarium and migrate to surround each successive 16-cell germline cyst (Margolis and Spradling, 1995; Tworoger et al., 1999; see Fig. 1B). During the next 2 days, cellular interactions within the germarium set the stage for a new follicle (also known as an egg chamber) to bud off that contains a single germline cyst, a monolayer of follicle cells and a spacer of specialized stalk cells. Polar cell precursors are simultaneously set aside and two polar cell pairs soon take up positions at the anterior and posterior poles of the new follicular epithelium (Fig. 1A). Genetic studies have implicated Notch signalling (Ruohola et al., 1991; Xu et al., 1992; Cummings and Cronmiller, 1994; Goode et al., 1996; Larkin et al., 1996; Grammont et al., 1997; Jordan et al., 2000), Hedgehog signalling (Forbes et al., 1996a; Forbes et al., 1996b; Liu and Montell, 1999; Zhang and Kalderon, 2000) and cadherin-mediated differential adhesion (Oda et al., 1997; Godt and Tepass, 1998; Gonzalez-Reyes and St Johnston, 1998b; Goode and Perrimon, 1997) in follicle formation, but many aspects of this process remain poorly

understood at the cellular level. For example, the role played by a subset of cells we term 'intercyst cells' (Fig. 1B), which are located just anterior to the budding follicle, has remained poorly defined. Intercyst cells lie close to forming polar and stalk cells, express specific genes, and respond to Notch signalling during the budding cycle, but their functional roles are unknown (Larkin et al., 1996; Tworoger et al., 1999).

Once formed, the follicular epithelium extensively participates in establishing both the anterior-posterior and dorsal-ventral egg axes (reviewed by van Eeden and St Johnston, 1999). Posterior follicle cell fates are defined after receipt of a local epidermal growth factor (EGF) receptor-mediated signal from the oocyte nucleus. These follicle cells communicate back to the oocyte and cause its microtubule cytoskeleton to reorganize, a process essential for stable localization of the posterior determinant Oskar, and for relocation of the oocyte nucleus to the future dorsal anterior corner. Disrupting Notch signalling (Ruohola et al., 1991), Cadherin (Godt and Tepass, 1998; Gonzalez-Reyes and St Johnston, 1998b) or Laminin A (Deng and Ruohola-Baker, 2000) in the follicle cells, or meiotic progression (Ghabrial et al., 1998) or cytoskeletal structure (Emmons et al., 1995; Manseau et al., 1996; Schulman et al., 2000) in the oocyte interferes with Oskar localization and downstream events. Subsequently, dorsal follicle cells receive another round of

EGF receptor-mediated signals from the re-positioned germinal vesicle; they respond by defining the dorsal-ventral axis and patterning additional sub-groups of follicle cells that will mediate the final morphogenesis of the egg (Schnorr and Berg, 1996; Wassermann and Freeman, 1998; Ghiglione et al., 1999). These terminal morphogenetic steps include the dumping of nurse cell contents into the oocyte (see Matova et al., 1999), egg elongation (see Edwards and Kiehart, 1996) and eggshell patterning (see Waring, 2000), events that are also controlled by germline steroid hormone levels (see Buszczak et al., 1999).

The structure and organization of the follicular epithelium are crucially important for it to properly proliferate, migrate, change shape, acquire new cell fates and to communicate with germ cells (Jackson and Berg, 1999; Bilder et al., 2000). *Drosophila* follicle cells are organized along the apical-basal axis, much like other epithelia (reviewed by Müller, 2000; see Fig. 1C). A prominent extracellular matrix (ECM) is secreted from the basal surface, while septate and adherens junctions hold neighboring cells together near their apical ends. Several genes are known to be required to maintain epithelial polarity, including armadillo (Peifer et al., 1991), DE-cadherin (Oda et al., 1997), egghead (Rubsam et al., 1998) and brainiac (Goode et al., 1996), while α-spectrin is needed to maintain the follicle cells in a monolayer (Lee et al., 1997; Zarnescu and Thomas, 1999). Mutations in two junction-associated membrane proteins, Discs-large and Scribble, disrupt follicle cell polarity, proliferation and development (Goode and Perrimon, 1997; Bilder and Perrimon, 2000).

Orthogonal to the apical-basal axis, many epithelial sheets are also polarized in the plane of the tissue. For example, wing cells produce an actin-rich hair and align them in parallel with their neighbors. Genetic studies in imaginal disc epithelia have identified a genetic pathway that is important for planar polarization (reviewed by Schulman et al., 1998). Whether spherical tissues such as ovarian follicles exhibit planar polarization has never been addressed. Basal bundles of parallel microfilaments in the follicle cells of stage 9-13 egg chambers have been described (Gutzeit, 1990). Laminin, a major component of the extracellular matrix, is also reported to occur in parallel rows within the basement membrane that adjoins the basal surface of follicle cells (Gutzeit et al., 1991). In females bearing *kugelei* mutations, the actin and laminin alignment is lost and eggs frequently fail to elongate along the anterior-posterior axis, leading the authors to propose that the basal cytoskeleton serves as a molecular corset that resists the forces of elongation (Gutzeit et al., 1991). However, the molecular nature of *kugelei* remains unknown and whether basal actin alignment marks a follicle cell planar polarity has not been addressed.

The actin cytoskeleton provides the structural basis for cell polarity in many systems. Recently, an important role has been found for protein complexes containing Arp2/3, WASP/SCAR or ENA/VASP in controlling actin polymerization (reviewed by Hu and Reichardt, 1999; Cooper and Schafer, 2000). In outgrowing axons, where the function of these complexes is well established, the 'Leukocyte antigen related' gene (*Lar*), which encodes a receptor-like tyrosine phosphatase, signals to control the activity of these complexes (Krueger et al., 1996; Wills et al., 1999a; Wills et al., 1999b; reviewed by Lanier and Gertler, 2000). Recently, a *Drosophila* homolog of the yeast

cyclase-associated protein has been identified (Benlali et al., 2000) and been shown to regulate actin polymerization in developing egg chambers (Baum et al., 2000), suggesting that similar complexes act in follicle cells.

We now identify female sterile mutations in *Lar* that disrupt multiple steps in follicle development and patterning. We show that *Lar* is required in posterior follicle cells to maintain Oskar localization in the oocyte. In addition, *Lar* plays an important role in aligning follicle cell actin relative to the polar cells to promote egg elongation along the anterior-posterior axis. We propose that Lar interacts closely with the extracellular matrix to facilitate the planar polarization of epithelial layers.

MATERIALS AND METHODS

Drosophila strains

Drosophila stocks were maintained on standard media at 22-25°C. The Lar^{bola} mutant strains were identified in a P-element mutagenesis screen (Karpen and Spradling, 1992), and were originally designated fs(2)00937 (=bola1) and fs(2)06448 (=bola2). The EMS-induced $Lar^{5.5}$ and $Lar^{13.2}$ alleles, and three *scw* alleles have been described previously (Krueger et al., 1996). Deficiencies in the *Lar* region: *Df(2L)TW84; Df(2L)TW50; Df(2L)TW2; Df(2L)TW9; Df(2L)TW65; Df(2L)TW161; Df(2L)TW1* were used for complementation tests. The screw (*scw*) gene is located within a Lar intron (Arora et al., 1994) but mutants complemented Lar^{bola} alleles (data not shown). The $LanA^{9-32}$ FRT 18A was provided by W.-M. Deng. For clonal analysis we recombined $Lar^{5.5}$ and $Lar^{13.2}$ into an FRT 40A chromosome and crossed to hs-FLP; arm-lacZ FRT 40A stock (Lecuit and Cohen, 1997). Ectopic expression of hedgehog used hs-hhM11 (Ingham, 1993).

Antibodies and reagents

The following antisera were used at the indicated dilutions: rabbit polyclonal anti-Laminin A 329 (Gutzeit et al., 1991), rabbit polyclonal anti-Oskar (Rongo et al., 1995) (1:3000), rabbit polyclonal anti-β-galactosidase antibody (Cappel) (1:3000) or mouse monoclonal anti-β-galactosidase antibody (Promega) (1:2000), and 7G10 anti-FasIII mouse monoclonal antibody (Patel et al., 1987) (1:10). Secondary antibodies were goat anti-mouse or goat anti-rabbit IgG conjugated to Alexa488 or Alexa568 (Molecular Probes) (1:400). Actin was stained with Alexa488 or Alexa568 (Molecular Probes) conjugated to phalloidin (1:200).

Antibodies against different segments of Lar protein were generated as follows: 11 GST (pGEX, Amershan Pharmacia Biotech) fusion constructs were made against pieces of Lar that varied from 150 to 200 amino acids in length. Together the fusions spanned the entire protein. Fusion proteins were purified according to Ausubel et al. (Ausubel et al., 1987). Rats were immunized with the material at Covance Research Products. Three antisera recognized a protein of correct size on western blots (data not shown), but none specifically labeled Lar protein in tissue.

Clonal analysis

Mitotic clones were generated according to Xu and Rubin (Xu and Rubin, 1993). One- to 3-day-old females of the genotype *hs-FLP*; $Lar^{5.5}$ or $Lar^{13.2}$/*Arm-lacZ* were heat shocked for 1 hour at 37°C twice a day for 3 days. Flies were transferred to fresh yeasted food. *Armadillo-lacZ*, which is strongly expressed in all cells in the ovariole (Xie and Spradling, 1998), was used as a clonal marker. In all figures, the green cells are Lar+ and blank cells are Lar−. All the clonal analysis data presented is from flies aged 7-14 days after heat shock regimen. This ensures that clones analyzed are stem cell clones (Margolis and Spradling, 1995).

Hs-hh induction of ectopic polar cells

Adult females containing the hsp70-hedgehog (hs-hh) transgene were subjected to cycles of heat shock at 37°C followed as described by Forbes et al. (Forbes et al., 1996). Ovaries were removed after 3 days of treatment and the number of polar cells analyzed by staining with anti-FasIII antibodies.

Actin labeling and immunofluorescence microscopy

For proper fixation of cytoskeleton, egg chambers were isolated in a solution of equal osmolarity (Tilney et al., 1996). For both dissection and fixation, 1× Grace's medium (GIBCO BRL) was used. The presence of phalloidin in the fixative also stabilizes F-actin. All steps were carried out at room temperature. Ovaries were dissected in 1× Grace's medium and immediately fixed for 20 minutes in freshly prepared F buffer (four parts Grace's Medium, one part fresh EM grade 16% formaldehyde, 2% Triton-X-100, 1 U/ml of phalloidin). This was followed by two 20 minute washes in phosphate-buffered saline (PBS) containing 0.2% Triton X-100 and 1 U/ml of phalloidin, and another 20 minute wash in PBS containing 0.2% Triton X-100 but without phalloidin. The ovaries were then rinsed in PBS alone, after which they were ready for antibody staining. Immunofluorescence microscopy was carried out as described previously (deCuevas et al., 1996). Stained ovaries were mounted and analyzed using a Leica NTS-confocal microscope.

Scanning electron microscopy

Drosophila eggs were fixed in 3% glutaraldehyde/1% formaldehyde 0.1 M cacodylate pH 7.4 overnight. After an ethanol dehydration series, specimens were stabilized in hexamethyldisilizane, coated with platinum/palladium and imaged in JEOL SEM 35 microscope.

Molecular analysis of *Lar^bola* alleles

The location of the P insertions *Lar^bola1* and *Lar^bola2* was determined by polytene chromosome in situ hybridization as described (Karpen and Spradling, 1992) and mapped to 37F2-38A2. Their location on the genomic sequence was determined by plasmid rescuing flanking genomic DNA as described (Karpen and Spradling, 1992), and sequencing across the junction from the 5′ P element end. Both flanking sequences gave a unique match to genomic DNA sequences from region 37F2-38A2, at nucleotides 149,050 and 175,640, respectively, of contig AE03663.1 (version 1). The annotated 5′ end of the Lar transcript in the same coordinate system is at 170,056 and the end of the first exon is at 170,203.

Northern blot analysis

Total ovary RNA was obtained, size fractionated and blotted as described by Schneider and Spradling (Schneider and Spradling, 1997), except that TRIzol™ (Gibco BRL) was used as extraction buffer. The blot was probed with a 1.5Kb *Eco*RI fragment from Dlar55 cDNA (Streuli et al., 1989), corresponding to the 5′ region of the gene. The blot was simultaneously probed with the 3.4 kb *Eco*RI-*Sac*I fragment isolated from the full-length cDNA of the *cup* gene (Keyes and Spradling, 1997) as a loading control.

Whole-mount in situ

Single strand digoxigenin-labeled sense and antisense cDNA probes from the clone Lar55 (Streuli et al., 1989) were generated by single strand PCR (Patel et al., 1992). Hybridization was carried out according to Suter and Steward (Suter and Steward, 1991).

RESULTS

Bola mutations block egg elongation and the planar polarization of basal actin filaments

Ovarian follicles are nearly round when first budded from the germarium, but elongate differentially along their anterior-
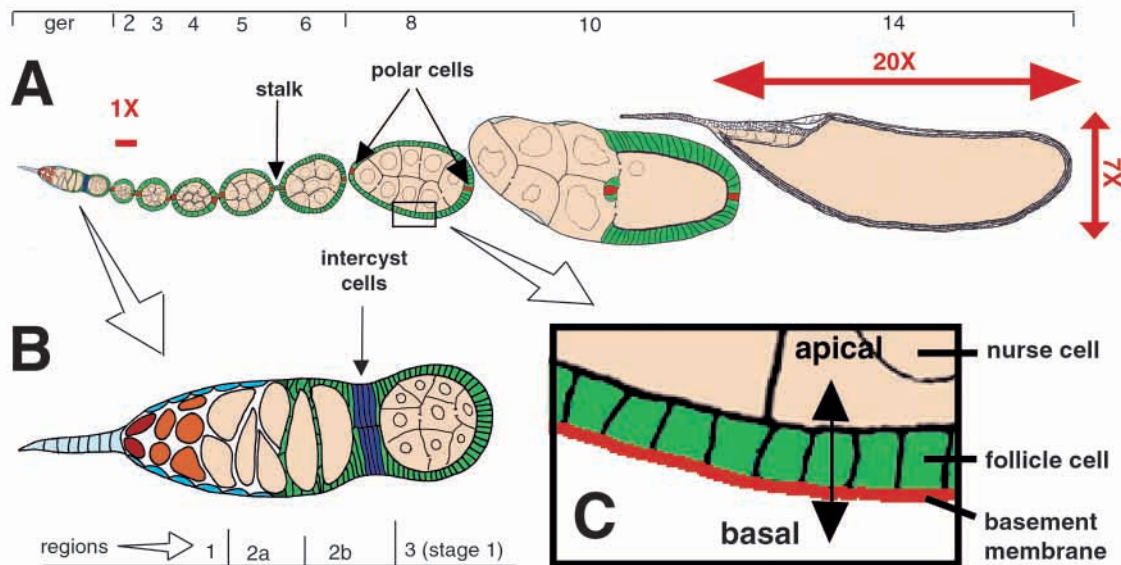

Fig. 1. Structure and development of *Drosophila* ovarian follicles. (A) A drawing of a *Drosophila* ovariole, showing the anterior germarium (ger) followed by a string of eight successively older ovarian follicles connected by interfollicular stalks. The position of the polar cells is shown (red). Each egg chamber stage is indicated above (see Spradling, 1993). As a result of egg elongation during stages 8-14, mature stage 14 egg chambers are 20-fold longer than stage 2 egg chambers in the AP axis, but only seven times wider in the DV axis. Somatic cells are shown in green, whereas nurse cells and the oocyte are tan; the germline stem cells and forming cysts are illustrated in red and orange, respectively. (B) A magnified view of the germarium showing regions 1, 2a, 2b and 3. Cell types are indicated by the same colors as in A, except that the intercyst cells at the end of region 2b (arrow) are in dark blue, while the non-dividing somatic cells that surround region 1 and 2a are shown in light blue. (C) A magnified view of the follicular epithelium from a stage 8 follicle. The apical and basal orientation of the follicle cells can be seen with respect to the basement membrane (red) and the nurse cells.

posterior axis during stages 8-14 (Figs 1A, 2A), in concert with individual follicle cells (Fig. 2B). Elongation of the oocyte proper accelerates after the nurse cells break down and dump their cytoplasm into the oocyte. Several factors are required for dumping: a force must be generated to squeeze the nurse cell contents through a properly constructed system of ring canals, and special nurse cell actin bundles must tether the nurse cell nuclei and prevent them from clogging up the canals (reviewed by Robinson and Cooley, 1997). Mutations that disrupt the stage 10 nurse cell actin bundles prevent nurse cell cytoplasmic transfer and oocyte elongation, indicating a possible role for actin-myosin networks in elongation. However, the failure of eggs to elongate in this class of mutations might alternatively be due to a checkpoint or be a physical requirement of the dumping process for the later events of elongation.

To distinguish the roles of actin filaments in dumping and egg elongation, we identified two allelic female sterile mutations, termed *bola1* and *bola2*. In mutant females 20-70% of the follicles fail to elongate, despite transferring their nurse cell contents normally (see Fig. 2A (inset),C). Unelongated follicles develop into eggs that are nearly round, resembling a soccer ball ('bola' in Portuguese), and contain unelongated follicle cells (Fig. 2C,D), but enclose a normal volume (112%±29% of wild type *n*=40). When stage 10A *bola* follicles were stained with Alexa-phalloidin to visualize actin filaments, normal microfilament bundles were observed in nurse cells, consistent with the ability of these eggs to normally transfer their cytoplasmic contents (Fig. 2G). Consequently, we analyzed the bundles of actin filaments located near the basal cortex of stage 8-13 follicle cells to determine if these structures were affected by the mutations.

Although basal actin filament bundles are known to be present and to be aligned perpendicular to the anterior-posterior (AP) axis of the egg chamber by stage 9 (Gutzeit, 1990), their behavior earlier in oogenesis has not been reported previously. Taking care to preserve actin morphology (see Materials and Methods), we fixed and stained wild-type and homozygous *bola* mutant ovaries, and studied the behavior of actin filaments in somatic cells. In the germarium, large actin bundles are located along the basal cortex of somatic cells that

line region 2b and 3 (Fig. 2E). These filaments are aligned in an organized fashion such that they run around the ovariole perpendicular to its AP axis. At the time a new follicle buds from the germarium, this orientation is lost (Fig. 2E, arrow). Newly formed stage 2 chambers typically retain some organized actin near their poles, but in the middle of the follicle, actin filaments swirl along the AP axis. Stage 4 egg chambers show no evident polarity or organization of their actin filaments (Fig. 2F). By stage 7, however, the basal actin
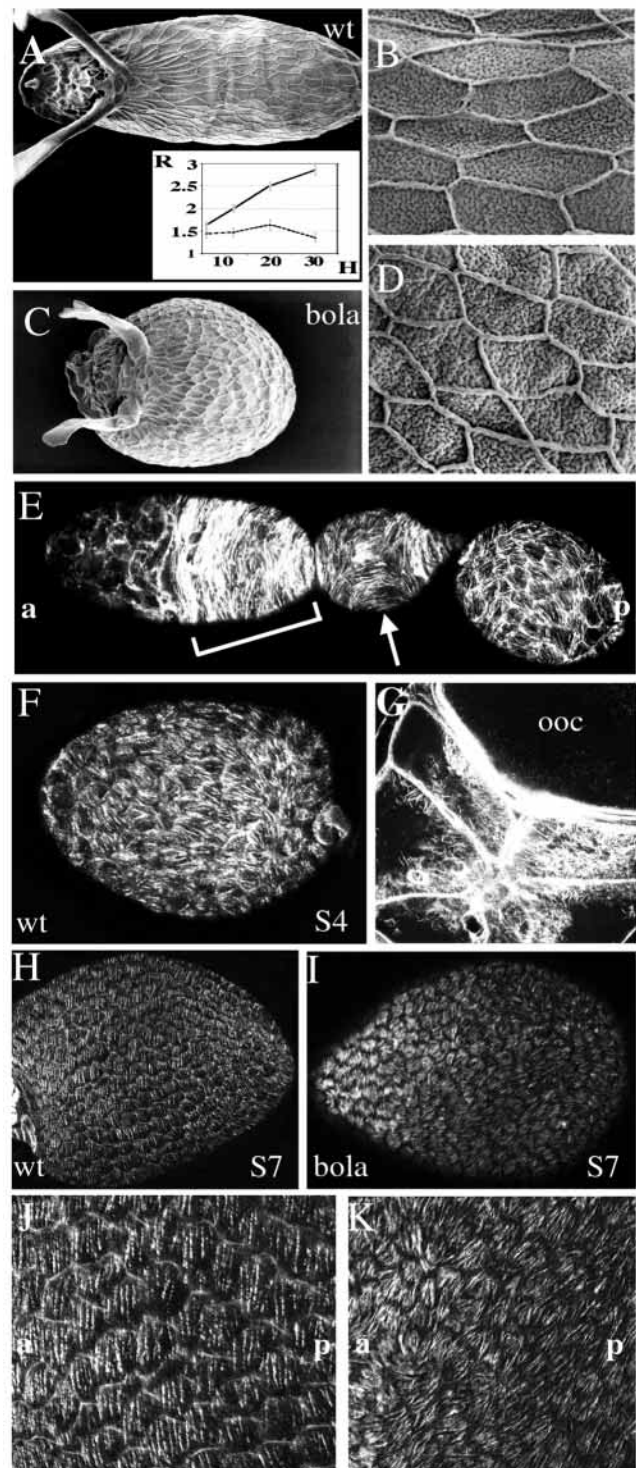

Fig. 2. *bola* mutations disrupt egg elongation and basal actin alignment. Scanning electron micrographs of wild type (A) and homozygous *bola2* mutant (C) stage 14 egg chambers illustrate that *bola* follicles remain round, instead of elongating like wild type. A graph of the ratio (R) of the AP axis to the DV axis in wild type (unbroken line) and *bola* (broken line) egg chambers is plotted versus the time in hours (H) after stage 7 (inset). Individual *bola* follicle cells fail to elongate as indicated by the shape of individual follicle cell imprints in the eggshell (compare B (wild type) with D (*bola*)). Alexa-phalloidin staining in the germarium (E) shows abundant basal actin fibers aligned perpendicular to the AP axis, but newly budded stage 2 follicles contain swirling, unpolarized actin (arrow). At stage 4 (F), the orientation of basal actin in follicle cells still lacks a coordinated pattern; however, by stage 7 (H), it is polarized perpendicular to the AP axis of the egg chamber (J). By contrast, in *bola* egg chambers, the basal actin at stage 7 (I) and later (not shown) usually does not become polarized (K). However, the nurse cell actin filaments in stage 10 *bola* follicles appear normal (G). a, anterior; ooc, oocyte; p, posterior; S4, stage 4; S7, stage 7; wt, wild type.

of all the follicle cells is aligned perpendicular to the AP axis (Fig. 2H,J).

In *bola* mutant follicles, the actin filaments were present at normal levels and their orientation was indistinguishable from wild type in the germarium and stage 2-4 egg chambers. However, in 30-90% of follicles, the filaments never became aligned perpendicular to the AP axis, although intracellularly the number and size of actin bundles appeared normal (Fig. 2I,K). Thus, a failure to globally polarize the microfilament arrays within individual follicle cells correlated with the failure of *bola* mutant follicles to elongate normally. The organized circumferential bands of actin filaments in wild-type chambers might resist expansion perpendicular to the AP axis, channeling expansionary forces to elongate individual cells and the egg in the AP direction. These observations establish a role for actin filaments in egg elongation that is independent of their role in nurse cell dumping.

bola mutations are alleles of *Lar*

A single P element insertion in each *bola* mutation was mapped to cytogenetic position 38A by polytene chromosome in situ hybridization (data not shown). Genomic DNA flanking both alleles was recovered by plasmid rescue and sequenced. The insertions in *bola1* and *bola2* were located 20 kb upstream and 5 kb downstream from the previously reported 5′ end of *Lar*, a previously characterized gene in region 37F2-38A2 (Fig. 3A). As the *Lar* transcription unit is known to extend 128 kb downstream from the 5′ end, rescue of the mutation with a genomic construct was impractical. However, excision of the *bola1* element reverted all the associated phenotypes (not shown). In addition, we obtained two previously characterized lethal *Lar* alleles, *Lar*$^{5.5}$ and *Lar*$^{13.2}$, that are caused by stop codons at amino acid positions 551 and 1055, respectively (Krueger et al., 1996). These EMS-induced mutations have been generated independently using a different genetic background from that of *Lar*^{bola1} and *Lar*^{bola2}. Transheterozygotes between sterile and lethal alleles, or between sterile alleles and known deficiencies that uncover *Lar* produced sterile females whose follicles showed the same defects in egg elongation and actin alignment as *Lar*^{bola1} and *Lar*^{bola2} homozygotes (see Materials and Methods). Northern analysis of RNA extracted from whole wild-type ovaries using a Lar cDNA probe revealed a single band of 8.4 kb, the same as the previously characterized Lar mRNA from nervous tissue (Kreuger et al., 1996). Lar transcripts were undetectable in a corresponding preparation of ovarian RNA from *Lar*^{bola1} or *Lar*^{bola2} homozygous females (Fig. 3B, and data not shown). We conclude that *Lar*^{bola1} and *Lar*^{bola2} are new alleles of the *Lar* gene.

To investigate *Lar* expression during oogenesis, we carried out whole-mount in situ hybridization to wild-type ovaries. Strong *Lar* expression was first observed in the somatic follicle cell precursors located in region 2b of the germarium (Fig. 3C).
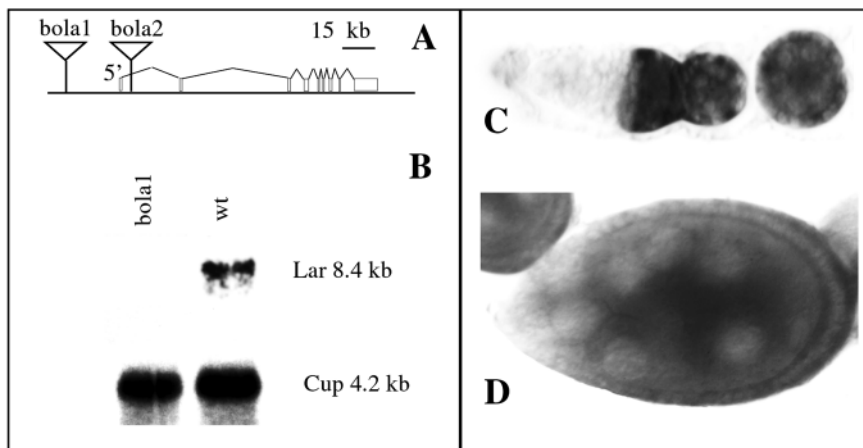

Fig. 3. *bola* mutations are viable alleles of *Lar*. The 129 kb *Lar* transcription unit is shown, along with the positions of the *bola1* and *bola2* P element insertions. *bola1* lies 21.0 kb upstream from the previously described 5′ end of *Lar*. *bola2* is located 5.4 kb downstream from the 3′ end of exon 1 in the first intron. (B) *bola* mutations abolish ovarian expression of *Lar*. A northern blot of ovarian RNA is shown that has been probed with Lar and cup cDNAs. The expression of the 8.4 kb *Lar* mRNA is specifically lost in the *Lar*^{bola1} ovaries. Similar results were obtained for *Lar*^{bola2} (not shown). (C,D) *Lar* is expressed in somatic and germline cells of the ovary. Whole-mount in situ hybridization was carried out of ovaries using a Lar cDNA probe. Both somatic cells and germ cells are labeled throughout much of oogenesis. In the germarium (C), strong expression starts in region 2b follicle cells. By stage 1 and in later egg chambers, *Lar* is expressed uniformly in both germ cells and follicle cells (C,D).

Weaker expression was apparent in germ cells at these stages as well. *Lar* expression continued as egg chambers budded from the germarium, and was robust in both somatic and germline cells of older follicles (Fig. 3C,D), in agreement with a previous survey (Fitzpatrick et al., 1995). Several antibodies were prepared against both intracellular and extracellular Lar domains (see Materials and Methods). Like the experience of other investigators, however, we found that none of these antisera would specifically label Lar in tissue (data not shown).

Polar cells and egg chamber planar polarity

A pair of specialized polar follicle cells lie at the anterior and posterior poles of ovarian egg chambers beginning at stage 3, and possibly earlier (Brower et al., 1981). Polar cells can be visualized by virtue of their high Fasciclin III (FasIII) expression levels (Patel et al., 1987) in stage 3 and later follicles. Polar cells must be specified even earlier, as they cease division in the germarium (Margolis and Spradling, 1995; Tworoger et al., 1999); however, markers specific for early polar cells are not available. To investigate whether polar cells define the poles of the basal actin pattern, we fixed and labeled wild-type egg chambers with Alexa-conjugated phalloidin and with anti-FasIII antibodies to reveal actin filaments and polar cells. These studies showed that the actin circles at the poles of stage 7 and later follicles were precisely organized around the polar cell pairs (Fig. 4A). In addition, the actin polarization was observed to arise gradually during egg chamber development proceeding from the poles toward the center. As a result, in stage 5-6 egg chambers, the actin was organized in the polar regions (Fig. 4B, arrow) but still not organized toward the center of the follicle (Fig. 4B, lower right). The observations that the polar cell pairs act as

Fig. 4. *La*r is required for follicle formation, polar cell development and oocyte patterning. (A) Actin fibers are polarized relative to the polar cells. A stage 7 egg chamber is shown that was stained with anti-FasIII (red) to mark polar cells (see also inset) and phalloidin to mark actin (green). Actin fibers circle the polar cell pair like parallels of latitude. (B) The actin orientation around the polar cells develops gradually during stage 5-6. A stage 5 egg chamber is shown in which the basal actin fibers circle the polar region (arrowhead), but remain disoriented in the middle of the chamber (lower right). (C) In a stage 7 *bola* egg chamber, actin remains unoriented with respect to polar cells. (E,F) *bola* follicles contain extra and ectopic polar cells. A stage 7 *bola* follicle containing three posterior polar cells (F, magnified in inset), and a stage 8 follicle containing three polar cell pairs are shown (E). (D,G) Ectopic polar cells generated by Hedgehog misexpression can influence actin polarization. A pair of ectopic polar cells (red staining) has oriented actin in the stage 7 follicle shown in D, as shown in the magnified regions (d',d''). Not all ectopic polar cell pairs (red staining) alter actin orientation, as shown in a different follicle (G). (I,K). Two germaria from homozygous *Lar^bola2* females stained for FasIII (red) and actin (green). In region 2b (brackets), germline cysts fail to acquire a lens shape or to span the width of the ovariole. Budding is frequently abnormal: one cell from the anterior cyst has been pinched off (K, arrow) and, rarely, long stalks form (I, arrow). (H,J) *Lar* is required for Oskar localization. Stage 9-10 wild-type (H) or *Lar^bola2* (J) follicles were stained with Oskar. Normally, Oskar protein accumulates at the posterior of the oocyte (J), but in more than 50% of *Lar^bola2* follicles, posterior Oskar localization was grossly abnormal (I)
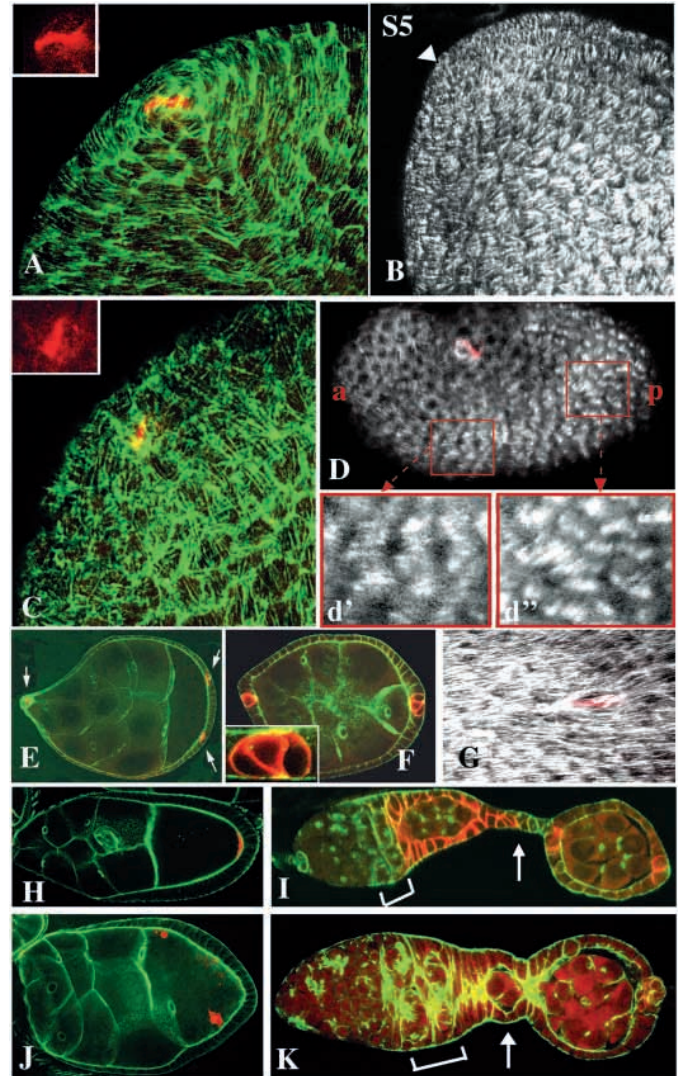
polarization centers, and that polarization proceeds from the poles towards the center of the follicle, suggests that the polar cells produce or stimulate production of a polarizing signal that is received by nearby cells and used to re-orient their planar cell polarity.

We have carried out similar studies on mutant egg chambers to investigate whether abnormalities in polar cell differentiation or function might be responsible for the failure of actin filaments to polarize in a large fraction of mutant egg chambers. Nearly half (47%) of the *Lar^bola* follicles contained extra polar cells (Fig. 4F). Instead of possessing exactly two FasIII-positive polar cells at each end, *bola* follicles contain an average of 2.3 anterior polar cells and 2.6 posterior polar cells (*n*=91). Moreover, 3.3% of the follicles contained an ectopic polar cell cluster of from 2-12 cells (Fig. 4E). All the follicles with extra polar cells, whether located at the poles or ectopically, failed to undergo actin alignment. However, the converse was not true. Many follicles were observed in which the number and location of polar cells was normal, but the actin fibers were not aligned (Fig. 4C). When actin alignment was observed in the mutant, it was always centered on the polar cells, as in wild type (data not shown). The correlation between extra polar cells and actin misalignment supports the idea that polar cell defects are responsible for the actin misalignment.

To further investigate whether polar cells act directly to organize actin polarity, we studied the effects of ectopic polar cells on actin organization. Misexpression of hedgehog protein (Hh) causes excess follicle cell proliferation and the production of ectopic polar cells (Forbes et al., 1996a: Forbes et al., 1996b; Zhang and Kalderon, 2000). We subjected flies carrying a heat shock promoter-hedgehog fusion gene to periodic heat shocks and analyzed their egg chambers for the presence of ectopic polar cells by FasIII staining and for actin polarity. As

expected, egg chambers bearing extra FasIII-positive cell pairs were found, and actin fibers sometimes circled around these ectopic cells, despite their location away from the true poles of the egg chamber (Fig. 4D). However, many other FasIII-positive cell pairs were found within fields of normally polarized actin filaments (Fig. 4G). The presence of these extra FasIII-positive cells did not usually cause a general disruption of actin organization, like that observed in egg chambers from *bola* mutant females. Most of the Hh-induced ectopic polar cells may not be fully differentiated, and consequently will lack actin polarizing activity.

bola mutations affect egg chamber production and oocyte polarity

Polar cells are normally specified in the germarium (Margolis and Spradling, 1995; Tworoger et al 1999). The presence of extra polar cells suggested that *bola* mutations might affect the process of egg chamber formation. Germaria from *Lar^bola* mutant females were found to frequently be abnormal (Fig. 4I,K). When wild-type germline cysts enter region 2b, they become lens-shaped and stretch to span the entire width of the germarium (see Fig. 1B). In 57% of germaria from *Lar^bola2*

females, the cysts in region 2b remain two-across and never become lens-shaped (Fig. 4I,K, bracket). Egg chamber budding is slowed and occurs abnormally (Fig. 4K, arrow), frequently causing additional round cysts to accumulate in region 3 of the germarium (see below), and occasionally producing composite follicles containing two cysts (not shown). Thus, defects caused by *Lar^bola* mutations are apparent as follicle cells begin to associate with germline cysts, and continue throughout the budding process These changes are likely to be related to the polar cell abnormalities and actin misalignment that become apparent later.

We suspected that egg chambers produced by *Lar^bola* females might also contain defects in oocyte polarity. To test this hypothesis, we stained egg chambers with antibodies specific for Oskar, whose localization at the posterior pole of stage 9-10 egg chambers represents a crucial step in posterior patterning and germ cell development. Strong staining of Oskar at the oocyte posterior was observed in all wild-type egg chambers (Fig. 4H), but in 20-30% of chambers from *Lar^bola* females, Oskar protein was present in one or more aggregates at other positions in the oocyte cytoplasm (Fig. 4J). The effects of the mutations on embryonic development could not be determined because the eggs produced by *Lar^bola* mothers never initiated embryonic development. The GV in *Lar^bola* follicles moved normally to the dorsal-anterior region and there were no defects in Gurken localization (data not shown), indicating that the defects were limited to the AP axis and possibly to the process of Oskar localization itself.

Lar function is required autonomously in posterior follicle cells for Oskar localization

To determine which cells require *Lar* function we carried out clonal analysis. Patches of marked *Lar* mutant cells were generated using FLP-mediated somatic recombination (see Materials and Methods) and the effects of different mutant cell groups on egg chamber development and polarity were determined. The effects of Lar mutation on Oskar localization are summarized in Fig. 5.

Oskar localization was found to depend on the genotype of the posterior follicle cells. When the approximately 70 posterior follicle cells were *Lar* positive, Oskar was always localized normally, regardless of the status of *Lar* function in the germline or in more anterior follicle cells (Fig. 5A). By contrast, when the posterior follicle cells lacked functional *Lar*, Oskar was normal only 15% of the time. In such chambers, a significant fraction of the Oskar protein was present as a central aggregate (Fig. 5B,C) or was absent altogether (Fig. 5D). We observed a number of follicles in which Oskar protein trailed from the posterior to a central aggregate (Fig. 5C), suggesting that posterior localization may have occurred normally, but not been maintained.

To address whether the action of posterior follicle cells in localizing or maintaining posterior Oskar was autonomous at the cellular level, we examined chambers that contained a clonal boundary within posterior follicle cells. In more than 70% of such cases, Oskar protein was found below the cells that were Lar$^+$, but was strongly reduced or absent under cells that bore a *Lar* mutation (Fig. 5F,G). The boundary of localized Oskar varied no more than one cell diameter from the position of the *Lar*$^+$ border.

Deng and Ruohola-Baker (Deng and Ruohola-Baker, 2000)

recently described similar effects of *LanA* clones in posterior follicle cells (Deng and Ruohola-Baker, 2000). As LanA has been reported to be a ligand for Lar in mammalian cells (O'Grady et al., 1998), we examined the distribution of LanA protein in developing egg chambers (Fig. 6J). LanA was abundant in the basal region of the follicle cells of stage 2-9 egg chambers. (Fig. 6J, arrowhead), but was not detected between the follicle cells and the oocyte (Gutzeit et al., 1991). Females mosaic for a lethal null allele of *LanA*, *LanA^9-32*, generated round eggs at a low frequency (Fig. 6J, inset), further supporting a link between LanA and Lar.

Lar function is not required autonomously in late stage follicle cells for planar actin polarization

Ovarioles mosaic for *Lar* exhibited all the actin alignment, extra polar cell and budding defects previously seen in homozygous *bola* mutants. We examined follicles at stage 7-8 to determine where *Lar* is required for actin polarization (Fig. 6, Table 1). There was no correlation between actin polarization and the genotype of the germline. Germline cysts mutant for *Lar* did not alter actin polarity if all or nearly all the follicle cells were *Lar* positive (Fig. 6A,B). By contrast, 52% of stage 7-8 egg chambers that did contain large patches (>33%) of *Lar* mutant follicle cells displayed severely abnormal actin polarity. Surprisingly, however, the position of the mutant cells did not correspond to the actin organization phenotype. Sometimes, actin polarized normally despite the presence of a large mutant clone (Fig. 6C,D). When actin was disrupted, the disruption did not stop at the boundary of the mutant follicle cells, as in the case of Oskar localization. Instead, both *Lar*-positive and *Lar*-negative cells were equally affected (Fig. 6E,F). The genotype of the polar cells was not an essential determinant, as we observed several cases in which actin was disorganized, despite the fact that the genotype of both polar regions was wild type. In other cases, actin fibers were organized normally around mutant polar cells (Fig. 6I). Although mutant follicle cells were required, no particular group of cells could be correlated with the actin defects in the stage 7-8 follicles.

A possible explanation for the lack of correlation between stage 7-8 follicle cell genotype and actin polarity is that *Lar* is required in cells that interact with polar cell precursors much earlier in development. Consequently, we examined the effects of *Lar* mutant germline or somatic cell clones in the germarium on cyst shape and follicle budding. When the germline lacked

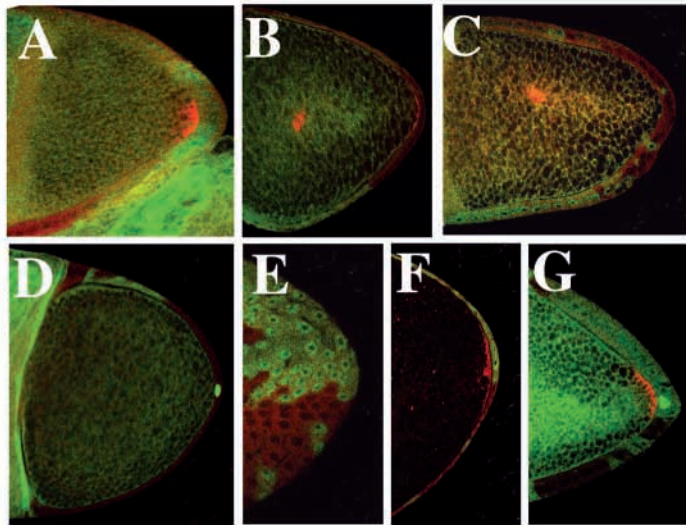### Table 1. Effect of *Lar* clones on follicle cell planar polarity

| Type of clone | Number | Polarization (% of egg chambers) | |
		Normal	Defective*
GL + FC+	46	100%	0%
GL − FC+‡	7	100%	0%
GL + FC$^{+/−}$§	46	82%	18%
GL + FC−¶	35	48%	52%

*Actin orientation deviates from the AP axis by more than 30° over > 20% of follicle surface.

‡Chambers were follicle cell (FC) positive if <20% of follicle cells were Lar⁻. Germ line (GL) clones in the absence of FC clones were infrequent, so this combination was rare.

§<33% of follicle cells were Lar⁻.
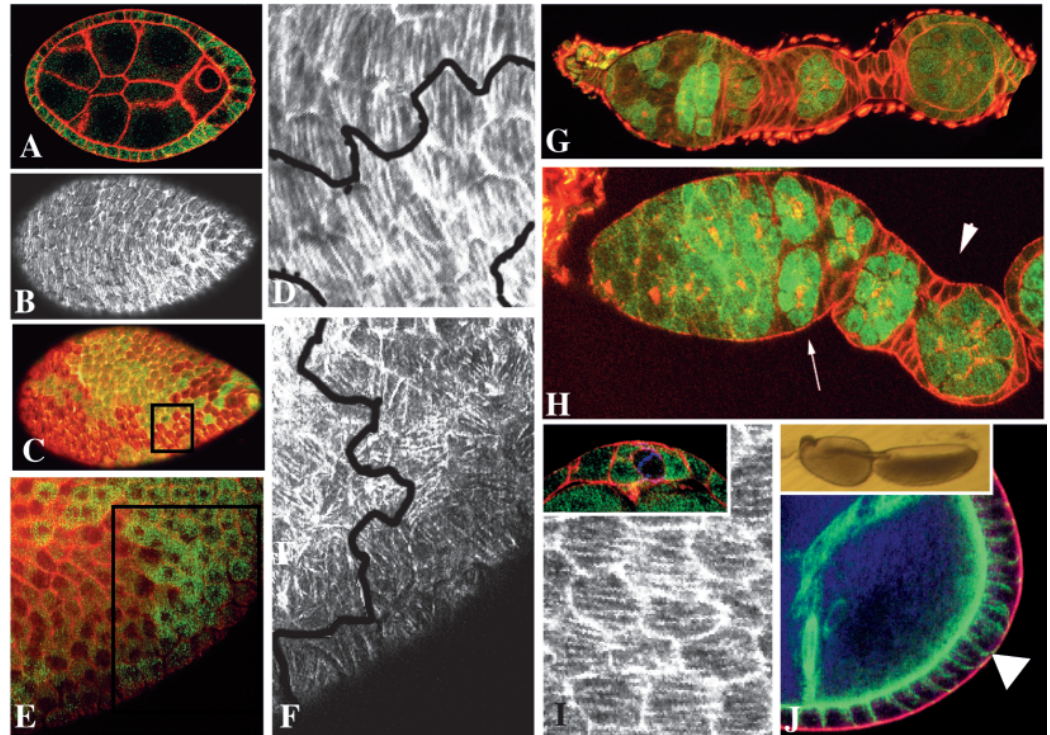
¶>33% of follicle cells were Lar⁻.

Fig. 5. *Lar* function is required autonomously in posterior follicle cells to localize Oskar. Egg chambers bearing follicle cell clones of *Lar*[5.5] were stained with antibodies for Oskar (red) and β-galactosidase (green). Normal posterior Oskar localization in stage 9 chambers was always observed if the 70 posterior-most follicle cells were wild type (A). By contrast, when posterior cells were mutant for *Lar*, Oskar was frequently present in a central aggregate (B,C) or absent altogether (D). When a clonal boundary split the posterior region (E), Oskar localization in the underlying oocyte was nearly cell autonomous (F; same chamber as E). The results are summarized in H. The types of clones and patterns of Oskar localization are indicated by drawings along the axes. NA, not applicable; *n*=83.

filaments within follicle cells during oogenesis. Although actin is known to be circumferentially aligned in stage 8-9 follicles (Gutzeit, 1990), little has been learned about the cellular and molecular mechanisms that are responsible. We found that actin filaments become disordered when follicles first formed by budding from the germarium. Polarization begins more than a day later during stage 5 and occurs gradually. First, actin orients around the polar cell pairs that lie at the anterior and posterior ends of the egg chambers. Polarization subsequently spreads toward the equator and eventually organizes the entire follicular layer.

There are several possible reasons for actin polarity to arise gradually. The follicle cells that surround a newly budded chamber derive from multiple sources, and have spent varying times in association with germ cells at the time of budding. Some follicle cells contact a germline cyst near the region 2a/2b junction or are born from them later by division and remain associated with the cyst cells. Other follicle cells likely migrate from the intercyst cell population, as it contains many more cells late in region 2b than the final number of stalk and polar cells (H. F. and A. S., data not shown). Some time may be required to globally polarize the cytoskeletal actin of follicle cells with these diverse origins. In addition, all follicle cells, except polar cells, continue to divide actively in early egg chambers, but neither their cell cycles nor division planes are synchronized or spatially coordinated. Consequently, full polarization might not be possible until the disruptive effects of mitotic divisions cease during stage 6.

Our observation that polar cells define the polarization axis of follicular actin suggests an additional reason that actin alignment develops gradually and after a substantial delay. The differentiation of polar cells themselves appears to be a gradual process. Polar cell precursors are specified in germarium region two, where they cease division long before the progenitors of main-body follicle cells (Margolis and Spradling, 1995; Tworoger et al., 1999). However, polar cell genes such as FasIII are not expressed specifically in these cells until much later, during stage 3. Polar cell differentiation may

Lar function, region 2b cysts never failed to become lens-shaped and region 3 never contained more than one round follicle in the process of budding. By contrast, when most of the follicle cells in region 2b and 3 were mutant (a relatively rare genotype), then 62% of the germaria contained such defects (Fig. 6G,H). Abnormal germaria frequently lacked *Lar* function in intercyst cells and contained an increased number of such cells (Fig. 6G,H). We suspected that polar cells were differentiating abnormally in such germaria, but this could not be directly addressed as markers that specifically label polar cell precursors in the germarium have not been found. Nonetheless, our experiments showed that lack of *Lar* function in region 2b-3 somatic cells frequently disrupts follicle formation and probably generates follicles with abnormal polar cells that fail to polarize normally during stage 6. By stage 7-8, the descendents of the crucial *Lar* mutant cells, which are not polar cell precursors themselves, may not occupy a consistent region of the follicle surface and cannot therefore be correlated with defective actin alignment.

DISCUSSION

The follicular epithelium becomes polarized gradually, beginning at the poles

Our studies clarify the mechanisms that order basal actin

Fig. 6. *Lar* function is not required autonomously in stage 7-8 follicle cells for planar polarity. Ovarioles bearing follicle cell clones of *Lar$^{5.5}$* were stained with Alexa-phalloidin (red) to reveal actin, and with anti-β-galactosidase (green) to mark *Lar*-positive cells. Actin polarization was unaffected by the Lar genotype of the germline (A,B). When egg chambers contained large clones of *Lar$^{5.5}$* follicle cells, actin polarity was either normal (C,D) or globally disrupted (E,F, see Table 1). Consequently, actin organization did not locally follow clonal boundaries. Defects in cyst shape (H, arrow) and follicle budding (G,H, arrowhead) were often seen when follicle cells in regions 2b and 3 were mutant. (I) A follicle with *Lar$^{5.5}$* mutant polar cells (FasIII$^+$lacZ$^-$ cell pair, inset). Despite this, the actin was normally polarized. (Actin, red; *lacZ*, green; FasIII, blue.) (J) Clones of *LanA$^{9-32}$* generate a low frequency of round eggs (inset). In all egg chambers, LanA protein was only observed on the basal side of the follicle cells (arrowhead), the location of the basement membrane. LanA, red; actin, green; vasa, blue.

be an ongoing process in early follicles and they may not acquire the capacity to organize actin filaments until stage 5, when alignment begins locally in nearby follicle cells. The behavior of ectopic polar cells generated by misexpressing Hedgehog was consistent with this interpretation. Some of the polar cell pairs induced local actin polarization while others had no effect (Fig. 4). Polar cell pairs that were able to orient actin in nearby cells may have been older, or more fully developed, than those that were ineffective.

Polarized follicle cell actin directs egg elongation

The molecular mechanisms that late in oogenesis cause eggs in many insect species to elongate along their anterior-posterior axis have long been a matter of speculation. Studies in *Drosophila* have suggested that egg elongation results when circumferential bands of follicle cell actin filaments (Gutzeit et al., 1991) cause forces generated at the time of nurse cell dumping to stretch the egg along the AP axis. 'Dumpless' mutations that disrupt the bundling of actin filaments (reviewed by Robinson and Cooley, 1995) or functioning of myosin II (Edwards and Kiehart, 1996; Wheatley et al., 1995) block both the transfer of nurse cell contents into the oocyte and egg elongation. *bola* mutations allow these effects to be dissociated. Nurse cell dumping, which depends on static actin bundles located within nurse cells, and probably requires actin-mediated contraction to drive nurse cell contents into the oocyte, is unaffected. However, follicles lacking somatic *bola* function frequently fail to elongate, indicating that oriented follicle cell actin filaments are crucially important for elongation. Eggs with an unpolarized follicular epithelium may fail to elongate because contractile forces are directed

isotropically, blocking any net shape change. Consistent with this model, the volume of the bola eggs was normal, and their shape changed little during or after dumping. Disruption of basal actin filament polarization in *kugelei* mutants also is associated with round eggs (Gutzeit et al., 1991), further supporting a functional role for actin polarization as a 'molecular corset' that directs egg elongation along the anterior-posterior axis. Identifying genes that cause round egg production in somatic cell clones is likely to define additional genes required for this polarization pathway.

Follicle cell actin polarization and planar cell polarity

Sheets of epithelial cells become polarized along both the apical-basal axis and in the plane of the epithelium (reviewed by Tepass, 1997). Planar cell polarity in epithelial tissues such as in the imaginal discs involves signals transmitted by an unknown ligand(s) that differentially activate the Frizzled receptor in nearby cells (reviewed by Schulman et al., 1998; Bray, 1999). For example, in the eye and wing discs, the planar polarity signal is sent by cells lying along the dorsal-ventral border. A gradient of Frizzled activity in cells lying different distances from the source cells transduces polarity information via a distinct pathway within the cell layer. In the eye disc, this involves differential activation of Notch signalling (Cooper and Bray, 1999; Tomlinson and Struhl, 1999). Differences in Notch activation control cell fate decisions that result in an inverted polarity of photoreceptor clusters with respect to the dorsal-ventral border (equator).

Biologically, the alignment of follicle cell basal actin filaments described here represents an analogous process of

planar polarity to those studied previously in imaginal disc derivatives. However, it remains to be determined to what extent these events use the same genetic pathways described in disc tissues. Polarizing a spherical tissue such as an ovarian follicular epithelium is likely to involve planar polarity signals from particular subregions whose receipt by follicle cells conveys positional information. Our data suggest that polarity signals are likely to derive from the poles of the follicle, rather than from a line of cells. Follicle cells may align their actin filaments towards other cells that receive the polarizing activity at an identical level. Our data make it unlikely that Lar acts as a primary receptor for polarity information in stage 6 and up follicles. Clones of $Lar^{5.5}$ and $Lar^{13.2}$ alleles produced ectopic veins in the wing, but there was little effect on the orientation of wing hairs (data not shown). Thus, the *Lar* pathway is redundant in this tissue.

Lar requirement for Oskar localization suggests a role for the ECM

The structure and properties of Lar that have been revealed by studies of both the *Drosophila* and mammalian counterparts suggest how it might act as a polarity transducer. Lar is a receptor-like tyrosine phosphatase that is important for axon pathfinding in *Drosophila* (reviewed by Lanier and Gertler, 2000). A family of closely related Lar-like phosphatases also exists in the mouse. Mouse Lar is required for the development of the mammary epithelium (Schaapveld et al., 1997), while the related PTPsigma functions in neuronal and epithelial development (Wallace et al., 1999). The Lar extracellular domain contains three immunoglobulin and several fibronectin type III domains and is thought to transduce signals via its cytoplasmic tyrosine phosphatase domains after activation by adhesion to the ECM or by small protein ligands. However, physiological Lar ligands have not been documented. In mammalian cells, binding of the ECM laminin-nidogen complex to a specific fibronectin domain in mammalian Lar causes changes in the actin cytoskeleton, suggesting that Lar can transduce information from the ECM (O'Grady et al., 1998).

A clue to the mechanism of *Lar* action comes from our studies on its role in Oskar localization. Posterior follicle cells must express *Lar* to ensure that Oskar is localized properly at the oocyte posterior (Fig. 5). Previously, Deng and Ruohola-Baker (Deng and Ruohola-Baker, 2000) reported that when posterior follicle cells lack the ECM component Laminin A (LanA), Oskar localization is usually disrupted. They suggest that *LanA* and ECM mediate the posterior follicle cell-oocyte signal. As Lar has been reported to bind to the laminin-nidogen complex (O'Grady et al., 1998), Lar might act as the LanA receptor in this pathway.

However, it remains less clear how a signal initiated by an interaction between LanA in the ECM and Lar on a posterior follicle cell would be transduced into the oocyte. Deng and Ruohola-Baker propose that some LanA-containing ECM resides between the apical surface of the posterior follicle cells and the oocyte, and that LanA interacts directly with the oocyte surface. We propose an alternative model. We only observed LanA on the basal side of the follicle cells, and observed that *LanA* clones could induce round eggs (Fig. 6J). These observations and the follicle cell autonomous requirement of *Lar* for Oskar localization argue that the LanA signal is received by Lar on the basal surface of the follicle cells and leads to some change in the receiving cells that is transduced to the oocyte. This could be via a secondary signal, or by changes in the structural or adhesive properties of the cells that can locally affect the oocyte surface with which they come into contact. Lar mutation did not affect the apical basal polarity of follicle cells, as the apical-basal asymmetry of actin staining was maintained and multiple-layered follicle cells were never observed.

Polar cells are likely to play a key role in polarizing actin in stage 5-6 follicle cells

Several of our observations support the idea that polar cells organize the actin planar polarity. Actin polarity focuses around both the anterior and posterior polar cell pairs, and spreads from the poles towards the equator of the follicle. Additionally, ectopic polar cells induced by Hh expression sometimes have actin polarizing activity. These findings suggest that polar cells send a signal that orients the actin alignment in circumferential direction. In follicles where actin fails to become aligned, the polar cell signal may have been blocked or reduced, despite the presence of morphologically recognizable polar cells. Not all the ectopic polar cells induced by Hh expression affected the actin alignment of nearby follicle cells, supporting the idea that polar cell can express differentiation markers, but still be incompetent as polarizing centers. However, Lar was not required in polar cells, because we observed follicles with normally oriented actin, despite the presence of a Lar mutant polar cell pair.

The apparent non-autonomy of Lar on actin polarity suggests an indirect action on polar cell differentiation

The autonomy of the effects of Lar on Oskar localization contrasts with its apparently non-autonomous action on follicle cell planar polarity at stages 6-8. There was no relationship between actin alignment and the Lar genotype of particular somatic cells in stage 7 or later follicles. This observation can be rationalized by postulating that Lar acts on a subset of cells that is required for polar cell specification and differentiation. Lar is required in region 2b of the germarium, a time when polar cells are not yet fully specified, and in its absence somatic cell behavior and follicle formation is compromised. We propose that the intercyst cells (Fig. 1B) interact with the polar cell precursors in a Lar-dependent manner. Intercyst cells mostly become main body follicle cells and do not remain a recognizable subpopulation; hence, it is not possible to infer their genotype from later stage egg chambers and compare it with the state of actin polarization. We did note a correlation between mutant intercyst cells and pinching defects (Fig. 6G,H). Thus, a relationship may exist between the intercyst cell genotype and actin polarization that cannot be followed with existing markers.

One possibility is that Lar acts in a similar manner in intercyst cells and in posterior follicle cells. The germarium contains peripheral somatic cells whose basal actin fibers are aligned perpendicular to the AP axis (Fig. 2E). Integrin is aligned in a similar manner, suggesting that the basement membrane is correspondingly organized (H. F and A. S., unpublished). As budding proceeds, Lar may be required to interpret polarity information from the basement membrane as

part of the process that partitions cells into main body and polar cells. Alternatively, Lar may act in a different manner within these intercyst cells to assist in polar cell specification and differentiation.

The Lar requirement for polar cell determination provides an explanation for another interesting fact. The phenotypic effects of Lar mutations we observed are very similar to weak mutations in Notch or other Notch-pathway genes. Notch mutants, like those in Lar, cause the production of extra polar cells, interfere with egg chamber budding and disrupt the anterior-posterior axis of the oocyte (Ruohola et al., 1991). Notch signalling is required for polar cell specification (Ruohola et al., 1991; Tworoger et al., 1999). Mutations in either Lar or Notch may cause similar disruptions in polar cell differentiation and hence similar downstream effects on egg chamber development and patterning.

The ECM may transiently store polarity information

Our experiments suggest a novel function for the ECM during follicle cell development – the storage of patterning information for later use. Somatic cells maintain an ECM that surrounds the germarium; when a follicle buds off, it contains a portion of this ECM in the basement membranes of its component cells. Our studies emphasize that this ECM may be a repository of polarity information that is used at critical times when polarization and cell specification are taking place. During follicle budding, the AP axis of the new chamber is correlated with the differentiation of two pairs of polar cells at each terminus. An interaction between posterior follicle cells and the oocyte ensures that the germline AP axis will correspond to the somatic axis (Godt and Tepass, 1998; Gonzelez-Reyes and St Johnston, 1998). We suggest that at about the same time, somatic cell interactions ensure that exactly four correctly positioned polar cells differentiate per follicle. This requires Lar-dependent readouts from the same ECM that the interacting cells contribute to polarizing. In stage 8, posterior follicle cells are likewise guided to maintain localized Oskar over an appropriately sized polar region. In both cases, cells that helped synthesize an ordered ECM later use it in a Lar-dependent manner for additional and possibly more refined patterning. The interactions we have studied may serve as a model for the roles of the ECM and of Lar signalling in the development of other epidermal and neural cells.

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